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Chair's introduction

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When I give an introductory lecture on anaphylaxis to medical students, I usually start with the story of a child in our neighbourhood who had a peanut allergy. While he was at a friend's home he was served ice cream. He read the ingredients on the carton, and saw that peanuts weren't mentioned. However, on his first mouthful he realized from the rapid onset of symptoms that peanuts must have been included. He was rushed to the emergency room, but there apparently was a delay before he received epinephrine (adrenaline) and he died. It turned out that while peanuts in fact were not listed on the carton, they were in the candy bar that was mentioned in the ice cream's ingredients. This phenomenon, anaphylaxis, a catastrophic and sometimes fatal allergic reaction to an otherwise innocuous substance, represents arguably the most grotesque imbalance between the cost and benefit of an immune response.

As Johannes Ring will tell us in detail, it turns out that observations of anaphylaxis extend far back in antiquity. For example, it is thought that Pharaoh Menes died from a reaction to a wasp sting some 4500 years ago. More recently, about 100 years ago, in an attempt to develop an anti-toxin to the venom of the Portuguese man-of-war (*Physalia physalis*), Charles R. Richet and Paul Jules Portier instead discovered anaphylaxis. They named the phenomenon anaphylaxis, taking 'phylaxis' from the Greek for 'immunity' or 'protection', and, according to one account, adding the 'ana' simply to make the term euphonic. This history is detailed in Estelle Simon's wonderful book *The Ancestors of Allergy* (Simons 1994). Apparently, receiving the Nobel Prize can induce paroxysms of modesty in those so honoured. Richet wrote that, 'The discovery of anaphylaxis is not at all the result of deep thinking, but of simple observation, almost accidental. It had no other merit than that of not refusing to see the facts which presented themselves before me completely evident.'

Since the original descriptions of anaphylaxis in the scientific literature 100 years ago, our understanding of this phenomenon has come a long way. There is no doubt that the activation of mast cells is a key event in many forms of anaphylaxis, and many investigators deserve credit for elucidating the mechanisms responsible for such mast cell (and basophil) activation in response

to environmental substances. Several investigators made important contributions to the discovery and characterization of the critical antibody isotype involved in anaphylactic reactions in humans, that was ultimately named IgE (Bennich et al 1968), including Kimishige and Teruko Ishizaka (Ishizaka et al 1966), S. G. O. (Gunnar) Johansson and Hans Bennich (1967) and Denis Stanworth (Stanworth et al 1967). Henry Metzger, who is a participant at this symposium, is responsible for defining the receptor for IgE as a single molecular entity (Metzger 1992) and he and Jean-Pierre Kinet cloned and characterized the genes for the three components of the tetrameric high affinity receptor for IgE, FcεRI (Metzger 1992, Turner & Kinet 1999). It is now known that the aggregation of the receptor, FcεRI, induces the activation of the mast cell, resulting in the release of diverse mediators, many of which can induce the pathophysiology of anaphylaxis (Metzger 1992, Turner & Kinet 1999).

As it turns out, IgE does not only 'sensitize' or 'prime' mast cells (and basophils) to undergo activation and to release mediators when the cells encounter the antigen for which that IgE has specificity. IgE also has the ability, independent of its antigen specificity, to enhance significantly the effector function of these cells. Two groups reported that there is a strong positive correlation in humans between levels of circulating IgE and levels of surface expression of FcεRI on blood basophils (Conroy et al 1977, Stallman et al 1977, Malveaux et al 1978). In 1985, using RBL (rat basophil leukaemia) cells, two groups reported that the presence of IgE in the media could cause a modest increase in the number of FcεRI expressed on these cells, by inhibiting the elimination of the receptor from the cell surface (Furuichi et al 1985, Quarto et al 1985). Subsequently, Don MacGlashan's group (Hsu & MacGlashan 1996) and my group (Yamaguchi et al 1997) reported that this IgE-dependent enhancement of FcεRI surface expression can be quite striking, quantitatively, in non-neoplastic *in vitro*-derived (Hsu & MacGlashan 1996, Yamaguchi et al 1997) or *in vivo*-derived (Yamaguchi et al 1997) mouse mast cells, and that this phenomenon has significant functional consequences. These include enhancing the capacity of the cells to bind more IgE, and thereby potentially to express sensitivity to an expanded panel of unrelated antigens, lowering the antigen concentration necessary to activate the cells, increasing the amounts of mediators released by the cells at a given concentration of antigen, and, at least in mouse mast cells, rendering the cells able to release additional products (including interleukin 4) that may not be detectably released by cells that express lower amounts of the receptor.

It is now clear that the basic findings regarding the IgE-dependent enhancement of FcεRI surface expression, and its functional consequences, first established in the mouse system, also occur in humans (Kawakami & Galli 2002, MacGlashan et al 1997, Yamaguchi et al 1999). The implications of these findings are that subjects with high levels of IgE, and therefore with high levels of expression of FcεRI on

the surface of mast cells and basophils, have key effector cells of anaphylaxis, namely, mast cells and basophils, that are primed to be more exquisitely sensitive to antigen, and to release larger amounts of mediators in response to antigen challenge, than are those in subjects with lower levels of IgE.

Thus, in the approximately 100 years since the description of anaphylaxis by Richet and Portier, it has become possible to outline a classical pathogenic sequence in the production of this reaction: once a subject produces IgE to a specific antigen, IgE-bearing mast cells (located in the tissues) and basophils (in the circulation) can recognize and undergo activation in response to that antigen upon its systemic distribution, and then can rapidly release large amounts of diverse mediators, resulting in the expression of significant pathology in multiple organ systems (Wasserman 2001).

However, a number of important issues are as yet unsettled, and several of these will be addressed in the discussions at this meeting. As we will hear, there is not yet an international consensus on the classification and nomenclature of anaphylaxis, especially when one considers those expressions of the disorder that reflect the activation of mast cells and other effector cells independently of IgE. Even if one considers only those examples of anaphylaxis that result from antigen-dependent activation of mast cells and basophils, it is not clear, in humans, whether some forms of this disorder may include a significant role for antibody isotypes other than IgE. In mice, by contrast, it is well known that either IgE or IgG1 antibodies, acting via FcεRI or FcεRIII, respectively, can induce potentially fatal anaphylactic reactions (Miyajima et al 1997, Strait et al 2002).

Are mast cells and basophils the only significant cellular sources of mediators in anaphylaxis? How do we explain the acute as opposed to late stages of the reaction? Why do only some individuals develop this kind of reactivity, and to what extent does this reflect genetic, as opposed to environmental, factors? Finally, what more can be done to discover improved treatments for this disorder, and, in the area of public policy, to use effectively what we already know about the causes, management and treatment of anaphylaxis to improve, and in some cases, to save, the lives of those afflicted with this devastating condition?

I expect that these questions, and other important topics in the field of anaphylaxis, will be addressed in detail by the speakers at this symposium. Each of the formal presentations will be followed by extensive periods of discussion. In addition, there also will be several general discussions throughout the meeting that will permit us to look at some of the major topics in more detail.

I will close these opening remarks by reminding us of the important contributions to this field of Mary Hewitt Loveless. Mary Loveless earned her undergraduate and medical degrees at Stanford and went on to perform her work on immunity and allergy in New York. By showing that injections of extracts of the venom sacs of stinging insects (Hymenoptera) could essentially cure patients of

fatal anaphylactic reactivity to the stings of such insects (Fackler & Loveless 1956), she opened the field of antigen-specific immunotherapy of anaphylaxis (and other disorders). Even though this important discovery was published almost 50 years ago, anaphylaxis unfortunately remains a major problem for many individuals, particularly those with food allergies. And, despite much recent progress in the understanding of the pathogenesis, pathophysiology, prevention and treatment of anaphylaxis, most tragically, some patients still die of this disorder. So, despite impressive progress in the understanding and management of anaphylaxis over the last 100 years, there is much work still left to do.

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History and classification of anaphylaxis

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Abstract. Anaphylaxis is the maximal variant of an acute allergic reaction involving several organ systems. The phenomenon itself is old, but it was recognized and named at the beginning of the 20th century by Richet and Portier. The clinical symptoms of anaphylaxis affect various organs, most commonly starting in the skin and proceeding to the respiratory tract, to gastrointestinal involvement and to cardiovascular symptoms, and finally to cardiac and/or respiratory arrest. Anaphylaxis *stricto sensu* is an immunological reaction, mostly mediated by IgE antibodies, but also by IgG or IgM antibodies (immune complex anaphylaxis). There are cases with similar clinical symptomatology without detectable immunological sensitization which are called pseudo-allergic or anaphylactoid reactions. In the newer nomenclature, some authors tend to include these under the heading of 'anaphylaxis' which has then to be defined as an acute systemic hypersensitivity reaction. The most common elicitors of anaphylaxis include drugs, foods, additives, but also other allergens as well as physical factors (cold, heat, UV radiation). The clinical outcome — the intensity of the reaction — is not only influenced by the degree of sensitization, but also by concomitant other factors: sometimes, individuals only develop anaphylaxis after simultaneous exposure to the allergen and an infection, physical exercise, psychological stress or concomitant medication (e.g. β blockers). The term 'summation anaphylaxis' has been proposed for this phenomenon which probably underlies many cases of so-called idiopathic anaphylaxis. In patients with insect venom anaphylaxis, decreased levels of plasma angiotensin have been measured in inverse correlation to the severity of the reaction. Certain differential diagnoses have to be distinguished from anaphylaxis. Every patient with a history of anaphylaxis should undergo allergy diagnosis with the aim to detect the eliciting agent, characterize the relevant pathomechanism (e.g. IgE-mediated reaction) and to offer a tolerable alternative (in food or drug allergy). In clear-cut IgE-mediated anaphylaxis, allergen-specific immunotherapy (hyposensitization) is the effective causal treatment, with success rates of 90% in insect venom anaphylaxis.

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Allergic diseases have been increasing in prevalence in most countries over the last few decades (Ring et al 2001) and are often not taken seriously because they are not regarded as contributory to increased mortality rates. This rather superficial

opinion has been contradicted by a variety of life-threatening emergencies in allergology (e.g. fatal asthma attack, anaphylaxis, laryngeal [angio-]oedema, severe serum sickness with vasculitis and nephritis, bullous drug eruptions like toxic epidermal necrolysis) among which anaphylaxis undoubtedly represents the most acute condition.

History

The phenomenon of anaphylaxis is old and has been described in ancient Greek and Chinese medical literature. The first documented anaphylactic patient might have been pharaoh Menes who died 2640 BC from the sting of a wasp, as hieroglyphs tell (Wadell 1930).

The phenomenon was only clearly recognized in 1901 when Charles Richet and Paul Portier were doing their experiments on the yacht of the prince of Monaco and later on in the laboratory in Paris, trying to immunize dogs with *Actinia* extracts (Portier & Richet 1902). When, contrary to the expectation, after repeated injections, the animal died under dramatic circumstances, Richet — who was called by Portier into the lab — immediately recognized that there was something new ('C'est un phénomène nouveau, il faut le baptiser!') and wanted to find a name for it. What he wanted to express was 'lack of protection' and should have been 'aphylaxis' (Greek α privativum = negation); however, for euphonic reasons, he preferred 'anaphylaxis', a term which rapidly spread all over the world; for its description Richet won the Nobel prize in 1913.

This discovery, describing an obvious damage by immunization — while earlier immunization was only connected with the positive and desired effect of protection against pathogenic organisms — subsequently led to the creation of the term 'allergy' by Clemens Freiherr von Pirquet in 1906 (von Pirquet 1906).

Later on, researchers realized that similar symptoms (Hanzlik & Karsner 1920) can be elicited by the injection of histamine in individuals or could occur in animals not previously sensitized ('anaphylactoid reactions') (Lorenz et al 1977, Kind et al 1972).

Epidemiology

There is limited knowledge about the exact prevalence and incidence of anaphylaxis in the general population and in different age groups. Some estimates of insect-sting anaphylaxis range between 1 and 3% (Müller 2001, Yocum et al 1999). For drug-induced anaphylaxis, different incidence rates have been reported for different drugs (e.g. prevalence of penicillin allergy 2%; fatal anaphylaxis 1:50 000–1:100 000).

Clinical symptoms

Clinically, anaphylaxis represents a syndrome of different symptoms involving various organs which may develop either alone or simultaneously or subsequently, most commonly

- starting in the skin (pruritus, flush, urticaria, angioedema) and the neighbouring mucous membranes (itchy palate, paraesthesia in pharynx, genital mucosa) are often the first symptoms
- proceeding to the respiratory tract (sneezing, rhinorrhoea, hoarseness, dysphonia, laryngeal oedema, cough, laryngeal obstruction, bronchospasm, respiratory arrest)
- abdominal symptoms (nausea, cramps, vomitus, defecation, diarrhoea, also miction and uterus cramps occur)
- and cardiovascular symptoms (tachycardia, blood pressure changes — not necessarily hypotension, but also transient-type hypertension has been observed as first symptom — arrhythmia, shock, cardiac arrest). Primary cardiac manifestation in anaphylaxis has been observed in ECG-changes (T-flattening, supraventricular arrhythmia, AV block) (Pavek et al 1982, Marone et al 1995). Marked changes of central venous pressure are common. During anaphylaxis, myocardial infarction has occurred (Cistero et al 1992, Wagdi et al 1994).

Prodromi of anaphylaxis comprise paraesthesia on palms and soles, a metallic 'fishy' taste, anxiety, sweating, headache or disorientation.

Several attempts have been made to develop grading scales for severity scoring of anaphylaxis which differ in some respects (Mueller 1966, Ring & Messmer 1977, Ansell 1990). We proposed in a study describing 248 anaphylactoid reactions and observing 200 906 intravenous infusions of colloid volume substitutes, a simple scoring system from I to IV which is immediately useful with regard to acute therapy without need for long reflection (Table 1).

Although the clinical symptoms of anaphylaxis are rather characteristic, some differential diagnoses have to be considered (Table 2).

Pathophysiology

Anaphylaxis *stricto sensu* is an immunological reaction mostly mediated by IgE antibodies on the surface of mast cells and basophil leukocytes which, after a bridging with an at least bivalent allergen, trigger the secretion of preformed and newly synthesized mediators. In spite of our knowledge of mast cell activation and IgE antibodies, the exact mechanisms of amplification are not yet understood

TABLE 1 Grading of anaphylactic reactions according to severity of clinical symptoms

	Symptoms			
Grade	*Dermal*	*Abdominal*	*Respiratory*	*Cardiovascular*
I	Pruritus Flush Urticaria Angioedema			
II	Pruritus Flush Urticaria Angioedema (not mandatory)	Nausea Cramping	Rhinorrhoea Hoarseness Dyspnoea	Tachycardia (> 20 bpm) Blood pressure change (> 20 mmHg systolic) Arrhythmia
III	Pruritus Flush Urticaria Angioedema (not mandatory)	Vomiting Defecation Diarrhoea	Laryngeal oedema Bronchospasm Cyanosis	Shock
IV	Pruritus Flush Urticaria Angioedema (not mandatory)	Vomiting Defecation Diarrhoea	Respiratory arrest	Cardiac arrest

bpm = beats per minute.

TABLE 2 Differential diagnoses of anaphylactoid reactions

Pharmacological/toxic drug effects
Hyperventilation
Vasovagal reaction
Globus hystericus
Syncope (cardial, cerebral)
Carcinoid syndrome
Seizure diseases
Bolus aspiration
Pulmonary embolism
Hypoglycaemia
Artificial anaphylaxis (Munchausen syndrome)

which allow a healthy individual to be killed by a few micrograms of an allergen within minutes.

Apart from IgE, other antibodies may also elicit anaphylaxis via immune complex formation and complement activation (immune complex anaphylaxis), (Smedegard et al 1979, Richter et al 1980, Ring 1978). Clinical examples are anaphylactic reactions to blood products, xenogeneic proteins as well as dextran (Ring & Messmer et al 1977, Hedin et al 1976).

Apart from these clear-cut immunologically mediated reaction patterns, there are cases with very similar clinical symptomatology of anaphylaxis without detectable immunological sensitization (antibodies or sensitized cells) which have been called pseudo-allergic or anaphylactoid reactions. The mechanisms of these reactions are much less well-understood (Table 3) and include direct liberation of vasoactive mediators (e.g. histamine), general mast cell or basophil activation with release of other mediators, activation of the complement or other plasma protein systems (coagulation, kallikrein-kinin) as well as neuropsychogenic reflex mechanisms. It is known that psychological stress alone can lead to increased plasma histamine levels (Irie et al 2002).

In the end phase of the anaphylactic reaction, similar pathophysiological changes occur which are relevant for the clinical symptoms with post-capillary plasma exudation, microcirculatory disturbance with decreased capillary pressure and perfusion and erythrocyte stasis (Withers et al 1998, Endrich et al 1979, Fisher 1986, Sudhakaran et al 1979). Mast cell dependent anaphylactic reactions go along with the secretion of mast cell tryptase — preferably β-tryptase — in the serum which still can be detected even hours (sometimes post mortem) after a reaction (Schwartz et al 1994, Brockow et al 1999).

The amount of mediator release from mast cells and basophils depends not only on the serum concentration of IgE antibodies or the concentration of allergen or other elicitors, but is influenced by non-specific factors like acute infection, physical exercise, psychological stress, concomitant medication, such as β blockers or angiotensin-converting enzyme (ACE) inhibitors. These influences

TABLE 3 Possible mechanisms of non-immune anaphylactic (pseudo-allergic) reactions

Direct release of mediators (e.g. histamine)
Direct activation of complement system
Activation of the coagulation system
Interaction with kallikrein-kinin system
Shift in eicosanoid metabolism toward leukotriene formation
Platelet activation
Psychoneurogenic reactions

may — by the action of cytokines, like interleukin 3, 4, 13 or others — influence the 'releasability' of mediator-secreting cells and help to explain the well-known clinical fact that sometimes patients only react under certain circumstances when several eliciting factors act simultaneously (e.g. infection+allergen, exercise+ allergen; Sheffer & Austen 1980, simultaneous exposure to different relevant allergens, etc). The term 'summation anaphylaxis' or 'augmentation anaphylaxis' has been proposed for this phenomenon which seems to be much more common than previously thought and probably underlies many cases of so-called 'idiopathic anaphylaxis' (Table 4).

Recently, some authors have included the non-immunologically mediated immediate-type reactions also under the heading 'anaphylaxis'; then, anaphylaxis would have to be defined as 'acute generalized immediate-type hypersensitivity reaction' (Johansson et al 2001).

Problems in terminology arise from the fact that classifications are attempted at different levels, either coming from clinical symptoms or from pathophysiology. So the terms may have different meanings and furthermore, our knowledge, especially regarding pseudo-allergic reactions, is so limited that classifications always remain speculative in nature. It should be stressed that the term 'pseudo-allergic' or 'non-immune' anaphylaxis is negatively defined in that it is not possible to detect immunological sensitization in the serum or at the cellular level. Possibly, with advanced technology, such reactions may be turned from pseudo-allergic anaphylactoid reactions into allergic anaphylactic reactions. From a clinical point of view, the broader meaning of 'anaphylaxis' seems acceptable and should not lead to confusion when the further distinction into immunologically mediated (IgE, IgG or others) or non-immunological (pseudo-allergic) is kept in mind!

During anaphylaxis, the organism has a variety of systems to counteract the untoward effects of the suprarenal hormones (stress), but also the rennin–angiotensin system. We could show that during drug-induced anaphylaxis under controlled conditions, angiotensin II concentrations sharply increase in urine together with clinical symptoms; this also could explain why sometimes initial

TABLE 4 Summation anaphylaxis (symptoms only after exposure to a combination of influencing factors)

Acute infection
Mental stress
Emotional stress
Physical exercise
Concomitant allergen exposure (indoor, outdoor, food)
Intake of medications (cyclo-oxygenase inhibitors, β-blocking agents)

hypertension is observed prior to hypotension in severe anaphylaxis (Rittweger et al 1994). In a series of patients with insect-venom anaphylaxis, we found significantly decreased plasma levels of components of the rennin–angiotensin system, and also in a patient with unexplained idiopathic anaphylaxis (Hermann & Ring 1993).

Allergens and elicitors

The most common elicitors of anaphylaxis are drugs, proteins, foods, aeroallergens, additives, body fluids, latex and microbial antigens, but also physical factors (Table 5). However, the total spectrum of elicitors is much broader, even anaphylaxis to ethanol has been described (Przybilla & Ring 1983). Rare cases of passive transfer by IgE antibodies via blood transfusion as well as attempted suicide (penicillin-allergic nurse) have been reported. Murder has been attempted by eliciting anaphylaxis in the detective literature. Also anaphylaxis factitia ('Munchausen's syndrome') exists (Ireland et al 1967). The eliciting agent may contact the organism via the air (fish allergens in volatile form around fish stores, latex allergens in operation theatres or rooms decorated with balloons), via the skin surface (contact anaphylaxis) (Ring et al 1986) but mostly after oral or parenteral intake.

TABLE 5 Elicitors of anaphylaxis (including anaphylactoid reactions)

Drugs
Foods
Drug and food additives
Occupational substances (e.g. latex)
Animal venoms
Aeroallergens
Seminal fluid
Contact urticariogens
Physical agents (cold, heat, ultraviolet radiation)
Exercise
Echinococcal cyst
Summation anaphylaxis
Underlying disease
Complement factor 1-inactivator deficiency
Systemic mastocytosis
Idiopathic (?)

Patient management

Every patient with a history of anaphylaxis should undergo allergy diagnosis which has to include three steps:

- detection of the eliciting agent
- characterization of the relevant pathophysiology
- offering a tolerable alternative (Ring & Behrendt 1999).

For prophylaxis, this means abstaining from polypragmatic pharmacotherapy. Equally important are endeavours of the pharmaceutical industry to produce better and less allergenic drugs. Predictive testing for these purposes (namely, characterization of IgE-inducing allergens) has to be improved.

Knowledge of possible complications is the basis of successful therapy. This implies education of the informed patient and his surroundings as well as improved declaration laws.

In clear-cut IgE mediated anaphylaxis, allergen-specific immunotherapy is the effective causal treatment with success rates of over 90% (Przybilla et al 1987). Attempts of 'hyposensitization' in certain types of drug allergy have been successful. In only few cases, specific induction of tolerance against xenogeneic horse immunoglobulin (Ring et al 1974, Jones et al 1976) or by hapten inhibition in dextran anaphylaxis have been proven successful (Laubenthal 1986).

Treatment of the acute anaphylactic episode follows the severity of symptoms (Messmer 1983) and includes the intramuscular use of epinephrine (adrenaline) as soon as severe respiratory involvement or hypotension occurs. However, it has to be recalled that epinephrine, even if used correctly, does not guarantee a successful outcome. In spite of early and adequate epinephrine, fatal anaphylaxis has been described (Lockey et al 1987). Furthermore, epinephrine may induce severe cardiac arrhythmia up to ventricular fibrillation, especially in elderly patients (Sullivan 1982).

Outlook: problems still to be solved

In spite of all our increasing knowledge in modern experimental and clinical allergology, anaphylaxis still represents a major problem both for researchers and clinicians.

Many more studies regarding pathophysiology, especially with regard to the non-IgE-mediated or IgE-independent mechanisms in the development of anaphylaxis, are needed.

Better techniques to study the involvement of different cell populations and mediators (differential release, such as increased histamine release with normal eicosanoid secretion or vice versa) have to be considered.

In diagnostic work, the detection of the eliciting agents is a major difficulty. Reliable skin tests or *in vitro* tests only exist for some protein allergens and few drugs as haptens. There is no *in vitro* or skin test for pseudo-allergic reactions.

Provocation tests under blinded conditions are the only reliable tool in many cases, but go along with a significant risk and, therefore, have to be performed under emergency and, preferably, in-patient conditions. Particularly difficult is the question of provocation tests in parenterally applied substances such as volume substitutes, radiographic contrast media or anaesthetic agents where the performance of a provocation in adequate dose poses also ethical questions.

There are major problems regarding the prognosis as well as the definition of risk factors for anaphylaxis. Up to now, apart from history, there is almost no reliable diagnostic test giving adequate information on the risk of future reactions after repeated contact. The question whether atopic individuals are at higher risk for anaphylaxis is still controversially discussed. Most likely, food anaphylaxis and anaphylactic reactions with predominantly respiratory involvement may occur more frequently in atopics, while parenterally elicited anaphylactic reactions (insect stings, penicillin, etc.) are not related to atopy.

Problems in acute treatment include the question as to who should use epinephrine, when and in what dosage. Novel approaches would be desirable. Application of angiotensin II may be an alternative for the future. In causal treatment approaches, avoidance is only possible if the patient is well-educated and the elicitors are declared in drugs, foods or other substances. Allergen-specific immunotherapy for many elicitors of anaphylaxis does not exist. Studies are needed for food allergy and many cases of drug allergies. Studies with biologicals such as monoclonal antibodies against IgE seem promising and have to be performed at a larger level (Leung et al 2003).

At the level of industry — not only pharmaceutical, but also general — the problem of predictive testing with regard to IgE-inducing allergens is still unsolved and deserves attention and research. Education of the public with regard to the nature of anaphylaxis and immediate first aid manoeuvres (e.g. posture) are mandatory!

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DISCUSSION

Galli: Could you expand on the concept of summation anaphylaxis? Have there been any prospective studies that have looked at defined combinations, or is this concept primarily based on clinical observations?

Ring: I'm not aware of good studies, except for summation in the sense of exercise-induced anaphylaxis. The combination of exercise plus food has been studied best.

Müller: There is a paper by Hepner et al (1990), which is a case–control study looking at the effect of β blockers in patients on allergen immunotherapy. They found no increase in the frequency of systemic allergic side effects in the group on β blockers but concede that side effects in these patients may be more severe and more difficult to treat.

Galli: Hugh Sampson, have you seen a summation effect in cases of food allergies that you have studied?

Sampson: As Johannes Ring mentioned, we see food-associated exercise-induced anaphylaxis. There are some children who had milk allergy early on, appear to have outgrown it, but retain evidence of IgE antibody. In some cases, if they consume milk and then exercise, they will experience anaphylactic symptoms whereas if they are not exercising they are fine. No one has worked out the mechanism underlying this phenomenon.

Galli: What is your speculation about the angiotensin II connection?

Ring: It's a fascinating observation. Novartis produces an angiotensin II drug called 'hypertensin'. It is rarely used, but it would be a useful addition to our emergency kit. It is independent of epinephrine.

Fisher: Have you looked in any groups other than Hymenoptera allergy?

Ring: Yes, we've looked at drug-induced anaphylaxis.

Fisher: Did they also have low levels of rennin/angiotensin?

Ring: No, they didn't have generally decreased angiotensin levels, but the angiotensin levels increased during anaphylaxis.

Marone: I was very impressed by your data showing that the renin–angiotensin system can be involved *in vivo* during systemic anaphylaxis. This is an important issue for several reasons. First, I remember that Urata et al (1993) showed several years ago that chymase can efficiently convert angiotensin I to angiotensin II. More recently we have shown that mast cell chymase released from immunologically activated cardiac mast cells can efficiently convert angiotensin I to angiotensin II. (Marone et al 1998). It is possible that certain mediators such as chymase released from mast cells can play an important role in the homeostatic control of anaphylaxis.

Schwartz: It is a fascinating observation that chymase could be a source for generating angiotensin II in tissues. It has been difficult for people doing research on this area to show that such an event is pathophysiologically important. Along the same lines, there is a second angiotensin converting enzyme (ACE), ACE2, that has now been described to counterbalance ACE. When those two enzymes are out of balance, problems ensue. ACE2 makes an inactive or less active form of angiotensin. It is a smaller peptide that has less activity, so it ends up reducing the potential amount of angiotensin II. Thus, the system has become more complex; I'm not sure what role chymase plays.

Ring: I should add that the patients I described had normal ACE levels, although they had reduced angiotensin I and II in the plasma.

Austen: There are two issues I would like to speak about briefly. The overall context to my comment is that I am dismayed by the idea that we would mix distinct biochemical and immunological disease mechanisms and that we would use the terms pseudo-allergic and anaphylactoid synonymously with the term 'anaphylaxis'. They can be used as separate terms for descriptive purposes. Anaphylaxis is an immunological term that

excludes, for example, the non-steroidal anti-inflammatory adverse reactions and also adverse reactions to the ACE inhibitors. With regard to the non-steroidal anti-inflammatory agents, we know that the adverse reactions which have clinical characteristics of anaphylaxis are precipitated by inhibition of cyclooxygenase type 1 resulting in attenuation of PGE2 generation and increased generation of cysteinyl leukotrienes; this adverse reaction can be blocked by preventing the generation of the cysteinyl leukotrienes, or by antagonism of their receptor-mediated action. That does not mean that there cannot be the occasional patient who recognizes an epitope in a true immunological reaction to the non-steroidal (NSAID) drug but the vast majority of adverse reactions to the NSAIDs are not structurally specific but rather share a common biochemical mechanism. This is a wonderful example of why one shouldn't lose sight of mechanism by either clinically or intellectually lumping a lot of different pathways together. We'll never sort them out if we do that.

As to life-threatening angioedema, targeted disruption of the inhibitor of the first complement component (C1INH) has shown that the augmented permeability is due to the elaboration of kinins. Indeed, a double 'knockout' lacking C1INH and a kinin receptor is protected (Han et al 2002). At the human level the data are not yet as far along, but a number of laboratories have shown elevated kinin levels in hereditary C1INH. The ACE inhibitors not only block the function for which they are named but also a kininase, which is responsible for the inactivation of the kinins. Furthermore, this pathway has been implicated in a subfraction of patients with idiopathic anaphylaxis. My point is that we will be able to dissect mechanistically clinically similar adverse reactions and then introduce rational management in biochemical terms. The adoption of a nomenclature that buries our thinking, both clinically and scientifically, is a mistake that neither serves our clinical field nor our patients.

Galli: Johannes, did I understand correctly that the proposal is to refer to all of the reactions that are clinically similar to anaphylaxis without attempting to break out anaphylactoid reactions?

Ring: That is right. I am a member of this task force and we have had a lot of discussion about this. I was in favour of Frank Austen's mechanistic distinction. For me, anaphylaxis is an immunological reaction. Yet, the argument from the other side is a clinical one. You see the patient and you have to write a letter to the relatives telling them, for example, how the patient died. You can only uncover the mechanism days or weeks later, so you have to use the term anaphylactoid irrespective of a mechanism.

Lee: Part of this debate about nomenclature stems from the fact that we don't understand the pathophysiological mechanisms. Management wise, it is critically important to know the mechanism, so if it is an IgE-mediated phenomenon we

now have anti-IgE therapies. In the discussions over the next few days we will be hearing a lot about the effector arm of the response. What we may not hear much about is the target tissue response. In other words, are there situations in very severe anaphylaxis or allergy whereby there is a hypersensitivity of the target tissue, caused for example by greatly enhanced receptor expression. I believe there is in aspirin intolerance (Sousa et al 2002). In order to provide insight on ways in which we can stop people dying from anaphylaxis we have to understand both sides of the equation.

Fisher: We have wrestled with this nomenclature issue for many years. In the first papers that we wrote we talked about severe histamine-mediated reactions, which is probably the first two or three minutes. Then we talked about clinical anaphylaxis. The problem in practice with the anaphylactoid/anaphylaxis classification is that often anaphylactoid can mean many things. It means that there is no immunological basis to that reaction, but what it really means is that there is no immunological basis that I have found to this reaction. It often means that I haven't looked, or that I haven't looked with the appropriate technology. There are documented cases where someone has assumed a reaction was anaphylactoid, or not immune mediated, on the basis of it occurring on first exposure. This has led to the deaths of patients. On the warning bracelet we write 'anaphylaxis'. Everyone knows to be frightened of that. But for scientific papers it is a different ball game.

Simons: I would like to comment on the issue of diagnosis. Johannes Ring mentioned that diagnosis of anaphylaxis is easy. However, I'd like to suggest that there is at least one group of patients in whom this isn't the case, namely infants and pre-school children. I was concerned for many years by the fact that few infants and young children were included in the retrospective studies of anaphylaxis episodes from all triggers in all ages that have been published (Yocum et al 1999, Kemp et al 1995). The mean age in these studies was 29–39 years. There are two small studies and one recent large study of anaphylaxis from all triggers in children (Dibs & Baker 1997, Novembre et al 1998, Simons et al 2003). Recently, we have completed a prospective surveillance study of anaphylaxis within the Canadian Pediatric Surveillance Program. During an 18 month period, 747 cases were reported, two thirds of which involved children under the age of six years. A new picture of anaphylaxis in the very young is emerging from this study. The fact is that infants and very young children often can't describe their symptoms and if they do describe them, they use a non-traditional vocabulary. Itching, for example, is described as burning, hurting, scratching, tickling, tingling or hot or is reported when caregivers observe rubbing, pulling or clawing at the itchy part by those too young to verbalize. I mention this in order to draw attention to the fact that diagnosis of anaphylaxis may not be easy in infants and young children (Simons et al 2003).

Pumphrey: I agree with that. Of patients that are referred to us that have been given treatment for anaphylaxis, over half have been misdiagnosed. For example, I do my anaesthetic reaction clinic with an experienced consultant anaesthetist, and in two-thirds of cases referred following a 'reaction' we find an alternative cause for the event and no evidence for anaphylaxis.

Galli: In these cases are the patients presenting with the clinical picture of anaphylaxis because of mast cell activation by a non-immunological mechanism, or do they have something else entirely?

Pumphrey: There are many causes during anaesthesia for someone's blood pressure falling rapidly or sudden difficulty with ventilation.

Galli: So this isn't simply a debate about whether to call it an anaphylactoid reaction or anaphylaxis — they have a completely different pathophysiological mechanism.

Pumphrey: Yes.

Lasser: For many years the reactions that resulted from X-ray contrast material injections were termed 'anaphylactoid', although clinically they were indistinguishable from other anaphylaxis reactions. In the last few years we have found something that will enable us rightfully to call these reactions anaphylactic and with good reason.

Ring: In response to Estelle Simons' comment, when I said diagnosis was easy I was speaking in relative terms. It can be difficult. Description is not just a problem in children. When adults describe their symptoms they use colourful terms. All doctors have a similar questionnaire with standard questions, but we don't let the patients tell us themselves what they have experienced. One patient described to me that she saw 'white mice'. They describe a lot of symptoms which we reject because they are outside of our usual thinking.

Sampson: Tak Lee mentioned about studying the target audience response. I think this is very important. One of the observations we have made over the years doing double-blind challenges is that in children who are allergic to more than a single food, we can see reproducible responses. For example, if someone is allergic to milk they may have a reproducible response in the skin and gastrointestinal (GI) tract, and if we then challenge them with egg which they are also allergic to, we might get a reproducible response in the lung and skin. One of the questions that we have always had is why, if they have IgE on the mast cells in all these target organs, do they have one reproducible target response with one food and another target response with other foods. In response to Estelle Simons' comment about young children, one thing that is evident is that young children respond in a certain way and as they get older their anaphylaxis becomes more apparent. There are many children in the USA who respond to peanut early on, often with skin and GI symptoms, yet if they are exposed again a few years later will exhibit a full systemic response. One of the problems in the young children is

that they may not manifest all target organ responses early, but it will become much more evident later on.

Schwartz: We published a case report about an infant once with underlying systemic mastocytosis who presented with recurrent spells of apnoea. We were able to observe one of those in the hospital. Mature β tryptase levels went up markedly in the blood during one of these episodes, and then fell to baseline. Thus, in an infant with anaphylaxis, one of the presenting manifestations might be apnoea.

Simons: In particular, if skin signs are absent, you can imagine the difficulty the parent or nursery school teacher might have in diagnosing anaphylaxis. In our paediatric series: about 10% of children didn't have skin signs. I have a question. I saw a case report in which constitutive hyperhistaminaemia played a role in increasing the susceptibility of adults to anaphylaxis (Hershko et al 2001). Does anyone else think this is significant?

Ring: This raises the question of histaminase deficiency induced by diamino-oxidase blockers. When patients take many drugs, some of them block this enzyme and then the plasma histamine increases. Dr Ohtsu, you have knockout mice which might address this.

Ohtsu: Yes, I made mice lacking histamine by knocking out histidine decarboxylase. These mice are completely opposite from what we would expect. Now I am making transgenic mice which produce a lot of histamine, but I haven't checked them yet. Perhaps we could look at diamino oxidase in our transgenic mice.

Ring: How do the mice that don't have histamine react to anaphylaxis?

Ohtsu: I'll present some data on this later in the meeting.

Galli: Estelle Simons, in the case of the patient with high levels of histamine, was the origin of the high levels of histamine completely obscure? Was the patient examined for abnormalities of diamino oxidase or histaminase?

Simons: This was a patient who had elevated plasma histamine levels and impaired urinary histamine clearance. He had experienced anaphylaxis after eating fish. On other occasions, he had anaphylaxis from unknown causes and on yet additional occasions, he was able to eat fish without getting any symptoms (Hershko et al 2001).

Galli: Getting back to Hugh Sampson's point about the organ specificity of responses to different foods, I know that Hannah Gould has studied local production of IgE. Hannah, would you like to comment on the possibility that the local production of IgE, that might not yet be reflected in systemic sensitization of mast cells could in part account for this?

Gould: We have carried out a number of studies on nasal biopsies and blood from hay fever patients. Several observations suggest that the nasal mucosa is the primary source of allergen-specific IgE antibodies in these patients. (1) We have

incubated the nasal biopsies and observed the synthesis of IgE and allergen-specific IgE *ex vivo* (Smurthwaite et al 2001). (2) Locally synthesized IgE contains a significantly higher ratio of specific/total IgE than serum IgE from the same patients. (3) The relative frequency of IgE-expressing B cells in the nasal mucosa is several orders of magnitude greater than the frequency in circulating B cells: 5% of $CD19^+$ B cells and 25% of $CD138^+$ plasma cells in the nasal mucosa, compared to one in ten thousand B cells in the circulation, express IgE (Kleinjan et al 2000). The differentially expanded population of IgE-expressing B cells in the nasal mucosa is also observed at the mRNA level (Durham et al 1997). (5) Probing the biopsies for molecular markers (germline gene transcripts and switch circle transcripts) has provided evidence for local class switching to IgE (P. Takhar, S.R. Durham and H.J. Gould, unpublished results), likely accounting for the selective expansion of IgE-expressing cells in the tissue. (4) Analysis of IgE VH cDNA sequences reveals the presence of clonal families of B cells in the nasal mucosa of hay fever patients (H.A. Coker, S.R. Durham and H.J. Gould, unpublished results), suggesting that B cells in the nasal mucosa are activated by allergens and undergo clonal selection, proliferation, somatic hypermutation and class switching *in situ*.

We have observed that the production of grass pollen allergen-specific IgE in the nasal mucosa of grass pollen-sensitive hay fever patients persists between seasons, suggesting that the plasma cells (or their clones) are long-lived residents of the tissue. Long-lived plasma cells may be able to continually re-sensitize mast cells in the tissue for an immediate response to the allergen that originally drove the selection. The rate of IgE synthesis out of season in the nasal mucosa of grass pollen-allergic hay fever patients is sufficient to maintain the hypersensitivity of the nasal mast cells, taking into consideration the number of mast cells and the rate of IgE synthesis/volume of tissue, the number of molecules of the high-affinity IgE receptor, FcεRI, per mast cell and the rate of dissociation of IgE from the mast cells in tissues (Gould et al 2003).

The organ specificity of IgE responses may therefore stem from the chance migration of a B cell expressing a specific antibody to the target organ, clonal expansion, somatic mutation and class switching of the immunoglobulin genes, and IgE antibody synthesis *in situ*. It may not be a problem (in explaining the organ specificity of food allergens) that IgE antibodies of other specificities are present in the serum if these antibodies were not produced in the target organ. The IgE antibodies produced at a particular site in the tissue would be more likely to occupy IgE receptors on the neighbouring mast cells than IgE diffusing into the tissue from the circulation.

It would be very interesting and feasible to biopsy the three tissues mentioned by Hugh Sampson, the skin, GI tract and lung of the milk- and egg-allergic individuals and assay the levels and specificities of the IgE synthesised *ex vivo* (Smurthwaite et al 2001). This would reveal whether local

IgE production can account for the observed organ specificity of the reactions to these allergens.

Vercelli: One of the common objections to local switching has been that while B cells can switch in an organ, in reality they engaged in the process elsewhere. Thus time is of the essence. What I found impressive is that, in still unpublished work, Hannah Gould's lab is now able to identify in the nasal mucosa molecular events that define switching as a very recent occurrence. It is not as if these cells have had a lot of time to go very far. In a sense, the window of opportunity for switching to occur out of the nodes is getting narrower and narrower. This is important to answer the potential objection that the cells undergoing switching in the nose have been triggered in some other tissue. It is very likely that they have not.

Finkelman: If you are looking for reasons for different responses in different organs to different foods, it might not just be differences in the sites where IgE is produced but also differences in the populations of mast cells that are present. In addition, the cytokine environment in different organs may amplify responses to mediators released by mast cells. Curiously, in the lung and gut different cytokines seem to be responsible for mastocytosis.

Gould: The sites of IgE synthesis are probably linked to the presence of mast cells.

Galli: One of the issues here is the assumption that some of us make that both the antigens and also the reactive immunoglobulins will be systemically distributed. If the same antibody and effector cells are involved, why would one food activate a process in one organ and another food in another? With the possible exception that there may be local production of IgE in an individual organ that has not yet resulted in enough systemic distribution of IgE to sensitize mast cells in other sites, these clinical observations are difficult to explain.

Sampson: These children in whom we have observed this have circulating levels of IgE to these specific foods that are often greater than 100 kilo units per litre. At least when we look systemically, therefore, we don't see anything. This doesn't rule out the possibility that some local plasma cells are producing even higher levels locally, so we are getting differential binding to these receptors.

Finkelman: Perhaps another way of looking at it would be differences in locally produced IgG and/or IgA antibodies that may have blocking capacity.

Galli: The phenomenon of local activation of a response deserves some sort of a local explanation. The question is, what is that?

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Rethinking Th2 antibody responses and allergic sensitization

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Abstract. Human Th2 cytokines (interleukins 4 and 13) induce co-expression of IgE and IgG4 through sequential switching. The regulation of IgG4 responses and the role of these responses in the pathogenesis of allergy have not been characterized. We are addressing these issues by comparing and contrasting the expression of allergen-specific IgE and IgG4 in a population of European children thoroughly defined for lifestyle, environmental exposures and allergic phenotypes. The current analysis focused exclusively on children from non-farming families ($n=493$) in order to avoid potential effects of exposure to microbial products abundant in farming environments. We found that allergens induce Th2-mediated IgG4 and/or IgE responses in the majority of the population. Approximately two-thirds of the children had allergen-specific IgG4 but not IgE, only a minority had both IgG4 and IgE, only a few were negative for both, and virtually none had only IgE. The prevalence of asthma and hay fever was dramatically higher in children with high IgG4 and IgE compared to children who only mounted IgG4 or low IgG4 and IgE responses. These results appear to recapitulate different stages of *in vivo* Th2-dependent sequential switching from IgG4 to IgE. These patterns of Th2-induced antibody responses may warrant a redefinition of the notion of allergen sensitization.

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Multiple lines of evidence implicate T helper (Th)2 responses in the initiation and/or amplification of the pathogenetic processes that result in human allergic disease. Expression of the Th2 cytokines, interleukin (IL)4 and IL13, has been linked to allergic lung inflammation, rhinitis, and atopic dermatitis, and to dysregulation of immunoglobulin (Ig)E responses (Oettgen & Geha 2001, Vercelli 2001, Vercelli 2002). The role of IgE as a central effector molecule of allergic reactions is undisputed (Gould et al 2003). Understanding the molecular events leading to class switch recombination (CSR) to IgE is therefore critical to develop effective strategies to control and possibly prevent allergic disorders.

In an attempt to define the mechanisms which regulate CSR to IgE in human B lymphocytes, a molecular assay to detect recombination between switch (S)μ and Sε regions was developed which also allowed cloning and sequencing of Sμ/Sε switch products (Shapira et al 1991, 1992). This approach led to the identification of genomic DNA fragments in which the Sμ and the Sε regions were separated by inserts derived from Sγ4 (Jabara et al 1993). These results suggested that human CSR can occur sequentially from IgM to IgE via IgG4, a notion that was later independently confirmed through a different approach (Zhang et al 1994). Sequential IgM/IgG4/IgE switching provided a mechanistic explanation for several hitherto unexplained findings, i.e. the presence of both IgE and IgG4 in IL4-stimulated cultures (Lundgren et al 1989), and the simultaneous production of IgM, IgG4 and IgE in clonal B cell populations stimulated with IL4 and anti-CD40 mAb or activated $CD4^+$ T cell clones (Gascan et al 1991a,b). These data also linked both IgE and IgG4 to Th2 responses regardless of the profound differences that exist between their effector functions. Indeed, IgG4 is functionally monovalent, does not fix complement and binds weakly to Fcε receptors (Aalberse et al 1983, Schuurman et al 1999, van der Zee et al 1986). Thus, unlike IgE, antigen binding by IgG4 is expected to have no harmful consequences.

IgE and IgG4 levels are known to be co-regulated *in vivo* in certain diseases, such as chronic parasitic infections. Of note, typical allergic reactions are rare in helminth-infected patients, even though FcεRI-bearing cells are sensitized with anti-parasite IgE and are exposed, often continuously, to parasite antigens (Vercelli et al 1998). Inhibition of allergic reactivity has been attributed to 'blocking antibodies', predominantly found in the IgG4 subclass (Hussain et al 1992). IgG4 are unusually predominant among anti-filarial antibodies, representing 50–95% of the total IgG response (Kurniawan et al 1993). Depletion of IgG4 by adsorption on anti-IgG4 affinity columns specifically removed the blocking activity from the sera of microfilaremic patients (Hussain et al 1992), and IgG4 inhibited the binding of anti-*Schistosoma mansoni* IgE by over 96% (Rihet et al 1992). Furthermore, a potential role of blocking IgG4 antibodies in allergen immunotherapy was recently suggested (Akdis et al 1998). Indeed, IgG4 with blocking activity is detectable in sera from patients receiving

immunotherapy for insect venom and house dust mite hypersensitivity (Akdis & Blaser 1999).

The discovery of sequential IgM/IgG4/IgE switching, and the possibility that IgG4 may act to block IgE-mediated reactions, prompted us to investigate the mechanisms which regulate allergen-specific IgE and IgG4 antibody responses *in vitro* and *in vivo*. Our studies on the molecular regulation of ε and $\gamma 4$ germline transcription have been discussed elsewhere (Agresti & Vercelli 1999, 2002, Monticelli et al 2002, Thienes et al 1997). Here, we present novel *in vitro* data in support of preferential IgG4 expression by IL4-stimulated B cells, as well as an *in vivo* analysis of Th2 antibody responses in a population from rural areas of Germany, Austria and Switzerland thoroughly defined for lifestyle, environmental exposures, and allergic phenotypes (Braun-Fahrlander et al 2002, Riedler et al 2001). Although the ALEX population includes both farmers and non-farmers, our analysis focused exclusively on children from non-farming families, so as to avoid complex effects of exposure to microbial products (Vercelli 2003) on allergen sensitization and type of antibody response. We examined the prevalence of allergen-specific IgE and IgG4 responses, the patterns of IgG4 and IgE co-expression, and the role of IgG4 antibodies in disease pathogenesis. To our surprise, we found that virtually every individual in the population mounted allergen-specific Th2 antibody responses, as indicated by the expression of IgG4, but only a minority of subjects concomitantly expressed IgE. Allergic disease was restricted to the latter group. The Th2 antibody response patterns revealed by our studies may warrant a redefinition of the notion of allergen sensitization.

Methods

Isolation of naïve surface(s) IgD$^+$ B cells

Peripheral blood mononuclear cells were isolated from normal non-allergic donors by density gradient centrifugation, resuspended at $5–10\times10^6$ cells/ml in RPMI1640–10% human AB$^+$ serum, and adhered overnight in plastic Petri dishes. Non-adherent cells ($5–10\times10^6$ cells/ml) were then incubated on ice for 30 min in the presence of anti-CD3 mAb (OKT-3), washed and incubated for 30 min with magnetic beads coated with goat anti-mouse IgG (Dynal, 8–10:1 bead:cell ratio) at 4 °C with slow rotation. CD3$^+$ cells were then removed using a magnet. This procedure was performed twice. Negative selection with mAb OKM-1 was used to remove CD11b$^+$ cells. The cell populations thus isolated contained 95% CD19$^+$ B cells, as assessed by immunofluorescence. To isolate sIgD$^+$ B cells, cells were washed, resuspended in labelling buffer (PBS, pH 7.2, 2 mM EDTA) and incubated on ice for 30 min with biotin-conjugated goat

anti-human IgD (Sigma: 10 μg/sample), washed with labelling buffer and then incubated for 20 min on ice with streptavidin-conjugated microbeads (Miltenyi: 10 μl/10^7 cells). After washing in separation buffer (PBS pH 7.2, 0.5% BSA, 2 mM EDTA), $sIgD^+$ cells were collected using a magnetic cell separator. Immunofluorescence analysis showed that the cell populations thus obtained contained 95% $sIgD^+$ B cells.

In vitro Ig production

$sIgD^+$ B cells were incubated with IL4 (R&D Systems, 10 ng/ml) and/or anti-CD40 mAb 626.1 (5 μg/ml) for 14 days. Culture supernatants were then harvested and assessed for Ig concentrations by enzyme-linked immunosorbent assay (ELISA). For IgE, 96-well plates were coated with anti-IgE mAb 7.12 and 4.15 (ATCC HB-236 and HB-235, 2 μg/ml in 0.1 M carbonate buffer, pH 9.0) overnight at room temperature. Wells were then washed twice with PBS–0.05% Tween and blocked with 2% milk–PBS–0.01% azide for 4 h at room temperature. After extensive washing with PBS–0.05% Tween, dilutions of an IgE standard curve (Hybritech) and samples were added to the wells overnight at room temperature. Following extensive washing with PBS–0.05% Tween, a horseradish peroxidase-conjugated rabbit anti-human IgE antiserum (DAKO, 1:1000 in PBS–1% milk–0.05% Tween) was added to the wells for 4 h at room temperature. After washing 10 times with PBS–0.05% Tween, the reaction was developed by incubation with ortho-phenylendiamine (Sigma) for approximately 20 min at room temperature in the dark. After stopping the reaction with 10% sulphuric acid, OD was read at 490 nm. Secretion of IgG subclasses was evaluated using commercially available ELISA kits (The Binding Site). The limits of sensitivity for the Ig assays were: 0.42 (IgG1), 7.35 (IgG2), 0.29 (IgG3), 0.44 (IgG4) and 0.2 (IgE) ng/ml. Control cultures for the evaluation of preformed Ig were set up in the presence of cycloheximide (100 μg/ml). Net Ig synthesis was calculated by subtracting the Ig concentrations detected in cycloheximide-treated cultures from the values found in untreated cultures.

Epidemiological studies

Subjects included in this study were participants in a cross-sectional survey conducted by the Allergy and Endotoxin (ALEX) Study Team in rural areas of Germany, Austria and Switzerland which included both farming and non-farming households (Braun-Fahrlander et al 2002, Riedler et al 2001). Briefly, parents of 3504 children in school grades 1–6 were invited to answer a questionnaire on respiratory and allergic diseases. 2618 (75%) of the parents elected to participate and were asked to consent to further testing. 1406 (54%)

consented. From this group, all children from farming families and a random sample of children from non-farming families from the same rural areas were invited to continue testing. The final group was restricted to children born in Germany, Austria or Switzerland and who were nationals of those countries ($n=812$). Farmers' children were defined as children whose parents answered 'yes' to the question 'does your child live on a farm?' The results reported here are limited to children of non-farming families ($n=493$).

For serum IgE measurements, each sample was first tested against a panel of aeroallergens (mixed-grass pollen, birch pollen, mugwort pollen, *Dermatophagoides pteronyssinus* (Derp), cat dander, dog dander and *Cladosporium herbarum*) by fluorescence enzyme immunoassay (FEIA, CAP, Pharmacia). In children who had a positive result to this panel, specific IgE responses to timothy grass pollen, cat dander and Derp were measured. Allergen-specific IgE were expressed as kU/L, and the limit of detectability was 0.35 kU/L (Platts-Mills et al 2003). Undetectable samples were assigned a value of 0.30 kU/L.

IgG4 antibodies specific for timothy grass pollen, cat dander and Derp were measured in 487 sera using FEIA (CAP, Pharmacia) and diluting samples 1:10 in diluent provided with the kit. Allergen-specific IgG4 were expressed as μg/L. The limit of sensitivity of the assay was 15 μg/L, and undetectable samples were assigned a value of 10 μg/L.

Disease was defined as 'ever hay fever' and 'ever asthma'.

Statistical analysis

This was performed using the Statistical Package for the Social Sciences (SPSS) for UNIX, version 6.1.3. Values of allergen-specific IgG4 were log-normally distributed and results are reported as geometric means. Due to the large number of undetectable allergen-specific IgE values, detectable IgE values were grouped into half-log intervals and analysed using χ-square analysis and contingency tables.

Results

The IgG4 subclass is preferentially induced by IL4 in human naïve B cells

The isotype specificity of IL4-dependent CSR in human B cells is controversial. The combination of IL4 and CD40 cross-linking was reported to induce IgE and all IgG subclasses, except IgG2, in tonsillar naïve B cells (Fujieda et al 1995). By contrast, molecular analysis identified IgG4 as the typical IL4-dependent γ subclass (Jabara et al 1993), even though other IgG isotypes appear to be occasionally

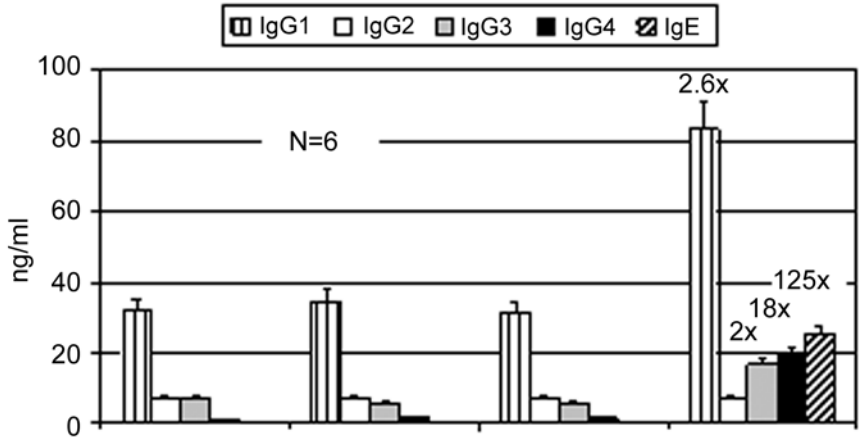

FIG. 1. The IgG4 subclass is preferentially induced by IL4 in human naïve B cells. Human $sIgD^+$ naïve B cells were isolated from peripheral blood by negative selection and stimulated *in vitro* with IL4 (10 ng/ml) and/or anti-CD40 mAb 626.1 (5 μg/ml) for 12 days. Culture supernatants were then harvested, and Ig secretion was assessed by ELISA. The figure shows the mean ± SEM of results obtained in six consecutive experiments.

targeted (Zhang et al 1994). We felt that the source of human B cells used for studies of CSR isotype specificity may be a critical variable. Most studies were performed using tonsil B cells. However, this tissue is a site of intense inflammation and cytokine production (Agren et al 1996). It is therefore conceivable that a significant proportion of tonsil B cells, while still expressing IgM and/or IgD on their membrane and thus naïve by commonly accepted criteria, may nonetheless be engaged in cytokine-dependent remodelling of the relevant immunoglobulin loci. In order to analyse CSR in truly resting human B cells, we isolated naïve $sIgD^+$ B lymphocytes from peripheral blood, rather than tonsils, and stimulated *in vitro* with IL4 and/or anti-CD40 mAb 626.1 for 12–14 days. Total IgE and IgG4 secretion in cell culture supernatants was assessed by ELISA. Figure 1 shows the mean SEM of results obtained in six consecutive experiments. Both IgG4 and IgE were strongly (18- and 125-fold, respectively) up-regulated in cultures treated with both IL4 and anti-CD40 mAb. In contrast, only modest enhancement was detected for IgG1 and IgG3, while IgG2 secretion remained unaffected. As expected, IL4 or CD40 cross-linking alone did not up-regulate Ig secretion. These results demonstrate that, consistent with previous molecular evidence (Jabara et al 1993), the IgG4 subclass is preferentially induced in naïve B cells in which both the IL4 and the CD40 receptor have been engaged.

Prevalence of allergen-specific Th2 antibody responses in the ALEX population

We then moved on to analyse the *in vivo* prevalence of antigen-specific IgG4 and IgE responses, the molecular signature of CSR induced by Th2 cytokines. To this end, we measured levels of IgE and IgG4 specific for three common inhalant allergens (timothy grass pollen, Derp and cat dander) in sera from ALEX children. The ALEX population includes both farmers and non-farmers (Braun-Fahrlander et al 2002, Riedler et al 2001). However, we limited our analysis to children from non-farming families ($n=493$) so as to avoid potential effects of farm-related environmental exposures. Table 1 (top) shows that 98% of children in the non-farming ALEX population produced IgG4 and/or IgE to one or more of the test allergens ('Any' group). Interestingly, the majority of the ALEX children (64.8%) had pure IgG4 responses, whereas only 33.5% of the population expressed both IgE and IgG4 to at least one allergen, and only one child mounted a pure IgE response. These results show allergen-specific, Th2-dependent antibody responses occur in the totality of the non-farming ALEX population. In two-thirds of the children, these responses are uncoupled, i.e. IgG4 antibodies are secreted in the absence of IgE.

We then investigated whether allergen-specific patterns could be detected in IgG4/IgE responses. Table 1 (bottom) demonstrates that only 10.1% of the ALEX children produced IgE as well as IgG4 to cat dander. By contrast, 26.3% of the children had both IgE and IgG4 to timothy grass pollen and 15% had both isotypes to Derp. Of note, timothy grass pollen was the only allergen against which a significant portion of the population (29.8%) failed to mount a Th2 antibody response. Thus the nature of the allergen, and/or the environmental context in which allergen exposure occurs, appear to have an impact on Th2 response

TABLE 1 Percentage of non-farming children in the ALEX population with specific IgE and IgG4 antibodies[a] to cat dander, Derp, timothy grass pollen and any of these three allergens

Antigen	*n*	*$IgG4^-/IgE^-$ % (n)*	*$IgG4^-/IgE^+$ % (n)*	*$IgG4^+/IgE^-$ % (n)*	*$IgG4^+/IgE^+$ % (n)*
Any	486	1.4 (7)	0.2 (1)	64.8 (315)	33.5 (163)
Cat	487	1.6 (8)	0.2 (1)	88.1 (429)	10.1 (49)
Derp	487	7.0 (34)	0.6 (3)	77.4 (377)	15.0 (73)
Tim	486	29.8 (145)	1.4 (7)	42.4 (206)	26.3 (128)

[a]The limit of sensitivity assigned to the assay was <0.35 kU/l for allergen-specific IgE, and 15 μg/l for allergen-specific IgG4.

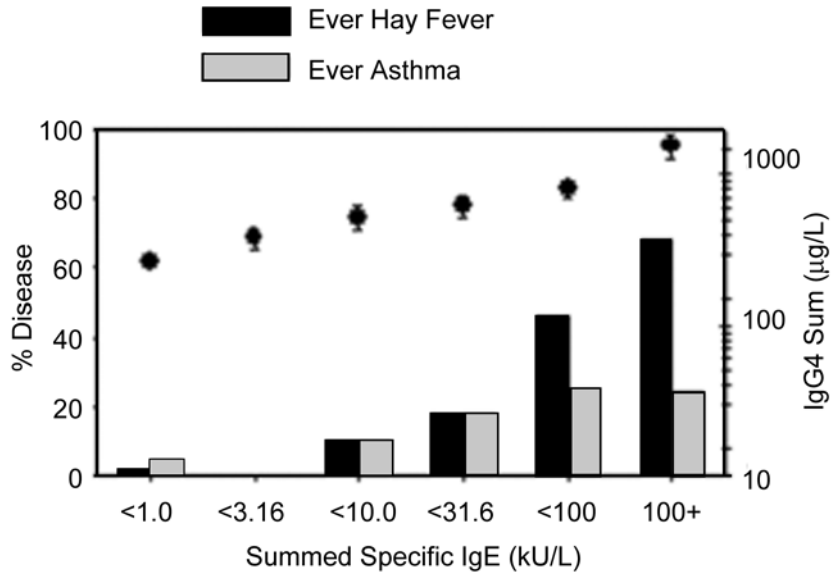

FIG. 2. Prevalence of ever hay fever and ever asthma and the geometric mean (±SEM) of IgG4 levels (μg/l) by half-log intervals of specific IgE (kU/l). Percentage of children in each summed specific IgE group with ever hay fever or ever asthma and their corresponding geometric mean summed specific IgG4. Summed IgE group $n=323, 38, 20, 28, 48, 25$ ($n=482$).

patterns. Despite these differences, however, isolated IgE responses were the exception rather than the rule for all three allergens tested.

Th2 antibody responses and disease

In an attempt to characterize the role of Th2 antibody responses in the pathogenesis of asthma and allergy, we then investigated the association between levels of allergen-specific Th2 antibodies and incidence of allergic disease in the non-farming ALEX population. Figure 2 shows that children with high levels of allergen-specific IgE (sum of IgE against the three test allergens) had high incidence of both hay fever and asthma. The IgG4 sum directly correlated with IgE sum (Spearman $r=0.51$, $P<0.001$), i.e. the highest IgG4 levels were found in the high IgE groups. However, strong IgG4 responses were also found in the absence of both IgE expression and disease. Overall these results indicate IgG4 responses are not pathogenic, but they are not protective either when coupled with vigorous IgE production.

Discussion

'Allergen sensitization' is commonly used as a synonymous term for allergen-specific IgE responses, and these are in turn considered as *the* outcome of antibody-mediated Th2 immunity. According to this view, Th2 responses would be both relatively infrequent and usually pathogenic. Our current results highlight a different scenario, in which Th2 antibody responses to allergens (whose signature is the expression of IgG4 as well as, or instead of, IgE) occur frequently and overall invariably in the population. Most importantly, Th2 responses are mostly restricted to the IgG4 isotype, and are non-pathogenic.

The scenario we propose has several implications. The traditional notion that allergens are antigens to which healthy individuals do not develop detectable responses is not supported by our data. Indeed, the majority of the ALEX children mounted IgG4 responses to the three allergens we tested, mostly in the absence of a concomitant IgE response and disease. These children would have been considered allergen non-responders, had we not measured allergen-specific IgG4. Similar conclusions were recently drawn upon examination of antibody expression profiles in individuals exposed to domestic animals. High levels of exposure to cat allergen were found to be accompanied by an IgG and IgG4 antibody response without allergic symptoms or risk of asthma (Platts-Mills et al 2001, Platts-Mills et al 2003). Of note, this Th2 response was interpreted as a form of tolerance (Platts-Mills et al 2001). However, in as much as tolerance represents a failure to respond to an antigen, we would argue that expression of allergen-specific IgG4 without IgE would rather represent an intermediate Th2 response, i.e. a situation in which IgM/IgG4/IgE sequential switching induced by Th2 cytokines is arrested at the IgG4 stage.

In this context, allergens may then be better defined as antigens which, because of molecular signatures we have not yet deciphered, evoke Th2 antibody responses (IgG4, with or without IgE). Most allergen-exposed individuals become 'sensitized' biologically, even though sensitization may remain clinically silent. That the immune system is quite prompt in mounting Th2 responses to common inhalants suggests a default pre-programming which may have been shaped by evolution, and is supported by recent evidence from animal models (Dabbagh et al 2002, Eisenbarth et al 2002).

The different clinical outcome of Th2 antibody responses begs the question, what determines whether the response will include both IgG4 and IgE (and will be potentially pathogenic), or only IgG4 (and will be harmless). The nature of the antigen appears to play a role. Furthermore, it is possible that gene–environment interactions, i.e. a complex interplay between genetic makeup and environmental exposure, may tip the balance between different kinds of Th2 responses. The ALEX population, with

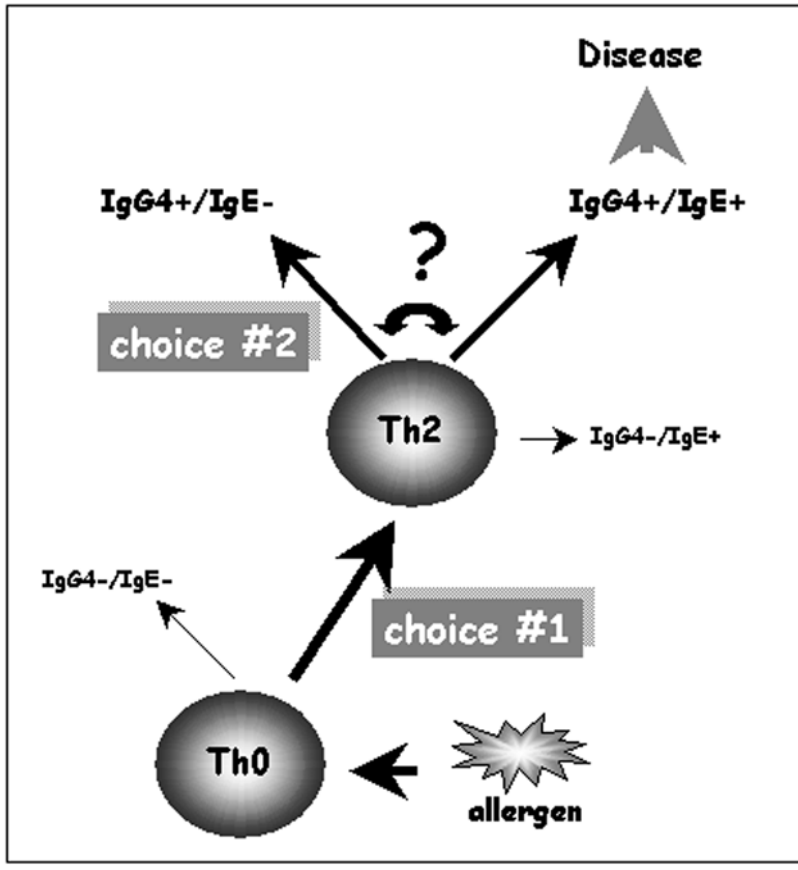

FIG. 3. A model of the development of allergen-specific, Th2-mediated antibody responses in humans.

its well characterized profiles of environmental exposures, will be ideal to test the multiple facets of these hypotheses.

One puzzling element emerging from our analysis is the lack of protection associated with IgG4 responses. This finding was unexpected, because the *in vivo* data from patients with chronic parasitic infections suggested a strong blocking effect of IgG4 (Hussain & Ottesen 1986, Hussain et al 1992, Ottesen et al 1985), and similar patterns were observed following bee venom (Akdis et al 1998) and birch pollen (Visco et al 1996) immunotherapy. This discrepancy may result from differences between the immunization routes, target organs, and regulatory networks engaged in these conditions. Furthermore, inhalant and parasite-derived

allergens are likely to differ in their biochemical structure and the biological context within which they are presented to the immune system.

We conclude by proposing a model for the generation of allergen-specific antibody responses predicated on our current results (Fig. 3). Allergen-specific, Th2-mediated antibody responses represent the outcome of two fundamental choices made by a developing $CD4^+$ Th cell precursor. The first choice is whether to differentiate along the Th1 or Th2 pathway. The fact that allergens frequently elicit Th2-mediated, IL4-dependent antibody responses of the IgE and/or IgG4 class is likely to reflect inherent biochemical properties of these molecules, as well as route and context of exposure. The second choice, perhaps a more complex one, is whether the ultimate outcome of allergen-specific Th2 responses will be expression of IgG4 only, or both IgG4 and IgE. In the first case, the response will be clinically silent; in the second case, disease may ensue. The molecular mechanisms which activate differential isotype switching to IgE and IgG4 in B lymphocytes remain undefined. Our results showing that potentially pathogenic IgE responses are not the inevitable outcome of Th2-mediated immunity should provide the impetus to define these mechanisms and devise approaches to redirect Th2 effector functions toward intermediate responses and expression of the non-pathogenic IgG4 isotype.

Acknowledgments

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DISCUSSION

Galli: Before I ask Hannah Gould to comment further on the local production of IgE, I'd like us to keep in mind a couple of observations. One is that some patients with anaphylaxis are not atopic. The second is to remind us about a point that Hugh Sampson mentioned earlier, regarding his studies of food allergy. Patients who developed what appeared to be distinct organ-specific patterns of symptoms to two different foods had detectable levels of circulating IgE against both of the foods: that is, it wasn't simply that there were not systemic levels of IgE for this food or that food. Now I would like Hannah Gould to comment on the antigen-specificity of local as opposed to systemic IgE.

Gould: It is true that some patients with anaphylaxis are not atopic. I said before that locally synthesized IgE contains a significantly higher ratio of specific/total IgE than serum IgE from the same patient. In fact in one of these patients no IgE antibody at all could be detected in serum whereas it represented a sizeable fraction of the IgE synthesized in biopsies from the nasal mucosa (Smurthwaite et al 2001). In earlier work it was shown that allergen-specific IgE could be often be measured in nasal secretions but not in serum from the same patients (reviewed in Durham 1997). IgE in nasal secretions was taken to represent IgE produced in the tissue.

This seems to have been justified in view of our results with the tissues themselves.

The recent results in our studies of asthma patients also support the local production of IgE. A sizeable proportion (up to 30%) of asthmatics are non-atopic or intrinsic asthmatics, with no detectable antibodies to any of the common allergens in the conventional skin prick tests or radioallergosorbent test (RAST). However, bronchial biopsies from these patients exhibit IgE ε heavy-chain mRNA and FcεRI α-chain mRNA (Ying et al 2001, Humbert et al 1999), implying that IgE is synthesized, secreted and bound to mast cells in the tissue.

I would like to mention another observation of possible relevance to the pathogenesis and localization of the allergic response. The studies of IgE VH sequences I mentioned earlier yielded another interesting result, the over-representation of a minor VH family, VH5, in the nasal biopsies from hay fever patients (H.A. Coker, S.R. Durham, H.J. Gould, unpublished results). The total repertoire of VH genes is around 50 and the VH3 family is the largest family, represented in 40% of the immunoglobulins expressed in normal human serum. The VH5 family has only 1 or (in half the population) 2 members and is represented by 3% of the immunoglobulins in serum. In contrast to normal immunoglobulins, VH5 is represented in 8% of serum IgE and in 18% of the IgE in the nasal biopsies of hay fever patients (H.A. Coker, S.R. Durham, H.J. Gould, unpublished results). Similar over-representation of VH5 has been observed in bronchial biopsies from an asthma patient (Snow et al 1997) and the circulation of patients with atopic dermatitis (van der Stoep et al 1993).

The distribution of replacement mutations, those which lead to amino acid substitutions, is also abnormal in the mucosal tissues, being skewed towards the framework regions rather than the complementarity-determining regions, as seen in the antibodies to conventional antigens. Together the over-representation of the expressed IgE VH5 sequences and the pattern of somatic mutations in VH5 suggest the activity of a local B cell superantigen that binds to VH5.

It is possible therefore that B cell superantigens are involved in the pathogenesis and localization of allergic disease. Whether allergens themselves are superantigens or the allergens are able to stimulate an IgE response in the tissues by virtue of local superantigens eliciting hypersensitivity (Genovese et al 2003) is an important question. This might relate to organ-specific responses, as it is conceivable that a complex between a specific allergens and a tissue-specific protein could constitute a superallergen and elicit organ-specific responses to food allergens.

Galli: The question for people doing this kind of investigation is whether there can be a detailed analysis and comparison of the antigen specificity and other

properties of the IgE antibodies that are present systemically, as opposed to those that are present locally. This is needed to answer the question of whether, given the types of allergens that may be present at a particular site, the locally represented IgE may provide a higher level of reactivity at that site, and thereby explain the local expression of disease. There are other possible explanations of the clinical phenomena, of course. Perhaps at this point such detailed comparative studies of the systemic and the local antibody have not been done.

Gould: The amount of information is perhaps limited, but as I have said before we have shown in our work with hay fever patients that the ratio of specific to total IgE is significantly greater in the IgE produced in the nasal mucosa than in serum IgE, and in some patients we could only detect specific IgE as a product of local IgE synthesis (Smurthwaite et al 2001). There is also the earlier evidence that in some patients IgE antibodies against allergens are undetectable in serum but detectable in nasal secretions (reviewed in Durham et al 1997). IgE produced in the tissue can diffuse out of the tissue in two possible directions, into the circulation and/or into the secretions. It is likely that the relative rate of diffusion in these two directions differs from one patient to the next, perhaps determined by the proximity of the particular B cells (or B cell clones) to each of the surfaces.

Further evidence for the production of specific IgE in the target organ comes from measurements in secretions versus serum as a function of time after exposure to allergens. It has been shown that the appearance of specific IgE antibodies in the serum is delayed relative to the appearance in nasal secretions (more representative of the local response than the serum) upon exposure to allergen after a period of allergen avoidance (Sensi et al 1994), pointing to the tissue as the source of specific IgE.

Marone: I would like to comment on the VH5 family, which is apparently overexpressed locally. This is a fascinating observation and also relates to the possibility, mentioned by Hannah Gould, that endogenous, bacterial or viral superantigens can activate IgE on the surface of human basophils and mast cells through the VH family. During the last years we have shown that several of the immunoglobulin superantigens, such as protein A, protein Fv and gp120, basically interact with IgE VH3+ (Genovese et al 2000, 2003, Patella et al 2000). This interaction induces the release of mediators and cytokines from human basophils and mast cells. Interestingly, the VH3 is the most represented VH family in the repertoire of human immunoglobulin. This opens the possibility that the *in vivo* exposure to certain immunoglobulin superantigens can induce a natural selection of the VH family.

Schwartz: A useful control experiment might be to look at another tissue that has been sensitized, such as the skin. If you do this, do you see the new Ig synthesis there in B cells, or is it really specific for the target organ?

Gould: This is an important question, which we would like to study. We would need to get ethical approval and then do the biopsies, so it would take a little time before we can answer that question.

MacGlashan: You said that the local specific to total IgE ratio was different to that in the circulation. The local was 30–70%; what did you find in the circulation?

Gould: We had about 10% in the circulation, and 30–70% in the nose.

Müller: It was mentioned just a few minutes ago that not all anaphylaxis is atopic. In fact, there have been quite a few studies on IgG4 in venom anaphylaxis, especially in relation to beekeepers. In highly exposed people like beekeepers, we find very high levels of IgG4 but little IgE. By passive immunotherapy with beekeeper γ globulin, several groups have shown that it is possible to protect even allergic patients. However, we have looked in a number of venom-allergic patients at IgG4-specific antibodies and the relationship between IgE and IgG4 in serum taken directly before a sting challenge. There we could not find a clear correlation between protection and IgG4.

Galli: Donata Vercelli, I'd like to raise the question of whether there are sufficient numbers of subjects in the ALEX study to do a meaningful study of anaphylaxis in that cohort. What are your views?

Vercelli: The ALEX group involves some 800 children, which may not be enough to achieve statistical power, given the low frequency of anaphylaxis. However, the same group is now recruiting another population, which will be named Parsifal, and will involve the same countries plus Sweden. They are intending to recruit a total of 6000 or so children, so it may well be possible to study anaphylaxis in this group.

Simons: Yes. In my presentation I will describe a study in which we tried to address the prevalence of anaphylaxis in a geographically defined population of children. We found that although there was some variation with age, 1.44% of the children had epinephrine dispensed for out-of-hospital use. The highest epinephrine dispensing rate, 5.3%, was found in boys aged 12–17 months (Simons et al 2002). This is really the only data set that exists on the prevalence of anaphylaxis from all triggers in children. As mentioned previously, children are seriously under-represented in retrospective studies of anaphylaxis from all triggers in all ages (Yocum et al 1999, Kemp et al 1995), and two of the three paediatric studies published of anaphylaxis from all triggers are small, involving 55 and 76 children, respectively (Dibs & Baker 1997, Novembre et al 1998). Anaphylaxis does seem to be increasing, and it is in the younger patients that this increase is most significant.

Lee: This sort of study, with large cohorts, is very powerful. But I would also like to encourage the collection of data on a longitudinal basis. Taking data in one snapshot of time can be misleading. When you have cohorts which you are

following up for a long time, it is enormously powerful to have the correlation of immunology with clinical patterns.

Vercelli: I couldn't agree more. In fact, we are going to do something very similar to the study I just showed for the ALEX group using a Tucson population that has been followed for 25 years. The children were enrolled before they were born, through their parents. The problem is that this is a somewhat smaller population, so there may be an issue of statistical power. But you are absolutely right, longitudinal studies are ideal.

Finkelman: It may not be clear that IgG4 is protective against allergy, but if you look at the reverse side of the coin, protection against worm infections, there are data in humans infected with schistosomiasis that indicate that the IgE:IgG4 ratio tends to correlate better with protection than IgE levels alone. This argues that IgG4 can have some mechanism of down-regulating allergic responses. As you know there is some analogy between IgG4 in humans and IgG1 in mice, in that both can be induced by IL4. With IgG1 there is evidence that *in vitro* it takes more IL4 to induce a good IgE response than to induce a good IgG1 response. Is this true for IgG4 versus IgE in humans? Related to that, I remember a paper suggesting that gamma interferon was more suppressive of IgE than IgG4 (Akdis et al 1997, Carballido et al 1994). This suggests that you'd see a higher G4:E ratio when both IL4 and IFNγ are being produced. Has this been replicated? Is it generally believed?

Vercelli: It's a complex situation. *In vitro* at least, when we induce IgE and IgG4 with anti-CD40 antibody and IL4, the response for IgE is much stronger. It goes from virtually zero to a significant amount, which ends up as about a 200-fold increase. However, serum IgG4 levels are much higher than IgE. Based on the data from the parasite and beekeeper studies, we were expecting some protection, and we were surprised when we did not find any. However we also saw very different patterns with different allergens. I think it is hard to answer this question. In a sense, IgG4 is more complex than IgE: if you take away IL4 in the mouse, IgG1 stays there. There are probably more ways to induce IgG1 than just IL4. IgG4 may have similar characteristics.

Finkelman: As you vary the amount of IL4 that you add to the *in vitro* culture with the CD40 stimulation, do you vary the IgG4:IgE ratios?

Vercelli: We haven't done this extensively. What you are asking me is a question about thresholds, and I don't have an answer yet.

Sampson: I wonder whether we need to go to another level of complexity. We looked at children who were allergic to eggs, and we were trying to do mapping of the IgE binding sites on ovomucoid. Children who had persistent egg allergy — that is, who did not appear to be able to outgrow it as most children do — had IgE binding to specific linear epitopes on the ovomucoid. When we looked at those children for IgG binding to those same epitopes, they bound IgG as well.

However, when we looked for IgG binding to linear epitopes in children who 'outgrew' their egg allergy, they had none. If we did an assay, however, in which we measured IgG and IgE to native ovomucoid, we saw these high levels. I almost wonder whether we have to go and look at specific epitopes to look for protection and non-protection.

Vercelli: I agree. This is probably the reason why there are discrepancies. It is possible that we don't see protection because not enough IgG4 is produced against the epitopes IgE reacts with. What I was intrigued by is that if I believe the data (and I do), the most important conclusion is that the complexity of Th2 responses *invivo* is much greater than initially anticipated, and their frequency is much higher. So this idea that there are only a few people who are Th2 responders is incorrect. Then the question becomes why is it that some people go one way or the other? This leads us straight into genetics. My anticipation is that there is a genetic component that shifts people one way or the other.

Schwartz: A critical issue here is how Th2 cells can educate a B cell to be either an IgG4 or an IgE producer. Could you expand more on this? One of the questions that comes to mind is if you take Th2 clones, can you find one clone that can educate B cells to switch to IgG4 and another one that would switch them to IgE? Or is this not where the regulation is taking place?

Vercelli: It is good that you are asking that: this is exactly what we are doing, trying to dissect this at a clonal level. To rephrase your question, what are the signals that make a response progress, or prevent it from progressing, from an IgG4 to an IgE response? A good candidate in the literature is IL10. This has been described as being a cytokine that is able to specifically suppress IgE expression or secretion and increase IgG4. IL10 has also been an interesting cytokine in patients reacting to parasites. One remarkable observation by Tom Nutman was that peripheral blood mononuclear cells from filaria-infected patients, stimulated with antigen *in vitro*, showed very little proliferation. However, if an anti-IL10 blocking antibody was added, proliferation went through the roof. If he added IL10 *in vitro*, IgE went down. So IL10 had a blocking effect. The effect that we see with IL10 is very complex: we are now studying this extensively. IL10 has effects at the T and B cell levels, and these effects are opposite. IL10 blocks CD40L induction but has a very strong enhancing effect on expression of IgE in a CD40-based system which bypasses CD40L. We don't know about IgG4 yet, but I think IL10 may have something to do with this. IL10 would also be a good candidate to mediate the effects of exposure, for obvious reasons: it is coming from APCs and macrophages and so on. It may be an interface between bacterial exposure and whatever mechanism it is that primes the immune response. Of course, IL10 is also produced by T regulatory cells, and these are likely to be critical to modulate allergic responses.

Gould: One thing that hasn't been mentioned is cell proliferation. IL4 is very good at driving cell proliferation. There is evidence in the literature that more cycles of cell replication are required for the switch to IgG than to IgE (Tangye et al 2002). This could be related to what Fred Finkelman was saying about the concentration of IL4, and whether you get IgG4 or IgE. At relatively low IL4 concentrations there may be fewer cycles of cell proliferation so IgG4 would be favoured. Another reason for the delay in class switching to IgE is likely to be the sequential switching through other isotypes (reviewed in Gould et al 2003).

Ring: We are looking for many influences, and you mentioned 'environmental factors'. What about the dose of allergen as the environmental factor? The more allergen around, the more IL2 or IL10 is formed. Low doses make IgE and high doses give rise to IgG4. This is the essence of immunotherapy.

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General discussion I

Galli: There are two topics that I would like us to address in this general discussion. One is the clinical differences in anaphylaxis between patients who develop anaphylaxis in the context of severe atopy, as opposed to those who have no evidence of atopy. The other topic is the controversy regarding the potential reclassification of anaphylaxis and the utility of the concept that everything that clinically looks like anaphylaxis shall be called anaphylaxis, as opposed to attempting to ferret out the different mechanistic causes of this phenomenon. Perhaps first we could consider anaphylaxis in atopic and non-atopic individuals.

Golden: I'll start by commenting on some findings we made some years ago in our epidemiological survey of insect sting anaphylaxis. We found a dissociation between symptoms and atopy (Golden et al 1989). The presence of IgE to inhalant allergens correlated clearly with the presence of IgE to venom allergens. But atopic history of rhinitis or asthma had no correlation to anaphylaxis to insect stings. It complicates the discussion about 'what is atopy?' and 'how do IgE level and atopy relate to the expression clinically of anaphylaxis?'.

Galli: I'd like to be more specific about my question. There seems to be no doubt that patients who do not fit the classical definition of atopy can develop anaphylaxis to, for example, venom and peanuts. The question is, however, when atopic individuals develop an anaphylactic reaction, is it in any way more severe or different than the anaphylaxis that develops in patients without atopy?

Ring: We did a study involving large numbers of patients which showed that insect venom anaphylaxis is not more frequent in atopics than in non-atopics. I think everyone agrees with this. But the concentration of IgE antibodies against bee venom was higher in atopics, independent of the severity. For practical purposes, in the atopic patients high IgE means less for their clinical symptomatology compared to non-atopic patients. An atopic patient may have class 5 or 6 without very severe symptoms, whereas if a non-atopic class 5 is probably more significant.

Golden: Is it clinically important? Do the non-atopics have more severe reactions with high IgE?

Ring: This is what we guess, but there is no good study.

Müller: There has been an interesting finding in beekeepers who are allergic to bee venom. In this group atopy is significantly more frequent than in non-beekeeper patients who are allergic to bee venom. There was a suggestion that

this is caused by the fact that atopic beekeepers inhale tiny amounts of the venom during their work in the beehive and thus get sensitized through the mucosal route (Miyachi et al 1979). If you look at symptoms, atopic patients with bee venom allergy more commonly have respiratory symptoms than non-atopics.

Vercelli: I have a general question. We have here a remarkable group of people who know everything about mast cells and basophils. From my point of view, if we think about potential genetic mechanisms, the question is, are there individuals with a genetically determined deregulation in their pathways that leads to degranulation and mediator release because of a lower threshold? In such individuals, stimuli that would normally be harmless may become pathogenic.

Galli: This is an interesting topic. Individual variation in the responsiveness of basophils and mast cells derived from different patients has been studied by a number of investigators, including Don MacGlashan and Gianni Marone. Perhaps we could discuss this after Larry Schwartz' presentation on the effector cells. However, getting back to one of the general discussion topics, I am still not certain as to whether we have a consensus on the issue of anaphylaxis being more or less severe in patients with atopy. Or does it depend on the circumstances?

Sampson: At least when we look at patients with food allergy, the patients who die virtually all have asthma and are atopic. Among the food allergic group atopy seems to be extremely harmful.

Galli: I would like to attempt to link this observation to one of the points that I made in my introduction. Mast cells and basophils in mice or humans with high levels of IgE have high levels of surface expression of the FcεRI, and these cells thereby have heightened ability to release mediators when activated by IgE-dependent mechanisms (Hsu & MacGlashan 1996, Yamaguchi et al 1997, 1999, Lantz et al 1997, Williams & Galli 2000, MacGlashan et al 1997). Specifically, *in vitro* studies indicate that cells with high levels of surface expression of FcεRI both undergo activation at lower concentrations of antigen and also, upon activation, can release larger amounts of pre-formed and lipid mediators, and cytokines (Yamaguchi et al 1997, 1999, Williams & Galli 2000, MacGlashan et al 1997). This could be one explanation for why people with very high levels of IgE may experience more severe anaphylaxis reactions to the same antigen than those with low levels of IgE.

Sampson: The one other observation that we have made is that we don't see a good correlation between the level of specific antibody and the severity of the allergic response. We see people with very low levels of food-specific IgE who die, and people with extremely high levels that get just some eczematous symptoms. We haven't seen a good correlation there, but there does seem to be this thing with asthma.

Pumphrey: Looking at the fatal cases and the cases we see in clinic, the severity of the responses does not seem to depend on the severity of allergy, but on

concomitant pathology. We recently had a striking illustration of this. A child at the age of six weeks was given cow's milk for the first time. He had a generalized allergic reaction with urticaria, local symptoms in the mouth and so on. That child then had bronchiolitis, went on to have viral-associated wheeze, and at the age of 5.5 months was given a similar dose of cow's milk, resulting in a fatal asthmatic anaphylactic reaction. I suggest the severity of the allergy hadn't changed; there was simply concomitant pathology that made the reaction more dangerous.

Galli: This certainly seems to be a well documented clinical observation. I am having a little trouble with the conclusion that perhaps the allergy hadn't changed in the interval, though. Isn't that an assumption? The child was allergic in the first instance and the second, but couldn't there have been some significant change in an aspect of the allergy during the intervening time?

Pumphrey: There are several possibilities. The level of IgE at the time of death was not outstandingly high (milk-specific 6 kU_A/L, total 22 kIU/L). Looking at that, there was no reason to assume that the child had extreme anaphylactic sensitivity, but it is possible that he died from an asthmatic reaction because of concomitant pathology in the lungs.

Golden: We have reported a number of times that there is not a consistent correlation between the level of venom-specific IgE and the outcome (Golden et al 2001). There is a paradox, if you will. However, there is a statistical correlation but it is the outliers that are the puzzle. It is the patient with barely detectable IgE who dies from sting anaphylaxis, or the patients who have late phase large local reaction to insect stings but rarely get systemic reactions despite markedly elevated venom IgE. I'd also like to put a slightly different spin on something that Hugh Sampson said. I'll suggest that there are at least two different phenotypes in anaphylaxis: the respiratory pattern and the vascular pattern. They can coexist, but perhaps what is occurring in the paediatric population is that they rarely get vascular symptoms — they are predominantly respiratory or cutaneous — and this may correlate more with atopy. The atopic children may have more of that respiratory pattern and fatal asthmatic deaths, whereas the vascular pattern which is more common in adults may not behave with that atopic or respiratory correlation.

Schwartz: In any definition one comes up with for anaphylaxis, it is just as important to consider the cells involved as it is to distinguish between whether it is IgE or non-IgE. It is important to decide whether anaphylaxis is mast cell-dependent or whether it depends upon other cell types. We participated in a study with John Yunginger looking at fatal anaphylaxis (Yunginger et al 1991). We divided the groups into those that had been exposed to the precipitating factor parenterally versus orally. The parenterally exposed subjects had low levels of antigen-specific IgE in their postmortem serum, but very high levels of mature tryptase. In those that succumbed to an oral challenge, they had high levels of

antigen-specific IgE, but low levels of mature tryptase. This suggests that there is a different magnitude of mast cell responsiveness depending on the route of administration. In a study that Peter van der Linden (van der Linden et al 1992), sensitive individuals were systemically challenged with insect stings; anaphylaxis occurred in a number of them. If you look at the baseline levels of total tryptase in their blood prior to anaphylaxis, there were higher baseline total tryptase levels in those who were destined to have a more severe anaphylactic reaction, perhaps reflecting higher mast cell numbers. There have been some reports in the literature suggesting that having a high baseline level of tryptase may be a risk factor for anaphylaxis. Although this remains to be confirmed, if true, does it reflect simply an increase in mast cell number, or instead some kind of priming or hyper-releasability of the mast cells?

MacGlashan: We have discussed several times the correlation (or lack of) for an antigen-specific IgE response and anaphylaxis. The other part of this is the ratio of the antigen-specific IgE to the total IgE. It is generally not the case that you can cross-calibrate between antigen-specific IgE, measured by radioallergosorbent test (RAST), and total IgE, measured by radioimmunosorbent test (RIST). In fact, the absolute ratio is never known (unless a laboratory explicitly cross-calibrates the two assays). In terms of the studies mentioned, we discuss this relationship between specific IgE and the anaphylactic response, but I take it it is not actually known what the specific to total IgE ratios are.

Golden: In the insect sting cases we have not systematically looked at the ratio of specific versus total serum IgE. We are in the process of doing that in our latest series of sting challenges, but I have no data yet.

Ring: Coming back to the relationship between atopy and anaphylaxis, I think the route of contact is crucial. Atopy is a hypersensitivity of the surface: the mucous membranes and the skin. Therefore, when the antigen comes via the surface, there is a connection. When the antigen comes parenterally, like a drug, there is no relation.

Lasser: It may be a mistake to look at death as an endpoint for the kinds of things that we are discussing. In contrast material reactions, for example, it is known that the incidence of total reactions is highest from the ages of 10–45. The incidence of total IgE is likewise highest in these ages, but death rates peak about age 70 (Lasser et al 1997). In other words, I think there are concomitant circumstances that go into the production of death at that time, versus earlier times. The patients are more fragile.

Galli: Most people would probably agree that if a patient who undergoes anaphylaxis also has significant pre-existing cardio-pulmonary problems, then it will be more likely that the anaphylactic reaction will be fatal. However, severe anaphylactic reactions resulting in death also occur in the younger age group. That outcome can occur both in the atopic and non-atopic populations. It is

probably fair to say that the most compelling data indicating that an atopic history is a predictor of a severe outcome are those from the food allergic patients. So, let's go back to the publication entitled 'A revised nomenclature for allergy' (Johansson et al 2001). I think this is the report that Johannes Ring was referring to in his paper when he mentioned the proposal that 'anaphylaxis' be used as a general clinical term. A number of you have commented that it is critical to understand the actual mechanisms that are responsible for producing this clinical picture in a number of different settings, but that these mechanisms may not always be known at the time when it may be necessary to explain the cause of death. Therefore, as I understand the recent proposal, it is to use the term anaphylaxis for all of these cases and then leave to the basic and clinical investigators the problem of ferreting out the mechanism(s) responsible. Would anyone like to comment on this proposal?

Metzger: As a non-clinician, I would opt for Frank Austen's position. It seems to me that at the autopsy or death report one shouldn't go beyond one's knowledge. If the patient died of respiratory death or shock, this should be put down. As soon as you use a term such as anaphylaxis you are implying something for which you may lack firm evidence. At one stage sarcoidosis was not distinguished from tuberculosis: you could make the same argument there. To the extent that we know at least one cause of a particular syndrome, it is useful to have a specific term for that syndrome caused by that pathology. That similar symptoms may occur due to other forms of pathology shouldn't justify the use of the same term.

Pumphrey: My understanding is that this new definition does allow for that. Anaphylaxis is divided into allergic and non-allergic, and the allergic is divided into IgE-mediated and non-IgE mediated. What you are talking about is IgE-mediated anaphylaxis, so you could possibly defend the European Academy of Allergology and Clinical Immunology definition.

Schwartz: How would you then classify the hypotensive shock that occurs from complement activation, due to dialysis membranes, for example? This doesn't involve mast cells or IgE. Would you call that anaphylaxis?

Pumphrey: That's a non-IgE-mediated anaphylaxis.

Marone: Frankly, I am a little confused by that position paper on the classification of allergic diseases. As previously mentioned by Frank Austen I fully understand the meaning of IgE-mediated anaphylaxis. It is more difficult for me to understand non-IgE-mediated anaphylaxis. Also in that paper it has been suggested to eliminate the use of the term 'anaphylactoid' reactions. We have to discuss this.

Galli: I have always liked that term, because it covers a whole variety of situations where we lack the information to say that something is anaphylaxis.

Mosbech: From a clinical point of view we have to keep things simple. We have heard that a lot of these patients are not treated as rapidly as they should be. If the

physicians have to think about whether this is one or the other, this might hinder prompt treatment. So it might be a good idea to call all these responses anaphylaxis.

Sampson: I can think of a scenario where that would be harmful to the patient. We have seen a syndrome in young children called milk-induced enterocolitis. These children present with severe repetitive vomiting, marked hypotension and look extremely sick. If you then diagnose that as anaphylaxis and give epinephrine, this will do nothing for them. What they need is large volume expansion. This is part of the therapy, but the first response would be to give them epinephrine, and this is not what these children need.

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The high affinity receptor for IgE, FcεRI

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Abstract. Progress in our understanding of the structure and function of the receptor with high affinity for IgE (FcεRI) is already being applied in attempts to mitigate allergic reactions, specifically by using specific anti-IgEs to prevent sensitization. Investigations of the complex network of intracellular signals generated by FcεRI and related receptors are proceeding. Although achieving a 'complete' description of the events will be a daunting task, the effort is likely to identify additional targets for therapeutic intervention along the way.

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As we learn more about the variety of surface proteins through which cells sense and respond to their environment it becomes increasingly apparent that the simplistic, linear conceptualizations we adopted during our initial experimental probes in which we focused on the cellular consequences of a single type of ligand–receptor interaction, are no longer adequate. We must now confront entire systems of molecules whose interactions with each other vary with time and with changes in the external milieu. In this discussion, I will use the receptor with high affinity for IgE as an example of such a conceptualization, emphasizing not so much the results that have been obtained so far, as the approaches that will be necessary to pursue a more holistic approach to the receptor's function.

This audience is undoubtedly familiar with many of the basic facts about the receptor (FcεRI) and up-to-date reviews have appeared regularly (Kinet 1999, Kawakami & Galli 2002, Metzger et al 1989), so I will sharply limit my discussion of these matters.

Status of structural analyses

X-ray diffraction structures of IgE, of the IgE-binding ectodomains of the receptor's α chain, and of the 1:1 complex of these structures have been determined (Wurzburg & Jardetzky 2002). The sites of interaction are

sufficiently well defined to assist in the rational development of small molecules that could act as inhibitors of the binding (Pietersz et al 2002). As noted below, the increasing evidence that anti-IgE antibodies that inhibit the interaction are clinically useful (Chang 2000, Metzger 2003), shows that in principle, this approach is worth pursuing.

Regrettably, there has been little progress in structurally defining the cytoplasmic domains of the β and γ subunits of the receptor, although there continues to be progress in clarifying the interactions of these domains with other cellular proteins before and after their phosphorylation. Interfering with such interactions is another potential target for low molecular compounds and it would be helpful if structural data comparable to that for the interaction between IgE and the receptor might be available.

Initial events

Aggregation as the initiating event

Considerable experimental data support a model in which it is the aggregation of the receptor induced by the interaction of a multivalent antigen with the receptor-bound IgE that initiates a variety of biochemical cascades (Metzger 1992). The ability of apparently monomeric IgE to likewise trigger similar events is likely a laboratory artefact due to the tendency of certain monoclonal IgE antibodies to aggregate (Asai et al 2001, Kawakami & Galli 2002) either spontaneously or possibly due to some cross reaction with an antigen in the medium or on the cell surface (see also below). Whether the 'anti-apoptotic' effect of the binding of IgE to FcεRI is similarly based on aggregation remains unclear (Asai et al 2001, Kawakami & Galli 2002).

Molecular consequences of aggregation

A variety of experiments have supported a mechanistic model in which a kinase (Lyn) constitutively associated with the C-terminal cytoplasmic extension of the unphosphorylated β chain *transphosphorylates* one or more γ chains on an alternate receptor that has become approximated by the binding of antigen (Pribluda et al 1994). Experimental data suggest that normally this initial event occurs in specialized lipid 'micro domains', but that that is an incidental occurrence rather than an absolute requirement. A somewhat different model proposes that the aggregation-induced movement of receptors into the micro domains is critical for the phosphorylation of β and γ because the micro domains are enriched in the kinase (*translocation model*) (Baird et al 1999).

Recent new findings have so-far not resolved the issue. On the one hand, studies using rigid bivalent ligands whose epitopes are thought (but not proven) to be

sufficiently distant from each other to make the transphosphorylation sterically impossible, found them capable of stimulating phosphorylation of the receptors, suggesting to the authors that the translocation model is correct (Paar et al 2002). On the other hand, experiments in which normal phosphorylation of the receptors was observed even though Lyn or constructs of Lyn that were incapable of entering the domains (Kovarova et al 2001, Vonakis et al 2001) were used, or the domains were disrupted (Yamashita et al 2000), seriously challenge the proposal that translocation is required for the initiating event. The subtle difference in these proposals is not irrelevant if one were to try to disrupt receptor-initiated responses by interfering with the proximal event following aggregation. On the basis of the transphosphorylation model, one would focus on inhibiting a specific protein–protein interaction; alternatively one would have to interfere with more non-specific protein–lipid domain interactions. Further exploration of these alternative models is warranted. The recent observation that a related kinase, Fyn, is also required during the initiating events has added a further level of complexity (Parravicini et al 2002).

Later events

Phosphorylation of FcεRI attracts a variety of additional proteins to the receptor aggregates. The composition of these signalling complexes, their lifetimes, and their topological movements are being actively pursued (Rivera 2002). That the propagation of signals requires the assembly of a multicomponent macromolecular 'machine' explains why at least some of the cellular responses reflect the lifetime of the receptor aggregates. This leads to a phenomenon called 'kinetic proofreading' (Hopfield 1974, McKeithan 1995), a mechanistic regime under which the length of the signalling pathway stimulated by different ligands will be inversely related to their rate of dissociation from their binding component. In the case of FcεRI, it is the rate of dissociation of the allergen from the receptor-bound IgE that determines the lifetime of the receptor in the aggregate, and therefore the length of time the aggregate can propagate the sequential downstream events.

We have explored the importance of kinetic proofreading in the IgE–FcεRI system and have uncovered two interesting aspects. The first was the phenomenon of non-competitive ligand inhibition. In this situation one ligand can inhibit the cellular stimulation by another ligand in the absence of direct competition for the ligand-binding sites (Sette et al 1994). The mechanism we uncovered was based upon the inhibitory ligand's ability to sequester the limited supply of a critical enzyme (Torigoe et al 1998). A second phenomenon, the details of which have just been published (Eglite et al 2003), relates to the ability of a weakly binding (rapidly dissociating) ligand that stimulates a relatively distal

response such as degranulation, poorly or not at all, to nevertheless effectively stimulate cellular responses that are even more delayed, e.g. transcription of the gene for the chemoattractant MCP-1. In this instance it appears that relative to its own lifetime, a critical messenger for stimulating the transcription, Ca^{2+}, is generated early enough in the cascade initiated by the receptor aggregates, that adequate concentrations of the ion can be stimulated even by the short-lived aggregates.

Future analyses

Ultimate goals

The goal(s) for those engaged in unravelling the ways particular cells respond to a variety of external signals is to '... understand as completely as possible the relationships between sets of inputs and outputs that vary both temporally and spatially. How does a cell respond appropriately to one voice when it must listen simultaneously to many, and how does it alter this response in the context of other concurrent or recent signalling events?'(Gilman et al 2002). Ultimately, of course, one wishes to use this information therapeutically, either to correct genetic defects or pathological responses. The more we know about the signalling pathways the better we will be able to pinpoint the appropriate targets for manipulation, but our understanding need not be exhaustive before such interventions can be attempted. A good example, already referred to above, is the use of anti-IgE to prevent sensitization. This approach was initiated even before many of the details about how IgE interacts with FcεRI were uncovered. Even now, the impact on the therapeutic effectiveness of the anti-IgE interacting with membrane IgE on B cells (but not with IgE bound to FcεRII on lymphocytes, monocytes and platelets) and the presence of IgE:anti-IgE complexes in the serum remain to be determined (Chang 2000).

What will be required and how will we get there?

To achieve the more complete understanding we seek, we must first identify all the critical molecules engaged in relevant signalling pathways, and characterize the temporal changes in their concentrations, locations, interactions, and modifications normally, and in pathological conditions. Second, these data must be incorporated into mechanistic and quantitative models that can be used to test whether the critical events are understood, and to predict the consequences of interventions.

Those who are following the progress in this area of cell biology are becoming increasingly impressed by the extraordinary complexity of these systems. Although there has been nothing uncovered so far that would justify a nihilistic

assessment of the likelihood for achieving any level of understanding we desire, the enormity of the task ahead cannot be minimized. It makes the unravelling of the human genome look relatively simple by comparison. This realization is prompting some novel proposals on the organization of research in this area. Perhaps the most ambitious proposal is the recently organized 'Alliance for Cellular Signaling' (AfCS) (Gilman et al 2002).

This consortium currently involves some 50 'participating investigators' from around 20 institutions who will organize their group and individual activities according to an initial organizational network (see Fig. 1 in Gilman et al 2002) that stipulates the interactions for decision-making with respect to research directions, development of new technologies, distribution of experimental findings and publication of significant new information.

The AfCS's initial experimental strategy is presented in Table 1. As can be seen there is nothing particularly novel in the individual components; all of the same elements have been used by individual investigators interested in particular cellular events. The unique feature will be the coordination and comprehensiveness of the efforts.

In their inaugural 'Overview' the group underscores two aspects of this endeavour. First, that this endeavour is itself an experiment in collaborative science that although not unprecedented in biology (e.g. the human genome project) is unusual in the basic research arena. There are implications to such efforts that go well beyond the experiments conceived, planned, executed, interpreted and published by committees. For example, the current reward systems in academic careers in the biological sciences put a premium on individual creativeness, a characteristic that will be difficult to tease out for any particular participant in such group efforts.

Second, only a modest contribution can be expected from even the substantial coterie of investigators assembled so far along with its $10 000 000 initial annual budget relative to the size of the problem. The present effort will be directed towards only two cells, mouse B lymphocytes and cardiac myocytes. The

TABLE 1 Overview of AfCS experimental strategy

1. Define basic scope, including pathways of interest, and initial list of molecules
2. Determine which molecules are present, their amounts and subcellular location over time
3. Expand list of molecules using broad screen to detect protein–protein interactions
4. Assess physiological validity of candidate interactions
5. Define flow of information quantitatively with selected inputs, intermediate responses and endpoints
6. Develop and test models

consortium of course hopes that their experiment in 'socialistic science' will be sufficiently productive to attract the (financial) interest of pharmaceutical firms, and to encourage additional investigators to use the AfCS approach as a research paradigm.

Quantitative modelling

I shall close this discussion with a brief consideration of the last element of this list on which my own group has focused in a long-term collaboration with a group in the Theoretical Biology and Biophysics Group of the Theoretical Division at Los Alamos. Although using the same overall strategy planned by the AfCS, our effort has of course been much more limited. We have varied only a single type of input (aggregation of FcεRI) and quantitatively examined the 'flow of information' through only a selected set of molecules that are engaged very early in the response. A presentation of the detailed mathematical model and its properties has just appeared (Faeder et al 2003).

Many of the molecular mechanisms that are modelled are based on experimental results from ourselves as well as several other groups, but also include a number of assumed aspects. The hope is that such modelling will allow one to test the validity of certain assumptions.

The current version models the phosphorylation of FcεRI and Syk following aggregation of FcεRI by addition of chemically cross-linked dimers of IgE — a surrogate input for aggregation of receptor-bound IgE by antigen. The model is a network which contains 354 discrete chemical species and the chemical reactions that connect them. Concentrations of the principal components (FcεRI, Lyn and Syk) are based on measurements using quantitative Western blotting. Twenty-one rate constants based on direct measurements or other observations are used: four constants relate to binding of the dimers to the receptors, four each to the association of Lyn and Syk kinase, eight to the phosphorylation of tyrosines in the various molecular aggregates and one for the rate of dephosphorylation. Among the simplifying assumptions is that multiple tyrosine residues on the subunits of the receptor and on Syk are treated as single units of phosphorylation. The network is then converted to a predictive model using a set of coupled differential equations. Quantitative outcomes were based on a computer program that generates the states and reaction network from the components and reaction rules. A second program uses the generated network, rate parameters and initial concentrations to generate and solve the set of 354 differential equations as a function of time.

I will not focus on the results of this modelling except to note a few findings. First, simulations of experiments where RBL cells were exposed to covalently cross-linked dimers of IgE reproduced the observed kinetics of phosphorylation

of the receptors' subunits over a period of an hour, and the predicted dose response curves followed closely those observed experimentally. The model also is consistent with the evidence for a kinetic proofreading constraint with respect to the phosphorylation of Syk kinase as observed experimentally. The model also makes a variety of other mechanistic predictions that can be experimentally tested in future experiments.

There is no reason to think that this approach would not be equally applicable to examine related systems such as those stimulated by other multi-subunit immune response receptors that initiate cellular responses using related molecular mechanisms. But, what is the likelihood that such an approach can be extended to more complete networks involving many more inputs simultaneously triggering multiple receptors thereby activating networks containing possibly hundreds of nodes?

The principal authors of this model at Los Alamos conclude on a pragmatic note: 'Ultimately we want sufficient knowledge for quantitative prediction of larger segments of signalling pathways. To have confidence in such predictions, our approach is to proceed step-wise, testing each extension of the model against many different forms of experimental data' (Faeder et al 2003).

The leaders of the AfCS believe that this incremental approach will be inadequate to deal with the complexity of cellular networks. 'For this goal [to model such networks] it will almost certainly be inappropriate to adopt the traditional reductionist approach of simply measuring *in vitro* all the K_d, K_m, and V_{max} values, cooperativity constants, rate constants and molar concentrations for all molecules involved' (Gilman et al 2002). They surmise that it will not be practical to mimic the milieu in which these reactions occur nor the multiple interactions of single molecules and that therefore it will not be possible to estimate accurately many of the avidity and rate constants. Finally, they consider the manipulation of the mass of data and the equations that relate them, 'daunting'. Certainly no one could argue with the latter assessment.

The alternative approach they propose is first to express the observed functional interactions in relative terms. For example, fractional activation of a particular molecule would be related to a fractional effect on a downstream molecule in order to arrive at a series of 'linkage functions'. This approach, albeit still demanding, is much less so than the explicit approach currently used by the Los Alamos team and others. But the strategy proposed by the AfCS also has a potential problem. That is, whereas such linkage functions can be manipulated to give reasonable quantitative predictive values over a given set of inputs, they may not be able to distinguish between substantially different mechanistic models. The Ptolemaic epicycles and a variety of other mathematical constructs were quite effective in rationalizing the wandering motions of the planets but of course bore no relationship to the reality of our solar system.

Closing comments

The investigation of cellular responses is proceeding from the state where the focus was on identifying individual molecules affected by individual receptors and the linear pathways that connected them, to a more physiologically relevant approach. This will try to describe the multitude of molecular responses that follow variations in the stimuli to the multitude of receptors that cells use to monitor their environment. We already know enough to be able to develop a realistic assessment of the enormity of the task, but despite this enormity there is nothing, so far, that suggests that the task is in principle undoable.

The hope is that such investigations will not only be enlightening but also useful. Specifically, in the networks that are currently known one can distinguish two types of molecular interaction. The members of one class involve 'nodes' in which a molecule engages in only a small number of interactions; alternatively other interactions occur in 'hubs' and involve a multitude of interactions. Pathological aberrations in nodes may be relatively easy to correct by manipulations that will create detours around them; defects in hubs may be much more challenging to overcome but on the other hand can serve as effective targets for disabling a whole set of responses.

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DISCUSSION

Lasser: I have a rather simplistic question. In what way does the anti-IgE molecule inhibit the binding of IgE? What is the mechanism?

Metzger: The simple answer is that it sterically inhibits: it binds to an area on the molecule that is necessary for it to interact with the receptor. It is a little bit like the princess and the pea: it doesn't necessarily have to interact directly with those atoms that are involved with the interaction; as long as it prevents the IgE and receptor from forming the van der Waal's contact, that is enough to prevent the binding.

Lasser: Why does it do that?

Metzger: Because this particular antibody is directed towards a region of the IgE, the binding to which interferes with the interaction between the IgE and the receptor. The critical thing is that those molecules that bind to the IgE and inhibit the binding don't at the same time bind to molecules of IgE that are already bound. That's a perfect way of triggering the cell: to use an anti-IgE that aggregates the IgE.

MacGlashan: In the modelling that you and Bryon and his team have done, have you identified a hub in that signalling network?

Metzger: To some extent Syk would be a hub, as would Lyn. The Lyn phosphorylates Syk which then phosphorylates other molecules.

MacGlashan: I know they have this interesting path analysis where they work out which species are actually dominant in the network.

Metzger: The current concept is that there is some sort of an initial signalling complex, and that is what that diagram showed. Almost any molecule in that initial complex might be an effective target. We know that in the case of the inhibitor of the intrinsic kinase of the EGF receptor, this is an approach that has some therapeutic possibilities. Whether that would be better than preventing the initial sensitization of the cells, which a small molecule equivalent of anti-IgE could do, I don't know. I suppose one could say that if we had a way of preventing the interaction with an intracellular hub, we could reverse an allergic reaction, which the anti-IgE can't do. But this is a much more complex approach.

Galli: There are some recent observations that IgE, prepared in a way to ensure as much as can be done experimentally that one is dealing with monomers, might have effects on mast cells survival and function that are independent of any agent added specifically to induce FcεRI aggregation. The two reports (Asai et al 2001, Kalesnikoff et al 2001) agreed on several points, one of which is that these IgEs would inhibit the development of apoptosis in mouse mast cells from which growth factors have been withdrawn. I will mention two reservations about these two studies. First, they were done entirely using mouse cells, and second, the work was done entirely *in vitro*. Moreover, there were other observations in the two papers that differed. In the study by Kalesnikoff et al (2001), the IgE antibody preparations induced the cells to release cytokines, interestingly without evidence that they were releasing histamine. In contrast, in the study by Asai et al (2001), the antibodies did not induce the release of cytokines.

Both groups of course were curious about why they had obtained apparently contradictory results. They discovered that, for the most part, they had been dealing with different panels of monoclonal IgE antibodies. Most of the IgE antibodies have now been tested in both labs. The simplest way to summarize our results is that when we tested the antibodies used by Kalesnikoff et al, we got results similar to those that they had reported. In other words, there are some antibodies that can induce an enhanced resistance to apoptosis without inducing detectable cytokine secretion, and there are some that both enhance the resistance to apoptosis and also induce cytokine secretion (Kitaura et al 2003). In our hands, the latter antibodies can also induce histamine release. We performed indirect tests on whether the antibodies can induce FcεRI aggregation, such as examining Syk dependence using Syk knockout mast cells, or using monovalent hapten to inhibit the reaction. These experiments showed that the IgE antibodies can induce

responses that are like those induced by aggregation of FcεRI, in that they are completely Syk dependent and they can be inhibited by monovalent haptens (Kitaura et al 2003). But there is, as yet, no formal proof that FcεRI aggregation actually occurs in this setting. If it does, we don't know how it happens, although it could be related to the specific structural properties of the individual IgE molecules. Moreover, some of the observations have been replicated using serum-free medium (Kalsnikoff et al 2001, Kitaura et al 2003). So it is unlikely that the phenomenon simply reflects a reaction of the IgE with an unknown factor present in serum. However, so far, these observations lack a mechanistic explanation. Note: since the conference was held, it has been reported that one of the IgE antibodies that can induce the strongest enhancement of mast cell survival and mediator secretion has an antigen binding site that exists in two different conformations, one of which binds DNP and the other of which binds an unrelated antigen (James et al 2003). The potential 'multispecificity' of IgE antibodies, based on the conformational diversity of the antigen binding site, represents an intriguing clue to the mechanism by which certain IgE antibodies might induce FcεRI aggregation in the absence of 'specific' antigen (James et al 2003, Foote 2003).

Metzger: In some unpublished work (C. Torigoe), the two different kinds of IgEs behave differently on acrylamide gels as if the stimulatory IgE might be an aggregate.

Galli: Even the antibodies that don't induce detectable cytokine or histamine release can enhance resistance to apoptosis. This effect can also be blocked by monovalent hapten. One might argue that enhanced resistance to apoptosis on the withdrawal of growth factors is one of the most sensitive ways the cell can indicate that something is happening with the receptor that is very much like aggregation, if not aggregation itself. If that is true, then mostly everything can be explained.

Lasser: Is it not the case that this IgE molecule might elicit anti anti-IgE molecules?

Sampson: As far as I know, no one has reported any antibodies to the antibody when it is given i.v. or subcutaneously.

MacGlashan: There was a report where it was inhaled. It wasn't really in patients receiving the drug.

Sampson: In the one case I know of it was inhaled, but it hasn't been seen in subcutaneous or i.v. administered antibody.

Kricek: I wanted to extend this one step further. There exist anti-IgE autobodies, which occur naturally in the serum and which are non-analaphylactogenic. There are not only those which see the receptor binding sites of IgE. The same holds true for anti-α chain antibodies. There are some which are anaphylactogenic, some which are not, and some which become anaphylactogenic if you remove IgE

from the receptor. The idea was always that a therapeutic anti-IgE would have to compete with a high affinity interaction. One knows that just a couple of receptors which are still able to be triggered by IgE are sufficient to induce anaphylactic reactions. People always said that such a therapeutic approach wouldn't really work, even using a high-affinity anti-IgE antibody. The antibody that has been developed by Novartis is only moderate. Nevertheless, clinical data show that it works. One of the explanations was the down-regulatory effect on the receptor of removing IgE from the circulation. Another aspect has not yet been discussed: what if you generate anti-idiotypic antibodies? These would then mimic the part of IgE that interacts with the receptor. They might bind to the receptor and potentiate the effect of the passive anti-IgE immunotherapy. I don't know whether anyone who has developed therapeutic anti-IgE antibodies has already looked into this. The consequence would be that we shouldn't stuff half a gram of this anti-IgE into the patient, but instead we might formulate it as a vaccine and get the same protective effect with much less material.

Gould: I wanted to clarify the statement concerning the comparison of the two IgEs on gel electrophoresis. Did you mean when you said that it acted as an aggregate that it had oligomers? Or could the fact that one is slower be due to differential glycosylation?

Metzger: That still needs to be looked into. It would have to be a very substantial difference in glycosylation.

Marone: We have approached the question of the binding of monomeric IgE from a different angle. In collaboration with Lars Björck of the University of Lund. They purified a protein (protein L) from a bacterium (*Peptostreptococcus magnus*) which has four binding domains (B1–B4) for κ light chains. Each binding domain, B1–B4, was originally described to have a single binding domain for the κ light chains. We found that protein L and B1–B4 were potent stimuli for the release of preformed and *de novo* synthesized mediators from human basophils and mast cells (Genovese et al 2003). Björck and collaborators recently produced the recombinant B1 that apparently had a single binding domain for κ light chains. We found that B1 is also a very important stimulus for the release of mediators from basophils and mast cells. However, Björck and collaborators went back and did more careful NMR studies. These revealed that there are two binding sites for κ light chains on each domain of B1–B4, which turned out to be the explanation for our results.

MacGlashan: The 'dog in the manger' idea is applicable in your experiments to two different antigen specificities, where one draws the Lyn kinase from the other's reaction, effectively. The lower-affinity one is using up the available Lyn kinase, so that the higher affinity interaction doesn't seem to take place. Hugh Sampson and I had a conversation earlier about whether or not that would apply when you flood the cell with a low affinity IgE to the same antigen to which there might be some

high affinity interaction. In effect, the same antigen is binding to a low affinity IgE and a high affinity IgE on the cell. If there was enough low affinity IgE it would act in the same way. The lower affinity interaction might not be able to trigger, but it would give you that 'dog in a manger' effect, so that it gives you a disproportionately poor response in the cell. Have you ever done an experiment like this, where you had IgEs of differing affinities to the same antigen?

Metzger: No, we have used cross-reacting antigens with different affinities. The 'dog in a manger' effect is done with two antigens, both of which react with the same IgE, but which have very different affinities.

MacGlashan: So that would be the application of this idea. Part of the anaphylactic story is whether or not, at any given moment, your affinity for the antigen has shifted to one that is dominantly low or dominantly high.

Metzger: As you know, this is an area that those interested in modifying the action of T cells are exploring. By using so-called 'ligand antagonists' they hope to prevent T cell stimulation.

Finkelman: There is a very similar phenomenon in the B cell world. This is that if B cells don't express IgM or some other surface immunoglobulin, they die. This is despite there not being any established ligand for the IgM or IgD on these B cells. The idea is that there is some weak affinity interaction that causes a tiny amount of cross-linking, but it is not really established that cross-linking is critical.

Galli: Trying to prove the presence or absence of a tiny amount of cross-linking is rather difficult.

Finkelman: You mentioned that you were using a univalent ligand as an inhibitor. Which one do you use for FcεRI?

Galli: Depending on the specificity of the IgE antibody being tested, we used DNP-lysine, or TNP-glutamate (Kitaura et al 2003).

Finkelman: You are inhibiting the aggregation then.

Galli: Presumably. This speaks to Henry's point about whether some low level of aggregation will also have an anti-apoptotic effect. If the important event in this phenomenon is a low level of FcεRI aggregation, then, yes, this can have an anti-apoptotic effect and it is inhibited by the monovalent hapten.

Metzger: The critical point, which is confusing, is that it suggests that spontaneous aggregation is occurring via the antibody combining sites. It is strange. They used a hapten that was specific for the specificity of that IgE.

Finkelman: Do you get binding of the IgE to plastic when you do the *in vitro* culture? One of the ways of promoting T cell signalling is to coat plastic in anti-CD3 antibody. Do you see the same thing with the IgE antibody?

Galli: I don't think that any of the experiments have involved an attempt to bind the IgE to plastic.

Finkelman: As an accident? Is there anything to prevent it from occurring?

Galli: I can't answer that. One would have to investigate this possibility directly.

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Effector cells of anaphylaxis: mast cells and basophils

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Abstract. Systemic anaphylaxis arises when mast cells, possibly along with other cell types, are provoked to secrete mediators that evoke a systemic response. Mast cells in perivascular, respiratory, gastrointestinal and cutaneous tissues are likely involved, regardless of whether IgE or non-IgE-dependent pathways are invoked. α/β tryptases are selectively and abundantly produced by mast cells. Tryptase levels in the circulation provide a precise indicator of mast cell involvement. Mature β tryptase is stored in secretory granules and is released when the cells are activated to degranulate, as occurs in anaphylaxis. α/β pro/pro' tryptases are spontaneously secreted by mast cells. Consequently, mature tryptase levels in serum (normally 1 ng/ml) are elevated in systemic anaphylaxis. Total tryptase levels (mature plus precursor forms), normally 1–15 ng/ml in baseline serum samples, are elevated in patients with systemic mastocytosis ($>$ 20 ng/ml), a disease that also predisposes one to anaphylactic reactions. The assessment of basophils in systemic anaphylactic reactions has been problematic, because an assay for a specific releasable marker from this cell type has not been developed. Nevertheless, in cases of anaphylaxis in which elevations of histamine, but not tryptase, have been detected, it is enticing to speculate that basophil-dependent anaphylaxis may have occurred.

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Definition

Systemic anaphylaxis arises when mast cells are provoked to secrete mediators that evoke a systemic response. Although mast cells in any organ system may be involved, depending on the distribution of the instigating stimulus, the principal targets include the cardiovascular, cutaneous, respiratory and gastrointestinal systems, sites where mast cells are most abundant. The terms anaphylactic and anaphylactoid, respectively, attempt to distinguish between mast cell activation initiated by allergen and IgE through FcεRI, classical immediate hypersensitivity, versus those that initiate mast cell activation by alternative pathways. Although the mediators elicited from mast cells will overlap extensively in anaphylaxis and anaphylactoid reactions, and thereby invoke

similar acute therapies, understanding differences in causation will likely impact on therapeutic interventions aimed at preventing future attacks. Cells other than mast cells also undoubtedly participate in systemic anaphylaxis, particularly those armed with antigen-specific IgE. Basophils, like mast cells, constitutively express substantial amounts of the tetrameric ($\alpha\beta\gamma_2$), high affinity receptor for IgE, FcεRI, and when activated through this pathway, also release mediators within minutes. Eosinophils, monocytes, antigen-presenting cells and epithelial cells may be induced to express this receptor, primarily in its trimeric ($\alpha\gamma_2$) form and thereby affect the intensity, duration or character of anaphylactic reactions. Whether some cases of systemic anaphylaxis occur through one or more of these cell types without involving mast cells is theoretically possible, but remains controversial.

Precipitating factors

Most IgE-dependent mast cell activation events occur at local sites, and result in local disease. For example, allergic conjunctivitis, allergic rhinitis or allergic asthma typically occurs when allergen lands on the corresponding mucosal surface of a sensitive individual. Systemic anaphylaxis presumably requires the allergen (or non-allergen agonist) to distribute systemically before mast cells at remote sites will be activated. This is most likely to occur with parenteral administration, less likely with administration by the oral route, by inhalation or by direct cutaneous or ocular contact. Activation of mast cells in perivascular locations should have the greatest effect on systemic vascular responses, even though large amounts of mediators released locally in theory could spill into the circulation and affect remote sites. However, the precise distributions of mast cells that are activated during anaphylactic reactions are undetermined. Also, the numbers of mast cells available to respond, or factors affecting mast cell releasability may have an impact on the severity of the response of a sensitive subject to an allergen challenge, and need to be better understood.

Allergens

The most common allergens causing systemic anaphylactic reactions include drugs, e.g. penicillin, insect venoms, foods, radiocontrast media, allergen immunotherapy injections, latex and autoantigens. Proteins or glycoproteins typically serve as complete antigens. In contrast, most drugs act as haptens after coupling to self proteins. Allergen multivalency is important, because cross-linking of IgE on the surface of cells brings together at least two FcεRI molecules that then transmit an activating signal into the cell. A monovalent antigen would block cross-linking and thereby should block activation.

An allergen exposure must lead to sensitization before an immediate hypersensitivity reaction can occur, a process involving antigen processing, presentation to Th2 cells and class-switching of allergen-specific B cells to IgE, a process that takes between one to two weeks. Consequently, anaphylaxis does not occur upon first exposure to an allergen, but may occur after subsequent exposures.

Non-IgE-dependent agonists

Most non-IgE-dependent foreign agents do not require antigen processing, and can elicit a mast cell-activation response upon first exposure. These would include radiocontrast dyes, narcotics such as codeine and morphine, and vancomycin. Endogenous mast cell activators include neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide, and the complement anaphylatoxin C5a. Whether a magnitude of mast cell activation sufficient to cause systemic anaphylaxis can result from endogenous secretion or generation of these peptides by themselves is unproven.

Aspirin hypersensitivity can manifest either at mucosal or cardiovascular sites. Most cases of aspirin hypersensitivity appear to be pharmacologically (not IgE) mediated, and in sensitive subjects can occur to any of the cyclooxygenase (COX)-1 inhibitors. A mechanism to explain mast cell activation has not yet emerged. COX-2-selective inhibitors are relatively safe in aspirin-sensitive asthmatics, but have not been adequately tested in aspirin-sensitive anaphylaxis subjects. The recent identification of COX-3 and COX-related enzymes (Chandrasekharan et al 2002) that may have distinct pharmacological profiles adds to the complexity of aspirin-mediated hypersensitivity reactions.

Autoimmunity

Some patients present with spontaneous episodes of anaphylaxis. In certain cases this may be an extension of a physical urticaria. Because chronic urticaria may be associated with IgG and IgM antibodies against FcεRI or IgE, an analogous autoimmune process might cause anaphylaxis.

Epidemiology

Assessing the annual incidence of systemic anaphylaxis and the prevalence of those at risk for systemic anaphylaxis are compromised by imprecise diagnostic measures. Approximately 1500 to 2000 deaths occur per year from systemic anaphylaxis in the USA (Neugut et al 2001). Non-fatal cases are much more common, estimated to occur in about 0.2% of the population per year. Further analyses suggest that between 3 and 43 million (1–15% of the population) may be

at risk for such reactions. Drug reactions account for the majority of cases. Food, insect sting and latex reactions account for many of the other cases.

Pathophysiology

Mast cells participate in both acquired and innate forms of immunity (Wedemeyer et al 2000). They develop in peripheral tissues from bone marrow progenitors primarily under the influence of stem cell factor, the ligand for the tyrosine kinase receptor called Kit. Armed with allergen-specific IgE, they are activated by multivalent allergens that cross-link IgE and associated FcεRI molecules on the mast cell surface. This may be important in the defence against certain parasites that elicit a strong IgE response. Experiments performed in rodents suggest that mast cells also can be directly activated by certain bacterial products, leading to the secretion of mediators that recruit neutrophils, and thereby restrain bacterial dissemination until a more potent acquired immune response develops. Activation of mast cells by endogenous peptides such as substance P or calcitonin gene-related peptide may influence basic biological processes such a wound healing and angiogenesis. Whether mast cells have a critical, non-redundant role in these biologic and immunological processes remains controversial. However, their central role in immediate hypersensitivity is clear.

Mediators released by mast cells include preformed mediators stored in secretory granules, newly generated products of arachidonic acid, and an array of cytokines and chemokines (Schwartz 2002). Histamine, formed from histidine by histidine decarboxylase, is the sole biogenic amine stored in all granules of human mast cells and human basophils. Histamine released by mast cells or basophils diffuses freely, and interacts with H1, H2 and H3 receptors. Histamine-mediated bronchial and gastrointestinal smooth muscle contraction, vascular smooth muscle relaxation and increased permeability of postcapillary venules account for many of the signs and symptoms of systemic anaphylaxis, primarily but not exclusively through H1 receptors. Excessive levels of histamine in the CNS may account for the sense of doom commonly experienced at the onset of anaphylaxis. Once secreted, histamine is rapidly metabolized to methyl histamine and indole acetic acid. Consequently, the plasma histamine level is not a practical test for anaphylaxis. Urinary histamine levels reflect the small portions of histamine not metabolized in the circulation before renal clearance, and are affected by ingested histamine-containing foods and histamine-producing mucosal bacteria, compromising the utility of this test.

Prostaglandin D_2 (PGD_2) is the principal COX-catalysed product of arachidonic acid secreted by activated mast cells, but is not made by basophils. It binds to the G protein-coupled receptors CRTH2 and DP (Hirai et al 2001). Both COX-1 and COX-2 are involved in PGD_2 production by mast cells. Consequently, a COX inhibitor that is bipotent might be better than one which is selective at

blocking PGD_2-mediated responses during anaphylaxis, which may include hypotension, bronchospasm and inhibition of platelet aggregation.

Leukotriene C_4 (LTC_4), is released by both mast cells and basophils after its formation from arachidonic acid and glutathione is sequentially catalysed first by 5-lipoxygenase and 5-lipoxygenase activating protein and then by LTC synthase. Conversion to LTD_4 and LTE_4, which also are bioactive, occurs in the extracellular space. These sulfidopeptide leukotrienes bind to $CysLT_1$ (smooth muscle, epithelial and endothelial cells, lung macrophages, eosinophils) (Evans 2002) and $CysLT_2$ (endothelial and epithelial cells), both G protein-coupled receptors. Sulfidopeptide leukotrienes cause bronchoconstriction, mucus secretion, eosinophil recruitment, increased vasopermeability, diminished cardiac contractility, vasoconstriction of coronary and peripheral arteries and vasodilation of venules.

Mast cells also are the principal source of heparin proteoglycan and the proteases α tryptase, β tryptase, chymase and mast cell carboxypeptidase. Like neutrophils and monocytes, they also contain cathepsin G. Basophils are relatively deficient in these enzymes. Mature tryptase is stored in the secretory granules of all mast cells, while the other proteases appear together in a subset of mast cells. Those with tryptase alone are called MC_T cells, and are the predominant type of mast cell in the lung and small intestinal mucosa, while those with all proteases are called MC_{TC} cells, and account for most of the mast cells in skin, intestinal submucosa, conjunctiva and blood vessel walls. The role(s) of these molecules in the pathophysiology of anaphylaxis are undefined.

Tryptase (EC3.4.21.59) is the most abundant protein product produced by human mast cells, accounting for about 20% of the cell protein, and is derived principally from two genes on chromosome 16, α tryptase and β tryptase. The product of the β tryptase gene(s) is autoprocessed from β protryptase to β pro'tryptase at acidic pH, optimally in the presence of heparin proteoglycan, and then to β tryptase by a dipeptidase, thought to be dipeptidyl peptidase I in humans (Sakai et al 1996). Tryptase in murine mast cells utilizes a different dipeptidase (Wolters et al 2001). Mature β tryptase is stored in secretory granules as an enzymatically active tetramer in a complex with heparin proteoglycan until the cells are activated to degranulate and release the protease–proteoglycan complex. In contrast, α protryptase may not undergo autoprocessing from α protryptase to α pro'tryptase, because a tryptase-resistant Q^{-3} rather than a tryptase-sensitive R^{-3} is present in the −3 position of the propeptide (Sakai et al 1996). Indeed, when mature α tryptase is produced *in vitro*, though it forms a tetramer, the protein appears to be enzymatically inactive (Huang et al 1999, Marquardt et al 2002, Selwood et al 2002).

The major form of tryptase found in normal blood fails to bind to the G5 mAb, which recognizes mature forms of rhα and rhβ tryptases, but not the

corresponding pro or pro' forms of tryptase (Sakai et al 1996, Schwartz et al 1995, 2003). Thus, an immunoassay using this mAb measures mature tryptase. Although mature tryptase levels are undetectable in normal serum (<1 ng/ml), they are elevated in the blood of most cases of systemic anaphylaxis with haemodynamic compromise, particularly when the precipitating agent is administered parenterally. In such cases, the magnitude of mast cell degranulation appears to be the primary determinant of clinical severity (Schwartz et al 1987, 1989, van der Linden et al 1992). In contrast, based on mAbs that recognize all forms of α and β tryptases, a total tryptase immunoassay was developed that detected levels of tryptase in baseline serum from essentially all individuals (mean$\pm$SD, 4.9 ± 2.3 ng/ml) (Schwartz et al 1994). So-called total tryptase levels are elevated in subjects with systemic mastocytosis, and reflect the total body burden of mast cells (Schwartz et al 1995). These observations can largely be explained by the *in vitro* observations that precursor forms of α and β tryptases are spontaneously secreted by unstimulated mast cells, while mature tryptase is preferentially stored in secretory granules and released by activated mast cells.

Approximately 25% of individuals lack a gene for α tryptase (Guida et al 2000, Soto et al 2002). Whether such a genetic difference affects the level of tryptase in the blood has been investigated. Preliminary data in healthy subjects indicates that a deficiency in the gene for α-tryptase does not influence the circulating levels of tryptase precursors in healthy subjects (Schwartz et al 2003). Finally, the hypothesis that mature forms of tryptase are preferentially stored in secretory granules, while immature forms of tryptase are preferentially selected for spontaneous secretion has not been directly examined.

Cytokines (TNFα, interleukins 4, 5, 6, 13 and 16, GM-CSF) and chemokines (interleukin 8, monocyte chemotactic protein 1, monocyte inflammatory protein 1α) represent another dimension of the mediators released by mast cells and basophils. Though not selectively produced by these cell types, the vasoactive and inflammatory potential of such mediators could impact the severity and duration of anaphylaxis. Although cytokine secretion typically occurs in association with granulation, experiments *in vitro* indicate that cytokine secretion also can be induced in the absence of degranulation. As selective antagonists of the relevant cytokines and chemokines become available and are tested for therapeutic benefits, their roles in the pathogenesis of anaphylaxis will be better understood.

Diagnosis and differential diagnosis

Systemic anaphylaxis, with various combinations of hypotension, tachycardia, urticaria, bronchoconstriction, laryngeal oedema, colics and diarrhoea, often associated with a sense of doom, beginning within minutes of the provoking

stimulus, can be precisely confirmed by demonstrating antigen-specific IgE (sensitization) and an elevated β tryptase level in serum (mast cell activation). Skin testing or *in vitro* measurements of antigen-specific IgE should be delayed for at least two weeks after the precipitating event to prevent false negative results. An increased level of mature tryptase in serum obtained within several hours following a hypotensive event, normal levels being undetectable, strongly suggests that mast cell activation occurred. During a study of experimental insect sting-induced anaphylaxis, the increased level of mature tryptase correlated closely with the drop in mean arterial pressure, indicating that the magnitude of mast cell activation is a primary determinant of clinical severity (van der Linden et al 1992). Objective criteria, such as an elevated level of mature tryptase, provide greater precision for the diagnosis of anaphylaxis than clinical signs and symptoms alone, and may be useful to distinguish anaphylaxis from other conditions. However, some cases of apparent IgE-mediated anaphylaxis, particularly some cases of food-induced anaphylaxis, are not associated with an elevated level of mature tryptase. This raises the question of whether there are anaphylactic pathways not involving mast cell activation, but instead perhaps basophil activation.

Anaphylaxis should be distinguished from a variety of disorders with overlapping presentations. Vasovagal syncope presents with diaphoresis, nausea, hypotension and bradycardia, but without urticaria. Flushing disorders may be benign and unrelated to anaphylaxis, or could be a manifestation of pathologic conditions such as the carcinoid syndrome, in which urticaria and profound hypotension are not typically associated, and phaeochromocytoma, which causes episodic hypertension. Precise detection of these latter conditions involves determining serum serotonin and urinary 5-hydroxyindole acetic acid, catecholamines and vanillylmandelic acid levels. Panic attacks and vocal cord dysfunction can be a challenge to distinguish from anaphylaxis, especially by history alone, but nevertheless must be considered. Acute attacks of hereditary and acquired angioedema due to C1 esterase inhibitor deficiency are not associated with pruritic urticaria, and persist longer than attacks of anaphylaxis. Shock due to complement activation by contaminated haemodialysis tubing, without involving mast cell activation, also has been reported. Scombroidosis occurs 5–90 min after ingestion of histamine in poorly-stored fish, and presents with flushing, palpitations, headache and gastrointestinal symptoms. The condition lasts several hours, both duration and severity depending on the amount of histamine ingested, and usually responds to H1 receptor and H2 receptor antihistamines, but occasionally requires epinephrine and intravenous fluids. Acute serum sickness, genetic cell activation syndromes, endotoxin-mediated septic shock and superantigen-mediated toxic shock syndromes present with fever, which is not characteristic of anaphylaxis by itself. Also,

hypoglycaemia, seizure and primary pulmonary or cardiac events should be considered.

In some cases, systemic anaphylaxis may occur together with another disorder. For example, a 65 year-old male after being stung by a wasp complained of dizziness and shortness of breath, was hypotensive with urticaria, responded to treatment with subcutaneous epinephrine, then complained of chest pressure, and had an EKG indicating an inferior wall infarction. Both β tryptase level and cardiac enzymes were elevated, indicating both anaphylaxis and myocardial infarction had occurred.

Systemic mastocytosis is an important condition to consider in the differential diagnosis of anaphylaxis (Schwartz 2001). In adults, somatic activating mutations in the gene for Kit in mast cell progenitors result in an excessive body burden of mast cells. In children with this disorder the disease may regress spontaneously, possibly due to the lack of this activating mutation. Patients with too many mast cells are at increased risk for anaphylaxis, and anaphylaxis may be a presenting manifestation of systemic mastocytosis. For example, anaphylaxis to an insect sting, particularly in the absence of venom-specific IgE, should suggest the possibility of systemic mastocytosis. Diagnostic tests for systemic mastocytosis might include a biopsy of a skin lesion suspected to be urticaria pigmentosa, a bone marrow biopsy stained for mast cells (anti-tryptase immunohistochemistry being most sensitive), detection of bone marrow mast cells expressing surface CD2 and CD25, and an elevated serum level of total tryptase (mature plus immature forms of α and β tryptases) during a non-acute interval (Valent et al 2001).

Prevention and therapy

Acutely, treatment of systemic anaphylaxis first requires that airway patency, blood pressure and cardiac status be addressed. Epinephrine administration is critical, the earlier during the course of an anaphylactic event the better. Glucagon may be used in patients taking a β blocker. Parenteral administration of H1 and H2 receptor antihistamines may prevent progression of some of the signs and symptoms. Glucocorticosteroids may reduce the risk of protracted or recurrent anaphylaxis, but are unlikely to be of benefit acutely.

Patients who have experienced an anaphylactic reaction are at greatest risk of suffering another episode. Elevated baseline levels of total tryptase may be another indicator of anaphylactic risk (Fricker et al 1997, Ludolph-Hauser et al 2001, Schwartz et al 1994). Individuals at risk should wear a Medic-Alert bracelet, carry epinephrine, and avoid β blockers and ACE inhibitors as well as agents to which they are sensitive. In subjects with recurrent anaphylaxis, prophylactic use of H1 and H2 receptor antihistamines is beneficial. A

leukotriene antagonist and cyclooxygenase inhibitor theoretically would provide additional benefit, but have not been systematically studied. Immunotherapy for venom-sensitive subjects, desensitization for certain cases of drug allergy, anti-IgE therapy for subjects at risk of food-induced anaphylaxis and cyclosporin A for recurrent anaphylaxis are considerations. Glucocorticosteroids, which do not inhibit experimental mast cell activation, are unlikely to provide a major benefit in most patients with recurrent anaphylaxis. More effective and long-lasting therapies for IgE- and non-IgE-mediated anaphylaxis are still needed.

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DISCUSSION

Galli: How many instances have you observed in which good specimens of blood had been obtained and yet there was evidence for histamine release without elevated tryptase? You showed details about one case.

Schwartz: We haven't done a systematic study of this. You never know when you are going to get that. In some of the fatal or near-fatal food anaphylactic patients that Hugh Sampson has reported, assays were performed in which there was no elevation of total or mature tryptase. There are a number of potential explanations. Some of these patients had prolonged hypotension. It is not clear to me whether the same pathogenesis that is involved in the initial event is involved in the persistence of that event. Another possibility is that different groups of mast cells may get activated through different routes of allergen administration. Intestinal mast cells may not have the same quantity of tryptase per cell as the mast cells that are in the skin and around the vasculature. They conceivably could be more distant from blood vessels. Although I still favour the possibility that mast cells may not be involved in some of those reactions, perhaps other cell types have to be considered.

Vercelli: What do you mean when you say that a significant proportion of affected individuals don't have the α tryptase gene?

Schwartz: There is a tryptase locus. Each haploid chromosome 16 has two α/β tryptase genes (one or two β tryptase genes and one or zero α tryptase genes). About 25% of individuals have all β tryptase genes and no α tryptase gene.

Vercelli: So it is not as if they have a truncated gene that may give rise to some functional dominant negative, or anything like that. Do we know that the gene is not there at all?

Schwartz: There is allelic variation. One can have one of the β tryptase subtypes or of the α tryptase subtypes at one site, and at the other site just the β tryptases. There are also polymorphisms among both α and β tryptases that have been documented.

Austen: Larry Schwartz, could you say more about the number of tryptase genes? How many do you think there are, and what are the alleles? What about the relevance of the γ and δ tryptase?

Schwartz: If we look at a peptide phylogram showing the relationships between tryptases, there are two principal α tryptase forms that vary by one or two amino acids, and three principal forms of β tryptase which vary by one to three amino acids. α and β tryptases are more closely related to one another than they are to any of the rodent or other animal tryptases. George Caughey has performed the best work on this. About 25% of people have no α tryptase gene in either of their chromosome 16s. There is also a tryptase δ, a tryptase γ and a tryptase ε. We didn't originally describe the δ, but we published a paper saying that this was probably a pseudogene (Min et al 2001). It is truncated 40 amino acids earlier than are α/β tryptases. We detected little to no mRNA for it in mast cells. Subsequent to this there has been a very nice paper from an Australian group which does detect the mRNA in mast cells and possibly other cell types and shows that the protein is also expressed in these cells (Wang et al 2002). Because of a stop codon it would be about 5–10 000 Da less than tryptase. In Western blots with our anti-tryptase mAbs, we don't see a product of that size in mast cells. Either our antibodies aren't detecting it, or the amount present compared to α/β tryptases is very low. Tryptase γ has been described by both Rick Stevens and George Caughey. It has a transmembrane portion to it. It is biochemically and immunologically quite distinct from α/β tryptases. From what I recall, tryptase ε also was a little bit less similar to the α/β tryptases.

Austen: There are papers claiming that in systemic mastocytosis there are some patients who do not have elevated tryptase. George Caughey and colleagues have provided substantial evidence for α null humans (Soto et al 2002). Does this account for tryptase-negative mastocytosis patients?

Schwartz: At the recent International Mastocytosis meeting, this issue wasn't raised. When I spoke to Peter Valent and Luis Escribano at the meeting in Vienna, they told me that they didn't have anyone with systemic mastocytosis without an elevated tryptase level. At that meeting, a total tryptase level greater than 20 ng/ml was adopted as one of the diagnostic criteria. I think those patients without an elevated tryptase that are said to have systemic mastocytosis need to be

looked at carefully and reported. One possibility would be that they are α tryptase deficient and for some reason don't secrete enough tryptase. However, our results with healthy subjects, show that total tryptase levels are unaffected by the presence or absence of the α tryptase gene. Whether there is an effect in mastocytosis is being examined. Another possibility is that early in the course of systemic mastocytosis, when the mast cell burden is still low, the total tryptase level would be normal. The more distantly related tryptic-like enzymes that have been named tryptase, e.g. NK cell tryptase and clara cell tryptase are unrelated to the α/β tryptases of mast cells.

Simons: With regard to the normal values for total tryptase of 1–15 ng/ml, were young children included in the population from which these norms were derived?

Schwartz: Young children haven't been looked at systematically. With adults we've examined 100 to 200 subjects on at least two occasions. The mean and two standard deviations gives a range of about 2–12 ng/ml. I have taken three standard deviations to go from 1 to 15 ng/ml, because we have seen some individuals who appear healthy in that upper range, so I arbitrarily decided to extend it to three standard deviations.

Simons: My point is that for so many normal values there are age-related differences between infants and prepubertal children versus teens and adults. This might be an interesting line of investigation to pursue.

Schwartz: I agree.

Finkelman: We have some mouse data which are possibly relevant to the mast cell versus basophil issue in anaphylaxis. We have looked at two phenomena: the development of anaphylaxis as assessed by changes in behaviour and hypothermia, versus increase in *in vivo* production of Th2 cytokines, following anti-IgE treatment of either wild-type or c-Kit deficient mice. There is a pretty strong dichotomy between the two phenomena. When c-Kit deficient mice are treated with anti-IgE they shown no change in behaviour and don't develop hypothermia. Nor do they show an increase in MMCP1 or histamine levels. Yet they show as great an increase in IL4 production as do wild-type mice. In the mouse there is a relatively simple situation: the only two cell types that are thought to have FcεRI are mast cells and basophils. So these responses are coming from one of these two cell types. If one were to find that mast cells require c-Kit for development and the basophils don't, then one could say that the anaphylaxis is not coming from basophils. Again, I don't know whether the mouse situation will be pertinent to the human, but it creates a bias in my mind.

Schwartz: Certainly within those experimental circumstances, I think your interpretation is reasonable. Mice normally seem to have very few basophils. If you did something to induce higher levels of basophils in your c-Kit-defective

animals — such as using certain immunization techniques — and see what happens then, this may be revealing.

Finkelman: These experiments were done in mice that had been immunized in a way that causes a Th2 response. This results in the production of a large number of cells that are variously described as basophils or immature basophils.

Galli: About five years ago, Choi et al (1998) reported that active fatal anaphylaxis could be elicited to penicillin V in mast cell-deficient W/W^v mice and that this response was IgE-dependent. Based on a pharmacological approach, the authors attributed the reaction to PAF production, presumably by basophils. Although there was no formal proof that basophils were the origin of the PAF, the authors provided evidence that this form of active anaphylaxis is an IgE-dependent response (Choi et al 1998, Park et al 1997). So there has been at least that one reported example of an IgE-dependent anaphylactic reaction in the mast cell-deficient mouse.

Finkelman: I would argue with you about that paper. I don't think there was good evidence that it was IgE mediated.

Galli: If it was not IgE-mediated, then that may be why the reaction could be elicited in W/W^v mice.

Fisher: In one of the studies of the mast cell tryptase postmortem, there were three patients, I think, where the diagnosis was myocardial infarction. Have you any explanation for that?

Schwartz: That's a good point. There have been a couple of interesting studies where elevated β tryptase levels are found without any evidence for IgE-mediated anaphylaxis. Some of these are in postmortem samples in adults, associated with trauma. About 1 in 20–25 knife murders or shootings end up having markedly elevated tryptase. There have also been some hospital postmortem cases where elevated levels were thought by the medical examiner not to be associated with an IgE-mediated reaction. But many of these patients received a variety of drugs close to the terminal event, so it is hard to sort this out. Then there is a series of sudden infant death syndrome patients, where about 40% of these infants had postmortem tryptase levels that were elevated compared with explained death controls (Platt et al 1994). This has been more or less reproduced in two out of three subsequent studies. All groups agree that there is no antigen-specific IgE detectable in blood. There may be some non-IgE-dependent ways of activating mast cells, and these need to be better understood.

Fisher: One of the things that interested us about the cardiac cases is that in the last eight cases of anaphylaxis we have seen have had elevated troponins when they were measured in four. This is supposed to be very specific for myocardial ischaemia, yet there has been no evidence of myocardial ischaemia in these patients.

Schwartz: This sounds fascinating.

Sampson: Could you comment on the paper by Lin et al (2000) in which they looked at 92 patients coming into the emergency room with anaphylaxis? Only 21% had elevated tryptase.

Schwartz: Those elevations were all near the limit of detection. Almost none of those patients were hypotensive. Clinically these were not severe episodes. I am not sure that those results were clinically significant.

Golden: With respect to insect sting anaphylaxis and mastocytosis, you commented on the absence of IgE. That raises an interesting question. Is it anaphylaxis or mastocytosis? Can they coexist if there is no IgE? If I remember Ulrich Müller's reports correctly, in virtually all cases of insect sting anaphylaxis with mastocytosis there is detectable IgE. My question on the case that you mentioned, is what laboratory did the assays? Could there have been a very low level of IgE that might have been detected in a sensitive assay?

Müller: In the paper you are mentioning (Fricker et al 1997) we describe 10 cases with urticaria pigmentosa and anaphylaxis to Hymenoptera stings. Eight had elevated basal serum tryptase levels. In two of the patients we had both negative i.c. skin tests at 1 g/ml and in five no venom specific IgE was detectable by Phadezym RAST. Failure to detect specific IgE in mastocytosis patients in spite of positive skin tests could be explained by absorption of most of the specific IgE to the abundant mast cells.

Schwartz: With a higher sink for IgE, it is conceivable you could have mast cells that have become sensitized. In such a scenario, you could have a positive skin test and a negative assay in the serum.

Golden: Since the redescription in the past two years of sting anaphylaxis with negative skin tests the situation has gotten better. In 90–95% of those cases with negative skin tests we were able to detect at least a trace of venom-specific IgE. It is really hard to find someone with insect allergy but without venom-specific IgE.

Galli: Ulrich Müller, did you interpret your cases as probably being independent of IgE?

Müller: We thought of direct mediator release by venom components such as melittin, MCD-peptide or mastoparan.

Schwartz: This is a distinct possibility.

Ring: Perhaps 5–10% of our patients have negative skin tests. In these cases we then look at histamine or release from basophils. When we get a positive result we know they are sensitized, even though we have not detected venom-specific IgE. It may be the basophil that is responsible. You showed us a lot about mast cells. Do we have a marker or basophil product similar to tryptase?

Schwartz: No. There have been two basophil-specific antibodies. Both recognize a granule component, but in neither case has the antigen been identified.

Galli: So your point, Johannes Ring, is that in the skin test and radioallergosorbent test (RAST)-negative patients with anaphylaxis, a large proportion give you *in vitro* basophil histamine release in response to allergen.

Ring: Yes. We do this routinely. If they are negative in skin test and RAST, then we look at histamine release. Very few of them are still negative, yet they have a very severe reaction.

Schwartz: I take it that normal basophils won't respond to venom.

Ring: That is correct, in the respective doses.

Ohtsu: You showed that tryptase levels are very high in systemic mastocytosis. Theoretically, if there is a lot of tryptase in the blood, tryptase receptors should be down-regulated. Are there any tryptase receptors?

Schwartz: It is not clear that there are. Because tryptase is a protease, it would have substrates that could be on a cell's surface. What these biological substrates might be is not clearly resolved.

Ohtsu: Even so, I would expect that there would be some sort of diminished response with time to these elevated tryptase levels. Is there any evidence in these mastocytosis patients that the occurrence of systemic anaphylaxis reaction is difficult.

Schwartz: What likely is being detected in the mastocytosis serum is the pro- and pro'- forms of tryptase, which are enzymatically inactive. Those forms of tryptase would not be able to carry out the enzymatic function(s) of the mature enzyme.

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Cytokine enhancement of anaphylaxis

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Abstract: Two distinct pathways of anaphylaxis in the mouse have similar clinical features: the classical pathway in which antigen cross-linking of IgE bound to FcεRI on mast cells induces histamine release, and a second pathway, in which cross-linking of macrophage FcγRIII by antigen–IgG complexes induces release of platelet activating factor. IgG antibodies are a double-edged sword, blocking the first pathway but mediating the second. Both anaphylaxis pathways are considerably enhanced by interleukin (IL)4 or IL13 through a Stat6-dependent, γ_c-independent mechanism. Enhancement is rapid, sensitive, and observed during infection with intestinal nematode parasites, where it probably contributes to parasite expulsion. Enhancement involves increased sensitivity to mediators (platelet activating factor, histamine, serotonin, and cysteinyl leukotrienes), rather than increased mediator production, and is mediated by a synergistic increase in vascular permeability by cytokine plus mediator. Basophil production of IL4 and IL13, which is more sensitive to FcεRI cross-linking than mast cell release of mediators, may sensitize target cells to mediators prior to their release. Inhibitors of IL4 and IL13 may ameliorate allergy by rapidly blocking the sensitizing effects of these cytokines on the effector phase of the allergic response, as well as by more slowly blocking the induction phase of this response.

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Two pathways of systemic anaphylaxis have been demonstrated in the mouse (Fig. 1) (Strait et al 2002). In the classical pathway, antigen-specific IgE bound to mast cell FcεRI is cross-linked by antigen and induces release of mediators, predominantly histamine and, to a lesser extent, platelet activating factor (PAF), that cause shock which is reflected by hypothermia. Spontaneous movement and core body temperatures of mice undergoing anaphylaxis typically reach their nadir after 20–40 minutes, after which the mice either recover or die. This pathway of anaphylaxis can be induced by challenging primed or unprimed mice with a

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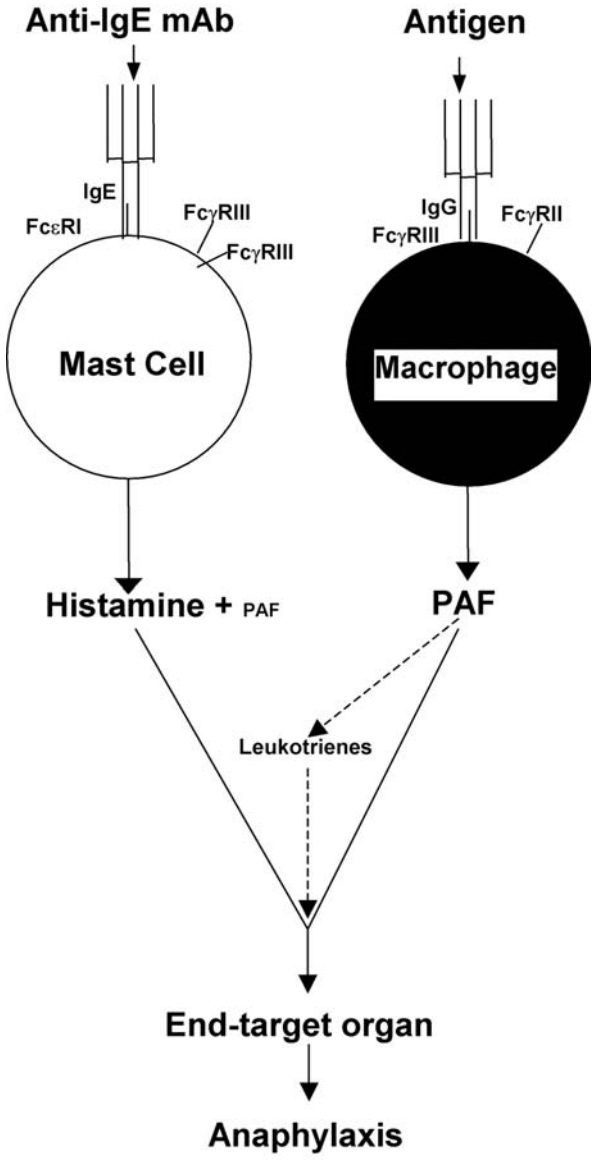

FIG. 1. Two pathways of anaphylaxis in the mouse. Mice primed with goat anti-mouse IgD antiserum, then challenged with either antigen (goat IgG) or an anti-IgE mAb develop anaphylaxis by two distinct pathways. The anti-IgE mAb challenge follows the classical pathway of IgE/FcεRI/mast cell/histamine. However, the antigen challenge follows an alternative pathway, IgG/FcγRIII/macrophage/platelet activating factor (PAF). There is minor involvement of PAF in the classical pathway and of leukotrienes in the antigen-induced pathway. Both pathways appear to involve the same end-organ targets.

monoclonal anti-IgE mAb and can be mimicked, to some extent, by injecting mice i.v. with histamine. The response to anti-IgE monoclonal antibody (mAb) is not observed in IgE-, FcεRI- or mast cell-deficient mice and is inhibited more by H1 receptor than by PAF receptor antagonists.

The second pathway of anaphylaxis is observed when mice hyperimmunized with an antigen are challenged i.v. with the same antigen. In our studies, mice were primed by a single injection of a goat anti-mouse IgD antiserum (GaMD), which induces large IgG1 and IgE anti-goat IgG responses as well as intestinal mastocytosis, and challenged i.v. two weeks later with normal goat IgG. Although the severity and kinetics of shock induced by intravenous challenge of GaMD-primed mice with goat IgG is similar to that induced by challenge with anti-IgE antibody, the mechanism involves goat IgG–anti-goat IgG complexes that bind to macrophage FcγRIII and induce these cells to secrete PAF. The response to goat IgG in GaMD-primed mice is mast cell-, IgE-, FcεRI-, and H1 receptor-independent, but is inhibited by depleting macrophages, blocking (or genetically deleting) FcγRIII, and inhibiting the PAF receptor. The IgG/macrophage/FcγRIII pathway of anaphylaxis can be mimicked, to some extent, by injecting mice with PAF or with a mAb that binds mouse FcγRII and FcγRIII. The two anaphylaxis pathways also differ in the way in which products of the enzyme 5-lipoxygenase (5-LO) affect the development of shock: GaMD-primed 5-LO-deficient mice (Chen et al 1994) develop less severe shock than wild-type mice when challenged with goat IgG or PAF, but more severe shock than wild-type mice when challenged with anti-IgE mAb or histamine.

The development of IgG/FcγRIII/macrophage/PAF-mediated anaphylaxis, rather than IgE/FcεRI/mast cell/histamine-mediated anaphylaxis when GaMD-primed mice are challenged with the relevant antigen (goat IgG) does not reflect a deficiency in IgE or mast cells; rather, the very high levels of IgG1 anti-goat IgG antibody induced by GaMD immunization appear to block the ability of injected goat IgG to reach mast cell-bound goat IgG-specific IgE. This is suggested by two observations. First, IgE-mediated anaphylaxis can be induced in GaMD-primed mice by increasing the quantity of challenge antigen 100-fold. Secondly, immunizing mice with TNP-GaMD, which induces the production of IgG anti-TNP antibody, blocks the ability of an IgE anti-TNP monoclonal antibody to mediate an anaphylactic response to TNP-ovalbumin. In contrast, immunization with unhaptenated GaMD antibody, which does not induce the production of anti-TNP antibody, does not inhibit the IgE anti-TNP antibody-mediated anaphylactic response to TNP-ovalbumin. Thus, the generation of IgG 'blocking antibody' can protect mice against systemic, IgE-mediated anaphylaxis, but at the possible price of substituting IgG-mediated anaphylaxis (Fig. 2).

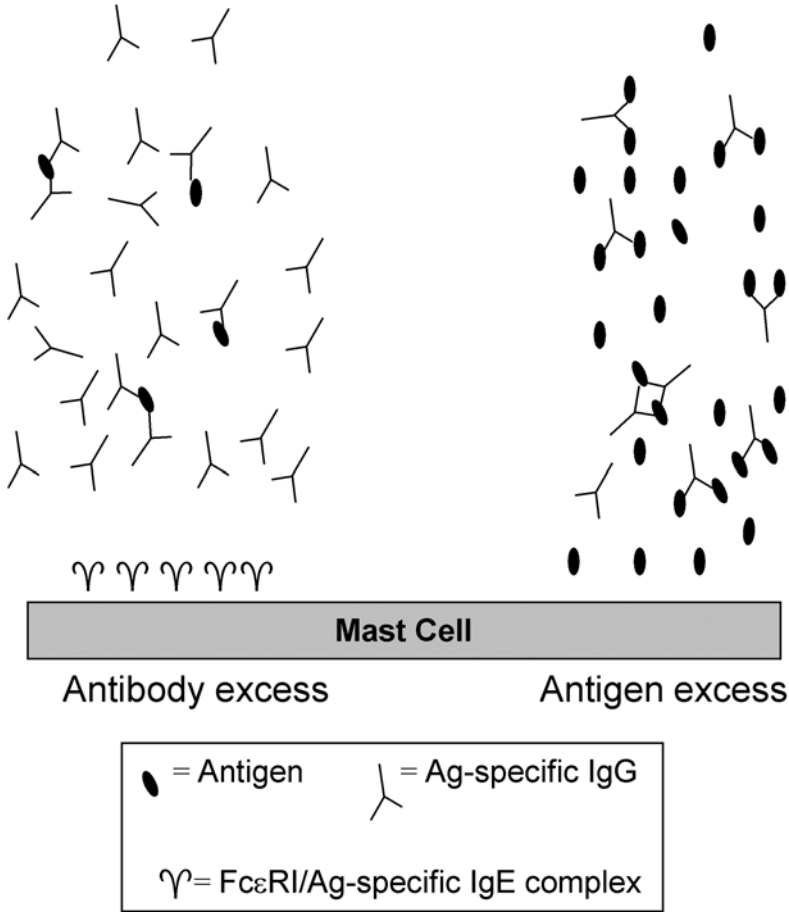

FIG. 2. IgG 'blocking' antibodies may protect against IgE-mediated anaphylaxis. Antigen (Ag)-specific IgG may bind antigen before it has a chance to bind to FcεRI/Ag-specific IgE complex (left side of figure). Meanwhile, Ag excess may be able to overwhelm antigen-specific IgG and reach the FcεRI/Ag-specific IgE complex to bind and induce IgE-mediated anaphylaxis (right side of figure).

IL4R*α* enhancement of anaphylaxis

The cytokines IL4 and IL13 have been implicated in many pro-allergic phenomena. Historically, interest has focused on the effects of these cytokines on the induction of allergic responses, including Th2 cell differentiation (Swain et al 1990, Le Gros et al 1990), B cell switching to IgE production (Coffman et al 1986, Finkelman et al 1988), induction of intestinal mastocytosis (Madden et al 1991,

Bischoff et al 1999), stimulation of chemokine production, and induction of adhesion molecule expression (Thornhill et al 1991). More recently, researchers have identified direct stimulatory effects of IL4 and/or IL13 on non-immune cells that contribute to allergic responses, including effects on epithelial cells that promote mucus secretion (Wills-Karp et al 1998, Cohn et al 1999) and effects on smooth muscle cells that increase responsiveness to contractile stimuli (Grunstein et al 2002). These observations suggested that IL4 and IL13 might also promote the effector phase, as well as the induction phase, of anaphylaxis.

To investigate this possibility, we evaluated whether pretreatment of BALB/c mice with IL4 would increase the severity of anaphylaxis that develops when unprimed mice are challenged with anti-IgE mAb or anti-FcγRII/RIII mAb. For convenience, most experiments used a long acting formulation of IL4 (IL4C) that is produced by mixing recombinant mouse IL4 with a neutralizing anti-IL4 mAb at a 2:1 molar ratio (Finkelman et al 1993). Pre-treatment with IL4C for as short a time as 1–2 hours increased the severity of both anti-IgE mAb- and anti-FcγRII/RIII mAb-induced anaphylaxis, as determined by drop in rectal temperature (Strait et al 2003). Pretreatment with IL4C for 24 hours had a considerably more substantial effect, causing stimuli that normally induce only a mild decrease in body temperature to induce lethal shock. Similar effects of IL4 on anaphylaxis were observed when GaMD-primed mice were challenged with goat IgG or when uncomplexed IL4, rather than IL4C, was used to sensitize mice, although the exacerbative effect of uncomplexed IL4, which has an *in vivo* half-life of a few minutes, abated several hours after injection while the effect of the IL4C, which has a 24 hour half-life, lasted for at least a few days (Strait et al 2003). IL4 enhancement of anaphylaxis is IL4Rα and Stat6 dependent. Because Stat6 is a transcription factor (Kaplan et al 1996), Stat6 dependence implies that IL4 exacerbation of anaphylaxis involves induction of transcription and synthesis of new proteins, even though this exacerbation occurs rapidly.

IL4 enhancement of anaphylaxis is a biologically relevant process

Even low doses of IL4 had dramatic effects on the severity of anaphylaxis. Doses barely capable of inducing an increase in B cell class II MHC expression, the most sensitive known *in vivo* effect of IL4 (Noelle et all 1984, Finkelman et al 1993), also increased the severity of anti-FcγRII/RIII mAb-induced anaphylaxis, as measured by an increased drop in rectal temperature (Strait et al 2003). The effect of IL4 pretreatment was dose-related; as the dose was increased, larger drops in rectal temperature, then increased mortality, then more rapid mortality were observed (Strait et al 2003). The sensitivity of the IL4 effect suggested that the IL4 (and/or IL13) that is produced endogenously during a worm infection (Urban et al 2000) would be sufficient to promote anaphylaxis. This was investigated by evaluating

the effects of infection with the nematode parasite *Trichinella spiralis* on the severity of shock induced by anti-FcγRII/RIII mAb. *Trichinella* infection changed anti-FcγRII/RIII mAb from an inducer of mild anaphylaxis to an inducer of rapidly lethal anaphylaxis. This effect was not observed when IL4Rα-deficient mice, which cannot respond to IL4 or IL13 (Finkelman et al 1999), were infected with *Trichinella*, even though IL4Rα-deficient mice develop a much more severe infection than wild-type mice (Urban et al 2000, Strait et al 2003). Thus, the quantity of IL4 and/or IL13 that is endogenously produced in a worm-infected animal is sufficient to exacerbate anaphylaxis.

IL4Rα-dependent enhancement of anaphylaxis appears to contribute to intestinal worm expulsion. *T. spiralis* is expelled through a process that requires IL4 or IL13 as well as mast cell degranulation (Urban et al 2000) and is accompanied by the development of a localized form of anaphylaxis, that is referred to as intestinal anaphylaxis. Chimeric mice that express IL4Rα on bone marrow-derived cells, including B cells, T cells, and mast cells, but not on non-bone marrow-derived cells, including cells intrinsic to the gut, fail to expel *T. spiralis*, even though they generate normal Th2 responses and exhibit normal or increased mast cell degranulation (Urban et al 2001). Consistent with this observation, IL4 enhancement of systemic anaphylaxis is B cell- and T cell-independent (Strait et al 2003). Thus, expulsion of *T. spiralis* appears to require IL4Rα-dependent amplification of the response to mast cell degranulation as well as mast cell degranulation itself.

IL4 exacerbates anaphylaxis by enhancing responses to vasoactive mediators

Anaphylaxis is a response to vasoactive mediators released by activated inflammatory cells. IL4 exacerbation of anaphylaxis might result from enhanced release of vasoactive mediators by these cells or enhanced responsiveness to these mediators. To distinguish between these possibilities, we evaluated the effects of IL4 pretreatment on histamine and mouse mast cell protease 1 release during anti-IgE mAb challenge and to PAF production during goat IgG challenge in GaMD-primed mice. None of these responses was increased by IL4 pretreatment. In contrast, IL4 pretreatment exacerbated the severity of shock induced by injection of histamine, PAF, leukotriene C4 (LTC_4), or serotonin (Strait et al 2003). Thus, IL4 appears to exacerbate anaphylaxis by increasing responsiveness to vasoactive mediators rather than vasoactive mediator production.

Although IL4 enhances the response to each of the four mediators tested, it does not enhance shock induced by each mediator to the same extent; responses to PAF and LTC_4 are increased more than responses to histamine or serotonin. However, the greater effect of IL4 on the responses to PAF, as opposed to histamine, does not

result from a greater increase in sensitivity to PAF. The greater effect of IL4 on the response to PAF parallels the steeper dose–response curve to PAF than to histamine; IL4 pretreatment shifts each curve to the same extent. Thus, even though IL4 enhances responses to different mediators to different extents, it may enhance responses to different mediators by the same mechanism. One likely mechanism is enhancement of expression of receptors for these mediators. Consistent with this, IL4 has been shown *in vitro* to increase cysteinyl leukotriene receptor expression by mouse spleen cells (Thivierge et al 2001) and PAF receptor expression by human peripheral blood mononuclear cells (Nguer et al 1992). However, the mechanism by which IL4-activated Stat6 could coordinately increase the expression of receptors for several different vasoactive mediators is not clear.

IL4 enhances mediator-induced increases in vascular permeability

Increased vascular permeability, which causes shock by depleting intravascular fluid volume through vascular leak, is an important mechanism in the induction of anaphylaxis by vasoactive mediators (Jancar et al 1991). Experimentally, vascular leak can be demonstrated as haemoconcentration, an increase in packed erythrocyte volume (haematocrit), because erythrocytes remain within blood vessels as fluid leaks out. If administered separately, histamine, PAF and LTC_4 all increase haematocrit, while IL4 has no effect. However, pretreatment with IL4 substantially increases haemoconcentration in response to each of the vasoactive mediators and the magnitude of the IL4 effect on the response to each mediator is related to the magnitude of the IL4 effect on mediator-induced shock (Strait et al 2003). These observations suggest that IL4 predominantly exacerbates the response to vasoactive mediators by enhancing their ability to increase vascular permeability and deplete intravascular volume. Consistent with this, mice treated with a normally lethal combination of IL4 followed by PAF are protected to a considerable extent by pretreatment with an intravenous bolus of albumin (Strait et al 2003). This treatment is not completely protective, however, so IL4 may also exacerbate the response to mediators through additional effects.

Effects of cytokines other than IL4 on anaphylaxis

To evaluate whether IL4 effects on anaphylaxis can be mimicked by other cytokines, we compared the abilities of IL2, IL4, IL5, IL9, IL13 and IL15 to exacerbate anaphylaxis induced by anti-FcγRII/RIII mAb. Only IL4 and IL13, which like IL4, interacts with a receptor that includes IL4Rα and activates Stat6 (Jiang et al 2000), exacerbated anaphylaxis. Consistent with the exacerbation of

anaphylaxis by both IL4 and IL13, IL4 induced anaphylaxis to the same extent in mice sufficient or deficient in γ_c, which is a component of the type 1 IL4R that responds only to IL4, but not a component of the type 2 IL4R that responds to both IL4 and IL13 (Jiang et al 2000, Strait et al 2003).

Because IL2 has been reported to enhance vascular permeability (Whittington & Faulds 1993), the failure of even a large dose of IL2 to exacerbate anaphylaxis was surprising. For this reason, we also evaluated the anaphylaxis-enhancing ability of IL2 that had been complexed with anti-IL2 mAb to increase its *in vivo* half-life. In contrast to free IL2, IL2/anti-IL2 mAb complexes had an anaphylaxis enhancing effect. The mechanism of this effect was different from that of IL4 in that the IL2 effect was γ_c dependent (γ_c is a component of the IL2R)(Johnston et al 1996) and Stat6 independent (Stat6 is not activated by IL2). Because enhancement of anaphylaxis by IL2, in contrast to enhancement by IL4, was only observed when a large dose of long-acting IL2 was used, we do not know whether IL2 enhancement of anaphylaxis is a physiologically relevant process.

Because interferon (IFN)γ frequently inhibits the effects of IL4, we evaluated whether IFNγ can inhibit IL4 exacerbation of anaphylaxis. Treatment with IL12 plus IL18 for 3 days was used to induce an *in vivo* IFNγ response. Treatment with IL12 plus IL18 blocked the ability of IL4 to enhance anaphylaxis induced by anti-FcγRII/RIII mAb and this effect of IL12 plus IL18 was itself blocked by treatment with anti-IFNγ mAb (Strait et al 2003). Thus, endogenously produced IFNγ can inhibit IL4 enhancement of anaphylaxis.

Basophils as a possible source of IL4 and IL13 for exacerbation of anaphylaxis

IL4 and IL13 produced by T cells in response to an intestinal nematode infection may be sufficient to enhance the intestinal anaphylactic response to nematode antigens. Alternatively, IL4 and IL13 produced by mast cells or basophils (Seder et al 1991) activated by these antigens might also contribute to this response. To evaluate this possibility, we used the *in vivo* cytokine capture assay (IVCCA)(Finkelman & Morris 1999) to quantitate IL4 and IL13 responses to activation of macrophages by anti-FcγRII/RIII mAb and activation of mast cells and basophils by anti-IgE mAb. Although anti-FcγRII/RIII mAb failed to induce a detectable IL4 response, large IL4 and IL13 responses were induced by injection of anti-IgE mAb. The IL4 response to anti-IgE mAb was IgE- and FcεRI-dependent, but occurred to the same extent in mast cell-deficient as in mast cell-sufficient mice. Thus, the IL4 response to anti-IgE mAb was probably generated predominantly by basophils, the only cell type in the mouse other than the mast cell that expresses FcεRI (Galli & Lantz 1999). It is not yet known whether the IL13 response to IgE cross-linking is similarly basophil derived.

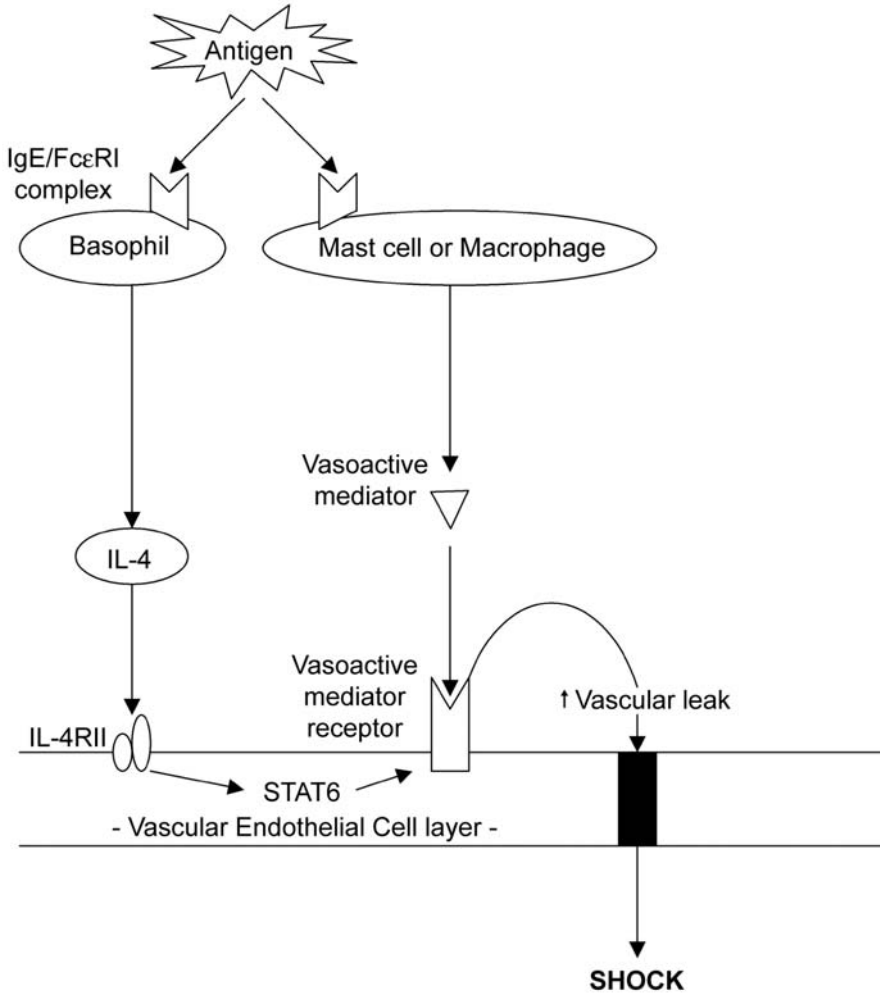

FIG. 3. IL4 enhancement of anaphylaxis. Antigen interacts with IgE bound to FcεRI on basophils to cause release of IL4 and with FcåRI on mast cells or with IgG to form complexes that interact with FcγRIII on macrophages, inducing the release of vasoactive mediators. Secreted IL4 activates the type 2 IL4 receptor, activating Stat6. Stat6, in turn, is hypothesized to increase the concentration of vascular endothelial cells receptors for vasoactive mediators. Vasoactive mediators interact with the increased number of vascular endothelial cell receptors, increasing vascular permeability, with the resultant extravasation of intravascular fluid and the development of shock.

Although basophils are the predominant source of the IL4 response to anti-IgE, mast cells are the source of the mediators that cause anaphylaxis in anti-IgE treated mice, because histamine release and anaphylaxis fail to develop in anti-IgE mAb-treated mast cell-deficient mice. Interestingly, a lower dose of anti-IgE mAb is required in wild-type mice to induce IL4 secretion than to induce anaphylaxis. These observations suggest that less IgE-mediated signalling is required to activate basophil synthesis and secretion of IL4 than to activate mast cell degranulation. Consequently, basophil synthesis of IL4 is probably triggered prior to mast cell degranulation during a worm infection, allowing IL4 time to sensitize intestinal cells to the effects of mast cell-produced mediators (Fig. 3).

Conclusions

In sum, anaphylaxis is induced in the mouse by two independent pathways, both of which are rapidly and potently enhanced by IL4 and IL13 through a mechanism that is IL4Rα and Stat6 dependent but B cell, T cell, NK cell and γ_c independent. IL4 enhancement of anaphylaxis occurs at concentrations that develop during worm infections and appears to contribute to worm expulsion. Enhancement of systemic anaphylaxis by IL4 is inhibited by IFNγ and results from increased sensitivity to vasoactive mediators, especially their effects on vascular permeability. IL4 secretion by IgE-activated basophils may precede mediator release by IgE-activated mast cells and sensitize target cells to these mediators. Thus, IL4 enhancement of anaphylaxis appears to be a physiologically important process that contributes to both host protection against pathogens and to the pathogenesis of allergic disorders. Studies to identify the mechanisms by which Stat6 activation enhances sensitivity to vasoactive mediators and the cell types relevant to IL4 effects on anaphylaxis, other allergic disorders, and host protection are in progress.

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DISCUSSION

Galli: Would you like to speculate on the phenomenon we discussed earlier about the organ-specific effects of different antigens, and whether this might be related to the cytokine environment?

Finkelman: I don't think this gives you an explanation as to why one food antigen will give you a response in one site, and another food antigen will give you a response in another site. It does give you an explanation as to why people with asthma may have more severe anaphylaxis, in that their lungs may be preconditioned by the higher IL4 and IL13 levels, which respond more than normal lungs.

Galli: Are you planning on examining the organ-specific expression of IL4 to test this in mice?

Finkelman: We are planning organ-specific expression of IL4 receptor, or STAT6. We already have about five different sets of transgenic mice to do those experiments.

Vercelli: How strain-specific is this model?

Finkelman: We have done most of our experiments in Balb/c mice. We have also done this in C57BL/6 mice and W^{v} mice. The Rag2 knockouts were in C57BL/6 mice. It seems to work in every strain.

Schwartz: Do you ever see a biphasic or late-phase reaction?

Finkelman: No, but we have never really looked for this. In some of these experiments the mice stay cold for a long time. It is strange. We have some experiments in which the mice still have rectal temperatures of around 30 degrees 8 hours later when they are running around again and appear to be fine. There is something that is keeping this part of the phenomenon going for a long period.

Lasser: Is there any known effect of IL4 on NO release? The reason I ask is because in our rat studies, we inject histamine and produce hypotension, but the greater part of this is due to NO and histamine itself releases NO via H1 and H2 receptors.

Finkelman: In general, IL4 seems to inhibit nitric oxide responses. It seems to push the use of arginine to the arginase pathway rather than the pathway that generates NO. There are some more complicated effects. Eosinophils can make NO through a different pathway, and IL4 can indirectly promote this by causing

an influx of eosinophils. We have looked at NO synthase expression in the gut and lungs of mice that were treated with IL4. It is reduced.

Galli: For those of you who don't work with mice, it is important to emphasize that you tend most readily to find what you specifically look for. We and Fred like to use the rectal temperature as an index of the overall response. But if you also search for additional potential features of the response, such as tachycardia or reduced pulmonary conductance, one can detect differences in IgE-dependent and IgG1-dependent components of anaphylaxis. These findings are summarized in Miyajima et al (1997). The bottom line is that the IgE-dependent anaphylaxis in the mouse is highly mast cell dependent, and it is usually associated with a rapid transient decrease in pulmonary conductance and dynamic compliance, and the very rapid development of tachycardia. In contrast, IgG1-dependent anaphylaxis has a slower development of the tachycardia and the pulmonary responses. Either type of anaphylaxis can kill the mouse, the first by a mechanism that requires mast cells and the second by one that doesn't. What we see in the typical antigen-induced anaphylaxis in an actively sensitized animal is a variable combination of both mechanisms. However, it is likely that the most rapid effects observed are related to mast cells, as well as some of the more prolonged effects. To protect these mice genetically against the development of this problem one can eliminate the FcR common γ chain, that will in turn eliminate signalling via both the high affinity IgE receptor and the FcγRIII. In many situations in which mice are actively immunized and challenged with antigen, both IgE- and IgG1-dependent processes are taking place, but the pathological mechanisms that are triggered by these pathways are in some respects quite distinct.

Finkelman: In this particular system, when we gave antigen we saw very little evidence of IgE-mediated anaphylaxis using our conventional lower 100 μg dose, which really is not such a low dose. I think this is because our system causes so much IgG antibody to be made. In the literature there are accounts in other systems of a combination of IgE and IgG responses to antigen challenge in primed mice. You mentioned IgG1. This is the predominant IgG in this particular system. But if we think about the IgG-mediated phenomenon working through FcγR3, then we realize that IgG2a and IgG2b also work very well. There is a confusing mouse literature that talks about IgG1 as having a special effect. This seems to be restricted to passive cutaneous anaphylaxis, where a subset of IgG1 has an increased ability to bind to mast cells.

Galli: What class do you think your blocking antibody is?

Finkelman: I think it was primarily IgG1, just because 90% of the IgG made in this immunization system is IgG1. We did an interesting 'half-experiment' in which we got some Balb/c IgG1-deficient mice and gave them the anti-IgD. They all died about seven days later without any further challenge. My guess is that IgG1 is acting as a blocking antibody in two ways. It may have blocked

what otherwise would have been an anaphylactic response induced by the IgE that was made to the goat anti-delta immunization in the absence of the IgG1 antibody, and maybe also blocking because IgG doesn't fix complement well and doesn't bind to FcγR1 well. Thus in the absence of IgG1, IgG2a and IgG2b may have induced more severe immune complex disease.

Galli: I have asked Rosetta Pedotti to make a brief presentation. Synthesized peptides are increasingly being used in approaches to modify the immune response. She and her colleagues have noticed an unexpected and unwanted association with this peptide immunotherapy approach.

Pedotti: I want to describe our work on anaphylaxis to self peptides in autoimmune diseases. The first of these diseases is experimental autoimmune encephalitis (EAE), which is an animal model for human multiple sclerosis, a chronic demyelinating autoimmune disease of the CNS. In this disease T cells orchestrate the process that leads to autoimmune brain inflammation and demyelination. Th1 cytokines, interferon γ and TNFα, play a critical role. The Th2 response seems to protect from EAE, and a shift of the immune response from Th1 to Th2 is a promising therapeutic strategy. A typical EAE model is the one induced by myelin proteolipid protein (PLP) fragment 139–151 in the SJL mice. After these mice are immunized with this peptide in complete Freund's adjuvant they become sick 10 d later, reach the peak of disease, and then go into a remission phase followed in some cases by relapses of the neurological symptoms. We gave these peptides in saline intraperitoneally at different time points during the disease. When the self-peptide was given during the acute phase, nothing happened. But when it was given 3–4 weeks after the induction of the disease, the majority of mice presented signs of anaphylactic shock. We confirmed the presence of anaphylactic shock by measuring body temperature and airway responsiveness. Mice that were going into anaphylactic shock, presented a dramatic drop in body temperature and an increase of airway resistance suggestive of the presence of bronchospasm. We studied the humoral response in these mice during the disease. There was a progressive increase in the titres of IgG1 specific for PLP139–151 compared with IgG2a while the titre of total IgE was not substantially changing during the course of the disease. Furthermore, there was a significant correlation between the presence of high titres of IgG1 against PLP139–151 in the serum of mice with EAE and the presence of anaphylactic shock upon challenge. This suggested that anaphylaxis to self peptide PLP139–151 was mediated by IgG1 antibodies. Mast cell studies that we performed showed very little degranulation, which further suggests that anaphylaxis to self was IgG1-mediated. We showed anaphylaxis also to another self myelin peptide, myelin olygodendrocyte glycoprotein (MOG) fragment 35–55. However, we saw no anaphylactic response to two further peptides, PLP178–191 and myelin basic protein (MBP) Ac1–11. One of the distinguishing features of these two sets of

peptides, PLP139–151 and MOG35–55 that cause anaphylaxis, and PLP178–191 and MBP Ac1–11 that do not, is their presence or absence in the thymus. These studies suggest that the presence of self-myelin peptides in the thymus is preventing anaphylaxis.

The second autoimmune disease I would like to mention is the model of human diabetes mellitus (T1DM) in the non-obese diabetic (NOD) mice. Similarly to EAE, this disease is considered to be mediated by Th1 cells which attack the pancreatic islets. Glutamic acid decarboxylase (GAD) 65 appears to play a key role in the autoimmune response that leads to diabetes. Also in this disease, peptide-specific therapy aimed to shift the Th1 response towards Th2 can suppress the progression to overt diabetes. It has already been shown that repeated injection of two insulin self peptides, B:9–23 and B:13–23, which are known to prevent diabetes, can induce fatal anaphylaxis in NOD mice. We have studied the presence of anaphylactic reactions to three immunodominant peptides of GAD65. We immunized NOD mice three times with these peptides in IFA and 4 weeks after the last immunization we gave these peptides intraperitoneally in saline. All the mice re-exposed to these self peptides had anaphylactic shock. The features of the anaphylactic reaction were very similar to those we observed in a model of IgE-dependent anaphylaxis induced in NOD mice by passive immunization with IgE anti-DNP antibodies followed 24 h later by challenge with DNP-HSA. The mortality rate in mice re-exposed to GAD65 peptide preparations was very high (around 84%). This percentage was even higher than what we observed in passively induced IgE-dependent anaphylaxis. The study of the humoral response in these mice revealed that IgG1 antibodies against GAD65 peptides increased much more than Ig2a. A slight, but significant, increase of total IgE was also observed.

In conclusion, the recognition of certain self peptides can induce allergy, both in the EAE model of MS and in the NOD mice, a model for T1DM. IgG1 antibodies seem to be involved in the expression of anaphylactic reaction. Furthermore, our findings also offer a cautionary note for peptide therapies aimed to shift the immune response towards Th2.

An altered peptide ligand (APL) derived from myelin basic protein (MBP) fragment 83–99, a peptide representing an immunodominant T-cell epitope of MBP, has been used in a phase II clinical trial in patients with MS. This trial has been suspended at a certain point because 9% of the patients experienced allergic reactions to the peptides. Although none of the patients exhibited anaphylaxis, this observation showed that also in humans Th2 immunity to self or altered self can induce allergy. Co-polymer 1 (glatiramer acetate), is another peptide now approved for use in relapsing–remitting MS. Also this peptide, which is not a self or altered self-peptide but induces an immune deviation toward Th2 responses, causes immediate-type hypersensitivity reactions in 10% of the patients.

Finkelman: In the studies that Edward Liu did, he found that he could block the anaphylaxis almost completely with a combination of antihistamine and a PAF antagonist (Liu et al 2002). If it should turn out that this therapy is useful in ameliorating diabetes or MS, then perhaps it could still be used along with a protective therapy against allergy.

Galli: I think that in general it will be challenging to argue successfully (e.g. with the FDA) that an agent that might induce anaphylaxis (or 'anaphylactoid' reactions) could nonetheless safely be used for therapy. It is likely that the agent would have to exhibit a very substantial therapeutic benefit to outweigh concerns about the possibility of the subject developing an anaphylactic or anaphylactoid reaction (e.g. if the person forgot to take the appropriate protective medication).

Ring: Are these reactions IgE mediated? In the mouse you measured IgG1. Did you look for IgE?

Pedotti: In the EAE model induced with PLP139–151 in the SJL we searched for specific anti-peptide IgE. With the ELISA method we used, we did not find specific IgE.

Ring: Has IgE been measured in the human trials you mentioned?

Pedotti: Yes. They measured IgE against the altered peptide ligand MPB83–99. They didn't find specific IgE, but they found high titres of specific IgG1.

Simons: In humans with allergic disease, there have been a number of trials of peptide immunotherapy. We performed a single-centre study of Fel d 1 peptides (Simons et al 1996). The adverse effects in these patients were quite different from those seen in allergic patients receiving conventional immunotherapy. No symptoms occurred during the first hour after injection. Between one and three hours but occasionally as late as 24 hours after injection, itching of the skin but no hives occurred in 23% of the patients, and rhinitis, chest tightness and a decrease in FEV_1 occurred in up to 14% of the patients. These were definitely not anaphylactic responses.

Galli: Of course, those were not self peptides.

Finkelman: Was there evidence of complement fixation?

Simons: This was not measured.

Finkelman: In the Liu studies (Liu et al 2002), he could also not detect specific IgE in serum. But he indirectly found that both IgE and IgG were mediating the response. In order to block it entirely, he had to block both antibodies.

Galli: Did he look for any morphological evidence of mast cell degranulation or histamine release, or anything to implicate mast cell activation in that model?

Finkelman: I don't remember. The only implication was that he could not block the mortality very well with PAF antagonist or histamine antagonist alone. But both together worked. Similarly, blocking with anti-IgE alone or anti-FcγR3 mAb alone did not work, whereas both together did.

Galli: It is interesting that although this seems to be reasonably good evidence that there is a contribution of IgE and mast cells to this process, there wasn't detectable serum IgE. This speaks to the issue of how little IgE may be enough to be significant clinically.

Finkelman: It can be difficult to detect low quantities of antigen-specific IgE in the mouse. However, in the models in which we have varied kinetics, often the IgE-mediated anaphylaxis works best when there is very little serum IgE. Presumably most of it is on mast cell FcεRI.

Lasser: Is it possible that these peptides could be activating the contact system, producing bradykinin?

Pedotti: It is an interesting question, but I don't have an answer.

Galli: When these peptides are injected multiple times in naïve mice they produce no detectable effect initially, but then on the fourth injection of the same amount of peptide three weeks after the last of the series of weekly injections there is a strong reaction.

Lasser: But as suggested earlier you could be progressively diminishing the inhibitors of the contact system.

Ring: Did they do skin tests with the peptides in the MS trial?

Pedotti: In that trial the administration of the drug was subcutaneous.

Ring: Was there a larger local reaction in the allergic patients?

Pedotti: In most of the patients who experienced allergic reactions the reactions were local in the skin.

Ring: It would be good to take a biopsy and look with immunofluorescence for the presence of immune complexes. If it is not IgE dependent then it might be immune complex anaphylaxis.

Golden: I can recall in the Johns Hopkins arm of the peptide study that Philip Norman and Lawrence Lichtenstein used the term 'T cell anaphylaxis'. Is there such a thing as this?

Simons: As you know, the trials with the Fel d 1 peptides were stopped, so the mechanism was never fully explored. Currently, in London, clinical trials are taking place with different peptides.

Galli: I understood that those reactions did not really represent clinical anaphylaxis.

Simons: They did not. They differed with regard to the timing and the clinical picture.

Galli: So the 'anaphylaxis' part of the term 'T cell anaphylaxis' doesn't seem to fit. There has been enough controversy already about the concept of anaphylaxis without introducing a concept of T cell anaphylaxis. I would vote against this.

Ring: What is the mechanism of the trick behind the anti-δ immunization? Why does it work so beautifully?

Finkelman: I can give you our explanation. B cells for the most part express IgD. When you inject this goat antibody it cross-links the IgD on the B cells and activates them. This induces an inflammatory response, and also you now have all these B cells that are presenting the antigen. They express increased class 2 and the B7 co-stimulatory molecules. Within hours after you inject this you see the B cell activation. A day or two later you start to see T cell activation. The T cells make a large cytokine response and IL4 is a very prominent part of that response. I suspect this is why you get such a strong Th2 response. As to why it induces a Th2 rather than a Th1 response, my best guess is that there is production of inflammatory cytokines such as IL1 which act as a co-stimulus to any kind of T cell activation. B cells don't make much IL12; they don't make much type 1 interferon — the cytokines that are needed to induce a Th1 response. In the absence of those agents the response defaults to Th2. These T cells then feedback on the antigen-presenting B cells, inducing them to differentiate into Ig-secreting cells. Because there is a lot of IL4 around, you get IgG1 and IgE.

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General discussion II

Galli: One of the things that has interested me about the presentations so far at this meeting has been the discussion of the exceptions: the small percentage of patients who, for example, have levels of antigen-specific IgE that are difficult to detect, or in the case of Larry Schwartz' paper, have anaphylaxis with evidence of little or no tryptase production. The question is, are we yet convinced that in allergen-specific anaphylaxis in humans there are antibodies other than IgE that can result in clinically-significant activation of mast cells? This may be another instance in which the effects of cytokines on mast cell phenotype may be important. Dean Metcalfe's group recently reported that interferon (IFN)γ can induce mast cells to express increased levels of FcγRI, and thereby express an increased ability to bind IgG_1 antibody and to undergo activation for mediator release upon stimulation with anti-human IgG (Okayama et al 2000, Woolhiser et al 2001). Or, effector cells other than mast cells and basophils could be recruited into the pathway in an allergen-specific manner.

Schwartz: We don't have any direct evidence for other cells. There are other cells that express high-affinity receptors for IgE, so even if you are talking about an IgE-mediated reaction, you could certainly invoke other cell types, whether it be monocytes, antigen-presenting cells or eosinophils. Whether those cells could invoke enough of a systemic response to cause the vascular collapse seen in anaphylaxis is unclear. There are certainly some macrophage activation syndromes that can present with collapse, but they usually have fever associated with them as well, and this is not a characteristic of anaphylaxis.

Galli: Hugh Sampson, you have searched pretty hard to find mediators associated with anaphylactic reactivity, including some of the cytokines. What do you think about the pathogenesis of the anaphylactic reactions in food-allergic children, particularly in those who exhibit little elevation of tryptase?

Sampson: We have thought about this a lot, but we don't have many answers. One of the things we tried to do was to look at basophils pre and post challenge. Somewhat simplistically, we looked at both number of basophils and total basophil histamine content, prior and following food challenge. We didn't see any change in basophil number or histamine content. To us this suggested that the basophil is not playing a significant role, at least early on. We really haven't got much beyond this.

Lee: When mast cells are activated in tissue, the tryptase has to find its way into the circulation. Are we comfortable with the idea that in mast cell degranulation at

the tissue level we always find tryptase in the circulation? We assume that this will happen, but we lack hard data on this.

Galli: That is a good point. It would be a difficult study to get approved because it would require biopsies. Isn't there enough evidence already that patients with clinically well documented anaphylaxis simply may not have a lot of tryptase present in the circulation? That is, it is useful if elevated levels of tryptase are present, but if there is no elevation of tryptase levels one can't conclude that anaphylaxis has not occurred.

Schwartz: It depends somewhat on the clinical circumstance. In insect sting anaphylaxis, which is the most systematically characterized form of human anaphylaxis, there is a good correlation between clinical severity and a rise in tryptase levels. If the patient has hypotension and you find tryptase levels within 2–4 hours that are negative, I would suggest that either the anaphylaxis wasn't severe enough or it was a misdiagnosis. In other forms, such as food anaphylaxis or drug reactions, I don't think we know enough about the dose–response relationship.

Galli: Leaving aside the actual levels of tryptase, if you examine a cohort of patients with well documented food allergy, aren't there a finite percentage of them who don't have detectable tryptase?

Schwartz: Yes.

Galli: From a clinical standpoint I understand that such patients have anaphylaxis that appears to be very similar to that in the subjects that do have elevation of serum tryptase. Tak Lee's point is that we may be at the threshold of detection in the serum of clinically significant levels of tryptase release. Similarly, IgE present on the mast cells is from an immunological standpoint more important than that in the circulation. So we may have enough IgE on the cells to activate them without having enough in the circulation to detect a high level of antigen-specific IgE. Perhaps there may be enough local release of mediators to induce anaphylaxis without a robust response detectable systemically.

Schwartz: There are several theoretical explanations. One is that the clinical reaction is often different with food allergen-induced reactions than with parenteral administration of allergen. With food-induced reactions, the respiratory tract and GI tract are typically involved. You may be dealing with a different subset of mast cells that have different mediator content. Another level of complexity is whether the mast cells are primed to degranulate or to release more newly generated mediators, both cytokines and lipids. If you release more newly generated mediators and less of the granule mediators you could have a similar clinical severity but low levels of tryptase. There are certainly good *in vitro* mechanisms for causing newly generated mediators to be released with very little granule mediator release. Finally, severe fatal reactions can result from laryngeal oedema without causing vascular collapse. In such a case, the reaction may be

mast cell-dependent, but the burden of mast cells activated may be small and inadequate to elevate tryptase levels in the circulation.

Golden: We have to raise the question of local versus systemic. By definition, anaphylaxis is systemic. If one is challenging by injection or ingestion, there may be an area of local mediator release, but how can it cause anaphylaxis if it doesn't become systemic? And if so, how come we can't detect it systemically?

Lee: I'm playing the devil's advocate here. Tryptase is being used as a marker for mast cell degranulation in most of clinical studies, but what role does it have in acute anaphylaxis? *In vivo* there may be differential distribution of mediators into systemic circulation from the site of release in the tissue, so that although tryptase is not measured systemically, other mast cell-derived mediators may still be the cause of the acute reaction. We mustn't confuse a marker with the pathophysiology.

Austen: In the mouse, but unfortunately not in the human, the point is made. One mast cell tryptase, mouse protease 6, remains bound to the released granule, whereas another, protease 7, diffuses away and may even enter the circulation. It is likely that the heparin in the mouse mast cell traps the more charged protease at the local site of degranulation. To the extent that we have studied the mouse we can show a difference in those two tryptases based upon trapping by the proteoglycans, as Tak Lee suggests. Protease 6 is enriched with arginine and lysine, whereas protease 7 binds to proteoglycans via histidines, which lose their cationic charge at the neutral pH of tissue.

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Patterns of anaphylaxis: acute and late phase features of allergic reactions

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Abstract. Anaphylaxis is usually defined as a multi-system allergic reaction, but includes isolated shock or airway obstruction. Hives do not occur in 20–30% of cases. Gastrointestinal (GI) symptoms are more common with foods. Cardiac anaphylaxis can cause arrhythmias, bradycardia or chest pain with ECG changes. Plasma histamine level correlates with hives and GI symptoms. Serum tryptase is elevated less often in food allergy, and correlates poorly with plasma histamine level. Anaphylaxis occurs in 30/100 000 population/year (mortality 1–2%) and is caused by foods (35%), drugs/biologicals (25%), insect stings (15%), exercise (5%) or is idiopathic (20%). Onset of anaphylaxis to stings or allergen injections is usually rapid: 70% begin in <20 minutes and 90% in <40 minutes. Food/ingestant anaphylaxis may have slower onset or slow progression. Rapid onset is associated with greater severity. Prolonged anaphylaxis can be resistant to epinephrine and i.v. fluids. Biphasic allergic reactions which recur some hours after the early phase of the reaction were reported in 25% of cases of fatal and near-fatal food reactions, and in 23% of drug/biological reactions. But they occurred in only 6% of anaphylaxis of mixed causes and are uncommon with insect stings. Late phase (biphasic) reactions rarely occur without initial hypotension or airway obstruction.

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Anaphylaxis has been difficult to define, but usually implies a multi-system allergic reaction. In its mildest form, a systemic allergic reaction may cause generalized urticaria, flushing, pruritus or angioedema, but not all experts would consider such a cutaneous systemic reaction as anaphylaxis. A definition of anaphylaxis as a systemic allergic reaction affecting 'one or more' systems has been used by some authors (Douglas et al 1994, Brazil & MacNamara 1998, and most studies of insect sting allergy). The definition may specify 'two or more' systems (Brady et al 1996), but in other published studies the authors define anaphylaxis as involving cutaneous signs plus one other system (Kemp et al 1995, Stark & Sullivan 1986, Yocum et al 1999). Many authors allow exceptions for isolated cardiovascular shock (e.g. after an insect sting), or isolated airway obstruction (e.g. after eating

peanut), which would usually be considered anaphylaxis even without involvement of other systems.

The severity of the reaction can also be graded in different ways. Although it is easy to agree on the mildest and most extreme reactions, there is less agreement on grading moderate and severe reactions. The classic grading scheme of Mueller (Mueller 1966) classifies the reaction according to the pattern of symptoms but does not necessarily reflect the severity. For example, a patient with dysphagia, hoarseness, nausea, swollen lip and anxiety would be Mueller grade 3, but a grade 2 reaction with severe dizziness, chest tightness and generalized urticaria could be more severe. The grading system of Dr Ring is quite similar, with the grade 2 and grade 3 reactions showing some transposition of the features listed in the system of Mueller (e.g. bronchospasm and dyspnoea) (Ring & Messmer 1977). Both of these grading systems do not seem to include the patient with isolated vascular shock if there are no other signs or symptoms. In some published studies of systemic allergic reactions, the severity of the reactions has been graded with more specific objective criteria. For example, in our studies of insect sting challenge, we have differentiated subjective from objective reactions, and have used specific criteria for the blood pressure and peak expiratory flow. However, this objective system still cannot be applied to historical reactions when there are no objective measurements available. Some reactions that would be classified as moderate in severity when they occur in the field are categorized as subjective during supervised sting challenge when we cannot find any abnormality on physical examination.

The epidemiology of anaphylaxis is not well studied. Early estimates ranged from 3.2 cases/100 000 per year (Sorensen et al 1989) to 90 cases/100 000 per year (Klein & Yocum 1995). A recent and well-designed study described all cases of anaphylaxis presenting to the emergency department in a well-defined community in Rochester, Minnesota (Yocum et al 1999). The authors found an occurrence rate of 30 cases/100 000 population per year, with the incidence of new cases being 21 cases/100 000 per year (i.e. some cases were recurrences of a known allergy). The mortality of anaphylaxis is estimated to be in the range of 1–2%.

The frequency of various causes of acute allergic reactions shown in various studies reflects the design of the study and the population studied. In one study (Kemp et al 1995), reactions to insect stings and allergen vaccines were excluded from the report. Studies of emergency department (Brady et al 1996) or consultation (Pumphrey & Stanworth 1996) cases would be unlikely to identify allergen vaccine injections as a significant cause (or might lump them in with reactions to drugs and biologicals; Yocum et al 1999). Also, some studies accept a presumptive diagnosis of food reaction, whereas others require positive skin test or food challenge. Taking the published studies together, we can estimate that

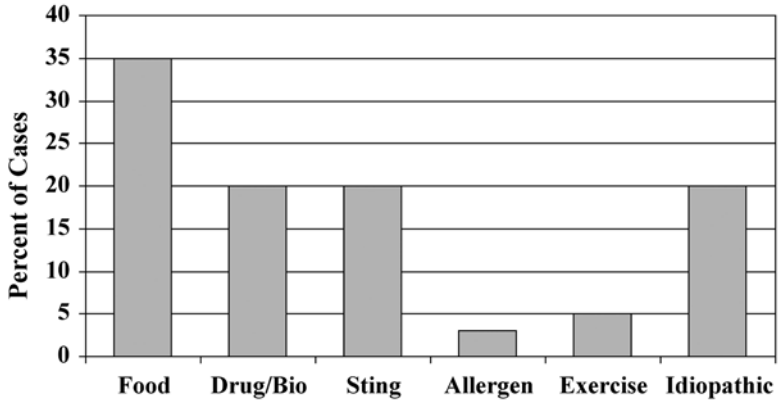

FIG. 1. The causes of anaphylaxis.

anaphylaxis is caused by foods in about 35% of cases, drugs and biologicals in 20%, insect stings in 20% (varies by season and geographic location), allergen vaccines in 3%, exercise in 5% and is idiopathic in 20% (Fig. 1).

Idiopathic anaphylaxis is an entity that has been extensively studied at Northwestern University in Chicago and the Mayo Clinic (Patterson et al 1995, Khan & Yocum 1994). Although some would say that all such cases will ultimately have a specific cause (if it can be discovered), there does appear to be an as-yet undefined intrinsic disorder that is associated with unpredictable reactions. The symptom pattern is the same as other types of anaphylaxis, in that each patient has a pattern of symptoms and signs that is quite reproducible, but can differ from other patients. However, more than a third of the reactions are nocturnal in this condition. The frequency of reactions varies, but is >6 per year in more than a third of patients. More than half of patients with idiopathic anaphylaxis have positive skin tests to food allergens, but their anaphylactic reactions show no clinical correlation to the suspected foods. Early in the study of idiopathic anaphylaxis it was said that no-one dies from this disease. Unfortunately, further years of study have revealed that the mortality may be similar to anaphylaxis in general. Pharmacological therapy of idiopathic anaphylaxis is often attempted but not often successful, in that the most severe cases require oral corticosteroids for control and prevention of acute episodes. Although more than half of such cases studied went into remission during several years of steroid therapy, other investigators have questioned whether this is simply the natural history of the disease.

There is a remarkable range of symptoms and signs in acute allergic reactions. In a combination of clinical studies, the most common signs and symptoms were cutaneous, with urticaria and angioedema occurring in more than 80% of patients. Upper or lower airway symptoms are next most common, occurring in 40% to 60% of cases. Symptoms of vascular insufficiency occur in less than 20% to 40% of reactions. There are a number of other symptoms and signs that occur in less than 20% of patients. These estimates are based on data compiled from several published reports. It is interesting to examine the specific data in individual studies. Cutaneous manifestations occur in more than 80% of cases, but may be limited to flush or pruritus in some cases, especially at the onset of reaction. Although more than 85% of cases include some generalized cutaneous manifestations, hives do not occur in 23–45% of cases (Lin et al 2000, Yocum et al 1999, Brady et al 1996). Gastrointestinal (GI) symptoms are more common with foods, but can occur with any cause. Cardiac anaphylaxis can cause arrhythmias, bradycardia, or chest pain with ECG changes. The difference in frequency of these manifestations in different reports is also probably related to the differences in causative agents in various studies. It is interesting to note that tachycardia is the expected observation in anaphylaxis, and that marked tachycardia is often one of the first signs of reaction, but that bradycardia can occur in approximately 4% of cases. In association with hypotension this could falsely suggest vaso-vagal reaction in rare cases.

It has been assumed that all systemic allergic reactions involve the release of mast cell mediators including histamine. Recent studies indicate that there are different patterns of mediator release in allergic reactions (Lin et al 2000). Plasma histamine level was elevated in 42 of 97 cases, and correlated with many of the symptoms and signs of allergic reactions, especially with hives and wheezing. In insect sting anaphylaxis, the highest levels occur in the worst reactions. Serum tryptase level was elevated in only 20 of 97 cases, and did not correlate significantly with most of the manifestations of reaction, except tachycardia and hives. Histamine and tryptase levels were not significantly correlated with each other. In patients with detectable β tryptase levels in plasma, the level of total tryptase did correlate significantly with serum histamine level ($P < 0.04$). Elevated serum tryptase is detected much less often in food allergy (Sampson et al 1992).

The onset and severity of allergic reactions varies with different causes, and in different patients. When allergen exposure is by the oral route, as in the case of food and some drug allergies, the reaction can be of rapid onset but some cases are of slower onset or more slowly progressive. The severity of food anaphylaxis may be worst with delayed onset or slow progression of symptoms, especially in the absence of urticaria (Sampson et al 1992). When allergen exposure occurs by injection of allergen, as with allergen vaccines, insect stings and some drug allergies, the reaction is usually of rapid onset, and the severity of reaction is worst with the most rapid onset. In the most extensive study of insect allergy, a

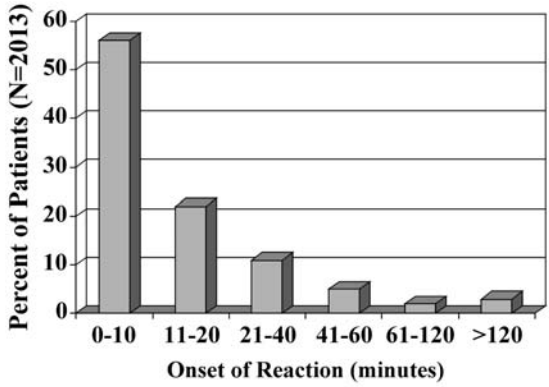

FIG. 2. Onset time of reaction in insect venom anaphylaxis. (From Lockey et al 1988, with permission.)

registry of over 3000 cases was collected and analysed by Dr Lockey under the auspices of the American Academy of Allergy Asthma and Immunology (Lockey et al 1988). In over 2000 cases, the data available permitted analysis of the onset of the reaction. Based on the first reported symptoms in these cases, the onset was generally quite rapid, with 56% of cases reporting symptoms in less than 10 minutes, 78% of reactions beginning in less than 20 minutes and 89% in less than 40 minutes after the sting (Fig. 2).

Of special interest in this presentation is the time course of allergic reactions. The Gell and Coombs classification of immunological reactions implies that allergic reactions are of the immediate hypersensitivity type. However, allergic reactions may be more than immediate. Four patterns of reaction have been described: immediate, biphasic, protracted and delayed (Stark & Sullivan 1986, Douglas et al 1994, Brazil & MacNamara 1998, Sampson et al 1992).

Biphasic anaphylaxis has an immediate phase with a period of improvement and response to initial therapy, but with recurrent symptoms 2–6 hours after the onset of the initial reaction. This pattern of reaction resembles the classic late-phase reactions observed in bronchial, nasal and cutaneous allergen challenge procedures, but may be somewhat accelerated in onset. Such late-phase reactions to allergen challenge have been associated with more severe reactions and with high dose challenge. Protracted anaphylaxis causes prolonged manifestations (usually respiratory distress or hypotensive shock) for 5–32 hours according to published reports, and is often resistant to treatment. Delayed allergic reactions, with no immediate phase, are quite unusual. One example I will discuss is the large local allergic reactions to insect stings. Delayed onset of anaphylaxis has been reported but only anecdotally.

The frequency of biphasic anaphylaxis varies in published estimates. This may be related to the different causes and severity of reactions described in different reports. The highest frequency of 20–23% was in fatal and near-fatal food reactions (Sampson et al 1992) and in predominantly severe drug reactions (Stark & Sullivan 1986). Brazil & MacNamara (1998) reported 18% biphasic reactions in assorted cases of anaphylaxis, many of which were due to insect stings. Brady et al (1996) reported 4% biphasic reactions: both of the cases developed recurrent urticaria several hours after clearing of the initial anaphylactic reaction to a sting. Douglas et al (1994) found 7% biphasic reactions in 59 hospital admissions for allergic reactions. Allergen injections seem to cause very few biphasic reactions: 5% in 44 cases reported by Douglas et al (1994). Kemp et al (1995) did not describe any cases of biphasic or protracted reactions in 266 patients with anaphylaxis. Late phase (biphasic) reactions rarely occur in milder cases when there is no significant hypotension or airway obstruction.

Protracted anaphylaxis has been described in 3 reports which included primarily the most severe anaphylactic reactions. The reports by Sampson et al (1992) and Stark & Sullivan (1986) both describe a high frequency of protracted anaphylaxis in addition to the frequent biphasic reactions mentioned before. Kemp et al (1995) did not find any cases of protracted (or biphasic) anaphylaxis among the 266 cases they reported, none of which were insect-related. In a paper I will describe in more detail, Smith et al (1980) reported on 3 cases of severe anaphylaxis (2 of which were protracted) among the 14 systemic reactions to challenge stings that occurred in a controlled clinical trial of venom immunotherapy (reported separately by Hunt et al 1978). The 14% frequency of protracted anaphylaxis in that study may have been related to the highly allergic population being studied and the intense nature of the challenge. In fact, other sting challenge studies have not reported biphasic or protracted anaphylaxis.

The frequency of protracted and biphasic anaphylaxis is also reflected indirectly in a published report by Korenblat et al (1999) in which he examined the need for a 2nd or 3rd dose of epinephrine in patients having anaphylaxis to allergen vaccine injections or to live sting challenge. Overall, 36 (36%) of 101 patients required additional doses of epinephrine. However, the frequency of repeated epinephrine doses was twice as high in grade II reactions (41%) as in grade I reactions (20%), and almost twice as high again in grade III reactions (72%). These data confirm that the more severe the anaphylactic reaction, the more likely there will be prolonged manifestations requiring additional treatment.

In the paper by Smith et al (1980), they described three cases of severe anaphylaxis. One patient responded rapidly to epinephrine, and hypotension resolved after a few minutes. In the second case, hypotensive shock showed no response to multiple doses of epinephrine, but seemed to respond better to norepinephrine given 40 minutes into the reaction. Note that in these cases, there

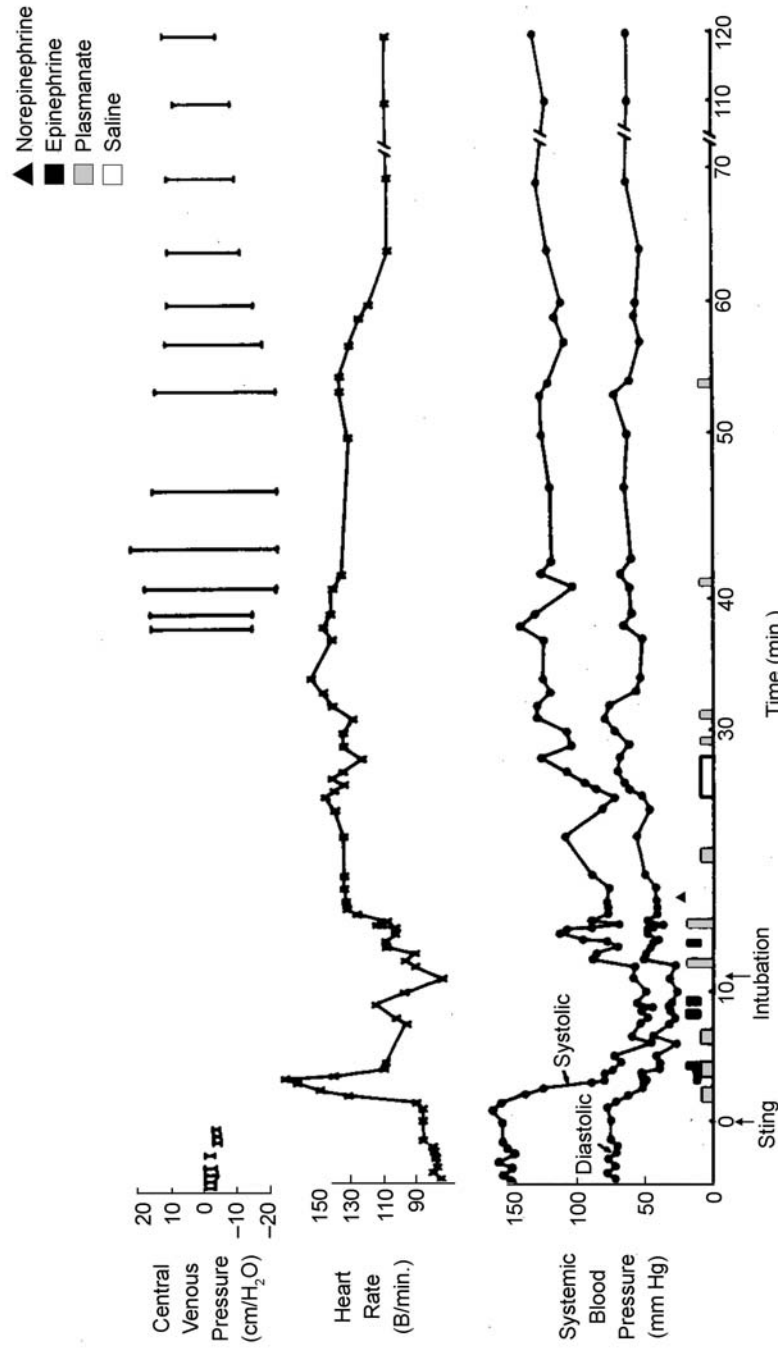

FIG. 3. Details of the third case of severe anaphylaxis described in Smith et al (1980). See text for details. (From Smith et al 1980, with permission.)

was a remarkable tachycardia coincident with the onset of reaction. In the third case, shown in Fig. 3, profound shock and respiratory insufficiency showed no response to rapid administration of multiple doses of epinephrine as well as both crystalloid and colloidal intravenous infusions. The patient was intubated after 11 minutes which resulted in transient and limited responses to several more doses of epinephrine and fluids. After norepinephrine was given at 18 minutes there was a very gradual improvement. These cases illustrate several important points. Anaphylaxis can be protracted especially when it is extremely severe. Epinephrine may be totally ineffective in cases of vascular collapse and anaphylactic shock. This observation was confirmed in an elegant animal model by Dr Simons, Bautista and co-workers, who reported last year that intramuscular epinephrine was ineffective and intravenous epinephrine was only transiently and mildly effective in reversing the most severe anaphylactic shock in dogs (Bautista et al 2002). To the clinician this is a reminder that anaphylaxis cannot always be managed easily with epinephrine and intravenous fluids.

There is very little in the literature regarding delayed allergic reactions. True delayed onset of anaphylaxis is most unusual. Of special interest to us in our studies of insect sting allergy has been the paradox of the large local reaction to a sting. These reactions are classical late-phase allergic reactions and about 80% of such patients have positive venom skin tests (Graft et al 1984, Mauriello et al 1984). In fact, their venom skin tests or radioallergosorbent test (RAST) often show stronger positive results than those of systemic reactors. In the small number of patients described in the literature, only 4%–10% had systemic reactions to subsequent stings. This is a very low frequency compared to systemic reactors who often have lower levels of IgE: a curious and unexplained observation. It would seem that the difference between large local and systemic reactors, if it could be identified, would reveal a great deal about the true nature of anaphylaxis.

There has been some evidence that allergen immunotherapy can suppress not only the early phase of the allergic reaction, but also the late phase reaction. Venom immunotherapy is not usually recommended for prevention of large local reactions because such patients have a low risk of anaphylaxis and because there is no evidence to support its efficacy. Because we encounter many patients who request venom immunotherapy for large local reactions, we have begun a pilot study (Hamilton et al 2001). In the 10 patients stung before and during venom immunotherapy, the size of the local reaction was reduced a mean of 48%, with more than 50% reduction reported by half the patients. The duration of the reaction was also reduced a mean of 59%, with 90% of the patients reporting more than 40% reduction. We plan to pursue this study in additional patients and, of course, with untreated control patients. If immunotherapy is effective in the majority of patients, it will be an interesting confirmation that late-phase allergic reactions can be prevented by specific immunotherapy.

In review of the published reports of biphasic and protracted anaphylaxis, there are several risk factors that are associated with prolonged reactions. As we have discussed, more rapid onset of anaphylaxis is associated with more severe and more prolonged reactions. The delayed use of epinephrine has been related to fatal outcome, and may lead to more prolonged and more severe reactions. Patients with pre-existing reactive airways are at higher risk for more severe and prolonged respiratory reactions, as are those on concurrent therapy with β blockers. The occurrence of laryngeal oedema with anaphylaxis is associated with prolonged reactions. It is suspected that exercise can serve as a priming factor (to increase the chance of reaction) and as a complicating factor (to worsen and prolong the reaction) (Dohi et al 1991).

It has long been hoped that investigators would discover a marker that would accurately predict which patients are most likely to react and when the reaction will be more severe or prolonged. Although there is an association of such reactions with higher levels of sensitivity, the strength of diagnostic tests is not a reliable indicator. In a report by Dr Day, as in others focusing on insect sting allergy, the strength of the venom skin test was sometimes strong in patients with mild reactions, and sometimes weak, or even negative, in patients with severe life-threatening reactions (Day et al 1994).

In conclusion, acute allergic and anaphylactic reactions vary in pattern, according to the cause, the severity, the mediators released and the duration. Biphasic and protracted anaphylaxis occur in 5–20% of cases, especially those caused by oral allergen ingestion. These reactions may require repeated doses of epinephrine and prolonged medical support and observation. Clinicians should be alert for such reactions especially in cases with rapid onset and extreme severity. The scientific basis for these reactions requires further study which may reveal better predictors and better targets for therapeutic intervention.

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DISCUSSION

Galli: I'd like to ask Dr Ohtsu to describe some of his work on the histamine knockout mouse. I'll make one point first: that is, when we study anaphylaxis of differing severity in the mouse, tachychardia is the first evidence of the reaction, and the one that persists at very low levels of mast cell activation.

Ohtsu: The goal of my experiments was to knock out the mouse *Hdc* gene, which makes histamine. Histamine is synthesized from histidine by using a unique enzyme called histidine decarboxylase (HDC). To do this we deleted a part of the *Hdc* gene starting from intron 5 to exon 9 and replaced this with a neomycin resistance gene. On exon 8 there is a coenzyme binding site. The mice had no

HDC activity and lacked histamine in various organs. I am going to describe two examples of anaphylaxis in these mice. The first one is a passively sensitized and the second is actively synthesized anaphylaxis. In the first one, we injected anti-TNP IgE into the tail vein, and 24 h later we injected the allergen into the tail vein. We monitored the mouse's temperature, respiratory function and blood pressure in order to see which parameter is important for the action of histamine. First, body temperature is decreased 2 or 3 degrees in wild-type mice, but in the knockouts the temperature is fairly stable. Therefore histamine seems to be important for body temperature. To measure respiratory function we put the mice into a body box. Respiratory frequency drops in wild-type mice but not in knockout mice. The same is true for tidal volume. However, blood pressure drops in both the knockout and the wild-type mice, although there is some individual variation. We compared W/W^v mice with control mice. W/W^v mice are deficient in mast cells. These mice have a stable temperature compared with control mice. We know now that the source of histamine which involved in the drop in temperature is the mast cells. We can reproduce a similar pattern by injecting histamine into the tail vein of the knockout mice, and we get a similar curve to that of the wild-type mice. Next we looked in detail at which type of histamine receptor is important in this reaction. We used H1 and H2 receptor antagonists. Both of them affected the body temperature, although the initial drop of body temperature was still found using these two kinds of antihistamine drugs. In a second series of experiments we looked at active sensitization and bronchial hypersensitivity. First we treated the mice with OVA with alum and challenged with OVA, and followed the concentration of total IgE and the bronchial response to methacholine. The surprising finding is that we expected the knockout to show decreased sensitivity, but in fact knockout mice showed a higher sensitivity to methacholine. Compared to the control we produced the mice that were hypersensitive to methacholine. Next we saw which cell types are increased in BAL fluid. Most of the cellular components were increased by treatment by OVA. However, the number of eosinophils was decreased in knockout mice. Also, around the bronchial lumen eosinophilia is decreased in the knockouts. There is therefore a discrepancy between the hypersensitivity and eosinophilia. We wanted to see whether the production of eosinophilia is affected or not. In peripheral blood there is no difference between knockout and wild-type mouse in the eosinophil count. But in bone marrow there is a difference. To look into the cause of this eosinophilia we checked interleukin (IL)5 concentration and P selectin levels. There is a difference in P selectin expression at the protein level but not in IL5 concentration. Taken together, these results show that we made hypersensitivity in knockout mice and we saw the difference in the number of eosinophils. Also we see some difference in P selectin, but we don't know if these differences are functionally important.

Austen: I want to be sure I understand your results. You are saying that the *Hdc* knockout had greater sensitivity after sensitization and challenge to methacholine, and a lesser eosinophil response.

Ohtsu: Correct.

Austen: Does that suggest that the eosinophil might have a positive protective role to play? There is other evidence that made us think along these lines in the past.

Ohtsu: I haven't thought about this very much.

Galli: As Dr Austen has mentioned, there are data from other knockouts which show a dissociation between eosinophil infiltration and airway hyperreactivity. Again, we are speaking about the mouse and not the human. In your initial report of the *Hdc* knockout mouse you reported some abnormalities of the structure of the mast cell granules. In addition to lacking histamine, have you defined other abnormalities of the mediator content of mast cells that may be related to the abnormal granule structure?

Ohtsu: I haven't got any definite results showing the effect from other granule components *in vivo*. We should be careful about our assessment of the results because the granules are abnormal. The content of kinase is decreased in mast cells but not tryptase. Those proteases are very important for mast cell function, and we should check these levels in *in vivo* situations.

Vercelli: The first example you gave is an IgE-mediated phenomenon. Histamine receptors have been found to be expressed on subsets of T cells. Histamine has been knocked out functionally, as it were, in the sense that these animals are developing in the absence of it. Is there anything adaptive in the immune system that would explain what you are seeing? It may be a little more complex than just looking at the reaction: there may be some kind of developmental process going on.

Galli: In knockouts it is always true that there may be responses to the lack of the particular gene product that was eliminated that occur during the development of the animal. In terms of the pathophysiology analysed in this case, Dr Ohtsu's ability to reproduce the responses in the knockout mice by injecting histamine into the animals would suggest that developmental abnormalities may not have contributed significantly to the results. Going back to David Golden's presentation and the late phase of the anaphylactic response, have the clinical studies provided any insight into the mechanism of those late reactions? Could you specifically address the issue of eosinophils and other cells that we can identify in association with late phase responses, e.g. under conditions of allergen provocation?

Golden: All we can do is somewhat blindly draw parallels with nasal challenge and bronchial challenge studies that have been able to identify the cellular components. There are virtually no studies relating directly to anaphylaxis.

Sampson: I want to comment on the biphasic response. In food allergy we went over the medical record minute-by-minute. What struck me is that it was much earlier than 4–6 h: we were seeing it at 1–2 h. To me, this indicated that it wasn't the classic late-phase response. When you look at the clinical data, it is not as long as 4–6 h. I am not sure that the biphasic response really is the late-phase response. In fact, I think it isn't. The other question I have was that in the Smith et al (1980) study, they gave multiple injections of epinephrine subcutaneously. Estelle Simons has shown that subcutaneous epinephrine is unlikely to be effective for up to an hour after it is injected. Rather than these people responding to norepinephrine, were these people finally getting some epinephrine into their systems and recovering from the initial epinephrine injection?

Golden: Those are good points. It is also a question of whether they were finally just stabilizing. I am not so sure that it was the norepinephrine. In the recent paper by Bautista et al (2002) they used an animal model to confirm that in profound hypotensive shock epinephrine doesn't work. These cases would seem to be saying the same thing, but you are quite right that they were subcutaneous injections in the face of profound hypotensive shock, so the efficacy could be seriously questioned. With regard to the time course of the so-called biphasic reaction, you are quite right. That is what we see in the papers, that it wasn't 4–6 h. It has made me wonder whether that was simply the time frame of the initial epinephrine wearing off at 60–90 min.

Simons: It is important to note that when epinephrine is given subcutaneously, it is not that it doesn't work, but rather that it may not work as quickly. When the first dose is given to a patient who is already hypotensive, we perhaps shouldn't expect too much from epinephrine (Simons et al 1998, 2001).

Ring: Coming back to the delayed reaction, I think this is an important issue. I agree with Hugh Sampson: biphasic is different from delayed. These are distinct patterns.

Fisher: There is a symptom of anaphylaxis that hasn't been mentioned. It is unusual and it occurred in our series in 18 out of about 400 female survivors of severe anaphylaxis. On day 3 after the reaction they developed a profuse, distressing watery vaginal discharge. We haven't seen this in the literature. The reason that it is important is that it goes away in about three days, and if enthusiasts treat it with dilatation and curettage there is a very high incidence of a need for blood transfusion. I don't know why this isn't in the literature. A second point is that in one stage in our series we had six patients who had adequate fluid volumes and their blood pressure was not responding to epinephrine infusion. Three of these were switched to norepinephrine and survived; the other three perished. There is another study in the anaesthetic literature by Paul McKinnon, suggesting that if norepinephrine is not working then vasopressin may turn the patient round. We have also seen a few patients

where H2 blockers seem to make them easy to look after, but we couldn't say any more than that. The last issue is the tachycardia. Studies in the anaesthetic literature suggest that tachycardia in anaphylaxis is related to the catecholamine response, and has nothing to do with histamine. As everyone is aware, if you get anaphylaxis in a β-blocked patient, they get bradycardic, not tachycardic. Asthma and beta blockers were both mentioned as things that lead to worse outcomes and make the reaction more severe. Asthma is a condition where there is a major impairment in the catecholamine response. The other thing that is like this under anaesthesia is the presence of an epidural: this causes a major blockade of the sympathetic response. This was shown to be associated with a higher mortality. These three factors are all associated with a major blunting of the catecholamine response.

Müller: In insect venom allergy we have almost never seen biphasic reactions. But then we do not treat urticaria with epinephrine. We use antihistamines instead, which have a longer half-life. I would suggest that if you treat minor anaphylactic reactions with epinephrine alone, recurrence of symptoms may be due to the short duration of the effect of epinephrine.

Golden: In one of the reports that showed a couple of biphasic reactions in insect venom allergy, they were both cases of mild recurrent urticaria.

Lasser: I'd like to mention another possible reason for delayed or biphasic reactions following mast cell activation. We have been considering principally histamine as a mediator. I'd like to talk briefly about the formation of bradykinin. In contact activation following the formation of kallikrein, bradykinin is produced. In considering this reaction we have made an interesting observation. When factor XII is activated, possibly by mast cell heparin release, it results in the formation of kallikrein, which goes one of two major ways. It is either completely inhibited by the C1 esterase inhibitor or it binds to $\alpha 2$ macroglobulin. If it does the latter, the kallikrein continues to exert some kallikrein activity. We have found that the binding of kallikrein to $\alpha 2$ macroglobulin occurs to a greater extent at ambient temperature rather than at 37 °C. This was very surprising to us. The kallikrein activity under these circumstances is also greater at 23 than it is at 37 °C. The skin and the lungs are the two organs that are closer to ambient temperature than the rest of the body. Therefore I wonder whether some consideration should be given to late reactions in the skin and lungs based on the protracted and temperature dependent build-up of bradykinin.

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Fatal anaphylaxis in the UK, 1992–2001

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Abstract. Each year in the UK, around nine deaths are attributed to anaphylaxis to pharmaceuticals, six to food and four to stings. I have identified 214 deaths associated with anaphylaxis, and have sufficient information for 196 to determine that 88 deaths were due to shock, 96 to asphyxia. Five deaths followed epinephrine overdose, seven were complicated by disseminated intravascular coagulation. There will have been other unrecognized fatal antibiotic and asthmatic food reactions. For foods, peak age was 17–27 with a female and atopic predominance; the first arrest was commonly from asthma 25–35 minutes after the implicated food. For stings, peak age was 45–70 with male and non-atopic predominance; death was commonly from shock 10–15 minutes after the sting. A majority of deaths from pharmaceuticals in hospital took 5 minutes or less from dose to arrest; peak age was 60–75. Maximum time for any cause from trigger to first arrest was 6 hours. The danger of epinephrine overdose and its limitations in reversing anaphylaxis must be recognized. The patient should remain supine with legs raised throughout sting and other shock reactions. Prevention of fatal food reactions will depend on avoidance and optimal daily control of asthma.

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Background

This study followed a workshop I had organized in 1992 on management of patients who presented with a history of anaphylaxis. It became clear that neither consensus nor evidence-based guidelines would be possible without greater insight into what made anaphylaxis dangerous. The relative numbers of patients dying from each cause was not known — previous studies had focused either on foods, stings, specific drugs or contrast media. Nor was there good information on the rate of reactions — previous studies had taken the time from exposure to death whereas it seemed more relevant to know how long it took from exposure to first respiratory or cardiac arrest.

I therefore decided to study all the fatal reactions occurring in the UK, whatever the cause, to obtain data on which to base guidelines.

Methods

Initially this was intended as a retrospective study, identifying cases from the death register of the Office for National Statistics and from fatal drug reactions reported to the Medicines Control Agency. The study was conducted according to the recommendations of the Local Research Ethics Committee. I notified my allergist and histopathologist colleagues of the register, seeking to identify further cases. Before long, cases were referred at the time of death, allowing more contemporaneous collection of data. Discussion with Her Majesty's Coroners led to my involvement in an increasing number of inquests, which proved a rich source of information about the deceased.

When I looked into the background of deaths registered as due to anaphylaxis it became clear that in around 15% of cases this diagnosis was not the most likely (Pumphrey 2000). Some deaths were due to known pharmacological actions or interactions. Two spectacular overdoses of epinephrine in cases where the diagnosis of anaphylaxis was unlikely highlighted the lethal potential of this drug (Pumphrey 2000); lesser overdose in three other cases had clearly contributed to death. In cases where an alternative diagnosis seemed to me more likely, I compiled a detailed anonymous history and sought the opinion of two or more experts in the appropriate field.

The data collected are summarized in Table 1. Throughout this account, the term 'arrest' is used to indicate first cessation of cardiac output or respiration.

Cause of fatal reactions

The likely cause of fatal reactions is summarized in Table 2. Using the European Academy of Allergology and Clinical Immunology revised nomenclature (Johansson et al 2001), 157 reactions were likely to have been IgE-mediated, 7 non-IgE and 3 non-allergic anaphylaxis; for 45 there was insufficient information to assign the reaction to one of these categories. In most cases, the cause of the reaction could readily be identified. In some the cause identified in the death certificate did not seem the most likely when all the available facts were considered. This was a particular problem with a third of deaths attributed to food allergy, similar to the problem with patients referred to our clinics with a history of reactions attributed to food allergy: in these too, the attribution is often unsupported by adequate evidence and challenge tests may be negative.

The more certain causes matched those reported previously in other studies (reviewed in Roberts & Pumphrey 2000) except for the absence of soybean allergy (Foucard & Malmheden Yman 1999). As with other food-allergic fatal asthma, such cases may well have been missed. More cases of fatal antibiotic anaphylaxis were identified than had been reported as adverse drug reactions: it

TABLE 1 Data collected

Death certificate	identity data location of death inquest verdict	
Reaction	date trigger circumstances	
	treatment	where, by whom epinephrine: when, route, rate
	progression	minutes to first arrest hours to death
	estimate of probability	trigger correctly identified death from anaphylaxis
Autopsy	macroscopic tryptase, IgE	
Previous medical history	allergic history	investigated?
	previous reactions	severity epinephrine kit?
	asthma treatment, severity	steroid compliance β2 agonist use
Blame		

TABLE 2 Suspected cause of 212 reactions

Sting	47	29 wasp, 4 bee, 14 unidentified
Nuts	32	2 almond, 2 brazil, 1 hazel, 10 peanut, 6 walnut, 11 mixed or unidentified
Food	13	1 banana, 2 chickpea, 2 fish, 5 milk, 2 crustacean, 1 snail
Food?	18	1 ?fish, 5 during meal, 1 ?grape, 3 ?milk, 3 ?nut, 1 ?sherbet, 1 ?strawberry, 1 ?yeast, 1 ?nectarine
Antibiotic	27	1 benzypenicillin, 10 aminopenicillin, 12 cephalosporin, 1 ciprofloxacin, 1 vancomycin, 2 amphotericin
Anaesthetic	35	19 suxamethonium, 7 vecuronium, 6 atracurium, 7 at induction
Other drug	15	3 ACE inhibitor, 6 NSAID, 5 gelatines, 2 protamine, 2 vitamin K, 1 Diamox (acetazolamide), 1 etoposide, 1 pethidine, 1 heroin, 1 kabikinase, 1 local anaesthetic
Contrast media	11	9 iodinated, 1 technetium, 1 fluorescein
Other	3	1 latex, 1 hair dye, 1 hydatid, 1 idiopathic

seems that antibiotic anaphylaxis is considered so well recognized that it no longer needs to be reported (Pumphrey & Davis 1999).

Despite concerns about latex allergy, only one fatality was identified, and that had nothing to do with healthcare exposure, being caused by application of hair weaving and bonding adhesive (Pumphrey et al 2001).

Some cases were identified where anaphylaxis was not recorded as the cause of death but was evidently the most likely cause from the details of the clinical history. Several reasons leading to this misclassification were identified:

- Negative autopsy findings. When patients die rapidly from anaphylaxis there may be no characteristic macroscopic findings (Pumphrey & Roberts 2000). The cause of death may then be recorded as, for example, the infection that led to the antibiotic being taken rather than the anaphylactic reaction it caused.
- Fatal acute asthma is regarded as a natural cause of death, so coroners may not wish to look for a cause for the attack. My own study of the 4851 death certificates 1993–1998 including codes for asthma indicates that there are 360 deaths each year in the UK from acute attacks of asthma in those under the age of 65. I was able to identify a few anaphylactic reactions to foods and antibiotics from within this group but suspect there were many more.
- Myocardial ischaemia is common in anaphylactic shock and has been mistaken for myocardial infarction due to arterial disease.

Both asthma and myocardial infarction are so much more common than anaphylaxis that it is not practicable to attempt retrospective identification of anaphylactic deaths from within these populations.

Mode of death

The mode of death is summarized in Table 3. Deaths from acute asthma during anaphylaxis occurred almost exclusively in those with pre-existing asthma — see below under *concurrent disease*. Death from upper airways occlusion by angioedema was the least common mode: approximately half the instances were due to food reactions and a quarter each to stings and drugs.

Rapidly fatal shock reactions often had no other symptoms. Perhaps rapid onset of shock precluded the leakage of sufficient fluid to cause angioedema or urticaria. There was a striking pattern in the slower shock deaths: the postural history is known for 10 patients; four patients collapsed within seconds of a change to a more upright posture and six died while supported sitting after loss of consciousness (Pumphrey 2003).

There may be a logical explanation for these sudden deaths on change to a more upright posture. While lying down, despite the greatly increased capacity of veins

TABLE 3 Mode of death

	Drug	*Sting*	*Food*	*Food?*	*Male*	*Female*
Lower airways	11	3	24	11	21	26
Upper+lower airways	6	4	13	3	5	19
Upper airways	7	8	5	3	16	12
Shock+asphyxia	21	4	2		12	15
Shock	32	18	2		23	29
Disseminated intravascular coagulation	5	1	1		2	4

'Food' indicates fatal anaphylactic reactions with a high probability of being due to the suspected food, 'food?' includes reactions where there was some doubt about the relationship between reaction and the suspected food cause: an unknown fraction of these may have been due to idiopathic anaphylaxis or idiopathic angioedema.

and capillaries during anaphylactic shock, sufficient blood returns from the vena cava to maintain a reduced cardiac output. On standing up, venous return from dependent parts of the body stops and the vena cava may become empty, preventing any filling of the right side of the heart. After a few contractions, no blood returns from the lungs, and with no ventricular filling, pulseless electrical activity ensues. Coronary flow depends on the pulse pressure, so the lack of pulse leads to myocardial ischaemia. Only one of the six supported sitting after loss of consciousness had an ECG: this showed changes of myocardial ischaemia similar to other non-fatal cases reported previously (Ceyhan et al 2001, Levine 1976). Myocardial infarction was diagnosed in none of the asphyxial anaphylactic deaths but seven other shock deaths.

Rate of reaction

First arrest in fatal food reactions was most commonly 25–35 minutes after exposure, slower than for stings (10–15 minutes) or drugs (five minutes or less in hospital and 10–20 outside hospital) (Fig. 1).

Risk factors

Age

Anaphylactic deaths occurred at all ages from 5½ months to 88 years. The median age at death was characteristic for each cause, with food allergy affecting younger people and drug allergy older (Fig. 2).

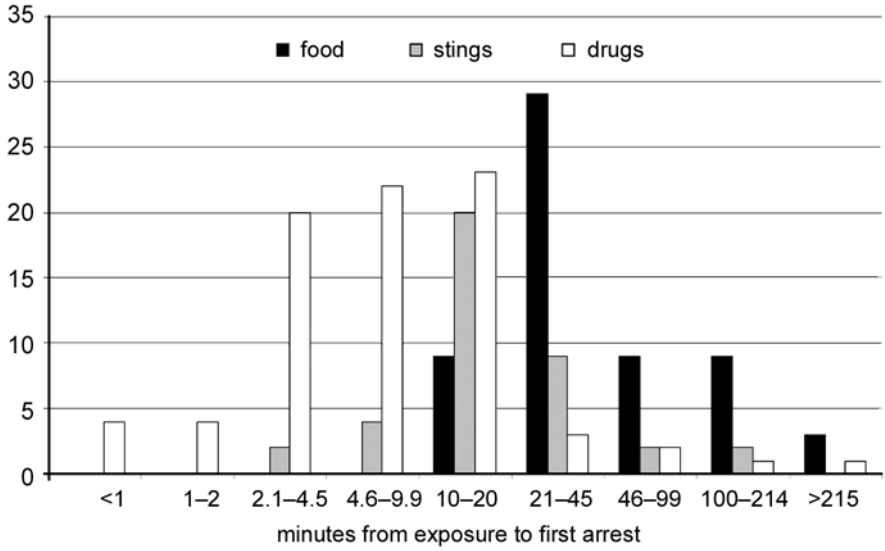

FIG. 1. Interval from exposure to first arrest. Drug reactions were fastest, mostly taking less than 5 minutes.

Sex

There was a slight preponderance of females (116:95=55%) overall, but the female:male ratio varied from 24:8 for nuts to 2:9 for contrast media. The previously reported (Lenler-Petersen et al 1995) strong male predominance for contrast media reactions was particularly striking in view of the female predominance in non-fatal contrast media reactions (Lang et al 1995). The tryptase level was very high when it was measured in this group, suggesting that fatal contrast media reactions involve mast cell activation and might be IgE-mediated. Among those dying from food allergy, the pattern of deaths suggests milk allergy is most dangerous in boys and peanut allergy in young adult females.

Race

The numbers were not sufficiently large for statistical significance: there were deaths in most racial groups, possibly in proportion to the general population.

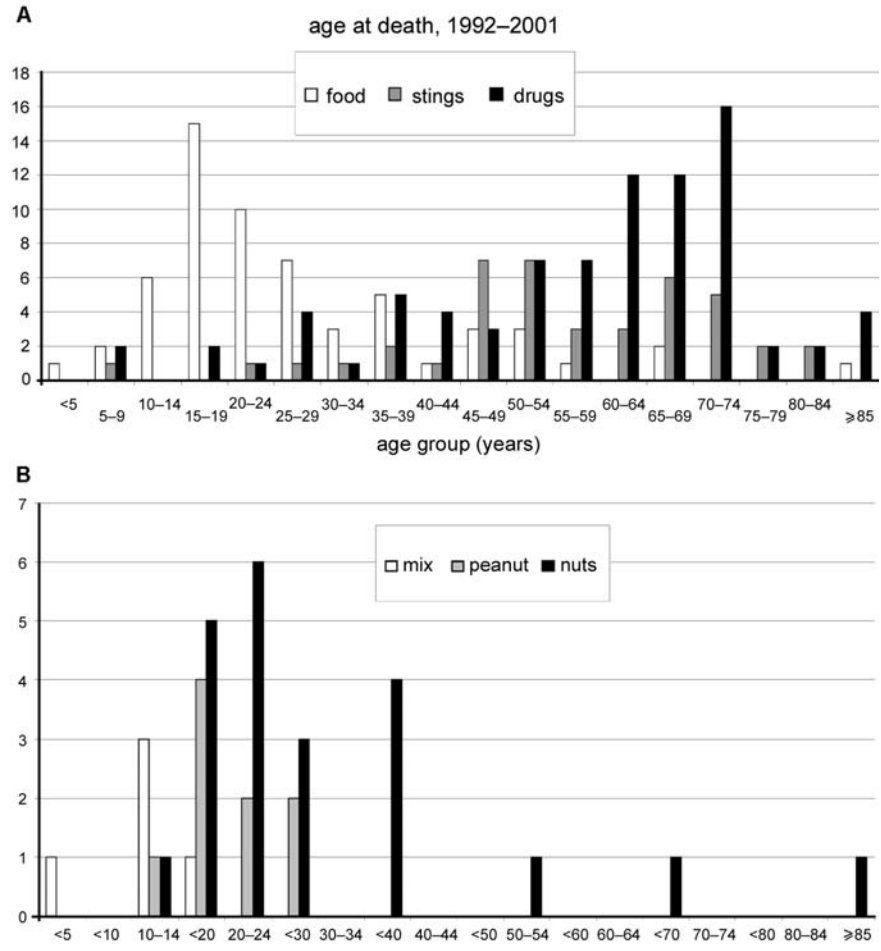

FIG. 2. Age at death from anaphylaxis. (A) The median age at death from food allergy was 21 (mode 19), for stings 56 and for drugs 61 (mode 74). (B) For fatal food allergy, the age at death varied according to the food; milk affected the youngest and peanuts probably affects younger people than tree nuts, though this is less certain because there may be some peanut deaths included among the 'nut' deaths.

Atopy

Deaths from presumed IgE-mediated food allergy were almost exclusively in individuals assessed to be atopic by their history of allergic symptoms; skin test results and specific IgE measurements were available for only a minority. Estimation of the rate of atopy in other anaphylactic deaths was insufficiently reliable to determine whether it differed from the national rate.

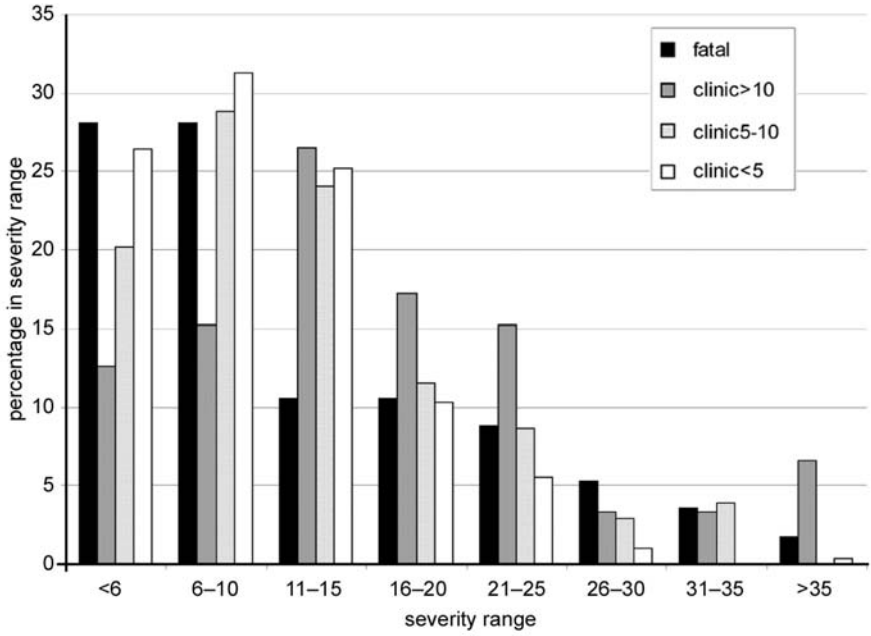

FIG. 3. Severity of previous nut reactions. The severity was calculated from weighted severity assessments for each component symptom during the reaction (Pumphrey & Stanworth 1996: reactions scoring <6 are very mild, those scoring >30 are usually considered life threatening). The previous worst reaction in clinic attendees has a distribution at least as severe as those who died from anaphylaxis — particularly when older clinic attendees are considered. The severity of reactions can therefore not be used to assess who is at risk of a fatal reaction.

Previous reactions

Many of those dying from anaphylaxis had only minor previous reactions. For nut allergy, the worst previous reaction of those dying had a distribution (Fig. 3) of severity (Pumphrey & Stanworth 1996) that was milder than that of patients presenting to my allergy clinic for advice following acute reactions to nuts: severity of previous reactions is therefore no guide to who is at risk of a fatal reaction, and should not be one of the factors determining who should be advised to carry epinephrine for self-treatment. This was even clearer for those dying following wasp stings, where the previous sting had often caused no generalized reaction. One would deduce that very few of those desensitized to stings will have their lives saved by this treatment.

In some cases, repeated previous mild reactions to one agent were followed by a fatal reaction to an agent with a slightly different chemical structure: e.g. a fatal reaction to hair dye based on *p*-toluenediamine after repeated lesser reactions to

one based on *p*-phenylenediamine, or a fatal reaction to cefaclor following repeated reactions to amoxicillin (Pumphrey & Roberts 2000).

Circumstances of exposure

Food. Commercial catering caused 68% of nut-related reactions; details of events leading up to the fatal exposure to nuts prove that asking for a meal without nuts is not a successful avoidance strategy. Neither the person serving nor in some instances the caterer realized that the food contained nuts. For other foods, the sources were distributed more evenly between whole foods, domestic cooking, commercial cooking and packaged foods.

Stings. Fatal stings occurred in a wide range of situations including 15% at work, 32% in the garden, 34% in the house at home and even 5% in bed.

Pharmaceuticals. Eighty-six per cent of iatrogenic reactions occurred in hospital and 61% in operating theatres where the patient was monitored and full emergency treatment at hand. Even so, reactions were fatal, suggesting that even with optimal treatment rescue will not always be possible.

Concurrent disease. Many examples could be found amongst those dying from anaphylaxis where concurrent disease had modified the course of the reaction to make it fatal. This was seen very clearly in the case of a 5-month-old where a reaction at age 6 weeks had caused generalized urticaria with local symptoms in the mouth and vomiting. Following bronchiolitis at age 7 weeks, he had repeated attacks of wheezing, and his next reaction to milk caused fatal broncho-constriction and mucus plugging; the lung histology at autopsy revealed chronic inflammation and remodelling similar to chronic asthma. All fatal asthmatic reactions to foods, two of the three fatal asthmatic sting reactions, both asthmatic fatal NSAID reactions and four out of six fatal asthmatic reactions to antibiotics were in those taking daily treatment for asthma; for many of those with food allergy, the history suggested that they had not been taking daily inhaled steroid and some of these had clearly been using more than the recommended rate of self-treatment with short-acting $\beta 2$ agonist.

Unlike some other series (Sasvary & Müller 1994), the fatal sting reactions did not have a predominance of those with coronary artery disease. Only one of 18 shock reactions to stings had been taking an ACE inhibitor and one a β blocker.

Previous 'idiopathic angioedema' was noted in at least four of the seven 'food allergy' deaths with upper airway asphyxia where the association between the suspected food and reaction seemed tenuous. The possibility that mild food allergy could act as a trigger factor for attacks of angioedema in such patients

with an underlying tendency to idiopathic angioedema has to be considered. One of these thought she was allergic to milk and had 0.5 kU_A/L specific IgE to milk with a total IgE of 35 kIU/L. Although this seems low to have caused a reaction, it was the highest level detected in over 500 patients with suspected milk allergy and total IgE under 100 kIU/L tested for milk-specific IgE at her age.

Two of the fatalities had mastocytosis. In one, the reaction was triggered by a bee sting; this man was not known to be allergic to bee stings. In the other (Vaughan & Jones 1998) the reaction occurred during an anaesthetic and may have been due to non-specific mediator release triggered by an opioid.

Threshold and dose

Meta-analysis of threshold for reaction to foods (Bindslev-Jensen et al 2002) shows a lognormal distribution extending three–four orders of magnitude either side of the median. Clinical experience suggests that there may be similar lognormal distribution for haptenic antigens such as β lactam antibiotics. The estimated dose for fatal nut reactions varies from a few milligrams to several tens of grams; fatal milk reactions show a similar range. Ninety per cent of fatal sting reactions followed a single sting, the remainder two–seven stings. Fatal antibiotic reactions followed doses of 0.25–1 g.

It seems most likely that the rate and severity of reaction increases as the amount by which trigger dose exceeds the threshold increases. For some allergens this threshold may be stable over months or years, others may vary rapidly — such as for sting allergy in the weeks following a sting. Very rapid reactions are seen in cases where a large bolus injection is given to a patient with extreme sensitivity: some patients were reported to have arrested within 30 seconds. In other cases, the reaction progressed more slowly, possibly due to a dose closer to the threshold, possibly due to delayed absorption.

The slowest reactions were those where there had been partial treatment: one man with brazil nut allergy had repeated injections of epinephrine on his way to hospital, where he was observed for some time until he suddenly collapsed and died 6 hours after the ingestion.

Geographical distribution

The distribution of cases around England and Wales was approximately in line with the distribution of population, but fewer cases than expected were retrieved from Scotland and Northern Ireland. This seems more likely to be due to detection rates rather than differences in occurrence.

Lessons for management of anaphylaxis

Recognition of anaphylactic reactions

The presentation of patients with anaphylaxis outside hospital in some cases confused first responders. Asphyxial anaphylactic reactions were mistaken for simple acute asthma, and shock reactions were mistaken for myocardial infarction; ECG changes of myocardial ischaemia contributed to this confusion. One might conclude that paramedics should be trained to enquire about a possible allergic cause before following protocols for these alternative diagnoses.

In hospital, side effects of drugs given to treat reactions have also been misinterpreted as a worsening of the anaphylaxis, leading to further inappropriate treatment. Fatalities occurred when blanching from epinephrine was misinterpreted as shock, and when pulmonary oedema from epinephrine overdose mistaken as a symptom of anaphylaxis: neither case had significant symptoms before treatment and in both cases further treatment with epinephrine caused a deterioration leading to death.

Symptoms during panic attacks and breathing difficulty due to food allergy may appear similar. In one case, the general practitioner attending the patient considered up until the time of fatal collapse that the symptoms were mostly due to panic; because of this, epinephrine was not given.

Treatment of the acute reaction

I have previously reported both fatal and damaging non-fatal effects from inappropriate use of epinephrine (Pumphrey 2000). Further instances continue to occur, commonly due to confusion between the use of epinephrine for cardiopulmonary resuscitation and for anaphylaxis (Gompels et al 2002). A report on food allergic reactions in children and adolescents suggested that recovery from an anaphylactic reaction is most likely if epinephrine is given within 30 minutes (Sampson et al 1992). In the cases reported here, arrest occurred at 30 minutes or earlier in 87% of venom reactions and 59% of nut reactions.

Reducing risk of exposure

In every case where there was a previous history of reactions, precautions had been taken to avoid re-exposure. For some, there had been a lapse in vigilance following a long interval since the last reaction. For others, precautions failed due to a variety of reasons, usually including failure of communication.

Reducing severity of reactions

Hyposensitization is known to reduce risk from stings, but few of those dying from sting reactions had a history of previous reactions. Suboptimal daily treatment for asthma was the rule among those with fatal asthmatic reactions due to food allergy; compliance with guidelines for daily inhaled steroids (British Thoracic Society & Scottish Intercollegiate Guidelines Network 2003) would reduce severity of many reactions.

Rescue medication

No features were apparent in the previous histories of those dying from anaphylaxis that would distinguish those who were at greatest risk. In particular, there would be no logic in using severity of previous reactions as an indication for epinephrine for self-treatment. Our clinical experience suggests that only a fraction of those dispensed epinephrine for self-treatment will have it with them at the time of a reaction and know when and how to use it, despite repeated training. Even in cases where it was used correctly this treatment did not always work.

Conclusion

Anaphylactic reactions are unexpected medical emergencies. The circumstances of these reactions indicate that a prospective controlled trial of treatment for anaphylactic reactions will not be feasible. Half occur during medical interventions; the other half have no immediate access to emergency medical care. Because there is a very low rate of fatality outside hospital even for untreated reactions, and because reactions are unexpectedly difficult to diagnose, recommendations for treatment of anaphylaxis must be safe for the majority of cases where the diagnosis is mistaken.

This study has revealed how avoidance, self-treatment and medical management failed to prevent anaphylactic death. The difficulty diagnosing anaphylactic reactions and their unpredictable development explain why epinephrine was seldom used in the early phase of the reaction. The findings suggest that deaths from food allergy could be reduced by improved compliance with existing recommendations for daily asthma management, and that shock deaths outside hospital might be prevented by the simplest of first aid.

Acknowledgements

I thank the Anaphylaxis Campaign for early notification of many fatalities, and all Her Majesty's Coroners, pathologists and others who have helped collect these data.

Research ethics approval

This study was carried out in accordance with advice from the Central Manchester Research Ethics Committee.

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DISCUSSION

Galli: Do people from other countries want to comment on the patterns of fatal anaphylaxis reported by Professor Pumphrey for subjects in the UK?

Sampson: We have similar findings to Richard Pumphrey in the USA. I have a comment based on a statement David Golden made about idiopathic anaphylaxis. I know this issue of individuals needing to eat more than one food to bring about a reaction is proposed all the time, but I am not aware of anyone we have seen who has to ingest two foods simultaneously in order to have a reaction. We certainly see this with exercise plus food or non-steroidal anti-inflammatory drugs (NSAIDs) plus a food, but never with two foods. Are you aware of any?

Pumphrey: No.

Sampson: I am not suggesting that it never happens; rather that is far less frequent than some people who suggest this is a cause for idiopathic anaphylaxis seem to think.

Pumphrey: Let me describe one of these idiopathic cases. This illustrates some of the difficulty here. This was a woman who was aged about 50 and who had been seen by one of my colleagues and treated for idiopathic angioedema. She had a number of swellings, often starting during the night. She had decided she was allergic to milk, which she had been avoiding. Then she had a cup of tea with milk in it, and within half an hour had difficulty breathing that became progressively worse. She didn't respond to inhaler or antihistamines, and died with upper airway obstruction. We measured her milk-specific IgE, and it was 0.5 with a total IgE of 35. This was the highest level of milk-specific IgE that I have found in a patient of that age with that low a total IgE, out of 500 cases. She thought she had milk allergy, and the reaction happened after she had milk, but she had a history of idiopathic angioedema. I can't tell whether what happened to her was simply coincidence or whether her milk allergy was a trigger for setting off one of these attacks of angioedema.

Simons: With regard to the two-food issue, I think the major problem is that often patients don't know exactly what they have eaten. Contamination of a food with traces of another food is a problem, as is substitution of one food for another food. Unless we actually analyse a sample of the food that supposedly has triggered the death, we may be just speculating.

Golden: What Hugh is saying is that in non-fatal cases I don't know that any of us in the room have ever confirmed or challenged a patient that required sequential foods to develop a reaction. I have had one case in 20 years who was diagnosed by history and skin test, but I never challenged them.

Sampson: We have tried doing this, when people have suggested particular foods. We have done this in sequence. We have actually brought in restaurant meals that they are sure they always react to. We can't validate this by challenge. I'm not saying it can't happen, but we have to remain sceptical about this.

Lasser: I am struck by the fact that in my own experience of contrast media anaphylaxis, the history of food allergy, other than that of fish allergy, almost never comes up. This makes me wonder whether there are two different

mechanisms. Dr Pumphrey, have you found that people who have a history of food allergy then go on to have a bad reaction with drugs, and specifically contrast media? In other words, how often in your experience has food allergy been in the history when a drug allergy has been the immediate incident?

Pumphrey: In the contrast media anaphylaxis patients, none had a history of previous food allergy.

Fisher: In our anaesthetic data, 8% of the non-reacting anaesthetic population have a history of allergy, but 40% of the people who have reactions have a history of allergy. Half of this 40% have a food allergy. This is sometimes used by lawyers in court cases to say that the patients should have been tested. If 20% of people have a history of food allergy, then 80% don't, so it's not a predictor.

Schwartz: I'd like to compliment Dr Pumphrey on a really heroic effort to gather all of these data. I take care of patients with recurrent anaphylaxis. I have always instructed them to get their epinephrine and then lay down and elevate their legs. I tell them not to get up: if they must go somewhere, crawl. I have done this from common sense, but it is nice to see supporting data. Where it becomes controversial is whether or not to tell them to go to the emergency room. Just the act of getting up to get into the car and sitting while being driven to the emergency room, is probably more dangerous than staying at home legs elevated.

Pumphrey: We would advise them to call for an ambulance. The problem is that quite often paramedics will then put the patient in a chair to carry them downstairs. We have had a case where the patient was alive at the top of the stairs dead at the bottom.

Ring: These are important data. There is so little solid information. Everyone of us is aware of many cases that have never been published. They are in files. When there is a forensic issue we write an assessment and are not allowed to publish the story. The problem is much larger than is commonly thought. Your case of the lady with the antibiotic (described in Pumphrey & Roberts 2000) was really moving and showed us several things. We have to improve the education of doctors, and even the pathologists who frequently misdiagnose anaphylaxis. We also have to educate the patients! This was a clear case of anaphylaxis, but this sort of thing happens commonly. Werner Pichler in Bern only prescribes the EpiPen to a patient when the patient has shown that they are able to inject a needle into their own skin. He gives them a needle and tells them to put it into their skin, and only if they dare to do this will he prescribe. Even if patients have an EpiPen they are often afraid to do it if they haven't already really injected a needle into their skin.

Müller: I am always fascinated by the high numbers of nut allergies in Anglo–Saxon countries. Certainly, in my country and continental European countries nuts are further down the list, in third or fourth place as the precipitating allergens.

Ring: There are few hard data in Germany.

Sampson: I agree, there are few hard data. But there are definite patterns in different countries. In the UK, USA and Australia, peanuts and nuts are first. In Italy and France nuts aren't the main food allergen. In Israel it is sesame seed. It depends on the exposure. In China, where they eat as much peanut as we do in the USA, there is virtually no peanut allergy. However, the peanuts eaten in China are in a different form. In the USA we get very early exposure to peanut through peanut butter. Breast-feeding mothers eat a lot of peanut products because it is considered a source of high-quality protein. The peanuts we eat are mainly dry roasted, which alters the structure of the peanut in such a way as to make it more allergenic than the forms eaten in Africa and China where it is boiled. In fact, in a mouse model we have developed of peanut anaphylaxis, if we sensitize with dry roasted peanut we can make the mice anaphylactic. However, boiled peanut doesn't work. We think this is something to do with the effect of the preparation method on the structure and subsequent allergenicity of the protein.

Golden: With regard to the pathology that the coroners report, I agree that all forms of anaphylaxis and fatal reactions are probably underestimated. I can recall one case in the USA in which the patient was found dead on the ground. They had been working in the barn, and there was a note on the bench saying 'stung by bee'. The coroner signed it out as death from myocardial infarction. We need to educate coroners. I think the pathology at post-mortem is most interesting, and the inability to find anything. Dr Austen described much of this some years ago.

Austen: That paper is so old it is not on the internet (James & Austen 1964)!

Golden: If I remember correctly there were a fair number of patients in whom there was no abnormal finding.

Austen: The study was done with a pathologist and limited to cases with a full clinical history and complete autopsy that included the larynx. No abnormal findings meant that the upper (larynx) and lower (lung) respiratory tracts were unremarkable, implying that the hypoxia was not direct but a consequence of cardiovascular collapse.

Mosbech: Richard Pumphrey, you showed that patients with asthma were at increased risk of severe reaction. I agree completely. Then you mentioned that they should use minimal amounts of $\beta 2$ agonists. This could be misunderstood. The point is not that they should not use $\beta 2$ agonists, but that they should use something else in addition.

Pumphrey: That is what I meant: that they should have optimal asthma management so that they didn't have to rely on large daily doses of $\beta 2$ agonists.

Finkelman: I am fascinated. I had not been aware of the association of asthma with death from anaphylaxis. I was thinking of some of the pathogenic mechanisms that might be implied. Richard Pumphrey showed the idea that you already have partial bronchoconstriction, so a little bit more will do much more damage than it would do in someone who didn't have this to begin with. Perhaps

there are some additional factors involved. I am struck by the finding that you see this in food anaphylaxis but not so much in insect-sting anaphylaxis. If it were just an increment in bronchoconstriction you should see the same thing in both perhaps. Perhaps what is going on is that this association between the shared immunity of different mucosal organs suggest that there is similar production of IgE in similar mast cell populations in the respiratory mucosa and in the gastrointestinal mucosa. Then, when you ingest a food allergen you will not just have the local reaction in the gut but also a local reaction in the bronchi. You wouldn't expect to see the same thing if you had more systemic inoculation with the allergens seen in insect venom allergy. Is there any evidence for this idea?

Pumphrey: Of the three sting deaths that were asthmatic deaths, two were taking daily treatment for asthma. My point was not that the bronchi are already partially constricted, but rather that there is remodelling: the whole structure of the bronchi is different in someone with chronic asthma. It is this change in structure that is important. There is doubling of the smooth muscle, inflammatory infiltrate in the mucosa and the elastic lamina is thickened and more rigid.

Galli: A related point is that many patients with food allergy have other evidence of atopic disease, including asthma. In contrast, insect allergy can occur with or without atopy.

Simons: Both papers this morning have touched on the subject of idiopathic anaphylaxis. I am among those physicians who think that if we search hard enough, we may be able to identify the anaphylaxis trigger in some patients with so-called idiopathic anaphylaxis. For example, David Golden mentioned anaphylaxis deaths in the middle of the night. This is a situation in which anaphylaxis from painless and unwitnessed insect bites may rarely occur. This is a relatively unexplored area due to lack of diagnostic agents (Beckett et al 2003).

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The human heart as a shock organ in anaphylaxis

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Abstract. Human mast cells, by elaborating vasoactive mediators and cytokines, are the primary effector cells of anaphylaxis. A body of evidence implicates human heart mast cells (HHMCs) in anaphylaxis. These cells have been identified perivascularly, in close proximity to myocytes and in the arterial intima in human heart tissue. The membrane surface of mast cells from human heart tissue of patients undergoing cardiac transplantation expresses the high affinity receptors for IgE (FcεRI) and C5a receptors. Activation of HHMCs *in vitro* with anti-IgE or anti-FcεRI induced the release of preformed mediators (histamine, tryptase and chymase) and the *de novo* synthesis of LTC_4 ($\cong 18\,ng/10^6$ cells) and PGD_2 ($\cong 18\,ng/10^6$ cells). Complement activation and anaphylatoxin formation occur during anaphylaxis in human. C5a caused rapid release of histamine and tryptase from HHMCs. These cells are activated *in vitro* by therapeutic (general anaesthetics, protamine, etc.) and diagnostic agents (radio contrast media, etc.) that may cause non-IgE-mediated anaphylactic reactions. Administration of low concentrations of histamine and cysteinyl leukotrienes in subjects undergoing diagnostic catheterization caused significant systemic and coronary haemodynamic effects. Taken together, these results indicate that the human heart can be both the site and the target of anaphylactic reactions.

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At the beginning of the 1900s, William Osler, an influential physician and academician, affirmed that acute attacks of bronchial asthma never caused death (Osler 1901). As a consequence, generations of physicians were taught that death from asthma essentially never occurs. The dogma remained firmly in place until 1922 when death as a complication of asthma was demonstrated in a pathological study (Huber et al 1922). Subsequently, reports of asthma-induced deaths began to appear (Bullen 1952, Williams 1953, Houston et al 1953, Earle 1953). In the United Kingdom the reported mortality from asthma among young patients rose between 1959 and 1966 and asthma became a common cause of death in this age group (Speizer et al 1968). Increased risk of death from asthma has also been documented in several long-term studies (Weiss & Wagener 1990, Lang &

Polansky 1994, Silverstein et al 1994). More recently, in a prospective study self-reported asthma was associated with an excess of mortality (Lange et al 1996).

Systemic anaphylaxis represents the most dramatic and potentially fatal manifestation of immediate hypersensitivity. Pathological observations have demonstrated that lesions of the cardiovascular system are the cause of death in patients who died from anaphylaxis (Carswell 1985). There is now compelling evidence that the heart is directly and/or indirectly involved in several forms of anaphylaxis in humans (Bochner & Lichtenstein 1991, Sampson et al 1992, Kemp & Lockey 2002). It is also well established that anaphylaxis can be associated with fatalities that account for more than 500 deaths annually (Bochner & Lichtenstein 1991). Despite these alarming findings, there is a surprisingly modest interest and lack of information on the involvement of the cardiovascular system in fatal and near fatal allergic diseases.

The purpose of this review is twofold: first, we will describe the possible roles of cardiac mast cells and their mediators during anaphylactic reactions in human; second, we will briefly review the growing evidence of the cardiovascular effects of mast cell-derived mediators *in vivo*.

Cardiovascular alterations in anaphylaxis

Patients with systemic anaphylaxis have cardiovascular alterations. Anaphylactic shock is frequently accompanied by such electrocardiographic (ECG) alterations as ischaemic ST-waves, arrhythmias and atrial fibrillations (Bernreiter 1959, Hanashiro et al 1967, Booth & Patterson 1970, Criep & Woehler 1971, Sullivan 1982, Ferrari et al 1996). Anaphylactic reactions after insect sting (Levine 1976) can be associated with coronary spasm or acute myocardial infarction (Bristow et al 1982). Moreover profound myocardial depression can be found in patients with systemic anaphylaxis presumably due to the negative inotropic effects of mast cell-derived mediators (Raper & Fisher 1988).

Myocardial infarction can also occur as a consequence of idiopathic anaphylaxis (Wong et al 1990). Pathological observations have demonstrated that the cardiovascular system is the site most affected in patients who died from anaphylactic shock (Delage & Irey 1972). In particular, myocardial lesions could represent the anatomic basis for the development of irreversible cardiac failure that is occasionally associated with systemic anaphylaxis (Delage & Irey 1972).

Mast cells in normal and diseased human heart

Mast cells have been identified around blood vessels and between myocardial fibres in all sections of human hearts (Patella et al 1995a,b). They are also present in normal and atherosclerotic human arterial intima (Forman et al 1985, Kamat et al

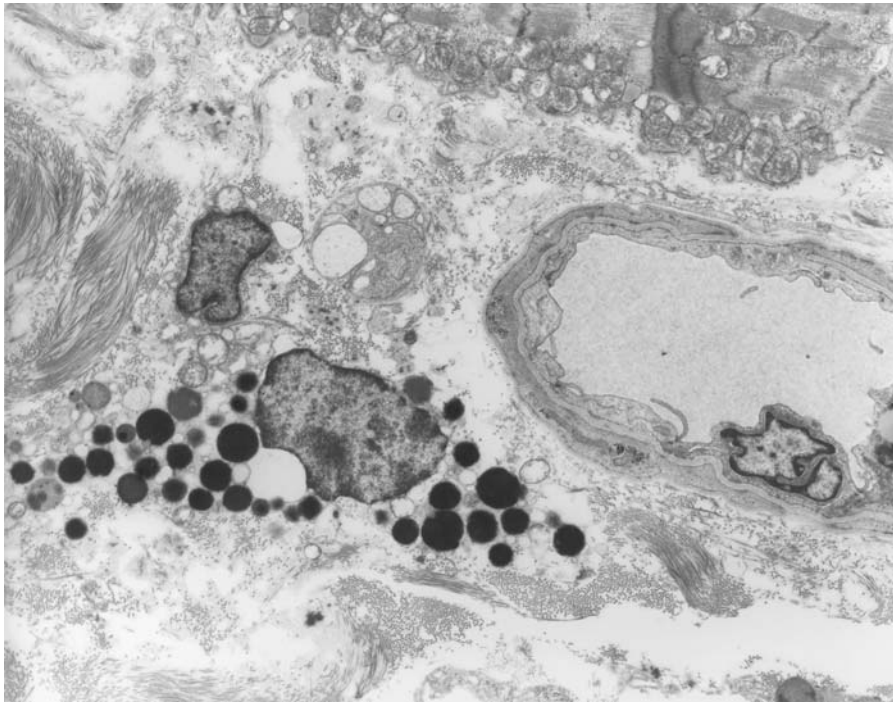

FIG. 1. Electron micrograph of a mast cell in human heart tissue. Note the elongated shape and the cytoplasm containing secretory granules. The mast cell is adjacent to a coronary blood vessel, surrounded by collagen fibres and is close to a myocyte (uranyl acetate and lead citrate stained; original magnification × 12 000).

1987, Kaartinen et al 1994a,b). *In situ* electron microscopy of cardiac mast cells revealed a small percentage (≅5%) of activated, i.e. partially degranulated mast cells (Patella et al 1995a, Laine et al 2000). This observation is clinically relevant because it implies that immunological and non-immunological stimuli can activate human heart mast cells to release vasoactive and proinflammatory mediators (Marone et al 1999).

Cardiac mast cells are frequently found close to coronary vessels (see Fig. 1). This finding suggests that circulating antigens, autoantibodies (anti-IgE, anti-FcεRI, etc.), drugs (e.g. general anaesthetics, protamine, etc.), and agents used for diagnostic purposes (e.g. radio contrast media, etc.) can easily reach the perivascular human heart mast cells (HHMCs). Activated mast cells can in turn release vasoactive substances (e.g. histamine, cysteinyl leukotrienes, PGD_2, PAF, etc.) that can affect blood vessels.

We isolated and partially purified cardiac mast cells from patients undergoing heart transplantation and in some cases from victims of car accidents. Using this technique we studied *in vitro* various aspects of HHMC biology (Patella et al 1995a,b, 1997). We have compared the cardiac mast cell density, the concentration of mast cell-derived mediators (histamine and tryptase) and the immunological and non-immunological release of mediators from mast cells isolated from failing hearts obtained from patients with idiopathic dilated (DCM) and ischaemic cardiomyopathy (ICM) undergoing heart transplantation and from controls without cardiovascular disease (CH) who died in accidents (Patella et al 1998). Cardiac mast cell-density and the histamine and tryptase contents of DCM and ICM hearts were higher than in CH. Immunological activation of HHMCs induced a significantly greater release of histamine, tryptase and LTC_4 in patients with failing hearts compared with CH. The increased cardiac mast cell density and the greater release of mediators might suggest that anaphylactic reactions could be particularly severe in patients with certain underlying cardiovascular diseases.

Preformed mediators synthesized by HHMCs

The histamine content of isolated HHMCs ($\cong 3$ pg/cell) was comparable to the content of lung parenchymal and skin mast cells, but higher than human basophils ($\cong 1$ pg/cell). All human mast cells contain tryptase: the mean tryptase content of HHMCs ($\cong 24\ \mu g/10^6$ cells) is lower than skin mast cells ($\cong 35\ \mu g/10^6$ cells) and higher than lung mast cells ($\cong 10\ \mu g/10^6$ cells). IgE-mediated activation of HHMCs caused the release of tryptase in parallel to the secretion of histamine (Patella et al 1995a).

Using a polyclonal anti-chymase antiserum (Schechter et al 1990) and the immunogold technique, we showed that HHMCs contain chymase as well as tryptase (Patella et al 1995a). This is particularly important because human heart chymase generates the vasoactive peptide angiotensin II from angiotensin I, thereby acting as an angiotensin-converting enzyme (Urata et al 1990). Supernatants of HHMCs challenged *in vitro* with anti-IgE can convert angiotensin I into angiotensin II, suggesting that chymase released from immunologically challenged HHMCs could also play a role in the homeostatic control of blood pressure. These findings might indicate that activation of HHMCs and perivascular mast cells and release of chymase can serve for the homeostatic control of blood pressure during anaphylaxis, and can modulate several cardiovascular functions through the activation of the angiotensin system.

Recent evidence from our laboratory shows that the supernatants of HHMCs activated by anti-IgE can convert a synthetic substrate of big-endothelin to endothelin 1. The latter observation is particularly relevant because endothelin 1 is a potent bronchoconstrictor in allergic subjects (Advenier et al 1990).

Lipid mediators *de novo* synthesized by HHMCs

Immunologically challenged HHMCs led to the *de novo* synthesis of prostaglandin D_2 (PGD_2) ($\cong 18\ ng/10^6$ cells) through cyclooxygenase activity (Patella et al 1995a, 1995b). The relevance of this finding lies in the fact that PGD_2 is a potent coronary constrictor (Hattori & Levi 1986). Therefore, the *in vivo* release of PGD_2 from HHMCs can cause coronary vasoconstriction in man. Activation of HHMCs with anti-IgE or anti-FcεRI induced the *de novo* synthesis of cysteinyl leukotriene (LTC_4) ($\cong 18\ ng/10^6$ cells).

This set of studies showed that immunologically activated HHMCs release PGD_2 and LTC_4. Interestingly, intravenous and intracoronary administration of traces of LTC_4 and LTD_4 may have several cardiovascular and metabolic effects (Vigorito et al 1997).

Cytokines synthesized by HHMCs

Immunological responses mediated by cytokines have been implicated in the pathogenesis of the development of heart failure in a variety of diseases (Sasayama et al 1999). Studies are beginning to focus on the presence and the possible roles of cardiac mast cell-derived cytokines. Tumour necrosis factor (TNF)α, an important mediator of inflammatory reactions, has been found in mast cells of human coronary atheromas (Kaartinen et al 1996). We have ultrastructurally localized the granule-associated stem cell factor (SCF) in HHMCs in patients with dilated cardiomyopathy (Patella et al 1998). Stem cell factor is the principal growth, differentiating, chemotactic and activating factor for human mast cells (Tsai et al 1991, Columbo et al 1992). This observation raises the tantalizing possibility that SCF present and released by HHMC is an autocrine factor that contributes to mast cell hyperplasia in dilated cardiomyopathy.

Cytokines modulate several cardiovascular functions by a variety of mechanisms (Ferrari et al 1995, Torre-Amione et al 1996, Sasayama et al 1999). Additional studies are necessary to clarify the roles, if any, of cytokines in anaphylactic reactions and the contribution of cardiac mast cells to their production.

Immunological and non-immunological stimuli that activate HHMCs *in vitro*

IgE cross-linking on mast cells dispersed from human heart tissue can be induced by antigen, anti-IgE or anti-FcεRI. Figures 2 and 3 show the effects of increasing concentrations of anti-IgE and anti-FcεRI on histamine secretion from HHMCs from several donors. It is important to emphasize that the percentage of release induced by the same concentrations of stimuli varies among different mast cell

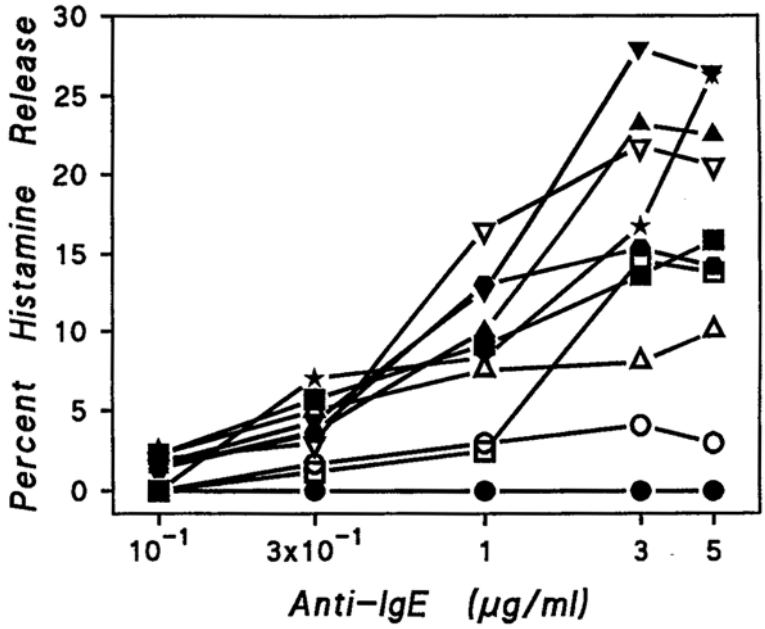

FIG. 2. Effects of increasing concentrations of anti-IgE on histamine secretion from HHMC obtained from patients undergoing heart transplantation. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations. (Reprinted with permission from Patella et al 1995a.)

preparations. This might well be due to the mast cell releasability effect (Marone et al 1986a,b, Casolaro et al 1989) and an understanding of the different responses to the *in vivo* exposure to immunological stimuli might be clinically relevant. Activation of HHMCs by anti-IgE and by a monoclonal antibody against an epitope of the α-chain of FcεRI may also be clinically relevant. In fact, histamine-releasing autoantibodies against IgE (anti-IgE) or the α subunit of FcεRI are present in the circulation of some patients with bronchial asthma, atopic dermatitis and chronic urticaria (Marone et al 1989, Hide et al 1993).

Complement activation and anaphylatoxin formation (C3a and C5a) occur during cardiac (del Balzo et al 1988) and systemic anaphylaxis (Smith et al 1980). Furthermore, complement deposition has been documented in infarcted areas of the human heart (Schäfer et al 1986). There is also experimental evidence that C5a causes several cardiovascular derangements directly or through the release of vasoactive mediators (del Balzo et al 1985, 1989). We found that C5a caused rapid, dose-dependent release of histamine from HHMC (Patella et al 1995a). Interestingly, C5a does not activate human lung mast cells, whereas human skin

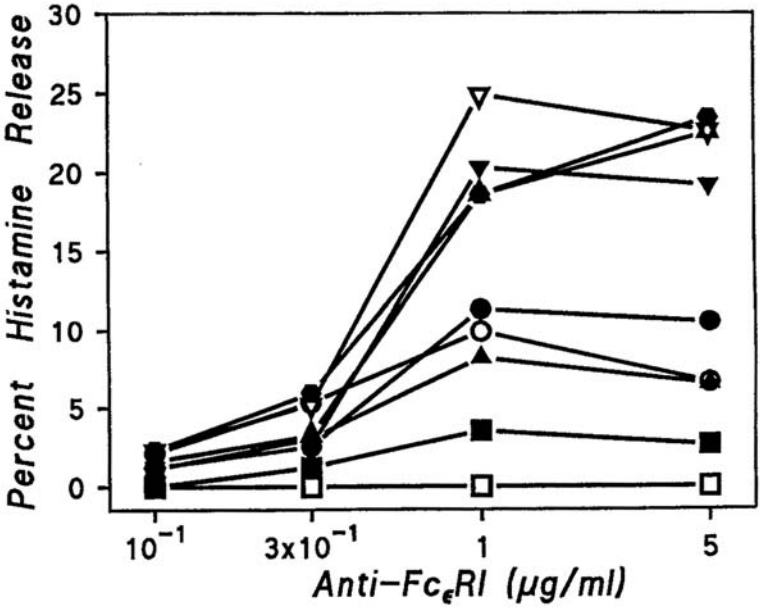

FIG. 3. Effects of increasing concentrations of anti-FcεRI on histamine secretion from HHMCs obtained from patients undergoing heart transplantation. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations. (Reprinted with permission from Patella et al 1995a.)

mast cells are responsive to C5a (Patella et al 1995b), suggesting that HHMC and HSMC are the only mast cells possessing C5a receptors.

HHMC are also responsive to SCF (de Paulis et al 1992). Interestingly, SCF is found in the secretory granules of HHMCs and is released upon immunological activation of these cells (de Paulis et al 1999).

HHMCs are also responsive to a variety of non-immunological stimuli. Some of these have clinical relevance because they might explain certain of the adverse effects observed *in vivo* when these compounds are used for diagnostic (contrast media, etc.) or therapeutic purposes (general anaesthetics, protamine, etc.) (Patella et al 1997). For example, protamine, widely used to neutralize heparin, can induce histamine release from HHMCs (Patella et al 1997). Figures 4 and 5 show that certain general anaesthetics (propofol and atracurium) can cause histamine release from HHMCs *in vitro*. Finally, radio contrast media, injected into the coronary arteries for diagnostic purposes, can activate HHMCs *in vitro* (Patella et al 1997). The close proximity of HHMCs to coronary blood vessels and the presence of mast cells in human coronary atheromas (Kaartinen et al

1994a,b), suggest that the intracoronary injection of high doses of contrast media can activate mast cells and induce the *in vivo* release of vasoactive mediators. This activation of HHMC may well explain some of the cardiac effects of these agents particularly in patients with underlying cardiovascular diseases (Vigorito et al 1986, 1987, Patella et al 1998). Interestingly, in a multicentre study of 20 patients who experienced immediate reactions to the injection of radio contrast media, there was an increased concentration of plasma histamine and tryptase (Laroche et al 1998). It is important to note that three of these patients had cardiac arrest.

Role of HHMCs in systemic and cardiac anaphylaxis

Levi and co-workers have provided evidence that the heart is directly involved in experimental anaphylaxis (Capurro & Levi 1975, del Balzo et al 1985) through the release of chemical mediators from cardiac mast cells (Marone et al 1985, 1999).

There is evidence of cardiac involvement in human anaphylaxis (Smith et al 1980) and this has been attributed to mediators originating from the lung and reaching the heart. Mast cells are present around coronary arteries and in human coronary atheromas, particularly in patients with ischaemic heart disease (Kaartinen et al 1994a,b). Therefore, the local release of vasoactive mediators by cardiac mast cells can contribute to cardiovascular derangements during anaphylaxis. In addition, complement activation and C5a formation have been documented during anaphylaxis in humans (Smith et al 1980). The *in vitro* immunological activation of human heart tissue and of isolated mast cells induces the release of preformed and *de novo* synthesized chemical mediators (Marone et al 1985, Patella et al 1995a,b). HHMCs possess FcεRI and IgE bound to their membrane surface and C5a receptors. Therefore, it is likely that IgE- and C5a-mediated activation of these cells may play a role in systemic and cardiac anaphylaxis in humans.

HHMCs can be also directly activated by many of the agents used intravenously for therapeutic (general anaesthetics, protamine, etc.) or diagnostic purposes (contrast media, etc.) that may cause non IgE-mediated anaphylactic reactions *in vitro* (Patella et al 1997, Stellato et al 1996) and *in vivo* (Laroche et al 1998). Therefore, the release of vasoactive mediators from perivascular, intimal and interstitial cardiac mast cells may well be relevant to anaphylactic reactions arising in relation to these agents.

Cardiovascular effects of histamine infusion in humans

Together with our colleagues of the Division of Cardiology of the University of Naples Federico II, we investigated the effects of mast cell-derived preformed

(histamine) and *de novo* synthesized mediators (LTC_4 and LTD_4) on peripheral and coronary haemodynamics in humans. In a first study conducted to cast light on the haemodynamic changes produced by endogenous histamine release, we infused histamine (0.4 μg/kg/min) in four patients with normal left ventricular (LV) function undergoing diagnostic cardiac catheterization (Vigorito et al 1983). We observed a significant fall in systolic, diastolic, and mean aortic pressure, systemic vascular resistance, LV end-diastolic pressure, and stroke index, and a significant rise in heart rate, cardiac output, and LV/dP/dt_{max} with small changes in mean pulmonary vascular resistance. During infusion there was also a significant rise in plasma histamine, epinephrine, and norepinephrine. All haemodynamic changes started 1–2 min after beginning histamine infusion and reverted to normal within 5 min after the infusion. In one subject there was a transient progression from first to third degree atrioventricular block, with prompt recovery of 1:1 atrioventricular conduction at the end of the infusion.

Thus, exogenous histamine administration in man produces significant and transient haemodynamic changes, mainly represented by systemic hypotension, tachycardia and increased LV performance. These changes can be attributed to the associated increase in sympathoadrenergic activity, although it cannot be excluded that histamine exerts a direct cardiac effect.

Effects of activation of the H1 receptor on coronary haemodynamics in human

We also evaluated the effects of selective activation of histamine H1 receptors on coronary haemodynamics in two groups of patients: group A, patients with atypical chest pain and normal coronary arteries, and group B, patients with vasospastic angina (Vigorito et al 1986). H1 receptor stimulation was achieved by infusing histamine intravenously (0.5 μg/kg/min) for 5 min after pretreatment with cimetidine to antagonize the H2 receptors. Heart rate was maintained constant (100 beat/min) by coronary sinus pacing and coronary blood flow (CBF) was measured by thermodilution. In group A, during histamine infusion mean aortic pressure fell, coronary vascular resistance (CVR) decreased and CBF and myocardial oxygen consumption remained unchanged. No patient of this group developed angina during histamine infusion. In group B, 40% of the patients developed angina during histamine infusion, accompanied by a decrease in CBF and an increase in CVR. In one of these two patients circumflex coronary arterial spasm was angiographically demonstrated during histamine-induced angina. These results suggest that stimulation of the H1 receptor in subjects with normal coronary arteries induces a reduction of CVR, probably resulting from vasodilation of small coronary resistance vessels. This response is also found in

approximately 60% of patients with vasospastic angina. However, in a considerable percentage of patients ($\cong$40%) with vasospastic angina, H1 receptor activation can cause vasoconstriction of large capacitance coronary arteries. These findings contribute to our understanding of the pathophysiological effects of histamine on CBF in humans and may have practical relevance in patients undergoing treatment with H2 receptor-blocking drugs. In fact, our results support the hypothesis that the endogenous release of histamine, which is a feature of anaphylactic reactions that occur during therapeutic or diagnostic interventions (Stellato et al 1996, Patella et al 1997), may precipitate coronary spasm in a subset of patients with vasospastic angina. This concept is substantiated by the finding that premedication with H2 receptor antagonist increases the risk of heart block in patients who develop anaphylaxis (Patterson & Milne 1999).

Data from *in vivo* studies have started to clarify the role of histamine H1 and H2 receptors in the cardiovascular manifestations of anaphylaxis. However, histamine can act also through activation of H3 and H4 receptors (Lovenberg et al 1999, Oda et al 2000). The H3 receptors were discovered as inhibitory autoreceptors in the central histaminergic pathway (Arrang et al 1983). Levi and co-workers have identified H3 receptors as inhibitory heteroreceptors in cardiac adrenergic nerve endings (Endou et al 1994, Imamura et al 1995, 1996). This discovery provides a mechanism whereby endogenous histamine can activate the release of norepinephrine in both normal and ischaemic conditions (Silver at al 2001, 2002). The functional identification of H3 receptors in the human heart (Imamura et al 1995) opens the possibility that these receptors could be directly and/or indirectly involved in the cardiovascular manifestations of anaphylactic reactions.

Metabolic and haemodynamic effects of cysteinyl leukotriene C_4 and D_4 in human

In a third study we evaluated the time course effects of intravenous and intracoronary administration of cysteinyl leukotrienes (LTC_4 and LTD_4) on metabolic parameters and on systemic and coronary haemodynamics in patients with normal coronary arteries (Vigorito et al 1997). LTC_4 (2 nmol given as a bolus intravenous injection) induced an early fall (at 2 min) in mean arterial pressure associated with a rise in heart rate and in plasma levels of epinephrine and norepinephrine, but without significant changes in CBF or CVR. Mean arterial pressure, heart rate, norepinephrine and epinephrine returned to baseline values 10 min after LTC_4 administration. In contrast, at 10 min post LTC_4, with coronary blood flow and myocardial oxygen consumption unchanged, there was an increase in coronary vascular resistance and in

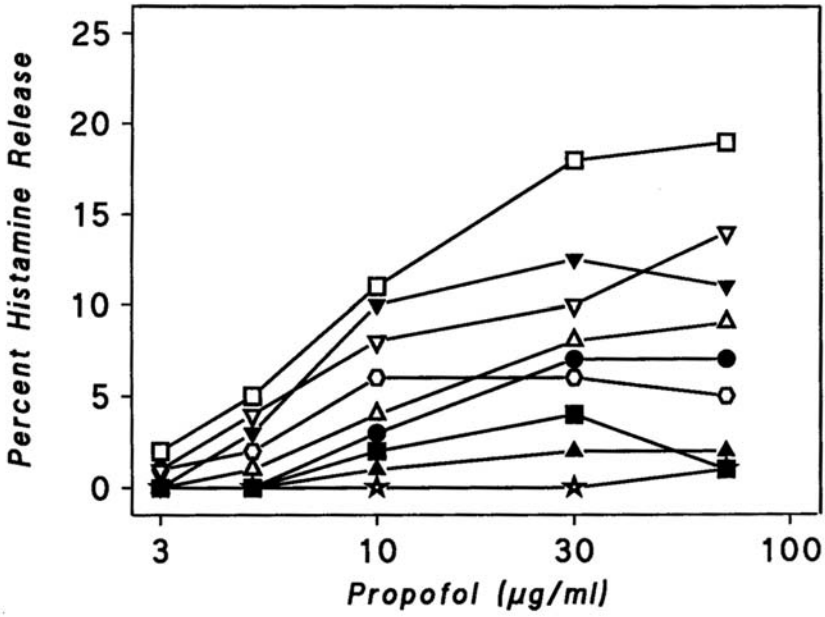

FIG. 4. Effects of increasing concentrations of propofol on histamine secretion from HHMCs obtained from patients undergoing heart transplantation. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations.

myocardial oxygen extraction, which returned to baseline values at 20 min post LTC_4. LTD_4 (3 nmol, given in the left coronary artery) induced an early (20 s) and transient fall in mean arterial pressure paralleled by a rise in heart rate and plasma levels of epinephrine and norepinephrine, all of which returned to baseline at 10 min. CVR increased at 10 and 15 min and myocardial oxygen extraction at 15 min. These results suggest that small doses of cysteinyl leukotrienes induce both an early and transient fall in mean arterial pressure associated with secondary sympathoadrenergic activation, and late increase in small coronary arteriolar resistance.

Cysteinyl leukotrienes exert potent biological effects through the activation of at least two classes of receptors, i.e. $CysLT_1$ and $CysLT_2$. $CysLT_2$ mRNA was recently detected at high levels in the human atrium, ventricles and the coronary artery, whereas $CysLT_1$ mRNA was barely detected (Kamohara et al 2001). Although the data available do not clarify which receptor is involved in the cardiovascular and metabolic effects of cysteinyl leukotriene injection, our findings may contribute to a better understanding of the cardiovascular changes occurring during anaphylaxis that is associated with leukotriene release.

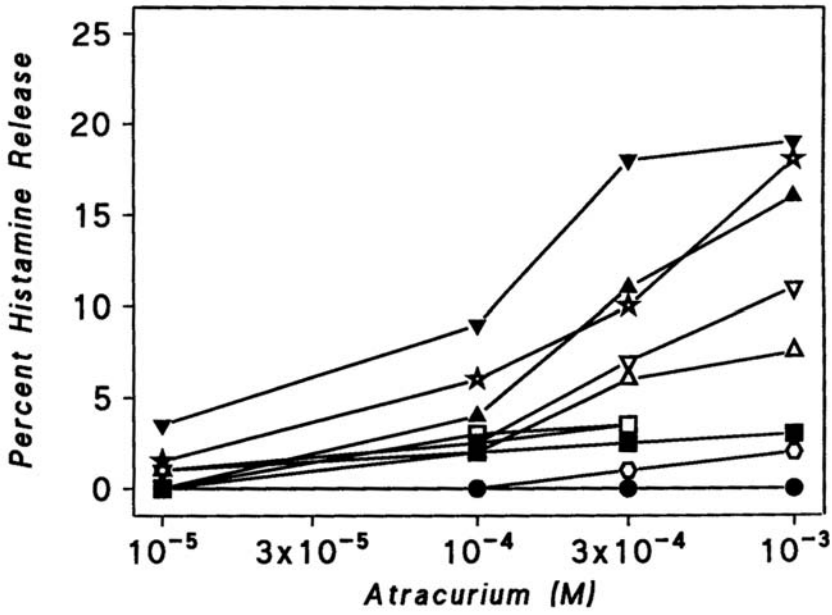

FIG. 5. Effects of increasing concentrations of atracurium on histamine secretion from HHMC obtained from patients undergoing heart transplantation. Each symbol represents the results obtained with a different donor. Each point represents the mean of duplicate determinations.

Conclusions and implications

Systemic anaphylaxis is the most dramatic and potentially catastrophic manifestation of allergic disorder. Anaphylactic reactions can be either IgE- or non-IgE-mediated (Johansson et al 2001). This syndrome can affect virtually any organ including the cardiovascular system.

The cardiovascular collapse and hypotensive shock that can occur in anaphylaxis have been attributed to peripheral vasodilation, enhanced vascular permeability and leakage of plasma, rather than to a direct effect on the myocardium. However, increasing experimental and clinical evidence indicates that the human heart can be the site and the target of anaphylaxis.

Mast cells are present in normal and even more abundant in diseased human heart tissue (Forman et al 1985, Patella et al 1995a,b, 1998, de Paulis et al 1999). Within heart tissue, mast cells lie between myocytes and are in close contact with blood vessels. Mast cells are also localized in the coronary adventitia and in the shoulder regions of a coronary atheroma (Kaartinen et al 1994a,b). The density of cardiac mast cells is higher in patients with dilated and ischaemic cardiomyopathy than in accident victims without cardiovascular diseases (Patella

et al 1998). More importantly, in some of these conditions there is *in situ* evidence of mast cell activation (Patella et al 1995a, Kaartinen et al 1996).

Direct activation of HHMCs by circulating antigens, autoantibodies (anti-IgE, anti-FcεRI, etc.), therapeutic (e.g. protamine, general anaesthetics) and diagnostic substances (e.g. radio contrast media) injected intravenously can explain some of the anaphylactic reactions caused by these agents. HHMCs possess FcεRI and IgE bound to the surface and C5a receptors, which could explain the direct involvement of cardiac mast cells in systemic and cardiac anaphylaxis. Our results showing increased cardiac mast cell-density and increased release of vasoactive mediators in patients with dilated cardiomyopathy (Patella et al 1998) might also have clinical relevance given the marked cardiovascular effects of histamine, cysteinyl leukotrienes and PGD_2 (Hattori et al 1986, Vigorito et al 1983, 1986, 1987, 1997).

A series of clinical studies have started to shed light on the cardiovascular and metabolic effects caused by such mast cell-derived mediators as histamine and cysteinyl leukotrienes. These studies provided the important information that the haemodynamic effects of mediators depend on both the underlying cardiovascular conditions and the pharmacological treatment (e.g. H2 blockade). For instance, exogenous histamine causes a transient fall in blood pressure, a rise in heart rate and cardiac output, with small changes in pulmonary vascular resistance (Vigorito et al 1983). Interestingly, however, in one patient there was a progression from first to third-degree atrioventricular block. In patients with normal coronary arteries histamine induces a dilation of the small resistance coronary arteries, which is predominantly, but not completely mediated by H1 receptors (Vigorito et al 1986, 1987). In contrast, in a significant percentage of patients with vasospastic angina activation of H1 receptors can cause vasoconstriction of large capacitance coronary arteries (Vigorito et al 1986). Finally, the injection of small doses of cysteinyl leukotrienes can induce both an early and transient fall in arterial pressure associated with sympathoadrenergic activation, and a late increase in small coronary arteriolar resistance (Vigorito et al 1997). Given the availability of specific antagonists of $CysLT_1$ and $CysLT_2$ (Kamohara et al 2001) it is now possible to evaluate the contribution of each receptor to the cardiovascular effects of these mediators.

Taken together, the results arising from these *in vitro* and *in vivo* investigations clearly indicate that the human heart can be viewed as both a site and a target in anaphylaxis.

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DISCUSSION

Vercelli: Could you speculate about the mechanism underlying the biological difference you see between HHMCs and populations in other tissue? And what is the mechanism underlying the difference in mast cell releasability that you have seen?

Marone: There is compelling evidence that human mast cells isolated and purified from different anatomical sites can be functionally different (Marone et al 2001). They display different surface receptors and they can also secrete different mediators and cytokines. It is not surprising that there are striking differences in the responses to different non-IgE-mediated stimuli or C5a. For example, it has been known for years that C5a can selectively activate skin mast cells but not lung mast cells. The only addition we have made is that cardiac mast cells express the C5a receptor (Patella et al 1995). The engagement of this receptor can probably play some role in systemic anaphylaxis. With regard to your second point about the difference in releasability, we have the expert on this at this meeting, Don MacGlashan. It is well established that when a basophil or mast cell is challenged

immunologically, there is great variation among the different donors. In this particular case, the variation is clinically important. It might explain why, for example, the injection of radiocontrast media and general anaesthetics is without significant adverse effects in the majority of patients, yet in certain patients with high releasability there is release of high concentrations of vasoactive mediators which is deleterious.

Vercelli: What I meant was, are there signals coming from the heart microenvironment which make a mast cell a heart mast cell, for example?

Marone: Yes.

Galli: I would like to ask a question about your experimental system. This is not the type of experimental system in which you can get volunteers in the laboratory to donate tissue! Are the hearts with idiopathic or ischaemic myopathy end-stage? I assume this is the case because that patient will be getting a transplant.

Marone: The majority of the so-called donors were patients undergoing cardiac transplantation for dilated cardiomyopathy. However, we also examined a number of 'normal' hearts from subjects who died in car accidents without cardiovascular disorders (Patella et al 1998).

Galli: For the most part, is the pattern of responsiveness to C5a and general anaesthetics similar for the mast cells derived from the normal hearts?

Marone: We didn't see significant differences. Unfortunately, we don't know from the histories how many of those patients were atopic.

Fisher: I have a problem with the heart as a target organ in anaphylaxis for several reasons. The first is that Chenoweth originally showed that C5a is activated at the end of bypass (Chenoweth et al 1981). We then showed that the heparin–protamine complex caused significant classical pathway activation at the end of bypass, when the patient was off the pump. Yet we couldn't show that it affected the heart in any way (Best et al 1984). Most of the studies of young, fit people who have had anaphylaxis have shown that the problem really is that there is no venous return, rather than a problem of contractility. In Jamie Cooper's study on histamine infusion impairing cardiac contractility only worked if he β blocked the patients. The theory we embrace is studies of the heart generally take out the sympathetic response, because they are *in vitro*, or the patients are either not very sick or are β blocked. Could it be that in anaphylaxis the sympathoadrenal response stabilizes the mast cells or reverses these changes so we don't see them clinically? It is common practice in anaesthetic anaphylaxis to perform echocardiography as a diagnostic aid. We find a heart that is normally contractile but empty. If we see an impaired heart we generally think of something different, such as cardiomyopathy, ischaemia, or pulmonary embolism.

Marone: These are excellent questions. I forgot to mention that protamine can probably act through different mechanisms. I just wanted to give an example of how protamine can eventually induce the release of mediators. I agree, it has been

clearly shown that protamine can cause adverse reactions through different mechanisms. Concerning your second point, I tried to emphasize that some of the cardiovascular effects of the mast cell-derived mediators are very transient. They only last a few minutes. This might be critically important, because it is possible that you do not detect the arrhythmias, or the transient progression to complete AV block. Also, the coronary spasm can be a transient phenomenon. Concerning the sympatoadrenergic activation, I agree with you. In the paper that we published we also measured the catecholamines (Vigorito et al 1983). There is no doubt that histamine infusion is associated with sympatoadrenergic activation.

Müller: Some years ago we looked at 1463 case histories of insect venom allergic patients for documented cardiac symptoms. We found 31 patients who had some documented symptoms. Most were arrhythmias, including three cases of cardiac arrest due to ventricular fibrillation and seven patients had typical angina pectoris, two were even acute myocardial infarction developing during anaphylaxis. These were mostly elderly people with pre-existing coronary heart disease.

Marone: This fits in with our findings.

Galli: This illustrates the important point that there may be significant differences in the effects of anaphylaxis on the hearts of otherwise healthy individuals as opposed to those of subjects with existing cardiac disease.

Golden: I'm interested in the possible significance of the increased number of mast cells that are observed in these patients with cardiomyopathy. One issue that comes up, which I am sure doesn't apply to the majority of those patients, is that one would have to distinguish whether there is an underlying mastocytosis. Did they have an elevated baseline serum tryptase level?

Marone: Unfortunately, we didn't measure tryptase. I would like to emphasize that we have detected two types of mast cell hyperplasia. One is a diffuse hyperplasia which is found in all sections of human heart tissue in patients with dilated cardiomyopathy (Patella et al 1998). In contrast, in coronary sparks and at sites of thrombosis there is localized mast cell hyperplasia.

Golden: Given that there are these types of patients who have increased mast cells and the possibility of a range of factors that might affect those mast cells, is there a possible role for therapy in these patients with leukotriene modifiers or mast cell stabilizing agents? Would they have any benefit? This is well outside anaphylaxis, but I wonder if this was ever considered.

Marone: There is the possibility for using specific antagonists of the cysteinyl leukotriene receptor type 1 and type 2, to distinguish the contributions of the two receptor subtypes on the cardiovascular system. This is what we are planning to do. Obviously, there is the potential that certain specific antagonists may prove useful prophylactically.

Fisher: Until about 1985, most of the cases of anaphylaxis which had detailed haemodynamic monitoring had shown no evidence of cardiac depression. The

one that had was Carol Pavek's case having anaphylaxis to protamine in the middle of a correction of a Fallot's tetralogy (Pavek et al 1982). In 186 patients with anaphylaxis we looked at cardiac rhythms (Fisher 1986). The majority had a supraventricular tachycardia (SVT). When this work was published referees asked why we did not distinguish nodal from sinus tachycardia. This was because they were the observations of anaesthetists in a state of total panic, and we didn't think they could distinguish this accurately. If we broke these patients down into those who had cardiac disease and those who didn't, it was extraordinarily unusual for the patients with no cardiac disease to develop significant arrhythmias, although some did. In the patients with cardiac disease it was very unusual for them to just have SVT: they generally developed other pathological arrhythmias. Similarly, with filling pressures these were elevated in patients with cardiac disease whereas this was rare in patients without cardiac disease to have anything other than an empty heart. Indeed, when they started using ECGs in anaesthesia Mike Roizen said that in the absence of cardiac disease the pathology of anaphylaxis is an empty contracting heart. As soon as we published this, along came a woman with anaphylaxis to alcuronium. This could have been related to the massive doses of catechols, but very early on she was shown to have severe biventricular hypokinesis and was dying of impaired cardiac function. We put a balloon pump in and we were able to reverse it. We then saw another patient who had a wasp sting where they developed a global cardiac problem that seemed to be the primary problem. Interestingly, this patient has now, 15 years later, just been diagnosed with a cardiomyopathy that we couldn't find at the time. Our hypothesis is that in young, healthy people, in spite of all these things that happen in the heart, they will overcome this. But in a diseased heart all these things that Gianni Marone has described become significant and very important.

Schwartz: Gianni Marone, the percentage of non-releasing mast cells looks to be around 20%. Have you been able to address the potential mechanism(s) for this? Is it comparable to what you would find for skin mast cells?

Marone: That's correct: there are about 20% of non releasers. But this is exactly what we found working with mast cells isolated from lung parenchyma and skin tissue, and also with basophils. It is not a big surprise.

Galli: If the cells are non-releasers when stimulated with anti-IgE, are they generally non-releasers when stimulated by non-immunological mechanisms? In other words, do some mast cells release with any stimulus and others don't?

Marone: We have never examined this in detail. We did a number of experiments to rule out the possibility that the harsh procedure for isolating the cells can affect the releasability. For example, we incubated the cells with an excess of IgE. We also incubated the cells with stem cell factor. It appears that there is no real change in the releasability parameters. There are a certain number of donors who are non-releasers.

Schwartz: Could you elaborate on the differences between skin and cardiac mast cells? You mentioned that there was a different response to radiocontrast dyes.

Marone: It is clear that there are certain stimuli, such as radiocontrast media, that are able to activate cardiac mast cells but not skin mast cells. Another example is the effect of morphine. This is a potent histamine releaser from skin mast cells but has no effect on cardiac mast cells (Tharp et al 1987, Stellato et al 1992). There are a number of divergent stimuli.

Ring: I like the angiotensin data. You have shown that together with histamine, at the same time angiotensin II is released from the mast cells, if I understand correctly.

Marone: The angiotensin-converting enzyme activity is released from human cardiac mast cells immunologically activated *in vitro*.

Ring: This will finally lead to angiotensin II formation, which we have shown during anaphylaxis. One could therefore speculate that this belongs to a counterbalancing system. It is physiologically active to counteract the untoward cardiovascular effects of mast cell activation. It might help to explain why not every allergic individual reacts every time, but only in 60% of the cases of allergen contact. There were two main themes in your talk: the heart mast cells, and the heart as an effector organ with the cardiomyocyte having histamine receptors. This second theme is distinct from the role of mast cells in the heart. The histamine floating around from elsewhere (mast cells in the skin, lung or gut) also has an effect on the heart, perhaps producing these arrhythmias that you have shown.

Marone: I agree. There is the possibility that kinase, which is locally released by cardiac mast cells, can convert angiotensin I to angiotensin II, which plays a role in homeostatic control in severe anaphylaxis. Concerning the other possibility, you are right that there is circulating histamine. I also wanted to emphasize that there is the possibility that histamine released from cardiac mast cells by immunological and non-immunological stimuli can affect the human cardiovascular system.

Schwartz: It is always an interesting process trying to translate *in vitro* results to an *in vivo* setting. Has anyone added chymase to plasma, and shown it can convert angiotensin I to II?

Marone: In the test tube, recombinant chymase can certainly convert angiotensin I to II. I am not aware of any experiments in plasma.

Schwartz: There is going to be a balance *in vivo* between how fast chymase can convert angiotensin I to II and how fast inhibition of chymase occurs. It would be nice to see *in vitro* experiments that more closely approach the *in vivo* setting.

Galli: One might also include experiments conducted in solutions that represent attempts to mimic the composition of interstitial fluid. The interstitium represents another potential site where the postulated reaction may occur, in addition to the plasma.

Sampson: In food-induced anaphylaxis it is frequently commented that dysrhythmias occur associated with these reactions. On the basis of what you said about H2 blockers, should we be thinking about using these more often in these people?

Marone: It might be the reverse. The picture is probably more complicated. Recently Roberto Levi described the presence of histamine H3 receptors in human heart (Imamura et al 1994, Endou et al 1994). It appears that the activation of the H3 receptor under specific experimental conditions can have certain positive effects. The data that I presented indicated that when we block the H2 receptor, this can probably produce a non-H2 effect and induce coronary spasm in a percentage of patients with vasospastic angina. There are a number of observations in the literature showing that treatment with H2 blockers alone can have adverse effects in anaphylaxis (Patterson & Milne 1999).

Sampson: One of the patients we reviewed in our initial fatal and near-fatal series was a 14 year old girl with protracted anaphylaxis. She was in hospital for three weeks and during this time she had a severe drop in her cardiac output. They had put her on the heart transplant list and had biopsied her heart, finding no abnormality. After three weeks the reaction stopped and her cardiac output returned to normal levels. What might have been happening here?

Marone: A couple of similar cases were reported by Malcolm Fisher in the *Lancet* several years ago (Raper & Fisher 1988). In these patients profound myocardial depression developed after severe anaphylactic reactions, in one patient following the induction of anaesthesia and in the other case after insect sting. Interestingly neither patient had pre-existent cardiovascular diseases.

Simons: A H3 antihistamine called Perceptin is now available for human trials. It might be worth repeating the experiments in which you blocked the H2 receptors and block the H3 receptors as well (Wulff et al 2002).

Marone: The story of the H3 receptor in the human heart will grow. In my paper I didn't say that the cardiovascular effects of histamine infusion in the presence of H2 blockade was due to the activation of H1 receptors because there is the distinct possibility that two receptors can be activated.

Simons: I suppose there's a potential role for H4 receptors also, but as yet there are no H4 antihistamines (Nakamura et al 2000).

Marone: To my knowledge there is no evidence of H4 receptors in the heart in humans or animals.

Simons: But they are present on circulating blood cells.

Lasser: We participated in a large national study of cardiovascular disease, with something like 1500 patients in it (Enright et al 1996). In this population, 9% of those over 65 were asthmatic. This is about the same incidence as occurs in younger patients. In these asthmatic patients there was no increase in the incidence of cardiovascular events.

Lee: In the radiocontrast experiments, are the lung mast cells activated?

Marone: Yes. I would like to emphasize that we used three types of radiocontrast media of different osmolality (Stellato et al 1996). We have not seen any correlation between the osmolality and the release of mediators. There are additional factors that can influence the release of mediators from mast cells induced by radiocontrast media.

Fisher: Coming back to morphine, the fantastic thing about the studies on differential feats of mast cells is that every anaesthetic textbook for 30 years has said that morphine causes asthma. It doesn't, because the histamine comes from the skin. Not much gets to the lung. The piece of the puzzle in anaesthesia, which you solved for us, is that if you make people release histamine directly it is extraordinarily unusual for them to get asthma. But when we look at the patients who have non-immunological asthma under anaesthesia, it is almost always due to one or two of the drugs that you have identified that work directly on the lung mast cell, not the skin mast cell. We think this is a very important mechanism.

Marone: The problem with morphine is highly relevant. When morphine was used in very high doses in cardiac surgery it was not unusual to see anaphylactoid reactions to the administration of morphine. Some years ago we published a collaborative paper looking at the effects of morphine on different types of mast cells and basophils (Tharp et al 1987). We found that morphine is a selective agonist of skin mast cells. It is likely that when high doses of morphine are injected, the release of histamine of skin mast cells can induce an anaphylactoid reaction.

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General discussion III

Galli: In this general discussion, I'll begin by asking David Golden to present some information about factors that may be used to predict which insect venom-allergic patients will develop anaphylaxis when stung.

Golden: Some of you here are aware of our efforts over the last several years to perform insect sting challenge of untreated adults with a history of systemic reactions to determine why some people react or don't on any given occasion. It has become very complex, because it can involve many different factors including the type of insect or the amount of venom injected. In our preliminary analysis of the data we have been quite disappointed. The only thing that was intriguing was the possibility that leukotriene release but not histamine release from basophils in these patients was predictive of whether a patient would react or not. There were some common sense things that seemed to correlate with the frequency of reaction. The ability of histamine-releasing factor (HRF) to induce release of mediators in these patients' blood leukocytes was statistically correlated with a higher frequency of reaction. I should emphasize that these are very preliminary results. What was most striking is that this was all we found. They were not strong correlations.

Galli: What was eliminated as a cause? What had no bearing on the frequency of reaction?

Golden: I think we are looking in all the wrong places. There is a focus on looking at basophils and the release of mediators, as well as looking at antibody levels. Actually, the IgG level was more predictive than the IgE level in these patients. It correlated with a lack of reaction. The concept of blocking antibodies may resurface. We have just started looking at cytokine release and responses in these patients. It has not been part of the first five year phase.

MacGlashan: The ability of the basophil to release leukotrienes and respond to HRF are both generally indicators of the releasability of the cell—the sensitivity of the cell to stimulation. We found a loose association many years ago between leukotriene release and the number of cross-links required to stimulate these cells. It takes fewer cross-links in the basophils of highly sensitive basophils to get a response, and this seems to be connected somehow with the ability of the cell to release leukotrienes. Susan MacDonald published recently that SHIP levels in these basophils are low (Vonakis et al 2001). This would be a regulator that down-regulates the response of the cell. Low levels lead to a hyperresponsive cell. The ability to respond to HRF is somehow connected to this hyperresponsiveness.

Again, this is highly speculative, but it is interesting that a couple of parameters that have popped out are things that go in the right direction for predicting whether or not there is a strong response. This notion of releasability is one that is worth discussing. It is a parameter that both mast cells and basophils have. They may have equal numbers of IgE molecules on the surface and yet the ability of the cell to respond to these can vary by at least 30-fold among individuals in the population.

Galli: Does the releasability as detected by studies in basophils correlate with the releasability of mast cells in the same population?

MacGlashan: This has never been studied, other than anecdotal reports. The inability of anti-IgE to induce a response in cells which demonstrably have a lot of surface IgE is known as non-release (but only from the standpoint of an IgE-mediated response). There are anecdotal reports that some of the basophil preparations that show no release to anti-IgE may have skin test positivity to certain antigens. This has never been systematically examined, and the precise relationship is not known.

Marone: When Enzo Casolaro was working with us in Naples he compared the releasability of basophils purified from peripheral blood to mast cells present in the bronchoalveolar lavage (BAL). This is one of the few studies in which it has been possible to compare the releasability from two types of cells from the same donors. There was no correlation between the BAL mast cell and basophil releasability.

MacGlashan: BAL mast cells are very odd cells in the sense that they are overly responsive in a lot of ways. They have different pharmacological response profiles. There is a caveat to that study.

Ring: Things are even more complicated. When we talk about releasability, this is not a general phenomenon in that a particular cell has a higher 'releasability'. It is specific for the stimulus and for the mediator released. You may have increased releasability to C5a but at the same time decreased releasability to anti-IgE. There may be increased leukotriene secretion and at the same time decreased histamine release.

Galli: To tie this back to David's comment, the releasability he was referring to was specifically to anti-IgE, HRF and antigen, but not C5a.

Golden: We looked at C5a in the first year but we there were no preliminary signs of any usefulness, so we stopped doing it.

Dubois: To add to the confusion, if you add adenosine receptor antagonists to mast cells, it greatly decreases IgE-mediated degranulation. These antagonists impair the responsiveness to IgE cross-linking.

Marone: We described the adenosine receptor on human lymphocyte and basophils some time ago (Marone et al 1978). We found that adenosine and various adenosine analogues are important inhibitors of the release of histamine and *de novo* synthesized mediators from immunologically activated basophils (Marone et al 1979). In contrast, we found that low concentrations of adenosine

potentiate the release of mediators from human lung mast cells (Peachell et al 1988). This is another nice example of how human basophils and mast cells differ pharmacologically.

Galli: Two themes are developing in our discussion of the search for a simple predictive test of whether a particular sensitized individual is likely to develop anaphylaxis: the heterogeneity of mast cells and the differences between mast cells and basophils. To address one more aspect of this study, are the correlations that are being developed between various measures of basophil releasability and the likelihood of developing anaphylaxis specific for venom anaphylaxis?

Golden: It strikes me that the kind of mechanism, such as releasablility, that might underlie the anaphylactic potential of the patient would be unlikely to be specific for certain causes, but might be specific for certain phenotypes of anaphylaxis (e.g. vascular shock or airway obstruction), or certain routes of exposure (e.g. GI or by injection).

Sampson: We have looked at histamine releasability in a food-allergic population, using HRF. The food-allergic population releases much higher levels of histamine through HRF generated by mononuclear cells from these patients. We did not see a correlation between this and severity of a reaction. This was mainly a young group.

Galli: So food allergics have higher releasability in general, but within that group it doesn't correlate with severity.

Sampson: There were two observations. One was that especially in this population who were ingesting foods prior to diagnosis and had severe atopic dermatitis, we found high spontaneous basophil histamine release. In looking at that we were then able to demonstrate that this population actually generated a histamine-releasing factor out of the PBMC (peripheral blood mononuclear cell) population. When we took this factor out of the supernatant and placed it with other food-allergic patient basophils, we got massive release of histamine, in the order of 80–90%. Looking at the severity of the challenges, which may not be a fair comparison, we didn't see a correlation with severity. We have also looked very carefully at absolute values of IgE and have shown that if someone exceeds certain levels we can tell that they are going to have a reaction. Again, there is no correlation with severity and the amount of IgE that is seen in food allergic patients.

Galli: Would you say that it is fair to conclude that the features of individual responsiveness that can predict which of the patients will develop anaphylaxis have yet to be discovered?

Sampson: Yes. David Golden may be correct in that we could be looking at the wrong things.

Golden: To add to the questions more so than the answers, does releasability vary over time in the same individual?

MacGlashan: No. One of the interesting things about releasability is that it is often quite stable over many years. There are a few individuals in whom there is cycling, but we only have this kind of information at the level of the basophil. This takes place in a timeframe of months to years.

Golden: What kinds of individuals have this cycling?

MacGlashan: It is generally unclassified.

Lee: This is a very important area. It seems to me that to look for one predictive factor that will determine anaphylaxis is unrealistic. It seems likely that there will be multiple factors. Have the mathematical models used to look at this been sufficiently robust to take a multivariate approach?

Golden: In a sense we are doing this, using a linear regression approach and trying to look for an index or combination of factors. I agree that it is not going to be one factor. In terms of patterns of anaphylaxis, we may have to take into account that this is not a single homogeneous condition. Whatever factors might react to certain types of anaphylaxis might not relate to others, and not just as far as the cause, but as to whether it is hypotensive or respiratory, for example.

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Food-induced anaphylaxis

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Abstract. Food allergies affect ~2% of the population and are the single leading cause of anaphylaxis occurring outside of hospitals in westernized countries. Given the frequency of IgE-mediated food allergy, it is imperative that physicians appropriately diagnose food-allergic patients, educate them in the appropriate measures to prevent accidental ingestion of food allergens, teach them to recognize early signs of anaphylaxis, and arm them with medications and a treatment plan to utilize in case of the 'almost inevitable' accidental ingestion.

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Although the first fatal allergic reaction was reported over 4500 years ago (Cohen & Saavedra-Delgado 1989) it was not until this century that the syndrome of anaphylaxis was fully characterized. In their classic studies, Portier and Richet (1902) described the rapid death of several dogs, which they were attempting to immunize against the toxic sting of the sea anemone (Portier & Richet 1902). Shortly thereafter Schlossman (1905) reported a patient who developed acute shock after the ingestion of cow's milk, but it was not until 1969 that Golbert and colleagues published the first series of food-induced anaphylaxis in humans (Golbert et al 1969). The reports by Yunginger et al (1988), Sampson et al (1992) and Bock et al (2001) further characterized the natural course of fatal and near-fatal food-induced anaphylactic reactions.

Prevalence

The actual prevalence of food-induced anaphylaxis is unknown since there is no requirement to report such reactions to any national registry. In addition, it is likely that many cases are misdiagnosed. Klein & Yocum (1995) reviewed all cases of anaphylaxis treated in the Mayo Clinic Emergency Department (Rochester, MN, USA) over a 3.5 year period and found that food allergy accounted for about one-third of the cases treated (Young et al 1994). This group also reviewed the medical records of Olmsted County inhabitants followed in the Rochester Epidemiology Study from 1983 to 1987 (Yocum et al 1999). Food allergy accounted for one-third

of the anaphylactic reactions recorded, an annual incidence of food-induced anaphylaxis of 7.6 cases per 100 000 person-years and a food-induced anaphylaxis occurrence rate of 10.8 per 100 000 person-years. Extrapolating the Olmsted County experience to project that of the USA (population 280 million), one could estimate that ~30 000 food-induced anaphylactic episodes are treated in emergency departments each year resulting in approximately 2000 hospitalizations and 150 deaths. Similar findings were reported by Pumphrey in the UK (Pumphrey & Stanworth 1996) and in France by Moneret-Vautrin and Kanny (1995). In Italy, Novembre et al (1998) reported that food allergy was responsible for about one-half of severe anaphylactic episodes in children treated in emergency departments. Similarly, a survey of South Australian preschool and school-age children revealed a parent-reported food-induced anaphylaxis rate of 0.43 per 100 school children, which accounted for over one-half of all cases of anaphylaxis in this age group (Boros et al 2000). A 5 year survey of anaphylactic reactions treated at the Children's Hospital of Philadelphia also found that food allergy was the most common cause of anaphylaxis outside of the hospital (Dibs & Baker 1997).

Clinical course

In 1988 Yunginger reported seven fatal cases of food-induced anaphylaxis that occurred over a 16-month period. In 1992 we analysed six fatal and seven near-fatal food-induced anaphylactic reactions in children (ages 2–17 years) that occurred in three metropolitan areas over a 14 month period (Sampson et al 1992). Common risk factors were noted in these cases: all patients had asthma (although most were well controlled); all patients were unaware that they were ingesting the food allergen; all patients had experienced previous allergic reactions to the incriminated food, although in most cases symptoms had been much milder; and all patients had immediate symptoms with about half experiencing a quiescent period prior to a major respiratory collapse. All patients had severe respiratory compromise and 11 of 13 had gastrointestinal symptoms including nausea, abdominal pain and vomiting. Surprisingly, only one of six patients who experienced a fatal reaction developed urticaria or angioedema. Four of the 13 patients developed a biphasic response, which occurred 1.5–3 hours after the initial reaction. Serum tryptase results were available in one post-mortem sample, in one patient with protracted anaphylaxis monitored over a three day period, and in three patients admitted to the intensive care unit (ICU) for food-induced anaphylaxis but not requiring intubation. No elevation in tryptase was seen. However, plasma histamine and tryptase levels were elevated in only 43% and 21%, respectively, of 92 patients presenting to emergency departments with anaphylaxis from any cause, raising some question about these mediators as

biomarkers of anaphylaxis (Lin et al 2000). More recently we analysed 32 cases of fatal food-induced anaphylaxis. The majority of patients dying were between 11 and 21 years of age, and peanuts and tree nuts accounted for about 90% of the fatalities. All but one of the patients was known to have asthma and only three of the individuals had epinephrine available at the time of their fatal reaction. The vast majority of reactions occurred away from home. Of the 32 fatal food-induced anaphylaxis cases reviewed, two of 32 individuals ($\sim$6%) received intramuscular epinephrine immediately but failed to respond. In an earlier series of 48 fatal cases reviewed by Pumphrey, three patients ($\sim$6%) died despite receiving epinephrine from a self-administration kit appropriately at the onset of their reaction (Pumphrey & Roberts 2000). In a registry of 200 fatal food-induced anaphylactic cases, Pumphrey noted that 25% of cases were due to food allergy, and of these most occurred away from home and over 85% were due to bronchospasm/asphyxia (Pumphrey & Roberts 2000, and personal communication).

The prevalence of food-dependent exercise-induced anaphylaxis appears to be increasing. Two forms of food-dependent exercise-induced anaphylaxis have been described: reactions following the ingestion of specific foods (e.g. milk, celery, shellfish, wheat) and rarely reactions following the ingestion of any food (Horan & Sheffer 1991, Romano et al 1995). Anaphylaxis typically occurs when a patient exercises within 2–4 hours of ingesting a food, but otherwise the patient can ingest the food without experiencing a reaction and can exercise without any apparent reaction as long as the specific food (or any food in the case of non-specific reactors) has not been ingested within the past several hours. There is a 2:1 female: male ratio and over 60% of cases occur in individuals less than 30 years of age. In a survey of 199 individuals experiencing exercise-induced anaphylaxis, food was felt to be a factor in the development of attacks in 54% of the cases (Horan & Sheffer 1991). Symptoms generally start with a sensation of generalized pruritus which progresses to urticaria and erythema, respiratory obstruction, and cardiovascular collapse. Patients with specific food-dependent exercise-induced anaphylaxis generally have positive prick skin tests to the food that provokes symptoms and occasionally have a history of reacting to the food when they were younger. Several factors appear to predispose an individual to food-induced anaphylaxis including a personal history of atopy, family history of atopy, age, and dietary exposure. Atopic patients with asthma are at increased risk of developing more severe food allergic reactions (Sampson et al 1992).

In the series of Yunginger et al (1988), Sampson et al (1992) and Bock et al 2001, the majority of individuals were highly atopic, and virtually all had histories of asthma. Interestingly, Lack recently noted that about one-half of children admitted to the St Mary's ICU and intubated for status asthmaticus were food allergic, compared to about 10% of age-matched asthmatic controls not requiring admission for their acute asthma attack (personal communication). Although

atopy reportedly does not predispose individuals to an increased risk of anaphylaxis (Settipane et al 1978), it does tend to predispose to more severe reactions. Age may play a factor in predisposing an individual to food-induced anaphylaxis. The incidence of food allergy appears greatest in the first two years of life and decreases with age. Consequently foods introduced during the first year (e.g. cow's milk, egg, soy, wheat and peanut [as peanut butter]) are more likely to induce hypersensitivity reactions. However, allergic reactions to milk, egg, soy and wheat are generally 'outgrown' in the first 3–5 years of life, whereas clinical sensitivity to peanuts, tree nuts, fish, and shellfish often persist, with only 15–20% of children diagnosed with peanut allergy early in life outgrowing their peanut allergy (Skolnick et al 2001).

Aetiology

A large variety of foods have been reported to precipitate anaphylactic reactions. However, depending upon the country, a limited number of foods account for the vast majority of severe anaphylactic reactions: peanuts, tree nuts (especially Brazil nuts) (Ortolani et al 1989, Donally 1930), fish and shellfish. In addition, these food sensitivities typically persist, in contrast to other foods such as milk, eggs, and soybeans (Sampson 1999). The potency of particular foods to induce an anaphylactic reaction appears to vary and is also dependent upon the sensitivity of the individual. In general, it appears that for some foods such as peanuts, microgram quantities may be sufficient to induce a reaction.

Prior exposure and sensitization to food allergens must predate the first food-induced anaphylactic reaction. However, there have been numerous reports of an anaphylactic reaction occurring after the first known exposure to a food substance. In one childhood series, about 80% of children reacted on their first known exposure to peanuts or tree nuts (Sicherer et al 1998). Several possibilities may account for this apparent paradox: most often infants are sensitized to foods passed in maternal breast milk during lactation (Sorva et al 1994, Jarvinen et al 1999, Vadas et al 2001). Sensitization may occur following an unknown exposure to a food antigen (e.g. milk formula given during the night in the newborn nursery), food given by another care-giver (e.g. baby sitter or grandparent), or food contained in another product which was not suspected of containing the antigen in question. There also has been recent data suggesting that sensitization may occur *in utero* (Warner et al 1994, Frank et al 1999).

Clinical features

Major food-induced anaphylactic symptoms develop slightly later than those from insect sting- or medication-induced anaphylaxis, although the time course of the

perception and appearance of symptoms and signs will differ among individuals. Almost invariably, at least some symptoms will begin within the first 5–30 minutes following the ingestion of a food allergen; generally the later the onset of anaphylactic signs and symptoms, the less severe the reaction. About 25–30% of patients will experience a biphasic reaction, where patients typically develop classical symptoms initially, appear to be recovering (and may become asymptomatic) and then experience the recurrence of significant, often catastrophic symptoms (Sampson et al 1992). The intervening quiescent period may last up to 1–3 hours. In the report by Sampson and colleagues, three of seven patients with near-fatal anaphylaxis experienced protracted anaphylaxis, with symptoms lasting from one day to three weeks. There are no data to indicate that the timing of epinephrine or early use of corticosteroids affects the prevalence of biphasic or protracted symptoms.

The first symptoms experienced often involve the oropharynx. Symptoms may include oedema and pruritus of the lips, oral mucosa, palate and pharynx. Young children may be seen scratching at their tongue, palate, anterior neck, or external auditory canals (presumably from referred pruritus of the posterior pharynx). Evidence of laryngeal oedema includes a 'dry staccato' or croupy cough and/or dysphonia and dysphagia. Gastrointestinal symptoms include nausea, vomiting, crampy abdominal pain and diarrhoea. Emesis generally contains large amounts of 'stringy' mucus. Respiratory symptoms may consist of a deep repetitive cough, stridor, dyspnea, and/or wheezing. Cutaneous symptoms of anaphylaxis may include flushing, urticaria, angioedema and/or an erythematous macular rash. The development of cardiovascular symptoms, along with airway obstruction, are of greatest concern in anaphylactic reactions. Cardiovascular symptoms include syncope, a feeling of faintness, and/or chest pain. Hypotension or shock may be the result of vascular collapse, cardiac arrhythmia, or asphyxia. Anaphylaxis may be complicated by myocardial ischaemia.

Other signs and symptoms reported frequently in anaphylaxis include periocular and nasal pruritus, sneezing, diaphoresis, disorientation, faecal or urinary urgency or incontinence, and uterine cramping (manifested as lower back pain similar to 'labour' pains). Patients often report an impending 'sense of doom.' The presenting sequence and severity of symptoms vary from one individual to the next. Additionally, one patient who experiences anaphylaxis to more than one type of food may experience a different sequence of symptoms with each food. While many patients experience similar allergic symptoms during subsequent reactions, patients with asthma and peanut and/or nut allergy seem to be less predictable. There are many cases of peanut allergic children who reacted with minimal cutaneous and gastrointestinal symptoms as a young child who later developed asthma and then experienced a catastrophic anaphylactic event after ingesting peanut in their teenage years.

Diagnosis

Due to its abrupt and dramatic nature, the diagnosis of systemic anaphylaxis is generally readily apparent, as discussed elsewhere in this Symposium. In many cases where a food is implicated, the inciting food is obvious from the temporal relationship between the ingestion and the onset of symptoms. The initial step in determining the cause of an episode of anaphylaxis is a very careful history. Specific questions to address include the type and quantity of food eaten, the last time the food was ingested, the time frame between ingestion and the development of symptoms, the nature of the food (cooked or uncooked), other times when similar symptoms occurred (and if the food in question was eaten on those occasions), and whether any other precipitating factors appear to be involved, e.g. exercise, alcohol, NSAIDs.

Basically, any food may precipitate an anaphylactic reaction, but there are a few specific foods that appear to be most often implicated in the aetiology of food-induced anaphylactic reactions: peanuts, tree nuts, fish and shellfish. In cases where the aetiology of the anaphylactic reaction is not apparent, a dietary history should review all ingredients of the suspected meal including any possible concealed ingredients or food additives. The food provoking the reaction may be merely a contaminant (knowingly or unknowingly) in the meal. For example, peanuts or peanut butter are frequently added to cookies, candies, pastries, gravies or various sauces such as chilli, spaghetti and barbecue sauces. Chinese restaurants frequently use peanut butter to hold together the overlapping ends of an egg roll, pressed or 'extruded' peanut oil in their cooking, and the same wok to cook a variety of different meals resulting in residual contaminant carry-over. Another infrequent (but not rare) cause of food contamination occurs during the manufacturing process. This contamination may happen with scraps of candy or dough that are 'reworked' into the next batch of candy or cookies, respectively, or in processing plants where there is a production change from one product to the next. A recent study by the FDA found that 25% of unlabelled processed foods produced in plants making products containing peanuts, milk or egg were contaminated with these allergens (*www.cfsan.fda.gov/~dms/alrgpart.html*).

Another source of contamination due to inadequately cleaned equipment would include milk-free desserts packaged in dairy plants (Gern et al 1991).

Some conditions may be confused with food anaphylaxis. Among these clinical problems are scromboid poisoning, vasovagal collapse and hyperventilation. In vasovagal syncope, patients may collapse following an injection or a painful or disturbing situation. The patient typically looks pale and complains of nausea prior to the syncopal episode, but does not complain of pruritus or become cyanotic. Pulse is typically slowed, but blood pressure is maintained and respiratory difficulty does not occur. Symptoms are almost immediately relieved

by recumbency. Hyperventilation may cause breathlessness and collapse, but usually is not associated with other signs and symptoms of anaphylaxis, except peripheral and perioral tingling sensations. Blood pressure and pulse are generally normal.

Laboratory evaluation

The laboratory evaluation of a food-induced anaphylaxis is generally focused on the identification of specific IgE antibodies to the suspected food(s). Limited prick skin testing or radioallergosorbent tests (RASTs) are necessary to demonstrate whether the patient possesses IgE antibodies to the suspected food. Although not absolutely proven in patients with anaphylaxis, a negative prick/puncture skin test is an excellent predictor for a negative IgE-mediated food reaction to the suspected food. In contrast, a positive prick skin test does not necessarily mean that the food is the inciting agent, but in a patient with a classic history of anaphylaxis to ingestion of an isolated food and a positive prick/puncture skin test to that food, this laboratory test appears to be a good positive predictor of allergic reactivity.

Possible causes of false-negative prick skin tests include improper skin test technique, concomitant use of antihistamines, or the use of food extracts with reduced or inadequate allergenic potential. With some foods, the processing of the food for commercial extracts may diminish antigenicity (Ortolani et al 1989). This is especially true for some fruits and vegetables. However, if there is a high index of suspicion that a food may have precipitated an anaphylactic reaction even though the prick skin test is negative, the patient should be tested with the natural food utilizing the 'prick-plus-prick' method to ensure an absence of detectable IgE antibody (Rosen et al 1994). Some caution should be exercised in doing this procedure since the amount of antigen on the prick device will not be controlled, and appropriate negative controls also should be performed. In cases where extreme hypersensitivity is suspected, alternative approaches may be warranted including the further dilution of the food extract prior to prick skin testing or the use of a food-specific *in vitro* test, e.g. RAST. Recent studies suggest that the CAP-System FEIA can give better predictive values for predicting a positive food challenge for at least milk, egg and peanut (Sampson 2001a).

Double-blind placebo-controlled food challenges are contraindicated in patients with a clear history of anaphylaxis following the isolated ingestion of a food to which they have evidence of significant IgE antibodies. However, if several foods were ingested and the patient has positive skin tests to several foods, it is essential that the responsible food be identified. Patients have been reported who experienced repeated anaphylactic reactions because physicians incorrectly assumed that they had identified the responsible food (Sampson et al 1992).

Young children who experience anaphylactic reactions to foods other than peanuts, tree nuts, fish and shellfish may eventually outgrow their clinical reactivity, so an oral challenge may be warranted following an extended period of food elimination with no history of reactions to accidental ingestions.

Treatment

Treatment of food-induced anaphylaxis involves acute and long-term management. While management of an acute attack is something physicians spend hours preparing for, the long-term measures provide the best quality of life for the food allergic patient.

Acute management

Acute management of anaphylaxis due to food allergy is no different than anaphylaxis of any cause. Initial therapy should be directed at the maintenance of an effective airway and circulatory system. Epinephrine (adrenaline) is the drug of choice in the treatment of anaphylaxis, as will be discussed in other sections of this symposium. A number of reports support the need for prompt use of epinephrine in the treatment of anaphylaxis. In general, patients who die from food-induced anaphylaxis do not receive epinephrine or receive an inadequate dose during their acute reaction (Yunginger et al 1988, Sampson et al 1992, Bock et al 2001, Donnally 1930, Miller et al 1992). In contrast, patients who survive near-fatal anaphylactic reactions are more likely to have received epinephrine early in the course of their reaction and many have received repeated doses of epinephrine. In a review of 94 food-allergic children who experienced 45 episodes of anaphylaxis, two of 13 children (14%) who received epinephrine promptly required hospitalization whereas 15 of 32 children (47%) not receiving epinephrine early required hospitalization (Gold & Sainsbury 2000). Once epinephrine has been administered, other therapeutic modalities may be of benefit, such as H1 antihistamines (i.e. diphenhydramine, 1 mg/kg up to 75 mg) and in some cases H2 antihistamines (i.e. 4 mg/kg up to 300 mg of cimetidine). The role of corticosteroids in the treatment of anaphylaxis remains unclear and does not appear to alter the likelihood of biphasic response. However many authorities recommend giving prednisone (1 mg/kg orally) for mild to moderate episodes of anaphylaxis and methylprednisolone (1–2 mg/kg intravenously) for severe anaphylaxis. Although still somewhat controversial, some authorities have suggested the use of activated charcoal in an attempt to prevent further absorption of food allergens from the gut. Patients who are at risk for food-induced anaphylaxis should have medical information concerning their condition available on them at all times, e.g. a Medic Alert bracelet. An emergency treatment

plan, such as that posted on the Food Allergy and Anaphylaxis Network's web site (*www.foodallergy.org*), should be available to anyone caring for the food allergic patient. This information may be lifesaving, as it will expedite the diagnosis and appropriate treatment of a patient experiencing an anaphylactic reaction.

Long-term management

The life-threatening nature of anaphylaxis makes prevention the cornerstone of therapy. If the causative food allergen is not clearly delineated, an evaluation to determine the aetiology should be promptly initiated so that a lethal reoccurrence can be prevented, as discussed above. The central focus of prevention of food-induced anaphylaxis requires the appropriate identification and complete dietary avoidance of the specific food allergen. An educational process is imperative to ensure the patient and family understand how to avoid all forms of the food allergen and the potential severity of a reaction if the food is inadvertently ingested. The Food Allergy and Anaphylaxis Network is a non-profit organization in Fairfax, Virginia, USA (phone: 800-929-4040; fax: 703-691-2713; *www.foodallergy.org*) and can assist in providing patients with information about food allergen avoidance, and several programmes for schools and parents of children with food allergies and anaphylaxis (including forms that can be downloaded from their website).

As discussed in the Symposium (p 248–264), a recent phase I/II trial indicated that the use of humanized, recombinant anti-IgE antibody in peanut allergic individuals can significantly increase the quantity of peanut necessary to induce an allergic response (Leung et al 2003). Future studies should demonstrate whether the prophylactic use of anti-IgE will prevent severe IgE-mediated food allergic reactions.

Prognosis

A number of studies suggest that food allergic patients will experience a number of allergic/anaphylactic reactions over their lifetime despite their efforts to avoid a specific food allergen. Consequently it is essential to educate them to recognize symptoms of anaphylaxis and to medicate themselves appropriately. As discussed elsewhere, a number of new therapeutic modalities are in development for the treatment and eventual prevention of food allergy. Some of these novel forms of treatment for allergic disease hold promise for the safe and effective treatment of food-allergic individuals and the prevention of food allergy in the future (Sampson 2001b).

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DISCUSSION

Leung: What is the mechanism of anaphylaxis in these patients? We know that tryptase is generally not elevated. In other causes of anaphylaxis we do see elevation of tryptase. Many asthmatics who have food allergy don't have anaphylaxis. Do you have any data on the reverse situation? I would corroborate that experience that when they have peanut anaphylaxis and asthma, there is a high risk for fatality, but what is the prevalence of fatal or near/fatal anaphylaxis in the general group of asthmatics, and why do you think tryptase is not elevated? Does this suggest that mast cells may not be as important as you think in peanut-induced anaphylaxis?

Sampson: I don't have most of those answers. In the challenges we have done in controlled settings we have been able to measure elevation in histamine. In some of the studies in which we have tried to see whether there is a difference in basophil

number and histamine content, we have not seen any change. It looks like it is coming from the mast cell. Why aren't we seeing tryptase? I'm not sure.

Simons: If I understood correctly, you make a clinical judgement as to which patients with food allergy should get self-injectable epinephrine. The Vander Leek et al (2000) data clearly show that 25% of children who previously had non-life-threatening reactions to peanut later developed life-threatening reactions to this allergen. How do you predict which patients will have increasingly severe reactions in the future and which patients will not?

Sampson: The key factor is whether or not they have had any form of wheezing at the time we were deciding whether to give them epinephrine. We have a large number of young children with atopic dermatitis who have no form of wheezing. Typically we don't give these patients the EpiPen.

Simons: What about cough?

Sampson: If in the meantime they develop asthma they are told to call us and they are given EpiPens. It is extremely rare for someone to have a fatal reaction without them also being asthmatic.

Simons: I accept this, but I'm concerned that the perception of signs and symptoms of asthma is not optimal; asthma is under-diagnosed.

Sampson: If there is a question, we err on the side of giving EpiPens. The problem is that there are a lot of little children who don't wheeze, who have, for example, egg allergy and get skin symptoms, but who never go beyond this.

Golden: You made a point advising patients when to use the EpiPen, and said that it should be used if they have wheezing or respiratory symptoms. But if I heard correctly, you said it depends on how severe their history was. If a patient has a history of severe anaphylaxis and starts to react, do you tell them to use their EpiPen or do you wait for their respiratory symptoms?

Sampson: For children who have had a severe reaction before, we suggest they use their EpiPen at the first sign. This population is more likely to have a similar reaction.

Golden: Do any of the children who you have seen have hypotension in their reactions?

Sampson: Yes. All the fatal/near fatal group were hypotensive. The question is, is it because of their severe respiratory reaction which then causes cardiac compromise, or is hypotension a primary response? It is interesting that in all the challenges we have done, which is well over 3000, we have very rarely encountered hypotension. We certainly see respiratory symptoms, so it doesn't look like hypotension is an early manifestation most of the time.

Pumphrey: In my paper there are some data about the previous worst reaction that people who have died from anaphylaxis have experienced. If we compare this with the previous worst reaction that is reported in the clinic patients, scored

on a severity system of 0–40, we can plot a distribution for the two groups of patients. This shows that the fatal cases have a less severe history of previous reactions than the average clinic patient. If you look at patients coming into the clinic, you cannot use how bad their reaction was to judge who should have an EpiPen. I don't think I can split my patients into groups who should and shouldn't have an EpiPen.

Galli: What about the possible association with asthma?

Pumphrey: It looks as though those people with asthma are at higher risk.

Galli: So you would agree with the recommendation that if there is a history of asthma, one should be prescribed an EpiPen.

Pumphrey: Yes. If you are going to say that, though, you have to give it to everyone with even the slightest bit of food allergy and asthma. I'm not sure that this is the right idea because there are so many cases where EpiPens have been abused by schoolchildren with quite serious adverse effects.

Galli: If one can't use the severity of prior incidents to make the decision about which individuals will be prescribed an EpiPen, how can that decision be made?

Pumphrey: In my clinic, I inform the patients as best I can about the risks of their allergy, the advantages and disadvantages of carrying their own epinephrine, and then if there are no contraindications, we let them make the choice.

Simons: Given a choice, most parents will prefer to have an EpiPen or EpiPen Jr available.

Sampson: In the USA it is interesting how many people go to the emergency room and don't get epinephrine. Many of the emergency room (ER) doctors use antihistamines and corticosteroids as their treatment for anaphylaxis.

Galli: Do you think that epinephrine is underused in ERs?

Sampson: Yes.

Galli: Isn't it part of the board certification in Emergency Medicine to know the proper treatment for anaphylaxis?

Sampson: One would think so, but it doesn't appear to be. David Golden, what do you see with insect sting anaphylaxis?

Golden: Exactly the same thing. I have done grand rounds for emergency departments. They have epinephrine phobia: they think it is the most dangerous thing they could give someone. Even in florid anaphylaxis they won't give it.

Galli: How is that situation being addressed?

Golden: We hope this will change in time. There is a core group of people who work in the USA on anaphylaxis, and through the Academy of Allergy we have tried to make a dent by addressing ER physicians directly, publishing papers in their journals and going to their meetings.

Simons: In the emergency medicine literature, there seem to be more papers on the use of diphenhydramine (Benadryl) in the Emergency Department for acute allergic reactions than papers on the use of epinephrine.

Fisher: In the last few years there have been some authoritative guidelines from emergency medicine people that have recommended epinephrine.

Muñoz-Furlong: We have worked with two of the ER physician organizations in the USA about this topic. We've learned that there is no universal definition for anaphylaxis. Therefore, patients are not treated the same from one ER to the other. The definition of anaphylaxis and the treatment recommendations are determined at the state level according to a source within the emergency medicine field. This is a huge problem affecting patient care. At the very least, FAAN would like to see these physicians get on board with referring patients to an allergist and prescribing an epinephrine auto-injector (EpiPen), to prevent future visits to the ER.

Golden: Hugh, did any of your patients die in an emergency department.

Sampson: Yes. There was a patient with a biphasic response. He was standing up to leave when he collapsed. He had appeared well for over an hour and a half.

Galli: A nuance to the question would be are you aware of deaths in the ER that appear to be related to reluctance to treat with epinephrine?

Sampson: I'm not sure I could answer that.

Lee: We have the same problem in the UK. In our hospital I have tried to do this in a number of different ways with very little success. The most hopeless approach is writing guidelines. They get circulated and no one ever follows them. We have embarked on a system of education. We go to the ER room personally and hold regular seminars on the treatment of anaphylaxis. The problem in the UK is that the person who treats the emergency tends to be the junior physician. The senior physician comes later if there is a problem. Junior physicians rotate, so you keep having to train new people every few months.

Ring: This is a real problem, because there are obviously also differences between the USA and some European countries. We share Hugh Sampson's opinions that if it is only grade I skin symptoms we do not give epinephrine. We put in an intravenous catheter and give antihistamines. In the USA doctors would be frightened to do this.

Galli: Is that in a patient who has had a previous anaphylactic reaction?

Ring: Yes. If a patient presents with hives and I think he will develop anaphylaxis, as long as he has only skin symptoms I don't give epinephrine. Most of the time this works out fine.

Galli: Does that group of patients include those who have already had very severe anaphylaxis?

Ring: Yes. Some of them experience anaphylaxis in front of me, in immunotherapy or under oral provocation by drugs. Some of us have injected epinephrine into patients: it is not a fun drug. We are a little hesitant in using it. We believe it is very helpful and may not cause problems in children, but in elderly patients with cardiac problems it can induce fatal ventricular fibrillation. It is also

not a miracle drug: people die in spite of receiving it. We should think of something better.

Simons: I agree completely that epinephrine is a far from perfect drug. The toxic and therapeutic doses are virtually the same. Almost everyone who gets epinephrine has some minor, transient toxicity such as pallor or tremor, even if they receive the appropriate dose. However, I have a question for you. In our Canadian Pediatric Surveillance study (Simons et al 2003) and in other studies of anaphylaxis from all triggers (Kemp et al 1995, Cianferoni et al 2001), about 10% of patients don't have any skin symptoms or signs, and approximately 5% of patients have itching but no visible skin signs. Does this complicate your approach?

Ring: If they don't have skin signs yet there are other serious symptoms, then they get epinephrine.

Simons: Is this regardless of their previous history?

Ring: When I see the reaction in front of me I don't care about previous history, but concentrate on the actual symptomatology. When I recommend the patient a self-administration kit the previous history is important. The critical issue is what should the patients do when they experience a reaction? It's hard for a patient to make a differential diagnosis, so therefore I follow history.

Golden: I don't think we are that different in that respect. One of the problems we are hearing about in the USA is a reluctance to use epinephrine at all. I have had cases of insect allergy where the patient was markedly hypotensive and dyspnoeic with angiodema and respiratory distress, but they were given only intravenous steroids and Benadryl.

Finkelman: Is it possible that some of the mast cells discharge almost directly into the gut lumen, or have leakage into the gut lumen? Has anyone looked to see whether there is any evidence of tryptase in diarrhoeal stools of people who present with diarrhoea?

Schwartz: With an allergen inhalation challenge we can find markedly elevated levels of tryptase in BAL fluid, with no change in serum levels. I don't know whether the same thing can happen in the gut, but I would offer this as one explanation.

Lasser: Are tryptase levels elevated in children with anaphylactic reactions other than food anaphylaxis?

Schwartz: Yes, in hypotensive anaphylaxis.

Simons: To put this into context, food allergy is overwhelmingly the most common cause of anaphylaxis in children. For example, 80% of the patients in our Canadian Pediatric Surveillance study experienced anaphylaxis from food (Simons et al 2003).

Lasser: I agree with Johannes Ring that the radiology community in the USA uses antihistamine in the presence of just hives, and will watch the patient to see whether something more develops before considering the use of epinephrine.

There may be other ways to treat these patients. NO appears to be responsible for the greater part of the pressure drop following histamine injections than does histamine itself. NO hypotension is easily and quickly reversed by the use of L-arginine analogues. In the allergy community, however, I find almost no mention of NO. It really has to be considered.

Sampson: When we do the double-blind challenges in our research unit we often use Benadryl. We have to look at what the data are. Johannes Ring points out that not everyone who gets epinephrine survives, but we know that only 10% of those who get epinephrine die, so 90% live. Also, when we look at the rate of hospitilazations in children, just 12% of those who get it early are admitted, compared with 50% of those who don't get it. This tells us something about trying to treat anaphylaxis out in the field, as opposed to our units or clinics where they have close observation.

Leung: I found the discussion about epinephrine totally confusing. It sounds as if there are two distinctions. One is the issue of should all patients get an EpiPen. On the basis of what Estelle Simons said, which is that you can't predict severity of future reactions, and what Hugh said about food allergy being the first symptom of allergy, perhaps everyone should have an EpiPen and we should then come up with better criteria about when they should use it.

Fisher: In the early stages of our studies, anaesthetists had epinephrine phobia. What changed their practice was making it known that court cases about anaphylaxis under anaesthesia were related to quality of treatment, and if you hadn't given epinephrine early on you had lost. This had a major impact on their practice.

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Anaphylaxis to insect venom

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Abstract. Systemic reactions to insect stings have a prevalence of about 1% in the adult population. The majority presents with urticaria and angioedema, and the number of deaths per year registered as due to insect stings is 0.1–0.5 per million in the general population. The venom contains peptides and low molecular weight substances such as histamine with an effect on blood vessels, smooth muscles and nerves. High molecular weight components in the venom often have enzyme activity and in addition can act as allergens. It can be difficult to separate allergic from vasovagal or psychogenic reactions based on the clinical history alone. Skin test reaction to venom and/or venom specific IgE in the blood indicate an allergy although a high proportion of people in the general population (up to 20%) with no previous reactions are positive in such tests. Test results can be used to identify the responsible insect species, since allergic reactions can occur against species-specific as well as common allergens in the venom. Following a systemic allergic reaction only about half of the patients will react systemically to a future sting. Immunotherapy with insect venom is effective but the duration of the treatment is still under debate.

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Venomous insects causing anaphylaxis primarily belong to the Apidae, Vespidae and Myrmicidae families, i.e. bees, wasps and ants. Other insects such as mosquitoes and flies can also cause allergic reactions due to components in their saliva introduced when they bite, but this is not a venom reaction and the offending substances are not as well described as for insect venom. Mueller (1990) has covered the topic most extensively.

Insect venom

Bees and wasps use stings to introduce the venom. In humans this occurs just for defence purposes, although some insects also use the venom to kill their prey. Ants either sting like bees and wasps, or bite and spray their venom in the wound. The amount of venom penetrating the skin is difficult to establish as it depends on the amount of venom in the venom sac, and the penetration and

TABLE 1 Main contents of Hymenoptera venoms (bees and wasps)

Type of substance	*Representative components*	*% dry weight*
Low molecular weight substances	Biogenic amines, sugars, amino acids, oligopeptides, phospholipids	20–25
Peptides	Mellitin, apamin, mast cell degranulating peptide, kinins	50–60
Proteins	Phospholipases, hyaluronidase, phosphatase, esterase, Antigen 5	15–30

duration of the sting (Schumacher et al 1994). The venom sac is often, especially following a honey-bee sting, left with the sting in the skin when the insect flies or is brushed off. Muscles on the venom sac can then continue to pump venom into the victim.

Insect venom contains a variety of components with biological activity (Table 1). The most important low molecular weight components are the amines, histamine and probably dopamine. The high molecular weight constituents are proteins mainly with enzymatic activity. In bee venom phospholipase A_2 comprises up to 15% of the dry weight. It is toxic to cell membranes. The less abundant hyaluronidase (common to bee and vespids) acts by increasing the penetration of the venom components into the connective tissue. Peptides constitute the major part of the venom. In bee venom 50% of the dry weight is mellitin. This peptide causes damage to membranes. It can result in cell death and liberation of intracellular membrane encapsulated enzymes. Other peptides are mast cell degranulating or act as neurotoxins. The allergenic components in the venom are primarily proteins: hyaluronidase, phosphatase and phospholipase, but in bee venom the peptide mellitin is also relevant. Antigen 5 is important in vespid venoms. Its biological function is unknown (King & Spangfort 2000).

The most important stinging ant, the fire ant (*Solenopsis*), also has phospholipase and hyaluronidase in its venom, but in much more limited amounts (Hoffman et al 1991). The smaller dialkylpiperidine molecules constitute the major part of the venom in this species. Dialkylpiperidines are strongly cytotoxic and can liberate histamine from mast cells. The molecules might also act as haptens and induce IgE production.

From an allergological point of view the similarity between various species in venom constituents reflects their taxonomic relationships. The allergens in various vespid venoms are rather similar although species-specific components exist. The same accounts for bee venoms, whereas venoms from bees and vespids have few allergenic components in common (Fig. 1).

FIG. 1. Cross reactivities among Hymenoptera venoms. (From Bousquet et al 1987 with permission.)

Pathophysiology and symptoms

The toxic components in insect venom will cause inflammation with pain, irritation, redness and swelling in all individuals. The intensity depends on the type and amount of venom, the location of the sting and the susceptibility of the victim. A certain tolerance can occur as can be seen in repeatedly stung non-allergic beekeepers who often only encounter very limited local sting reactions. Venoms with high toxicity such as from fire ants can induce pustules with subsequent scarring.

Allergic reactions involving IgE are classified as either isolated large-local or systemic. The systemic reactions have traditionally been further sub-grouped according to severity in four grades (Table 2).

TABLE 2 Classification of systemic sting reactions according to severity (an example)

Type of reaction	*Symptoms*
Minor	Itching, urticaria, oedema, malaise, anxiety
General	Chest tightness, palpitations, dizziness, nausea, abdominal pain
Severe	Somnolence, respiratory difficulties, vomiting, diarrhoea, incontinence
Anaphylactic shock	Drop in blood pressure, feeling of impending doom, cyanosis, confusion, unconsciousness, death

Less common are delayed allergic reactions and reactions involving immunological mechanisms other than IgE. To this category belong serum sickness and reactions specifically affecting blood, nervous system and kidneys. In most such cases however a toxic mechanism rather than allergy is suspected and severe reactions to multiple stings belong to this category.

Large local reactions

The mildest allergic reactions to insect venom are skin reactions limited to the sting location. Such large local reactions have been defined as reactions with a swelling of 10 cm or more in diameter, which remains for more than 24 hours. Accompanying lymphangitis is not infrequent. Large local reactions are unpleasant, but only dangerous if they cause airway obstruction. Specific IgE against the venom can be demonstrated in many cases, but even in such patients the risk for more severe reactions to future stings is limited and in general there is no need to apply any allergy testing here.

Skin reactions

Urticaria not limited to just the sting location is the most frequent generalized insect sting reaction. The reaction is, as for other similar inducers, caused by histamine liberated from mast cells in the skin and the appearance and accompanying itching is similar. Urticaria can occur within minutes and duration of hours up to a few days is a general finding. The prognosis is good. Some patients react similarly to future stings by the same insect, but progression to more severe reactions is very infrequent and allergological investigation will seldom have therapeutic consequences even though most of the patients have venom-specific IgE antibodies. Angioedema can occur alone but is often preceded/accompanied by urticaria. Duration is longer and the reaction may be dangerous if occurring in airways.

Respiratory reactions

Respiratory symptoms are severe and life threatening. They can occur due to obstruction of largest airways either if stung in or close to airways or if angioedema occurs as part of a generalized reaction. Allergy to insect sting may also result in brochoconstriction with asthmatic symptoms. The risk for such reaction is thought to be higher if the victim has asthma (due to other factors) beforehand.

Gastrointestinal reactions

Abdominal symptoms can be part of a more generalized allergic reaction with symptoms from several organs. In milder cases abdominal pain, nausea and vomiting occur. More severe anaphylactic reactions can include involuntary defecation and urination.

Cardiovascular reactions

Vasovagal reactions to insect stings simply induced by fear are not infrequent and can be difficult to distinguish from allergic reactions if not accompanied by symptoms from other organs and if no measurement of tryptase or blood pressure and pulse rate have been performed. In addition to the indirect effects on the heart induced by the hypotensive hypoxia in anaphylaxis, the mediators set free in the anaphylactic reaction might mediate a more direct toxic effect. Both can induce arrhythmia and infarction.

To complicate the picture further, the toxic components in venom can harm the heart muscles directly without involving an allergic pathway if several insects sting a victim simultaneously. As is the case for anaphylactic reactions, persons with preceding cardiovascular diseases probably are more sensitive to the direct toxic or hypoxic effects of stings. Patients with mastocytosis can have either allergic sting reactions like others or react to stings without detectable IgE antibodies (Fricker et al 1997).

Epidemiology and risk factors

The frequency of allergic sting reactions depends on the risk for stings in the population. This risk in an unselected population is influenced by factors such as climatic conditions, amount of bee keeping/gardening/other outdoor activity and vegetation, so figures from different regions are not necessarily comparable.

In Denmark with its temperate climate each year on average 0.2% of the population will contact an emergency department due to sting reactions (our own observations). An individual in the same population has been estimated to receive a sting every 10 years. Death certificates disclose a mortality of only 0.3 per million per year (Mosbech 1983). A mortality of 0.1–0.5 per million per year has been reported in other regions (Mueller 1990). The real figures are probably higher since a certain proportion of the deaths with no witnesses registered as sudden unexpected or even cardiac deaths might in fact have been caused by insect stings (Schwartz et al 1988).

In retrospective population studies the prevalence of systemic insect sting reactions ranged from 0.8% in youngsters (scouts in USA) to 2.3% in a rural Spanish population and 5% in Swiss blood donors. The large local reactions were

more frequent with prevalence from 2.3% in Canadian men to 7% in the Swiss blood donors (Fernandez et al 1999, Mueller 1990).

Abnormal reactions to insect stings seem to occur more frequently with increasing age. This could reflect that the risk for allergic sensitization increases with amount of exposure i.e. number of stings. Another explanation could be that sting reactions are less well-tolerated in victims with concurrent diseases as seen with increasing age. Probably both explanations are valid.

Although some studies have incriminated a link between the occurrence of insect allergy and atopy with hay fever, asthma or atopic dermatitis, several investigators have not been able to confirm this finding and in daily practice information of concurrent atopy or atopy in the patient's family neither supports nor excludes an allergic mechanism as the major cause of an insect sting reaction.

After a systemic reaction the overall risk for a new systemic reaction is about 50% if re-stung by the same insect. The severity of future reactions in insect sting allergic patients is closely related to the severity of their previous reactions and this is one of the most important prognostic markers. Patients with only local reactions tend to continue to present with such reactions. Urticaria patients will have urticaria if they react systemically to future stings, etc. The risk for a generalized reaction is in fact so low in patients with only large local reactions, irrespective of the occurrence of IgE against the venom, that further testing could be omitted. In patients reacting with urticaria, similarly, the risk for more severe reactions is limited and in most cases immunotherapy is superfluous.

Few long-term studies on untreated venom allergic patients exist, but the severity of the insect allergy seems to decrease at a slow rate if no re-sting occurs (Fig. 2).

Diagnosis

To be able to determine the risk for future sting reaction and give advice concerning treatment or prophylaxis, it is important for us to know if a sting reaction was elicited by allergic mechanisms or not. If allergy is likely, an additional identification of the insect venom at risk is relevant as well. The case history constitutes the primary and most important part of the diagnostic work. The additional tools: skin testing with and measurement of specific IgE against venom allergens and in special cases sting challenge can help to support a supposed diagnosis. However, 'false negative' results can occur if the interval from the severe sting reaction to the time of testing is long or very short and 'false positive' results are suspected when a high proportion of individuals with no previous systemic reaction presents with positive test results.

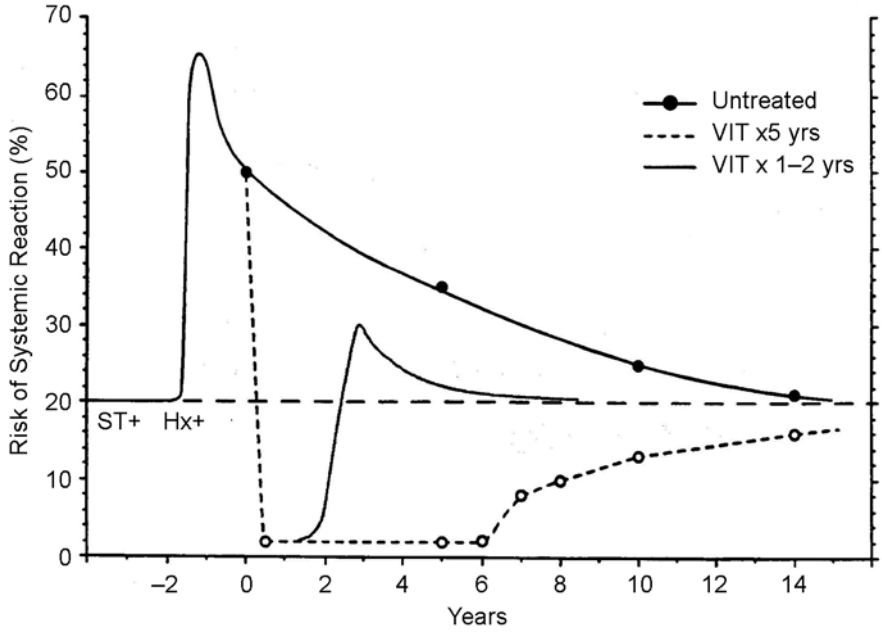

FIG. 2. Natural history of insect sting allergy showing the risk of systemic reaction to a sting in untreated patients (solid line) and in patients who received venom immunotherapy (dashed lines) for a duration of either 1 to 2 years or for a mean of 5 years. (From Golden et al 2000 with permission.)

Case history

Description of the circumstances and reaction to the sting can in many cases help to identify the culpable insect and classify the reaction as allergic or due to other reactions such as hyperventilation or vaso-vagal mechanisms. Furthermore, the allergic reactions are traditionally classified in four groups according to severity (Table 2).

Skin test

Insect venom extracts can be used for either skin prick testing or intracutaneous tests. The latter is the most sensitive, but also most prone to give non-specific positive results at high concentrations. Usually the low molecular components (histamine, etc.) have been removed from these test extracts to reduce the risk for local irritative effects. Previously extracts were prepared from whole insects. Nowadays only venom (or venom sacs) is used. This ensures a much higher

concentration of the relevant allergens in the extracts. Skin test reactions will diminish if test is performed many years after sting reaction.

Allergen-specific IgE

Measurement of IgE against allergens in venom of the various insect species can be used as a supplement to skin testing to ascertain the diagnosis especially if results are equivocal. The measurement is as an alternative to skin testing if test extracts for the relevant insect species are not at hand or available or if patient can't be tested due to ingestion of medicine, dermographism, etc.

The methodology for determining concentration of venom specific IgE varies between laboratories and subsequently test results are not identical, especially if levels are low or intermediate. The manufactures ought to declare the sensitivity and specificity or at least test results compared to skin reactions in groups of reactors and non-reactors. In general measurement of IgE is less sensitive than skin tests and the IgE level will decrease more quickly over time. A 60% reduction in three years has been reported (Mosbech et al 1986).

Specific IgE measurement (and skin test) can be done soon after a systemic sting reaction. However, in some patients tests will not be positive in the initial phase and (re-)testing is advised at least 4–6 weeks after the sting (Goldberg & Confino-Cohen 1997).

Sting challenge

Since a significant number of individuals with systemic sting reactions will not react to a subsequent sting and since our other diagnostic methods are at present not able to identify the reactors with certainty, sting challenge has been introduced as a kind of gold standard against which other tests should be compared. However it has drawbacks and for several reasons it is not adopted as general routine (Ruëff et al 1996). First of all it can be dangerous even if performed in intensive care units. Secondly, it is difficult to ensure that the insect gives a sufficient allergen dose. This can depend on the age and present function of the insect. In addition some insects, especially wasps, can spray venom into the air when caught and thereby reduce the content of their venom sac. Some patients tolerating sting challenge have in fact had reactions to subsequent stings (van Halteren et al 1996, Golden et al 2000).

Other tests

Histamine release from basophil granulocytes in the blood when exposed to the relevant venom can illustrate the degree of sensitization, but high allergen

concentration will give unspecific (toxic) histamine release and might be the reason why the predictive value of this test seems unsatisfactory (Mosbech et al 1993). Other cellular tests measuring leukotriene release (CAST, Cellular Allergen Stimulation Test), or surface markers indicative of activation have been applied (Binder et al 2002, Maly et al 1997, Sainte-Laudy et al 2000) but their usefulness in daily practice for diagnostic purpose has yet to be demonstrated. Venom-specific IgG will reflect exposure to the venom but not allergy.

Therapy

Prevention

Since the insects discussed here sting humans only in defence, the best preventive measure is to avoid the insects and only try to hit them if this can be done successfully at first attempt. If stung, it is important to remove the venom sac as quickly as possible to reduce the amount of venom injected (Schumacher et al 1994).

Symptomatic treatment

The treatment of full-blown anaphylactic insect sting reactions with epinephrine (adrenaline), antihistamine and corticosteroids does not differ from treatment of similar conditions due to other causes (Müller et al 1991). Patients at risk are often supplied with emergency kits containing epinephrine in a preloaded syringe, and antihistamine and corticosteroid tablets. The tablets should be taken as soon as possible if stung and epinephrine administered only if needed.

Angioedema in throat or larynx should be treated with adrenaline either inhaled or injected plus corticosteroid and antihistamine. Asthmatic reactions would benefit from inhaled $\beta 2$ agonists.

Patients who experience urticaria or just large local reactions should have antihistamine and in some cases also corticosteroid. For prevention, similar drugs could be taken as soon as possible after future stings.

Venom immunotherapy

Allergen-specific immunotherapy with venom extracts has shown effectiveness in treating patients with systemic allergic reactions to stings (Müller & Mosbech 1993). The degree of protection obtained depends on several factors (Table 3). Reviews have reported the risk for systemic reactions to re-sting after venom immunotherapy to be between 0 and 30% (Mueller 1990) with most studies at least with wasp venom giving a relapse rate close to 0%. In a mixed group of patients a 10% risk of reaction on each sting was observed at any time after

TABLE 3 Factors associated with reduced chance of beneficial effect of venom immunotherapy

Predictive parameter	*Decreases chance of success*
Severity of sting reaction pre-treatment	Severe (vs. moderate)
Age	Adults (vs. children)
Insect species	Bee (vs. wasp)
Side effects to venom immunotherapy	Present (vs. absent)
Duration	< 3 year (vs. $\geqslant 3$ years)
Co-morbidity	Mastocytosis

discontinuation of therapy and the systemic reactions were in general less severe (or equal to) the pre-treatment sting reactions (Golden et al 2000). Death due to insect stings after immunotherapy has been reported in case of the severe complicating co-factor, mastocytosis (Elberink et al 1997). Generally however immunotherapy has been successful also in insect allergic mastocytosis patients (Fricker et al 1997).

Various up-dosing regimens are equally effective. The most rapid schedule, ultra rush, takes only a few hours to reach maintenance dose and has to be performed under close supervision often in an intensive care unit. The same accounts for other rush regimens, which tend to have more side effects than slow up-dosing schedules (Mosbech & Müller 2000). The rapid regimens have the advantage of fewer visits to the doctor. This can be a great advantage if there is a long distance from patient to physician, if the patient is heavily exposed and needs rapid protection or simply if the patient has difficulties in finding the time necessary for many visits.

The protein content in the maintenance dose is most often 100 μg. This is equivalent to two or more average insect stings, dependent on the species (Hoffman & Jacobson 1984). Maintenance treatment is given at four to eight weeks intervals, normally for 3–5 years. The duration of the treatment and the subsequent protection have been discussed, but irrespective of the change in immunological parameters, a reasonable protection is seen at least 10 years after the treatment (Fig. 2)(Golden et al 2000). An insect venom skin test, which becomes negative, may or may not predict protection, but if both skin test and specific IgE becomes negative the risk of a generalized sting reaction is significantly reduced. A tolerated sting challenge does not guarantee that future stings will be tolerated without systemic reactions (Lerch & Müller 1998, Golden et al 2000) and as a consequence some centres recommend venom immunotherapy to be continued indefinitely in those individuals who have had life-threatening anaphylaxis (Committee on Insects 1998).

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DISCUSSION

Lee: What are your recommendations for follow-up patients with venom anaphylaxis after immunotherapy? What is your policy about epinephrine?

Mosbech: This is a hotly debated topic. The main point is that if the patient has had a very severe reaction, they should continue to carry the EpiPen. It depends on the risk factors. If you have had a severe reaction and a lot of risk factors, you have a risk of getting a severe reaction even during immunotherapy. In such extreme cases you should have an EpiPen. If you have had less severe reactions, my view is that it is not necessary to have an EpiPen. There is no hard and fast answer.

Lee: How often do you see patients after immunotherapy is completed?

Mosbech: Some I see once a year. You should see the patient if they are stung in the future. It is not important to see them once a year because we don't have a test to see whether they are protected or not. If I see them, it is to re-emphasize the importance of having an EpiPen and being sure they know how to use them.

Müller: I have a comment about prescribing EpiPens after the conclusion of immunotherapy. I have a slightly different opinion, but this could be because I'm from one of the few countries where honeybee allergy is more frequent than vespid allergy. Treatment success with immunotherapy is only complete in about 80% of our bee venom allergic patients as compared to $>95\%$ of *Vespula* venom allergic patients. Even after stopping successful immunotherapy there is a relapse of the allergy in 10–15% when they are re-stung according to prospective studies with an observation time up to 7 years after stopping. When we stop, I therefore tell the patient about this risk of relapse and for this reason recommend the continued use of the EpiPen. It is up to the patient to decide, though.

Golden: The risk factors are very important. Mainly the severity of the original reaction and whether or not there has been a systemic reaction during immunotherapy and honeybee versus vespids. There will be different approaches in different countries in the decision making process. In the USA fear of lawyers is often the overriding factor and not fear of medical consequences. I have become much more liberal in my prescribing of EpiPens for patients during or after immunotherapy. I used to say that they didn't need one unless they are going camping or hiking, whereas now especially after immunotherapy, once you are in a position to say to a patient that they have a chance of a systemic reaction greater than that of the general population, then in the USA lawyers will insist that you should have given a prescription for an EpiPen. After successful immunotherapy there is still a 5–10% chance of a systemic reaction, although it is most likely that this will not be severe. Many physicians would be rightfully comfortable not prescribing an EpiPen for those patients. I have a different question. How much can we say that stings to the head and neck are more risky than those to the extremities? This is more of a gut feeling, but I am not aware of any data on this.

Mosbech: You don't have the denominator in your question. I am referring to my own death certificate data. Some of these patients suffocated because they had local swelling. There is a higher risk of such reactions if you are stung on the head or neck.

Golden: This is why I always tell patients never to drink from a straw or a can. I have had many patients get stung in the tongue or throat this way.

Müller: We have such data, and they clearly show that patients with severe shock reactions more often have a sting in the head or neck region than those with stings in the extremities.

Golden: As Holger Mosbech points out, what is missing is the denominator here, because this is where most stings occur, in exposed areas.

Sampson: Have you made a distinction between patients that have asthma and don't have asthma with regard to severity of reaction, and whether you would prescribe an EpiPen?

Golden: Yes, by extrapolation. I am not aware of any data. We have the most difficulty with the respiratory component of the reactions in a number of ways. Was it really allergy or was it anxiety when they said that their throat felt tight and their chest felt funny?

Finkelman: Does prophylactic use of a long-acting antihistamine have any benefit? I could ask the same question in relation to food allergy, too.

Müller: There are a number of controlled double-blind studies on this topic which clearly show that you can reduce the side effects of immunotherapy by premedication with antihistamines.

Mosbech: These studies focus mainly on local side-effects.

Golden: Both Ulrich Müller's study and Brockow's study (Brockow et al 1997) showed reduced systemic reactions.

Muller: The rate of severe systemic reactions involving respiratory tract or cardiovascular system is so low that we didn't show significant differences with the numbers that can be included in such a study.

Fisher: There are three studies that I'm aware of on prevention of anaphylaxis to snake antivenom. Antihistamines do nothing. Subcutaneous epinephrine reduces the incidence of reaction. Both these plus steroids reduce the incidence by 24%.

Sampson: Carston Bindslev-Jensen did a study challenging patients who were either getting astemizole or not (Bindslev-Jensen et al 1991). He showed there was reduced cutaneous reaction and oral symptoms, but no difference in pulmonary symptoms in those receiving antihistamine. This was oral food challenge.

Simons: All the currently available second generation, non-sedating antihistamines, except acrivastine, are long-acting medications in that they have a duration of action of 24 hours and are given once a day. These newer medications are rather more useful in terms of benefit to risk ratio than any of the older first generation, sedating H1 antihistamine. Diphenhydramine is, however, one of the few H1 antihistamines that is water soluble and can be administered intravenously.

Golden: In an acute allergic reaction would you tell a patient to use diphenhydramine, or second- or third-generation antihistamines?

Simons: I am wary of recommending H1 antihistamines for anaphylaxis, because patients may use the antihistamine and not the epinephrine. The literature suggests that antihistamine use, coupled with a subsequent delay in epinephrine administration, may contribute to fatalities in anaphylaxis (Gold & Sainsbury 2000). It is important to differentiate between use of antihistamines in anaphylaxis *treatment* and *prophylaxis* of some forms of anaphylaxis by using H1 antihistamines. Radiocontrast media reactions, for example, can be prevented using a combination of a H1 antihistamine with other medications. This situation is quite different from sending patients forth with a H1 antihistamine and depending on it for treatment of anaphylaxis in a community setting.

Müller: Most people in Europe give venom-allergic patients tablets of antihistamine and corticosteroids with the instruction to take these tablets first when they are stung, then to use the EpiPen if symptoms arise. On the basis of your studies we recommend the use of a second-generation antihistamine with a rapid onset of action.

Simons: In one of your recent studies, you suggested that the use of the H1 antihistamine during venom immunotherapy improved the outcome as well as reducing the adverse effects (Müller et al 2001).

Müller: Our first controlled study began in 1989. Following increasing discussion about histamine receptors on T cells we went back to the long-term results of this double-blind study. In fact, of the 52 patients originally involved,

41 were re-exposed: 20 in the antihistamine group and 21 in the placebo group. Six patients developed a generalized allergic reaction, all of whom were in the placebo group. This is a retrospective study, of course, and we are now planning a prospective study with the sting challenge during the double-blind phase.

Golden: We were talking over lunch about whether there are some intrinsic differences between different causes of anaphylaxis, and in particular insect sting and food. One of the things that I think is different is the chance of progression. Although not all published studies agree on this, I sense some agreement that insect sting allergy usually does not progress in severity, whereas food allergy does seem to. Recent studies on peanut allergy indicate that there is a tendency for this to happen, with 20–40% of patients getting more severe reactions on the second or third exposure. If it is true that insect sting allergy usually does not progress, it makes life easier for us with regard to some of the clinical decisions we have been discussing.

Müller: I have seen these studies. I think with sting challenge studies in non-treated patients, there is a selection for those with less severe reactions. You are not going to sting challenge someone who had a very severe anaphylaxis and had to be intubated.

Golden: Of course, if they were already very severe then they can't get much worse. Do the milder ones get more severe?

Müller: We have experience from fatality studies that 42% had experienced a previous reaction.

Mosbech: The problem is that with insect sting we can't be sure of the dose. You might run into a swarm of wasps and get 10 stings: then you will not be protected.

Sampson: I want to make one point about food reactions. I wouldn't want people to be left with the impression that they always get progressively worse. We see that most children react very early on. Typically, they then avoid the allergen for a period, they develop asthma when they are three or four years old, and they then ingest a food when they are aged seven years old and have a more severe reaction. There was a time when people thought that oral cromolyn sodium would prevent food allergy. We did a study in which we challenged patients on and off with a four month differential (Burks & Sampson 1988). The challenges were virtually identical in dose and timing, so I think it is more an age-related phenomenon we are seeing with food as opposed to it just getting progressively worse every time.

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Anaphylaxis to anaesthetic drugs

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Abstract. Severe anaphylactic reactions during anaesthesia increased dramatically in incidence in the mid 1970s. Studies performed in our unit over the subsequent 25 years demonstrated the involvement of IgE in these reactions and the value and safety of intradermal and prick testing in the diagnosis and determination of the drug responsible. Radioimmunoassay studies demonstrated that neuromuscular blocking drugs produce anaphylaxis by cross-linking IgE molecules via their substituted ammonium groups. The IgE binding of these drugs leads to a high incidence of cross sensitivity. Mast cell tryptase measurement is highly sensitive and specific for anaphylaxis although it can be elevated in reactions due to direct histamine release. The reactions are unpredictable from the history. The heart is rarely a target organ in human anaphylaxis although the diseased heart is more likely to fail or produce serious arrhythmias than the normal heart. Colloid solutions produce a better response than crystalloid solutions in the treatment of hypotension. Anaesthetic allergy persists up to 27 years. Subsequent anaesthesia based on the findings of skin testing is usually safe.

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Direct release of histamine in response to drugs, or surgical and anaesthetic stimuli occurs commonly during anaesthesia but is transient and rarely life-threatening. In the 1970s there were increasing reports of severe reactions in many countries. This was partly due to the high frequency of reactions to alfathesin and propanidid, which were both withdrawn because of these reactions.

This paper describes the findings from studies performed in our unit between 1975 and 2001. Table 1 shows the nature of the reactions studied.

Incidence and outcome

The incidence of anaesthetic anaphylactic reactions (AAR) is between 1:900 and 1:20 000. About 30 million patients would need to be studied to establish an incidence with 5% confidence limits. Usage is a major determinant of incidence of reactions to specific drugs (Rose & Fisher 2001). The mortality in large series is 4%. An additional 2% of patients survive with severe brain damage.

TABLE 1 Reactions investigated 1974–2001

	General anaesthesia	*Local anaesthesia*	*Total*
Severe anaphylactic	744	6	750
Not anaphylactoid	191	283	474
Trivial	134		134
Delayed	50	2	52
Pre-operative	88		88
Histamine release	82		82
Total	1289	291	1580

Mechanisms of reactions

Direct histamine release (DHR)

There is a correlation between DHR due to anaesthetic drugs and cardiovascular, cutaneous and subjective symptoms (but not bronchospasm). Histamine may produce adverse effects during anaesthesia, which can be prevented by pretreatment with H1 and H2 blockers (Lorenz & Doenicke 1985). The degree of DHR produced by a particular drug correlates with its ability to produce minor but not severe reactions. DHR may rarely may produce severe reactions including death, particularly with large volume infusions, vancomycin, and in patients who are 'super-responders' to the DHR effects of neuromuscular blocking drugs (NMBDs).

Recent studies have shown that basophils and mast cells from different parts of the body show a different response to histamine releasing drugs (Genovese et al 1996). Morphine, for example, causes histamine release from skin mast cells but not lung, intestinal or cardiac mast cells or basophils. Although a potent releaser, very little histamine released from skin will reach the lungs, and bronchospasm is unlikely. In contrast there may be an increased incidence of non-allergic asthma from drugs which release histamine directly from lung mast cells such as atracurium, vecuronium and propofol.

IgE-mediated reactions (type I hypersensitivity)

The role of IgE-mediated reactions in severe reactions during anaesthesia was initially controversial, particularly to NMBDs, where previous exposure was unusual and DHR well documented. The demonstration of positive skin tests to a drug given immediately prior to the reaction in up to 80% of patients suggested IgE involvement, and this was initially confirmed by passive transfer tests, and

subsequently by radioimmunoassay (RIA) for drug-specific IgE. More recently elevated mast cell tryptase has confirmed the anaphylactic nature of the reactions.

Activation of the classical and alternate pathways of complement have been demonstrated after clinical anaphylaxis particularly due to alfathesin, contrast media, dextrans and protamine. The heparin-protamine complex activates the classical pathway but is rarely of clinical significance (Best et al 1984).

Predisposing factors

Allergy and atopy

There is a three to fivefold increased incidence of a history of allergy, atopy or asthma in patients who undergo anaphylactic reactions during anaesthesia compared with non-reacting patients, although the low prevalence of anaesthetic allergy make such a history a poor predictor of anaphylaxis (Fisher et al 1987).

Previous exposure

With thiopentone multiple uneventful exposures (usually more than five) are usual. With NMBDs a history of previous exposure occurs in less than 50% of IgE-mediated anaphylaxis. It is unlikely the antibody is formed in response to the NMBD. How sensitization to NMBDs occurs is unknown.

Drugs producing anaphylaxis

The drugs producing reactions in our series are shown in Table 2. Induction agents are currently a minor cause. The NMBDs still provide the major cause of life threatening anaphylactic reactions in all large series. Suxamethonium is the most common cause throughout the world. Cross-sensitivity to other NMBDs occurs in at least 60% of reactors with suxamethonium and gallamine, alcuronium and *d*-tubocurarine, and pancuronium and vecuronium the most common pairs (Baldo & Fisher 1983a). The NMBDs produce reactions by bridging IgE molecules via their substituted ammonium ion groups (Baldo & Fisher 1983b). Antibiotics are an increasing source of acute anaphylactic reactions during anaesthesia with cephalosporins the most common cause.

The third group causing reactions are solutions used for blood volume replacement especially synthetic colloids. All produce anaphylactoid reactions and there is no clear-cut evidence showing a higher incidence with a particular colloid. The reactions appear uncommon during shock. There is little convincing evidence of IgE involvement.

More recently attention has been drawn to reactions to the latex in gloves and anaesthetic equipment. These reactions are characteristically delayed more than 15

TABLE 2 Cause of life threatening clinical anaphylaxis during anaesthesia 1974–2001

Induction agents	88
Thiopentone	46
Alfathesin	29
Propanidid	8
Propofol	5
Other	2
Induction agent and relaxant	4
Neuromuscular blocking drugs	452
Suxamethonium	152
Alcuronium	137
Rocuronium	47
Atracurium	42
d-tubocurarine	24
Vecuronium	22
Gallamine	17
Pancuronium	14
Cisatracurium	2
Mivacurium	4
Decamethonium	1
Antibiotics	53
Cephalosporins	37
Penicillins	14
Vancomycin	2
Colloid solutions	40
Local anaesthetics	6
Protamine	11
Narcotics	16
Other drugs	27
No drug determined	56
Colloid solutions	40
Local anaesthetics	6
Protamine	11

minutes, and often occur in health care workers. While the diagnosis may be confirmed by skin testing and RIA for latex-specific IgE using commercial kits, we find a careful history is usually sufficient.

Allergy to local anaesthetics is extremely rare. The reactions tend to be painful swelling on the side of the dental block, collapse (usually related to dental injection) and bizarre neurological symptoms. The important clue to a vasovagal cause of collapse is that the patient recovered with minimal or no treatment. In many patients with bizarre neurological symptoms or cardiovascular collapse the syndrome may be precipitated by saline. In a study of 229 patients we found four patients with immediate allergy to locals and four patients with delayed reactions. A safe alternative was found for seven patients. The eighth patient reacted to all available locals and placebo. Progressive challenge is used to confirm or more usually exclude the diagnosis. Every effort should be made to exclude spurious histories of local anaesthetic allergy. In genuine allergy, antihistamines, β blockers, or narcotics may be used as local anaesthesia (Fisher & Bowey 1997a).

The clinical expression of anaphylaxis

Research on AARs has been disappointing in terms of studies elucidating the clinical response and response to treatment. Observations from anaesthetic anaphylaxis have produced a few haemodynamic studies of individual patients.

Some observations relevant to the pathophysiology and treatment of anaphylaxis from our series are:

- There is a significant blood volume deficit. Anaesthetic data favours the use of colloid rather than crystalloid in blood volume replacement (Fisher 1977, Waldhausen et al 1987).
- The heart is only rarely a target organ in human anaphylaxis in spite of *invitro* and animal studies showing direct effects of mediators on the isolated or animal heart. The catecholamine response is probably an important protector of the heart. Conditions in which the catecholamine response is impaired such as an epidural, beta blockade, or asthma make anaphylaxis more difficult to treat.
- Cardiac failure or arrhythmias other than supraventricular tachycardia are more likely to occur in patients with cardiac disease (Fisher 1986). In hypotension refractory to volume replacement and epinephrine (adrenaline), norepinephrine and vasopressin may be effective (Fisher 1986).
- When anaphylaxis occurs prior to cardiopulmonary bypass, bypass should be instituted as soon as possible and surgery should proceed (Ford et al 2001).

Investigation and follow-up: skin testing

There has been great controversy over the precise value of different tests which persists in spite of similar results from large studies in different units. Skin testing whether by prick or intradermal routes is the most valuable test and is mandatory in severe reactions (Fisher & Bowey 1997b). It detects a limited number of mechanisms (IgE and possibly IgG mediated reactions) but has the overwhelming advantage that it can be performed without special facilities. No fatal reactions have been described in such tests (not in anaesthetic skin testing: we have seen three easily treatable reactions in over 1200 tests). The data supports the safety of subsequent anaesthesia using skin test negative drugs in patients who are skin test-positive to other drugs, but this is not an absolute guarantee; we have seen two second reactions to skin and RIA test-negative drugs.

Skin testing is performed 4–6 weeks after the reaction. The high yield of positive results in published series reflects the high incidence of IgE involvement in severe reactions. For intradermal testing the drug is diluted and injected into the dermis, and prick testing in which the drug is introduced into the dermis by pricking the patient's skin through a drop of undiluted drug. Controls such as histamine or a high concentration of a narcotic are used to determine that histamine responsiveness and histamine releasibility are normal, and saline to exclude dermatographia (Fisher 1984).

Prick testing has the advantages of less trauma to the skin, ease of preparation, and probably safety. Prick tests are more likely to be successfully completed in children. Their disadvantage is that although they may be inherently more accurate than intradermal tests they tend to produce false negatives whereas intradermal tests tend to produce false positives. The consequences of a false negative test for subsequent anaesthesia are obviously greater than a false positive. In a prospective study, agreement as to the drug implicated between the tests in 212 occurred in 93% and if both tests were performed predictability improved by 7% (Fisher & Bowey 1997b). Skin tests are of little value in reactions to colloids, contrast media and blood products or in minor reactions.

With local anaesthetics the incidence of genuine reactions is so rare that the philosophy of diagnosis is changed, and the goal is to exclude allergy. A progressive challenge is used. Unless there was a clear cut history suggestive of anaphylaxis and a clear-cut positive wheal and flare reaction at 1:100 dilution of 0.5% local the dosage should be increased up to 2 cm^3 of undiluted local. If a psychological cause is suspected we aggressively challenge the patients with 2 cm^3 of radioimmunoassaysubcutaneous saline, which will often reproduce the symptoms (Fisher & Bowey 1997a).

Complement

As a diagnostic tool in clinical anaphylaxis complement levels have limited value although there is insufficient information published to enable a valid assessment.

Mast cell tryptase (MCT)

MCT is extremely useful in the diagnosis of AAR. Tryptase is a protease in mast cell granules released by activated mast cells. In AAR the serum levels are elevated for 1–5 hours after the beginning of the reaction, enabling the delay of sampling until resuscitation is over. Reliable results can be obtained in specimens taken post mortem (Fisher & Baldo 1993). During anaesthesia elevated MCT levels appear highly specific and sensitive for IgE mediated reactions. The difference in the incidence of positive tests for IgE antibodies in patients whose MCT levels were elevated compared to those whose MCT levels were not elevated was highly significant ($P < 0.000001$). Seven of 143 patients with IgE antibodies detected had no increase in MCT, and 33 of 158 patients with elevated MCT had no detectable IgE antibodies. (Fisher & Baldo 1998), although they are elevated in some states where IgE is unlikely to be involved such as reactions to contrast media, severe direct reactions to vancomycin, and in mastocytosis (our unpublished data).

Radioimmunoassay tests

Radioimmunoassay tests have been used to detect IgE drug-specific antibodies. These tests are only performed in a few laboratories, and are available for NMBDs, opioids and thiopentone (Baldo & Fisher 1993). Use of these tests in over 300 patients has shown:

- RIA tests will detect the drug responsible for a reaction with about the same frequency as skin tests if there is a RIA available for that drug. However, RIAs are only available for propofol, thiopentone, morphine, suxamethonium and alcuronium, vecuronium, pancuronium, gallamine and *d*-tubocurarine (Fisher & Baldo 1994).
- A combination of RIA and skin tests will detect the drug responsible better than either test alone (Fisher & Baldo 1994).
- There is generally agreement between the tests for the drugs responsible, but 50% disagreement for tests for other NMBDs when a battery of tests is used. Cross-sensitivity as determined by RIA is greater than for cutaneous testing, and those patients positive by RIA and negative on skin testing comprise both false-positive RIAs and false-negative skin tests (Fisher & Baldo 1994). The yield of positive results for NMBDs may be increased by using a RIA for

morphine (Fisher & Baldo 2000) or inhibition of the suxamethonium RIA (Laxenaire & Mertes 2001).

- In patients selected by any one test and studied with any alternative test the specificity of the alternative test increases. In practice when the results of tests disagree the patients should be warned off all positive drugs.

Other tests

Leukocyte and basophil histamine release tests have been used in specialized laboratories and give results similar to RIA.

After severe AAR a combination of available tests will determine the drug responsible in 90% of patients. In patients with severe reactions in whom no drug is detected alternative forms of anaesthesia (regional, volatile) should be considered.

The findings should be clearly documented and explained to the patient and the patient be instructed to carry a letter at all times in addition to a warning device. The letter is a vital adjunct because it gives subsequent practitioners the opportunity to assess the evidence for accuracy. Details of safe subsequent anaesthesia should be added to the letter. Such information is the best information upon which to base subsequent drug selection.

Anaesthetic allergy has been shown to persist up to 27 years, and few patients lose their sensitivity (Fisher & Baldo 1992).

Preoperative testing

It has been argued that patients presenting for anaesthesia should be preoperatively screened by RIA testing. The cost benefit of such an approach is questionable and the high incidence of cross-sensitivity between NMBDs would mean that the alternative drugs which may be given to a positive patient cannot be guaranteed as safe without secondary cutaneous testing.

Subsequent anaesthesia

We have records of subsequent anaesthesia in 290 patients diagnosed with four subsequent reactions due to cross-sensitivity and one bradycardic reaction, and in 70 subsequent anaesthetics in whom a diagnosis was not reached there has been one subsequent reaction.

The concept of pretreating patients allergic to NMBDs with monoquaternary compounds to competitively block the IgE receptors without bridging has been accomplished successfully in two French studies (Moneret-Vautrin et al 1993, Thomas et al 1988).

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DISCUSSION

Galli: You made a comment about patients with asthma. Could you review this for us? A theme seems to be developing that patients with asthma are at particular risk of anaphlyaxis, at least in food allergic patients.

Fisher: There are three identifiable patient groups who are at risk of bad outcomes from anaesthetic-induced anaphylaxis. Those who have had epidurals, β-blocked patients, and those with asthma. The hardest patients to treat are asthmatics who get anaphylaxis, because they will almost always have severe asthma. We have to treat them with everything we know. Three patients with allergic asthma were actually put on cardiac/pulmonary bypass because we couldn't reverse their asthma. Then there is transient bronchospasm which we think occurs when the peak levels of histamine hit the normal bronchus. In this case the anaesthetists report not being able to inflate. This is the second most common presentation. The other common one now is arterial desaturation. There is a relatively benign sort of asthma with direct histamine-releasing effects. Anaesthetists call this red-knob asthma: they just turn on the halothane for a while and the asthma goes away. This is almost certainly through direct histamine release and is due to the drugs that professor Marone has shown work on lung mast cells.

Golden: Why do epidurals increase the risk of anaphylaxis?

Fisher: I think it is because the catecholamine response is blocked. There is a higher incidence of anaphylaxis in response to colloid solutions administered for hypotension due to epidurals. In a Swedish study, Ljungstrom et al (1983) looked at reactions to dextran. The death rate was three times higher in patients who had an epidural. A very dangerous situation occurs in someone known to be allergic to suxamethonium and the epidural doesn't work. The resident has been taught that you must use suxamethonium in this situation. Although the patient is allergic suxamethonium is given. There have been two cases where this has happened and both patients died. Half our deaths have occurred in patients with epidurals when a general is administered.

Müller: In one of the French studies on perioperative anaphylaxis (Laxenaire 1993) they reported 12.6% of patients with latex sensitivity. Did you test your patients for this?

Fisher: We haven't seen a great deal of latex sensitivity. The interesting thing we have seen is in the three patients in that series who reacted to latex, the anaesthetist asked them preoperatively if they were allergic to anything and they said no. I asked

them the same question before I tested them, and they said no. Then when all the tests were negative I asked them whether they had ever had trouble blowing up balloons, and they all knew that they were allergic to latex. Also, in Australia we don't see the cross-sensitivity with fruit that is seen in Europe. We don't see a great deal of latex allergy. When I was an anaesthetic allergist, most anaesthetists asked whether patients were allergic to anything, but I used to ask whether they were allergic to any drugs, pills, tablets, potions, lotions, mouthwashes, gargles, enemas, skin preparations or latex. I got a higher incidence of allergy history than most anaesthetists do. The other thing that was a big problem in the USA was discase that is injected into the back. We have never seen a reaction to this in Australia. This has a package in it for testing people before it is administered. This says 'place one drop of solution A and one drop of solution B on forearm and prick'. This always caused a lot of merriment in the operating theatres.

Ring: From your paper I gathered that the prick test was always negative but the intradermal test is fine. Is that correct?

Fisher: No, they are about the same.

Ring: What concentration do you use for the intradermal administration?

Fisher: When we get a new drug we give it to 10 people to work out a concentration that doesn't cause a wheal and flare reaction in normals. We use morphine 1:10 for a control. We test narcotics at 1 in 100 000, induction agents, colloids and antibiotics at 1 in 100 and neuromuscular blockers 1 in 1000. For prick testing the only thing you have to dilute is morphine.

Ring: There are a lot of 'positives' in intradermal tests with neuromuscular blocking agents which might be irritative in nature.

Fisher: There is a catch in that because if they weren't positive we didn't know it was a neuromuscular blocker so they went into the undiagnosed group. Studies in Germany and France all reflect that. The recent paper by the French group shows that there is still a lot of argument about how good these tests are (Laxenaire & Mertes 2001).

Ring: We are in some disagreement to that, because we don't find so many positive reactions.

Fisher: The French do use higher concentrations than ours, but use the back and not the forearm.

Simons: You mentioned Lorenz. He advocates H1 and H2 prophylaxis intraoperatively (Lorenz et al 1994). Is this widely used?

Fisher: We have been mischievous because we wanted to make sure that the results are right. We have very rarely used prophylaxis in anyone. About 20% of those patients had a H1 blocker in their premedication, so they do nothing. Many of the things that Lorenz, Philbin and John Moss have shown are prevented by H1 and H2 blockers seem to us to be individual practices best avoided rather than giving antihistamine pre-treatment. H1 and H2 blockers have been shown in

anaesthesia to prevent reactions to *d*-tubocurarine (Moss et al 1981), reactions to 100 mg of morphine in β-blocked patients with cardiac disease (Philbin et al 1981) and in rapidly infused Haemaccel (gelatin) in non shocked volunteers (Lorenz et al 1977). It seems to us a more logical approach not to do those things to people. Willy Lorenz's major study on H1 and H2 blockers in haemodiluted patients undergoing major surgery is more compelling, but not something we can observe. There is a very interesting subset analysis from the study showing antibiotics (Lorenz et al 1994). In studies on histamine release with anaesthetic drugs, people often do all they can to maximize the effect. If you give your drug slowly you might not see these responses. With atracurium, however, there are these super-responders. This is not an immune phenomenon.

Lee: Is it possible to be sensitized to neuromuscular agents through diet?

Fisher: Why is muscle relaxant-anaphylaxis rare in the USA and Scandinavia, and unheard of in South Africa? There must be an environmental factor that is sensitizing people to these drugs. We have found all sorts of things that do not. Diet is an attractive option, because there is much more control of food in the USA than in other countries. Of the first eight patients we had in NZ, six of them came from Europe, three of whom were from Poland. This is very unusual in NZ. Most patients react on first exposure and have relaxant-specific IgE. Sensitization by some other factor is a possible explanation.

Schwartz: As Gianni Marone showed, some anaesthetic agents have the ability to activate mast cells directly. This would suggest that the rate of administration is critical. Do you see non-IgE-dependent anaphylaxis because agents are administered too quickly?

Fisher: We had a lot of trouble with the direct histamine releasing concept until the study on the selective effects on mast cells. For example, we have had a death from vancomycin with a tryptase of 400. This had been administered uneventfully over an hour, for about five days. It was then given over 10 minutes which caused the reaction. The big volume items such as Haemaccel which are given fast because of low blood pressure can do this. If you give *d*-tubocurarine the blood pressure drops due to direct histamine release. Apart from atracurium and vancomycin I don't know how often direct histamine release kills anybody. I think it is usually easy to fix and it is transient, particularly with the short-acting asthma that people get from vecuronium, propofol and some other drugs.

Schwartz: Have you had any experience with mastocytosis patients?

Fisher: If you do a literature search on chronic fatigue syndrome, multiple chemical sensitivities or mastocytosis and anaesthesia, you get nothing. It hasn't been well studied. We have had patients referred to us with mastocytosis. We have skin tested them, but we have only had two positive skin tests in all of that group. We tell people to avoid histamine-releasing drugs, give drugs slowly and H1/H2 block them preoperatively. They are not usually then a huge problem.

Marone: I would like to address the importance of the H1, H2 and possibly H3 receptors in the cardiovascular system in the prevention of anaphylactoid reactions to general anaesthetics. There are good studies showing that pretreatment with a combination of H1 and H2 antagonists can reduce the magnitude and prevalence of adverse reactions during general anaesthesia (Lorenz et al 1994). I believe that there is an alternative interpretation. There is now evidence that the histamine H3 receptors are present in the human heart. They probably play a protective role. When you have H1 and H2 blocking and there is an endogenous release of histamine, it is possible that this can activate the H3 receptor, playing a protective role.

Fisher: Certainly, there are good studies showing that the effects of H1 and H2 blockers will reduce the incidence of rashes, tachycardia and stuffy nose. There are data on radiocontrast media. The last study of contrast media by Lieberman which used a H1 blocker, a steroid and ephedrine reduced the incidence of reactions in the high risk, and adding a H2 blocker actually made things worse (Marshall & Lieberman 1991).We do pretreat patients with previous reactions to contrast media. I appreciate your point. We have just chosen to try to minimize those effects by using the drugs wisely rather than adding more drugs. When I first got involved in this you would see that patient who had thiopentone and a muscle relaxant go bright red. Now most of the patients have had six drugs before the reaction is noticed. It is much harder to sort out. After about three drugs in anaesthesia, I believe the incidence of adverse effects becomes exponentially higher. Yet when I had my anaesthetic I had six drugs and I felt fantastic.

Lasser: We did a very large prospective study involving over 6000 patients in which we pretreated the patients either with corticosteroids given 2 h before, or given 2 h and 12 h before injection of contrast material. We found that the 2 h pretreatment was indistinguishable from placebo, but the combined treatment gave very good protection (Lasser et al 1987).

Fisher: The real trouble we had with the contrast reagents was the study by Lalli (1975) which everyone quotes. There were (I think) 25 patients that had severe anaphylactic reactions to contrast media. He told them that didn't happen in his department, gave them contrast media and they got one rash. It was a bold study, but no one has ever explained (or repeated) it.

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General discussion IV

Physical causes of urticaria

Hölzle: I consider myself a dermatologist and a clinical allergist. I have done some research work on the effects of optical radiation on the skin, and I would like to share some of our observations on solar urticaria as an example of physical urticarias.

Solar urticaria is a rather rare disease. It is a type of physical uriticaria elicited by electromagnetic radiation, mostly UV radiation or visible light radiation. The action spectrum — the eliciting wavelengths — differs among patients. You might think this has nothing to do with anaphylaxis. This is not true. One of the definitions of anaphylaxis we discussed earlier includes a skin sign plus one other organ involvement, such as cardiovascular or pulmonary. Indeed, patients who expose larger areas of their skin will collapse; we think is due to the release of large amounts of histamine. This happens when, for example, the patients try to desensitize themselves in a suntan parlour, or when the treating dermatologist looks at the effect of UV light on the skin of the patient by putting them into a cabin for whole body irradiation. This is an anaphylactic reaction.

We can only hypothesize about the mechanisms involved. We think that in the skin of the patient a photoallergen is formed by irradiation. This means that a precursor molecule is somehow altered by a photochemical reaction thus forming an allergen. Then the patient develops specific IgE against this photoallergen that is bound to the mast cells. When the patient is re-exposed there is histamine release by IgE-dependent mechanisms. In 30% of patients we can find a 'serum factor'. That is, if we take the serum or the plasma of the patient, irradiate *in vitro* with the eliciting wavelengths and inject it back into the skin, we can produce the whealing reaction. This was the basis of the transfer experiments which we cannot do any longer for ethical reasons. This finding is the basis of one treatment modality used on our patients. It is plasmapheresis, which in some patients may achieve permanent cure.

We think that histamine plays an important role in the reaction, but there are also some doubts. We can find histamine in the draining venous blood of areas we have exposed, and after the wheal has formed we also see mast cells degranulate. On the other hand we know that H1 blockers are not very helpful in treating the patients. If you look at the mast cells in the early erythematous phase just a few minutes after exposure, even before the wheal is present, there is no degranulation. But we can detect very early signs, including aggregation of platelets in the vessels and cleft

formation between endothelial cells. With regard to treatment, in less severe cases, protection against the radiation is helpful in combination with H1 blockers. Skin protection is simple and effective, if the patient is sensitive to UV radiation because then we can use sunscreens. With visible light it is much more difficult. In a few patients we can find a plasma factor and then they might respond to plasmapheresis. In other patients we have to stick to induction of tolerance by hardening the skin through repeated exposures to the eliciting wavelength. We apply 2–5 exposures on the same day and then the repeatedly irradiated area remains tolerant.

We looked to see what the causes of this phenomenon could be. It turns out that it is not an exhaustion of the mediators that are released by mast cells, because if we inject histamine-releasing reagents whealing is again induced. Nor do we think it is a lack of the precursor or the allergen itself. If one repeatedly injects activated serum, again the whealing is prevented. We think that in this state of tolerance the IgE on mast cells remains occupied by the allergen, and unless new IgE is bound to the mast cells the skin is no longer reactive. Tolerance lasts for two or three days. If this hardening processes is achieved by a more complicated regimen, which we call photochemotherapy, tolerance lasts for much longer. This involves not only UV light but also a photosensitizer given systemically to the patient. This induces a phototoxic reaction in a controlled fashion. By doing this we find that the patient remains tolerant for a much longer period of time. They can maintain tolerance with photochemotherapy once a week for the entire sunny season.

Before I finish I would like to mention two very striking findings that we haven't yet been able to interpret. One is that from electron microscopic examinations in patients who had repeatedly been exposed and had experienced the whealing reaction, we found a degenerative effect in the nerve endings in the skin, with swelling of nerve fibres and decomposition of membranes. When we performed photochemotherapy, the nerve fibres became intact again. The other finding is that there is one variant of photo-urticaria, which we call fixed-solar urticaria. In this case the skin reacts only in certain areas, which remain stable for at least a couple of weeks. If we compare the morphology of mast cells in involved and non-involved skin, those in involved skin contain lipid droplets.

In summary, solar urticaria is a rare event, but it can lead to anaphylaxis. We think we understand some of the mechanisms, but there are also some striking findings which also might relate to other forms of urticaria.

Galli: In the treatment of the patients who develop anaphylaxis, do you use epinephrine?

Hölzle: No. Changing posture is enough. There have been no fatal cases. Antihistamines might speed this up.

Golden: Is solar urticaria chronic, self-limited and seasonal?

Hölzle: It is chronic. It is self-limited in some patients but takes 10–20 years to resolve. It is seasonal depending on the weather conditions and the climate. If there is continual exposure even to artificial light, it persists throughout the year.

Golden: With plasmapheresis you used the word 'cure'. Is that long-term?

Hölzle: Some patients can be cured. If you treat a group of patients who have this plasma factor, one-third will be cured, there will be a transient effect in a second third, and another third will not respond at all.

Golden: You said that the mechanism behind the phototherapy was not an exhaustion of the activated seroprotein or the substrate protein. You explained this by saying that you disproved this by giving an injection of the activated serum and there was still a reaction.

Hölzle: If you give activated serum there is no reaction in the skin site that is tolerant.

Golden: The other step I would try is to inject the non-activated serum protein and see whether the activating wavelength still activates it. In other words, you might have just depleted the substrate protein by phototherapy.

Hölzle: When we inject the activated allergen, we feel we apply the complete photoallergen and the reaction should occur in any case, unless the mast cell remains unresponsive. This unresponsiveness is obviously specific and we think it is caused by depletion of IgE on mast cells.

Galli: When you detect histamine, is tryptase also detectable?

Hölzle: We haven't looked.

Lasser: Do patients have an eosinophilia?

Hölzle: Not usually. Some of them are atopic. Some have other concomitant forms of physical urticaria.

Sampson: In patients who are cured by single plasmapheresis, what is the mechanism?

Hölzle: It is likely exhaustion of some precursor. Just a few patients who experienced transient amelioration with plasmapheresis were put on photochemotherapy. This led to many months, and in some cases years, without symptoms. Photochemotherapy possibly prevented new formation of specific IgE.

Mosbech: Did you try measuring antibody against the IgE receptor or the IgE molecule? Such autoantibodies have been found in patients with chronic idiopathic urticaria (Skov et al 2003).

Hölzle: No, we did not.

Galli: This has been reported in other forms of urticaria.

Hölzle: There are relatively few patients to study, and it is difficult to persuade them to stay in the hospital for several days for these experiments. But it certainly would be worthwhile to do.

Reference

Skov PS, Platzer MH, Poulsen LK, Bindslev-Jensen C 2003 Total histamine content in blood predicts the presence of autoantibodies against IgE/IgE-receptor in patients with chronic urticaria. J Allergy Clin Immunol 111:S1125 (abstr)

The radiocontrast molecule in anaphylaxis: a surprising antigen

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Abstract. X-ray contrast media are individually injected into human blood vessels in greater quantities than any other pharmacological substance. Adverse reactions to these substances have heretofore been considered anaphylactoid in nature. Others and we have demonstrated that the mechanisms involved are multifactorial and may involve activation of mast cells and basophils, activation of the complement system, activation of the contact system, and the conversion of L-arginine into nitric oxide. Appropriate pretreatment with corticosteriods will diminish the incidence of reactions. Most recently we have demonstrated that the contrast media function as 'pseudoantigens' (PsA). They can combine with antibodies, but cannot themselves produce antibodies. This property appears to be dependent on aggregation in high concentrations and varies with the individual media. It furthermore appears to be non-specific in relation to antibodies, and suggests that binding occurs to the Fc portion of immunoglobulins. We have now demonstrated that the least toxic of current media demonstrate the best antibody binding and in sufficient concentration can inhibit contrast induced mast cell activation and/or non-contrast antigen induced mast cell activation, apparently due to *in vivo* pseudoantigen excess. In lesser concentrations and/or lesser binding, the media can trigger mast cell activation.

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To our knowledge no other pharmaceutical agent is injected into human blood vessels in a single instance in as high a quantity as X-ray contrast media (CM). On the basis of quantity injected/adverse reactions, CM are among the safest of all drugs. Yet the very large number of individuals receiving these substances each year (in 1994 over 16 million intravascular studies were reported in the USA alone) accounts for an appreciable total number of adverse events and a continuing interest in gaining a full understanding of mechanisms involved. Although the incidence of severe reactions is low (about 40–60 per million) the incidence of lesser degrees of severity is more than 10 times higher (Lasser et al 1997).

Since the symptoms of CM reactions resemble those of true anaphylaxis but were (until recently) without convincing evidence of demonstrated antibody–antigen reactivity, they were generally referred to as 'anaphylactoid' in nature.

In this paper we will present data that indicates that the CM do in fact have the potential to act as antigens, but do not have the potential to induce antibody formation. We have therefore termed them 'pseudoantigens' (PsA) These will be shown to be non-specific in antibody activity, apparently binding to the Fc portions of immunoglobulins (Lasser 2000).

Some interesting data enter into any consideration of the dynamics of CM reactions. The chemical composition and size of the CM does not suggest that, *per se*, they could function as classical antigens. They are basically tri-iodinated, fully substituted aromatic rings existing in the ionic form as salts of Na or methylglucamine or made nonionic by coupling to one or more polyhydroxylated alkyl side chains. In either the ionic or nonionic form they may be formulated as monomers or dimers. Except for some early generation, incompletely substituted benzoates the protein binding capacity of these molecules does not suggest the capacity to act as haptens. It is therefore not surprising that no one has been able to induce antibodies by the injection of CM (Carr & Walker 1984). In this general context it is also of interest that CM reactors need not have received any previous CM and that allergic individuals and individuals with a history of a previous reaction are three to five times more likely to experience a severe reaction (Katayama et al 1990).

With this background we explored consecutively the potential roles of histamine release, complement activation, contact system activation and nitric oxide release.

Histamine release

Prior to the demonstration of CM-induced elevated histamine levels in experimental studies and in patients experiencing adverse reactions, there was strong clinical evidence that histamine release must play a role in reactions. The signs and symptoms of adverse CM events resembled those known to be dependent on mast cell and basophil activity, and the empirical use of antihistamines was associated with diminished toxicity. To obtain more specific data we carried out a series of studies in dogs (Lasser et al 1974). In these we noted that sequential intravascular monomeric ionic CM (Na or methylglucamine iothalamate) injections carried out approximately 12 minutes apart induced a potentiation of histamine release. A more interesting (and counterintuitive) observation was that comparisons of identical quantity injections lasting 2 s with injections lasting 39 s demonstrated higher levels of histamine release with the slower injection in over 80% of 27 determinations. The significance of this did not become clear until years later and will be discussed in a subsequent section. Another interesting finding in these studies was that the methylglucamine ion appeared to play a prominent part in histamine

release. This ion, in the form of methylglucamine chloride was capable of releasing histamine, but in combination with the CM anion more histamine was released. No nonionic CM studies were carried out.

Complement activation

Complement activation studies were carried out in view of the role played by this system in other forms of anaphylaxis. In these studies we noted that:

- CM *invitro* induced the activation of several complement components in normal, genetically C2 deficient and agammaglobulinaemic human sera. The activation was dose-dependent and was demonstrated by a reduction in whole complement as well as C4, C2, C3 and C5 haemolytic activities. Concomitant with the loss of C3 haemolytic activity was the appearance of C3 proteolytic cleavage products. Both the loss of C3 haemolytic activity and the production of C3 fragments occurred in the presence of EDTA indicating that CM induced C3 cleavage occurred without participation of the multicomponent C3/C5 convertases of either the classical or alternative complement pathways that require Mg^{2+} (Kolb et al 1978).
- *Invivo* studies in a group of patients (numbers in square brackets) reacting to CM injections and a group not reacting demonstrated that the baseline as well as post-injection serum samples for total complement (CH 50) in the reactors were significantly lower than in the non-reactors (baseline: 78.7 ± 6 units [14] vs. 99.7 ± 3.9 units [17], $P = 0.01$. post CM: 67.5 ± 5.8 units [18] vs. 94.8 ± 3.99 units [17] $P < 0.01$; two-tailed t test) (Lang et al 1976). One possible explanation for the CM induced complement activation in the absence of Mg^{2+} might be found in recent papers describing the actions of mannan-binding lectin. This member of the collectin family may bind directly to carbohydrates on the surfaces of potential microbial pathogens and this lectin and associated serine proteases can replace complement components C1q, C1r, and C1s of the classical complement pathway leading to cleavage of C4 and C2 of this pathway (Wallis & Dodd 2000, Tan et al 1996). For the moment, however, the notion that CM somehow activates the mannan-binding lectin system to produce complement activation must remain speculative.

Contact system activation

Activation of this system occurs when factor XII, the initiating factor of the intrinsic coagulation system, is activated to form factor XIIa. Sequential activation of prekallikrein to kallikrein and cleavage of high molecular weight kininogen to form bradykinin then results. The bradykinin thus formed has the potential to

metabolize arachidonic acid and produce leukotrienes and vasoactive prostaglandins via the respective lipoxygenase and cyclooxygenase pathways. To explore the possible role of these factors in CM adverse reactions we devised an assay that allowed us to determine the rate of transformation of plasma prekallikrein to kallikrein in plasma samples (Lasser et al 1981a). To accomplish this we added a known factor XII activator (500 kDa molecular weight dextran sulfate) to plasmas at 0 °C and then assayed for kallikrein. The 0 °C temperature obviated the influence of inhibitors. Using this assay or modifications of the assay we found that with 30 minutes incubation the production of kallikrein in known reactors significantly exceeded that in known non-reactors (440 ± 70 units [17] vs. 145 ± 10 units [22] $P < 0.002$; two-tailed t test). A group of patients with atopic asthma also produced kallikrein significantly faster than controls (Lasser et al 1983). At 30 minutes incubation the values were 420 ± 40[19] vs. 210 ± 10[19] $P < 0.002$; two-tailed t test. With this assay we also found that the addition of commercial heparin increased conversion rates. Since heparin as well as histamine is released by activated mast cells we then utilized an assay that would release endogenous heparin from plasma neutralizing proteins (Lasser et al 1987a). We found that the baseline samples of a group of patients with asthma contained significantly higher levels of an endogenous antithrombin heparin-like material than did non-allergic, non-asthmatic controls (1401 ± 340 units [17] vs. 210 ± 34 units [17] $P < 0.02$; two-tailed t test). To further explore these matters we similarly assayed the plasmas of six patients responding to inhalational antigen challenge. In three of these, challenge produced an immediate increase in a plasma heparin-like material concordant with similar increases in coagulation indexes and kallikrein activation that coincided with a fall in FEV_1 values (Fig. 1). Given all of the above, it suggests that the increased CM reactivity found in allergics and asthmatics may depend in part on the pre-existing presence of this heparin-like material, most likely representing material released from mast cells.

As in allergy, anaphylactic reactions in CM administration may have late, as well as immediate manifestations. These predominantly take the form of an urticarial-like rash but may occasionally be manifest as bronchospasm (Munechika et al 1998). We have found that the maximal complexing of α2-macroglobulin with kallikrein occurs at a temperature of 22–24 °C rather than at 37 °C and that the protease expressivity of the compound is also maximal at lower temperatures (Lasser et al 1991). In the same study we demonstrated that contact activated plasma containing α2-macroglobulin and high molecular weight kininogen, but no other contact system factor, produced sustained permeability changes when injected intradermally into guinea pigs. While α2-macroglobulin–kallikrein has weaker proteolytic activity than free kallikrein, the much longer persistence of the complex in tissues or in the circulation allows the complex to continue enzymatic activities towards factor XII and high molecular weight kininogen for sustained

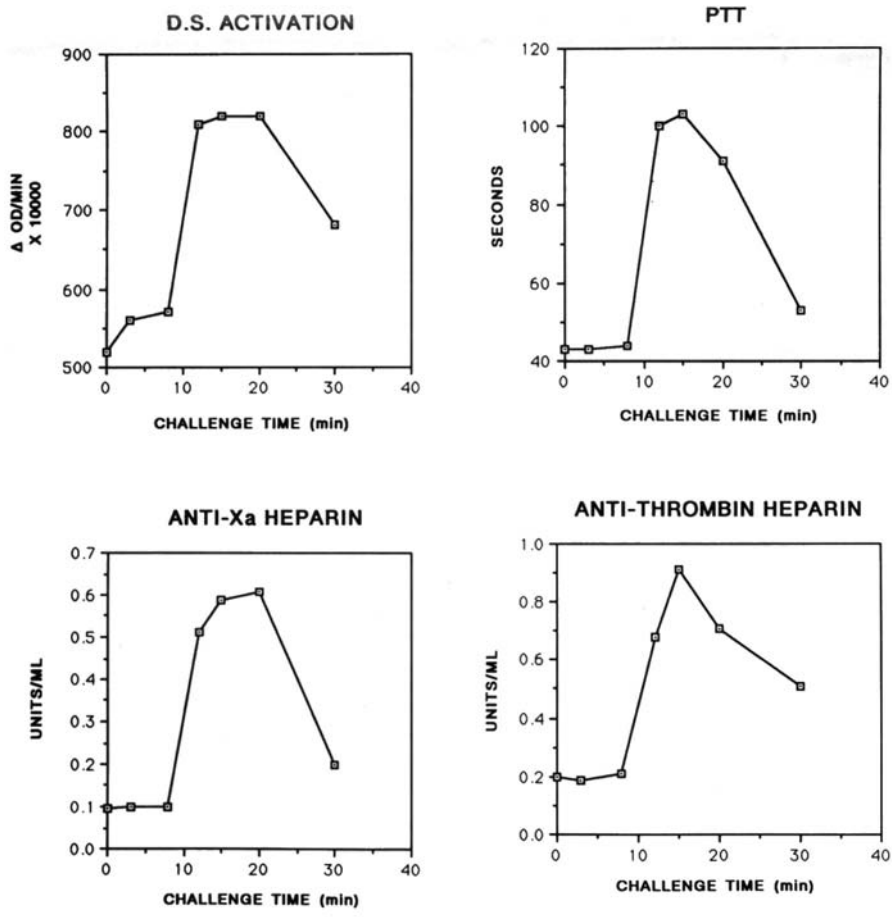


FIG. 1. A patient with a known allergy to rabbit dander was subjected to an inhalational challenge with that dander and arterial samples were collected at 10 minute intervals following the onset of bronchospasm and a fall in FEV_1 of 52%. The samples were analysed for heparin-like material active against thrombin and against factor Xa using a technique that displaced the heparin-like material from plasma binding proteins (Lasser et al 1987a). An anticoagulant assay (partial thromboplastin time) was also done. The dextran sulfate (D.S.) activation chart indicates the amount of free kallikrein present after 30 minutes incubation of the D.S. activated plasma samples at 0 °C. Note concordance of all the curves. This supports the concept that the anticoagulant heparin-like substance released at the onset of anaphylaxis is responsible for the contact system activity in antigen and in PsA-induced reactions and for a temporary increase in plasma anticoagulant activity.

periods that may outweigh the greater activity of free kallikrein (Vogt & Dugal 1976). With this in mind it is of interest that the skin and the bronchi are the two tissues in the body capable of maintaining temperatures that approach ambiance. It seems therefore possible that the permeability changes that occur in late and delayed reactions may in part be a manifestation of the enhanced α2-macroglobulin–kallikrein complexing and expressivity that occur at the lower temperatures.

Nitric oxide release

Since the release of NO produces a fall in blood pressure (and other reactions) and since it has been reported that the release of histamine can induce the release of NO via an effect on H2 receptors (Mannaioni et al 1997), it was thought worthwhile to study the possible role of NO in CM anaphylaxis. In addition to histamine-derived release, the synthesis of NO occurs in response to a number of other stimuli including bradykinin and flow increases. To study this effect we subjected both Brown-Norway and Sprague-Dawley ovalbumin-sensitized rats to infusions of either methylglucamine iothalamate (60% Conray; Mallinckrodt Pharmaceutical) or normal saline. The infusions were carried out at a rate of 1.5 ml of CM/min per kg and were continued until the cessation of respiration (Lasser & Lamkin 1994). Brown-Norway rats are known to be hyperimmune and when sensitized the rats exhibited an average IgE level of 2029 ng/ml. Sprague-Dawley rats when sensitized had an IgE level that was 10 times less than the non-sensitized level of the Brown-Norway rats. The CM infusions were accompanied by an infusion of either L-arginine analogues (L-NMMA, L-NAME, L-NA) or the same D-arginine analogues or normal saline injected 2 minutes before and in conjunction with the CM. Arginine is the major substrate for NO and L-arginine, but not D-arginine analogues inhibit NO release. All of the L-analogues (L-enantiomers) protected the Brown-Norway rats in these lethal dose studies, but had no effect on the Sprague-Dawley rats. (In 17 Brown-Norway rats the infusion of L-arginine analogues permitted 14.5 ± 1.5 g I/kg of Conray to be administered before LD_{100} levels were attained while infusions of D-arginine or saline in 10 Brown-Norway rats permitted only 10.5 ± 1.1 g I/kg to be administered before LD_{100} was reached, $P < 0.0005$.) H1 blockade also protected Brown-Norway. None of these measures altered the CM tolerance of the Sprague-Dawley rats.

How do the CM react with mast cells and basophils?

From the above data a case can be made that the primary event in CM anaphylaxis could be an action on mast cells and possibly basophils that results in the release of histamine and heparin. Histamine in turn releases nitric oxide and heparin activates

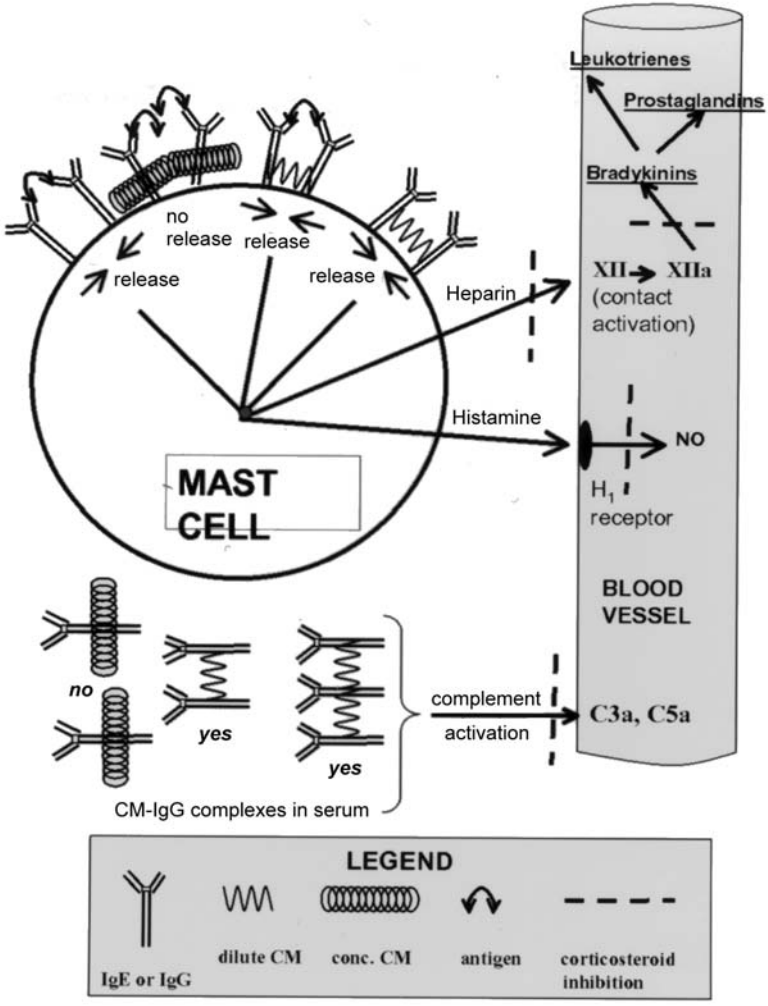


FIG. 2. Diagram of CM anaphylaxis depicts conceptual role of mast cell and CM acting in PsA–antibody equivalence and in PsA excess. Mast cell activation with the release of histamine and heparin is depicted in the presence of dilute CM (PsA) alone, in combination with an unrelated antigen, or with an unrelated antigen alone (converging arrows). In the presence of higher CM (PsA) concentrations and/or with greater antibody combining potential the PsA acts to either pre-empt antibody binding sites or to produce (by steric hindrance) dissociation of previously bound unrelated antigen, and no mast cell release occurs. The release of heparin and histamine accounts for the subsequent release of nitric oxide, leukotrienes and prostaglandins. Although the diagram depicts complement activation occurring (or not occurring) by virtue of PsA immunoglobulin aggregation, the aetiology of this activity remains obscure. Corticosteroid inhibition of CM related adverse events are depicted by the dotted lines.

the contact system. As noted earlier, however, CM molecular structures do not appear to be likely candidates as antigens or haptens.

Several years ago we conducted a study that has led to clarification of these issues. In passive RBC haemagglutination inhibition studies carried out with a number of ionic and nonionic CM we found that all the tested media at varying concentrations inhibited the specific interaction of RBCs sensitized with either ovalbumin or γ globulin with their respective antibodies (Lasser & Lamkin 1998). This is a highly sensitive assay and the surprising finding in this study was that the CM with the least clinical toxicity were better inhibitors than the more toxic CM. When testing the potential for CM inhibition of γ globulin binding to anti-γ globulin antibodies for example, we found that the lowest concentration of CM demonstrating inhibition of γ globulin binding at a 1/500 dilution of IgG anti-γ globulin varied from 8.0 mg I/ml to 28.2 mg I/ml. An ionic dimer inhibited binding at the lowest CM concentration, nonionic monomers were intermediate and ionic monomers necessitated the highest concentrations. (Nonionic dimers were not tested.) Since the CM best inhibiting the specific antibody–antigen binding did so by occupying the antibody site to the exclusion of the specific antigen, it should follow that the same CM would be the most likely to bind to IgE on mast cells and release the mediators involved in anaphylaxis. Exactly the opposite was true.

At this point, then, we were dealing with three counterintuitive data sets.

- CM molecules do not have the structural characteristics of multivalent antigens but exhibit the antibody binding of such.
- A faster injection of CM releases less histamine than a slower injection.
- CM that appear to bind to two quite divergent antibodies do so in a manner that is inverse to their histamine releasing potential.

The answer to data set 1 appears to be that the CM are now recognized to form aggregates that vary with the individual media, being greatest for the nonionic dimers, intermediate for the nonionic monomers, and least for the ionic monomers (Krause et al 1994, Speck et al 1998). Aggregation increases in high concentrations. The aggregates evidently have a morphology that more nearly approaches that of multivalent antigens and the CM showing the best binding potential are the same CM that show the greatest potential for aggregation.

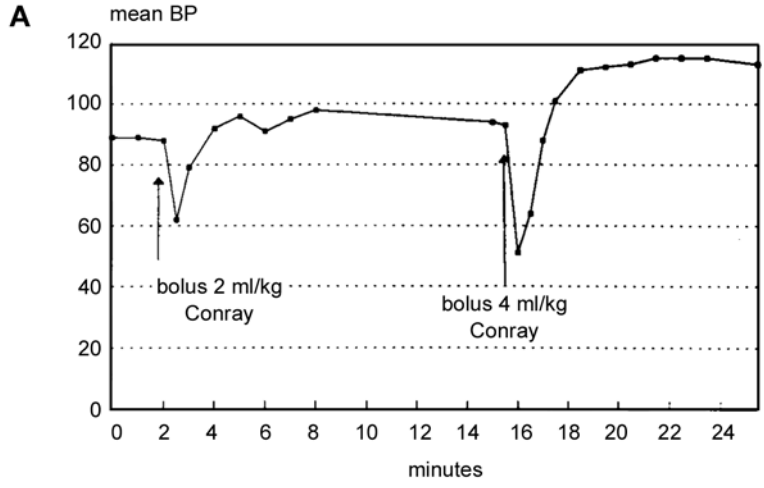
The answer to data set 2 is best found in bringing the concept of 'antigen excess' from *in vitro* to *in vivo*. To our knowledge this has not been done before, but no other pharmaceutical is given to patients intravascularly in the dosages used with the CM. With this concept the faster CM injection produced a higher concentration of the PsA and invoked a state of relative PsA–antibody excess, either diminishing or inhibiting mast cell activation. At slower injection rates the CM concentration

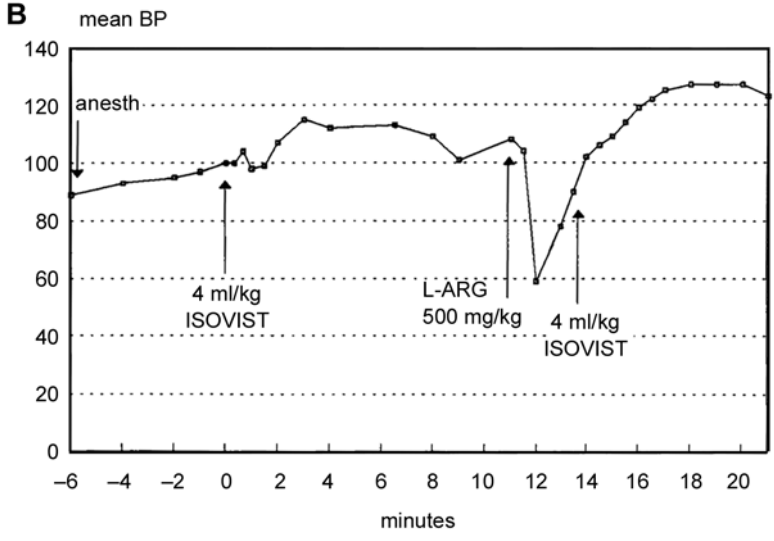
will be lower and in this circumstance there will be PsA–antibody equivalence rather than excess, resulting in mast cell activation (Fig. 3).
The answer to data set 3 is twofold.

- The binding to two divergent antibodies indicates that the binding is non-specific and must be to the Fc portion of the molecule. Additionally, a report indicated that three different tumour antigens exhibited reduced binding to their respective antibodies in the presence of CM (Watanabe et al 1998). While no explanation was forthcoming, it appears likely that this resulted from successful CM competition for the specific tumour antibodies. All of this suggests that the PsA fill the space between adjacent Fc areas on mast cells and in sufficient concentration exhibit steric hindrance to antigen bridging of the adjacent immunoglobulins, thus inhibiting mast cell release. Austen et al (1965) had earlier advanced a somewhat similar concept. These investigators indicated that the sensitization sites on mast cells could be pre-empted by anaphylactic antibody to naturally occurring antigens, or blocked due to steric hindrance by another immunoglobulin. In lesser concentrations, CM evidently promote the bridging of adjacent immunoglobulins since cell activation occurs (Fig. 2).
- The binding potential of the least toxic CM (the nonionic dimers), being greater than other CM molecules puts these CM in the best position to attain PsA excess at lesser concentrations than other media.

To further study the concept of PsA excess *invivo* we carried out a series of studies of Sprague-Dawley rat arterial blood pressures when subjected to bolus intravenous injections of CM or an H1 blocker (diphenhydramine) or a L-arginine analogue (L-NAME). We believed from earlier studies that the basal blood pressures reflected in part an ongoing release of histamine and nitric oxide probably mediated by endogenous antigens binding to mast cell IgE. This was substantiated by an immediate elevation of arterial pressure on injection of either of these substances (Lasser & Lamkin 2002). Injection of an ionic monomer (methylglucamine iothalamate, Conray: Mallinckrodt) produced a fall in pressure followed by an overshoot (Fig. 3A). Injection of an ionic dimer (ioxaglate, Hexabrix: Guerbet, Paris) or a nonionic dimer (iotrolan, Isovist: Schering AG, Berlin) produced an immediate elevation that simulated the patterns shown by the H1 blocker or the NO inhibitor. This elevation did not occur if nitric oxide production was inhibited by injection of a L-arginine analogue (Fig. 3B,C). Finally, we examined the effects of Hexabrix, an ionic dimer or a nonionic dimer (Visipaque, Iodixanol, Nycomed, Oslo) when injected in a bolus 45 minutes before, concomitant with, or 10 minutes after injection of an acute anaphylactic dose of ovalbumin in an ovalbumin-sensitized rat (Fig. 4A,B). In each instance, the blood pressure lowering effect of

BROWN NORWAY RAT #1496 – BLOOD PRESSURE CHANGES
WITH 2–4 ml/kg CONRAY IV





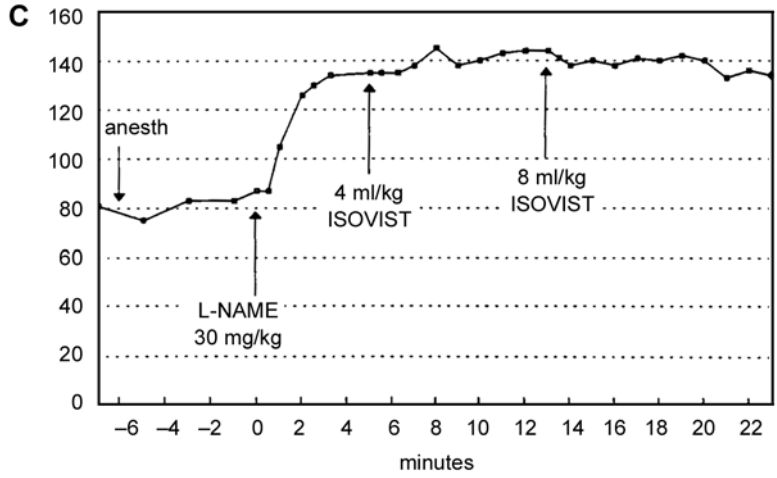


FIG. 3. Arterial mean blood pressures in rats were measured through a catheter inserted in the carotid artery. Bolus injections were into a tail vein. In 3A bolus injections of Conray, an ionic monomer, (methylglucamine iothalamate) produce an immediate fall in pressure followed by an overshoot that is proportional to the drop in pressure. In 3B the bolus injection of 4 ml/kg of Isovist, a nonionic dimer, produces a slight fall in pressure followed by a sustained rise. At 10 minutes an injection of L-arginine the substrate of nitric oxide (NO) produces a sharp fall in pressure and at 14 minutes a repeat bolus of Isovist produces a greater elevation in pressure (reproduced from Lasser & Lamkin 2000, with permission). In 3C an injection of L-NAME, an inhibitor of NO, produces an increase in pressure that then inhibits any further elevation of pressure with subsequent injections of Isovist. These findings support the concept that the dimeric nonionic PsA differ from the ionic monomeric PsA by not only failing to produce a significant pressure drop on injection, but actually producing an elevation in baseline pressure (which does not occur when the baseline pressure is already elevated by removing the influence of NO). It seems likely that the pressure elevation that follows the injection of dimeric CM is due to pre-emption of endogenous antigen binding to immunoglobulins on mast cells and the resultant steric hindrance of ongoing histamine/NO release.

the ovalbumin injection was ameliorated by the presence of the CM. When the CM was injected 10 minutes after the ovalbumin at the nadir of the blood pressure fall, the rise in pressure was immediate. This indicated that the CM PsA displaced bound ovalbumin from IgE mast cell binding sites and/or competed successfully with new ovalbumin for IgE binding sites.

Corticosteroid inhibition

There is good evidence that all of the adverse effects noted above can be diminished in occurrence when corticosteroids are administered at a sufficient interval prior to CM injection (at least 2 hours or more prior to CM injection) (Lasser et al 1987b).

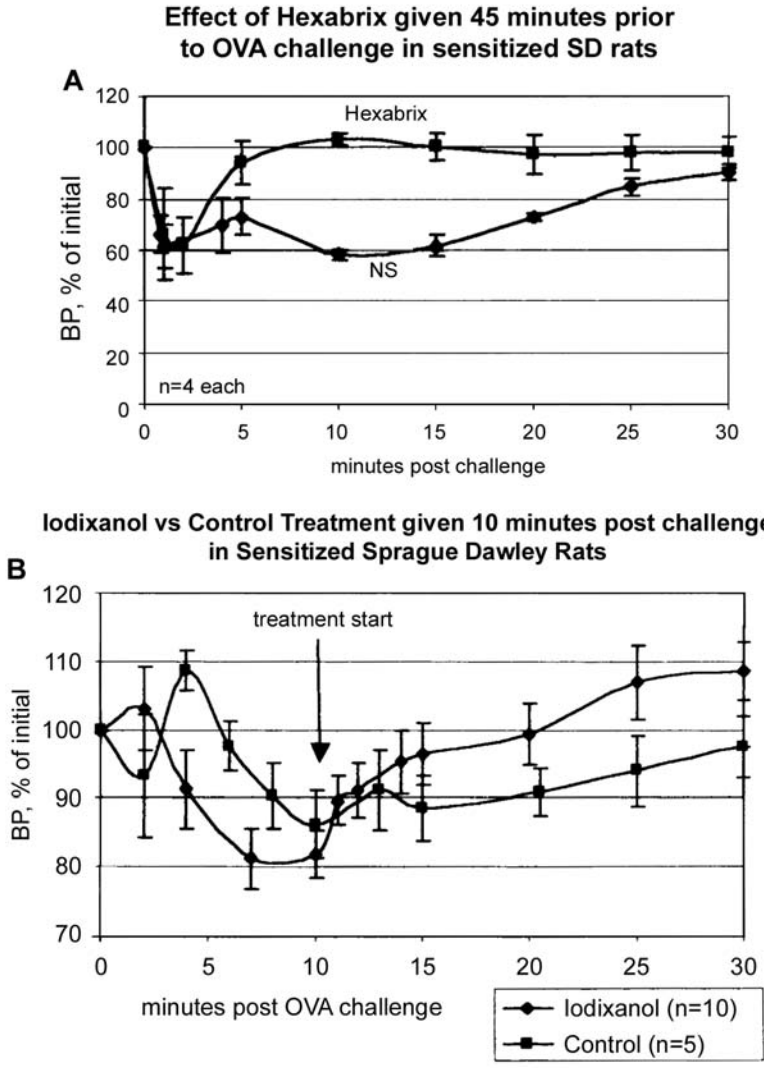


FIG. 4. Sprague-Dawley rats were sensitized to ovalbumin and challenged with that antigen 10–14 days later. Blood pressure effects are represented as percentage of initial mean arterial b.p. ± SEM. In 4A the effect of a 3 ml bolus of the ionic dimer Hexabrix (Na/methylglucamine ioxaglate) given 45 minutes prior to antigen challenge is seen in comparison to a 3 ml bolus of normal saline. In 4B the effect of a 3 ml bolus of Iodixanol (visipaque) a nonionic dimer, is compared with equiosmolar saline injected at 10 minutes post antigen challenge, when the blood pressure nadir from the challenge occurred. In both 4A and 4B it is evident that the dimers were responsible for increasing the antigen-induced fall in pressure to return towards normal more quickly. This appears to be another example of the PsA effect on the pre-emption of antigen-binding sites on antibodies bound to mast cells.

One mechanism for corticosteroid protection may be corticosteroid-induced elevation of C1 esterase inhibitor (Lasser et al 1981b), since this is the major inhibitor of the classical complement pathway and also the major inhibitor of the contact system.

Conclusion

X-ray CM reactions are multifactorial in nature, are launched by CM reactivity with IgE on mast cells releasing histamine, heparin and NO, and involve at a minimum, activation of the complement and contact systems. The CM act as PsA (they do not produce antibodies). The least toxic of the CM appear to act in PsA excess and inhibit mast cell activity by apparently binding to the Fc portions of IgE on mast cells and producing steric hindrance of bridging by adjacent immunoglobulins. NO, probably secondary to histamine release, may play a role in some reactions. Corticosteroids can diminish the incidence of reactions.

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DISCUSSION

Galli: Are you able to model *in vitro* the proposed interaction between the contrast agents and antibodies that induces mast cell activation? Are there *in vitro* data to support this?

Lasser: The only *in vitro* data we have are those of the haemagglutination inhibition assay. Many others have demonstrated histamine release on contrast media–mast cell incubations.

Austen: In view of your interesting data showing the interaction between the contrast material and immunoglobulin, and your data on the complement pathway without a cation requirement, am I correct in assuming that the contrast material is interfering with the *in vitro* assay?

Lasser: Our *in vitro* studies don't show this. I think something else is happening that allows complement activation in the absence of metals. It may be the third pathway that is now being talked about, involving mannose binding lectin.

Austen: That also has metal requirements.

Lasser: Yes, but it will also occur in the absence of the metal ions. There is a paper showing this (Kawasaki et al 1983). I don't know why complement is being activated, but is clearly not by direct action of contrast media.

Marone: I was really impressed by the transient decrease of C3 and C4 during the injection of radiocontrast media. This lasted for just a couple of hours. Might this transient decrease be due to an expansion of the plasma volume due to the injection of the hyperosmolar radiocontrast media?

Lasser: No, because IgM didn't show this transient decrease at all. Incidentally, C3 was converted to C3a.

Marone: Did you also evaluate the formation of C3a and C5a?

Lasser: Not C5a, but we did this for C3a and we found that this occurred.

Marone: Still, this was a very transient phenomenon.

Reference

Kawasaki N, Kawasaki T, Yamashina I 1983 Isolation and characterization of a mannan-binding protein from human serum. J Biochem (Tokyo) 94:937–947

General discussion V

Fatal course of *Vespula* venom immunotherapy: pretreatment withdrawal of the β blocker may have been involved

Müller: I want to describe a case that occurred in Switzerland about a year ago and caused quite a bit of concern. It was a 40 year-old woman who died during immunotherapy with vascular venom. I was asked by the local legal authorities to give an expert verdict on this case.

Before immunotherapy is started, it is generally recommended that β-blocking agents should be stopped because they might aggravate allergic reactions to allergen injections by interfering with endogenous defence mechanisms and medical treatment.

Case report

A 40 year-old patient was referred to an Allergy centre by her family doctor because of repeated generalized allergic reactions to *Vespula* stings, the last in 2001 with dypnoea, dizziness, tachycardia to 132/min, angioedema of the face and generalized erythema. Her i.c. skin test was positive at 0.01 g/ml, and *Vespula*-specific IgE were positive class 3 (3.5 kU/l). The patient suffered further from severe obesity and arterial hypertension, and because of ventricular arrhythmia (Lown class IVb) she had been receiving the β blocker bisoprolol, 5 mg daily since 1999.

In view of the repeated severe sting reactions and the documented sensitivity to *Vespula* venom, immunotherapy with *Vespula* venom was indicated. It was further recommended by the consulted allergist to replace the β-blocking agent. The family doctor chose indapamide, an antihypertensive diuretic for this replacement. Immunotherapy was started at the outpatient unit of a large hospital centre under antihistamine premedication (cetirizine 10 mg 30 min before injection) with 0.02 μg of a commercial aluminium precipitated extract injected s.c. in the upper arm. This first injection was tolerated without any problems. One week later the patient received, again under antihistamine premedication, the second injection of 0.04 μg s.c. Thirteen minutes later she complained about slight paresthesias on the head, felt thirsty and got some Coca-Cola. One minute later she collapsed and was found pulseless. Cardiopulmonary resuscitation was started immediately. The initial electrocardiogram showed ventricular fibrillation. The patient had to be defibrillated repeatedly, was

intubated and ventilated, received adrenaline, dimetinden and prednisolone i.v. as well as infusions with sodium chloride.

Her cardiovascular and respiratory situation was stabilized within 30 minutes. The patient, however, stayed in coma for the next few days. The tendon reflexes were increased and pyramidal signs bilaterally positive. Computed tomography revealed diffuse cerebral oedema and electroencephalography showed diffuse non-specific alterations, together indicating the presence of severe hypoxic brain damage.

The patient died 3 days later without regaining consciousness. Serum tryptase was 2.7 μg l 2 months before starting immunotherapy and 3.5 μg l 5 hours after the start of the adverse reaction.

Discussion

The cause of this fatal reaction cannot be determined unequivocally. Certainly, the absence of any allergen-specific symptoms, the lacking increase of serum tryptase and the extremely low venom dose applied are evidence against an anaphylactic reaction, without completely excluding it. On the other hand it must be considered that the withdrawal of the β-blocking agent in this patient with known severe ventricular arrhythmia contributed to the fatal outcome.

Conclusions

The withdrawal of β blockers in patients with cardiovascular disease, especially severe arrhythmia and coronary heart disease before immunotherapy has to be evaluated very carefully under consideration of the relative risk of the allergic versus the cardiac disease.

Epinephrine (adrenaline) in the first-aid, out-of-hospital treatment of anaphylaxis

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Abstract Epinephrine (adrenaline), the initial treatment of choice for systemic anaphylaxis, is an α- and β-adrenergic agonist with bidirectional, cyclic adenosine monophosphate-mediated pharmacological effects on target organs, and a narrow therapeutic index. In a recent study, 0.95% of a geographically-defined population was found to have had epinephrine dispensed for out-of-hospital use; dispensing rates within this population varied from 1.44% for individuals under age 17 years to 0.32% for those older than 65 years. Although epinephrine is widely available in the community, it is not necessarily given in a timely manner when anaphylaxis occurs. Individuals with anaphylaxis may fail to respond to first-aid treatment with epinephrine for a variety of reasons. These include: (1) delay in treatment (in an animal model, epinephrine injection at the nadir of shock fails to provide sustained haemodynamic recovery); (2) administration of epinephrine by sub-optimal routes such as subcutaneous injection or inhalation from a pressurized metered-dose inhaler instead of intramuscular injection; (3) administration of an inappropriately low epinephrine dose due to the limitations currently imposed by the availability of only two fixed-dose auto-injectors: EpiPen® Jr 0.15 mg or EpiPen® 0.3 mg; and (4) injection of 'outdated' epinephrine, with inadvertent administration of an inadequate dose. Additional fixed-dose formulations of epinephrine are needed to facilitate optimal first-aid dosing in patients of all ages and sizes.

2004 Anaphylaxis. Wiley, Chichester (Novartis Foundation Symposium 257) p 228–247

Pharmacology of epinephrine

Epinephrine (adrenaline) is the initial treatment of choice for systemic anaphylaxis. Most episodes of anaphylaxis occur outside of a health care setting (Simons et al 2003, Yocum et al 1999). Individuals in the community at risk for anaphylaxis, or those responsible for infants and children at risk for anaphylaxis, should be equipped with epinephrine for prompt injection if anaphylaxis recurs. The

TABLE 1 Pharmacology of epinephrine

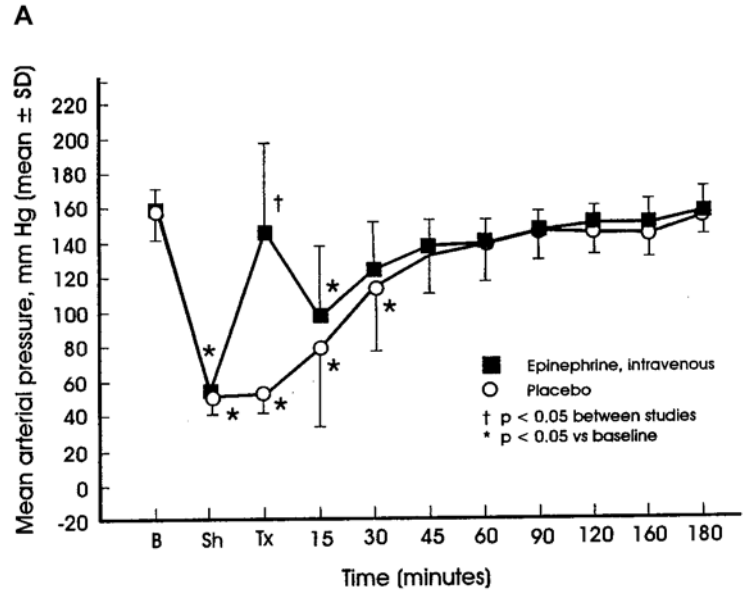
α_1	↑ vasoconstriction, ↑ peripheral vascular resistance, ↓ mucosal oedema
α_2	↓ insulin release, ↓ norepinephrine release
β_1	↑ inotropy, ↑ chronotropy
β_2	↑ bronchodilation, ↑ vasodilation, ↑ glycogenolysis, ↓ release of mediators (e.g. histamine, leukotrienes) from mast cells/basophils

Based on Hoffman (2001).

epinephrine should be prescribed in the context of a patient-specific anaphylaxis management plan that provides information about how to avoid trigger factors, recognize symptoms and signs, inject epinephrine and seek medical assistance after first-aid treatment with epinephrine (American Academy of Allergy Asthma & Immunology Board of Directors 1998, Ewan & Clark 2001).

Epinephrine is a sympathomimetic α- and *β*-adrenergic agonist with cyclic adenosine monophosphate-mediated, complex, bidirectional pharmacological effects on target organs. In anaphylaxis, its α_1-adrenergic effects (vasoconstriction, increased peripheral vascular resistance and decreased mucosal oedema) and some of its β_2-adrenergic effects (bronchodilation and decreased mediator release from mast cells and basophils) are of primary importance (Hoffman 2001) (Table 1). Achieving high plasma and tissue epinephrine concentrations rapidly appears to be critical for patient survival. *In vitro*, low concentrations of epinephrine paradoxically enhance release of histamine and other mediators of anaphylaxis from mast cells and basophils (Austen et al 1974). In an animal model, epinephrine given at the nadir of shock fails to produce sustained haemodynamic recovery despite elevation of plasma epinephrine concentrations (Bautista et al 2002) (Fig. 1). In humans, at very low epinephrine concentrations, the undesirable β_2-adrenergic effect of vasodilation has been demonstrated. In anaphylaxis treatment, an inappropriately low dose of epinephrine on a mg/kg basis might do more harm than good. Even if injected promptly, epinephrine is not always effective (Pumphrey 2000, Yunginger et al 1998, Sampson et al 1992, Bock et al 2001).

Epinephrine has a narrow toxic–therapeutic index (risk-to-benefit ratio). Administered systemically by any route, it commonly causes pharmacological adverse effects such as anxiety, fear, restlessness, headache, pallor, tremor, dizziness, or palpitations. Uncommonly, even in the recommended dose of 0.01 mg/kg to a maximum of 0.3 mg or 0.5 mg intramuscularly or subcutaneously, and especially if given in an overdose, it may lead to an increased QTc interval, ventricular arrhythmias, angina, myocardial infarction, pulmonary oedema, dramatic increase in blood pressure and intracranial haemorrhage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Time (minutes)
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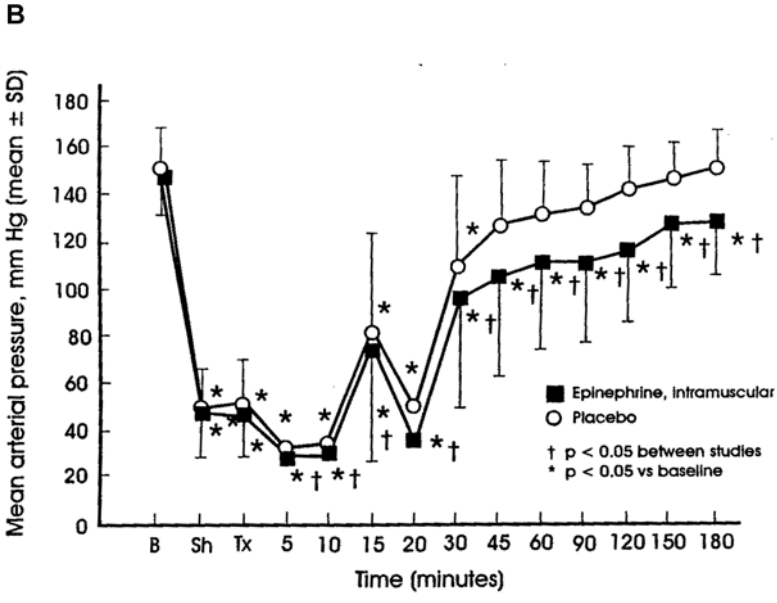
† p < 0.05 between studies
* p < 0.05 vs baseline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Epinephrine, intramuscular
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* p < 0.05 vs baseline

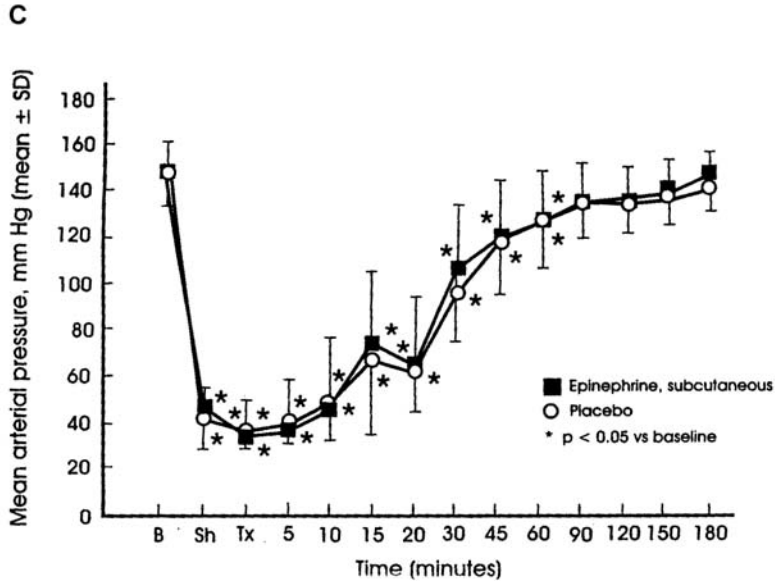


FIG. 1. In fully-developed canine anaphylactic shock, epinephrine failed to produce sustained haemodynamic recovery. In a prospective, randomized study of ragweed-sensitized dogs, epinephrine was administered intravenously (IV), intramuscularly (IM), or subcutaneously (SQ) at 5 week intervals. The animals were studied while ventilated and anaesthetized. Treatment occurred at maximum hypotension. Haemodynamics were followed for 3 h after shock. Mean arterial pressures (MAPs) are shown after (A) epinephrine IV; (B) epinephrine IM; and (C) epinephrine SQ. Compared to the no-treatment studies, epinephrine IV produced a transient immediate increase in MAP (144 vs. 52 mmHg, $P < 0.01$), stroke volume (32 vs 12 ml), and pulmonary wedge pressure (9 vs. 5 mmHg, $P < 0.01$), but no differences were observed 15 minutes after shock. Haemodynamics were not different between epinephrine IM or SQ and no treatment at any time point. Compared with the placebo study, plasma epinephrine concentrations were higher in the IV and IM studies but not in the SQ studies. B, baseline; SH, shock; Tx, treatment; $*P < 0.05$ vs. baseline; $\dagger P < 0.05$ between studies.

(Hoffman 2001). Individuals who are particularly prone to epinephrine adverse effects include those with pre-existing cardiovascular disease; cocaine users, in whom it stays at the site of action for a prolonged time; and patients with untreated hyperthyroidism, who have increased numbers of β-adrenergic receptors on the vasculature.

Epinephrine for first-aid treatment of anaphylaxis in the community

During the past few years, information about epinephrine availability and use in the first-aid, out-of-hospital treatment of anaphylaxis has been obtained by using a variety of research tools.

Population-based studies: epinephrine is widely dispensed

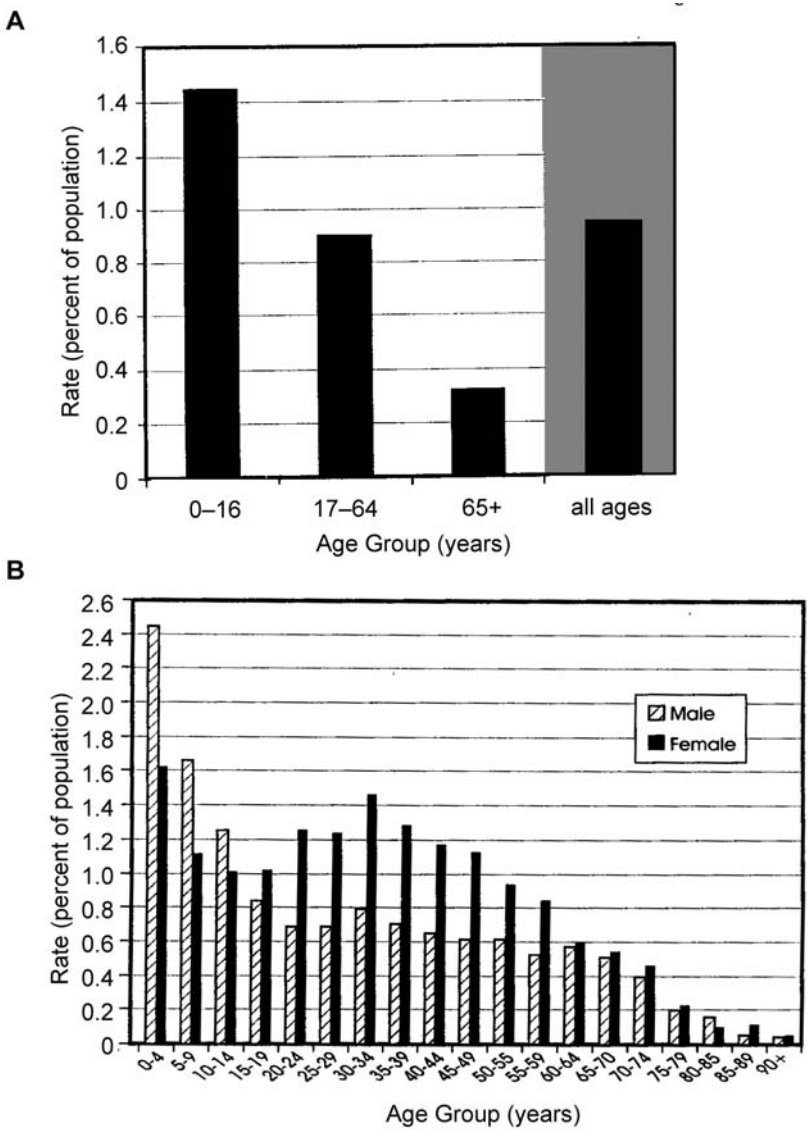
In a geographically defined population of 1.2 million individuals, a prescription administrative claims database involving real-time computer links with retail pharmacies was used to identify the epinephrine formulations dispensed in the community, along with the age and sex of persons for whom the dispensings were made (Simons et al 2002a). During a five year period, 0.95% of the general population had injectable epinephrine dispensed, with rates ranging from 1.44% for individuals younger than 17 years of age, 0.9% for those 17–64 years, to 0.32% for those age 65 years or older (Fig. 2A). In infancy, childhood and early adolescence, boys were more likely to have epinephrine dispensed than girls; indeed, the highest epinephrine dispensing rate, 5.3%, was found for boys age 12–17 months. Beginning at age 15 years and continuing into adulthood, girls and women were more likely to have epinephrine dispensed than boys and men. In the elderly, epinephrine dispensing rates did not differ between the sexes (Fig. 2B).

In the same geographically defined population, using the same administrative claims database, the mean age of transition from EpiPen® Jr (0.15 mg) to EpiPen® (0.3 mg) was found to be 6 years 6 months (range 1 year 10 months to 16 years 11 months) (Simons et al 2001a) (Fig. 3A,B). EpiPen® Jr and EpiPen® auto-injectors were both dispensed over almost the entire paediatric age range: EpiPen® Jr from 2 months to 16 years 10 months, and EpiPen® from 1 year 8 months to 16 years 11 months. In general, by the age of 6 years 6 months, fewer than 3% of children have attained a weight of 30 kg, and therefore, when treated with an EpiPen®, most children younger than 6 years 6 months receive a higher dose than the optimal dose of 0.01 mg/kg. Fixed-dose formulations of epinephrine of 0.05 mg, 0.1 mg, 0.2 mg and 0.25 mg are needed in addition to the 0.15 mg and 0.3 mg doses currently available, in order to facilitate accurate dosing of 0.01 mg/kg over the entire age and weight range of the paediatric population.

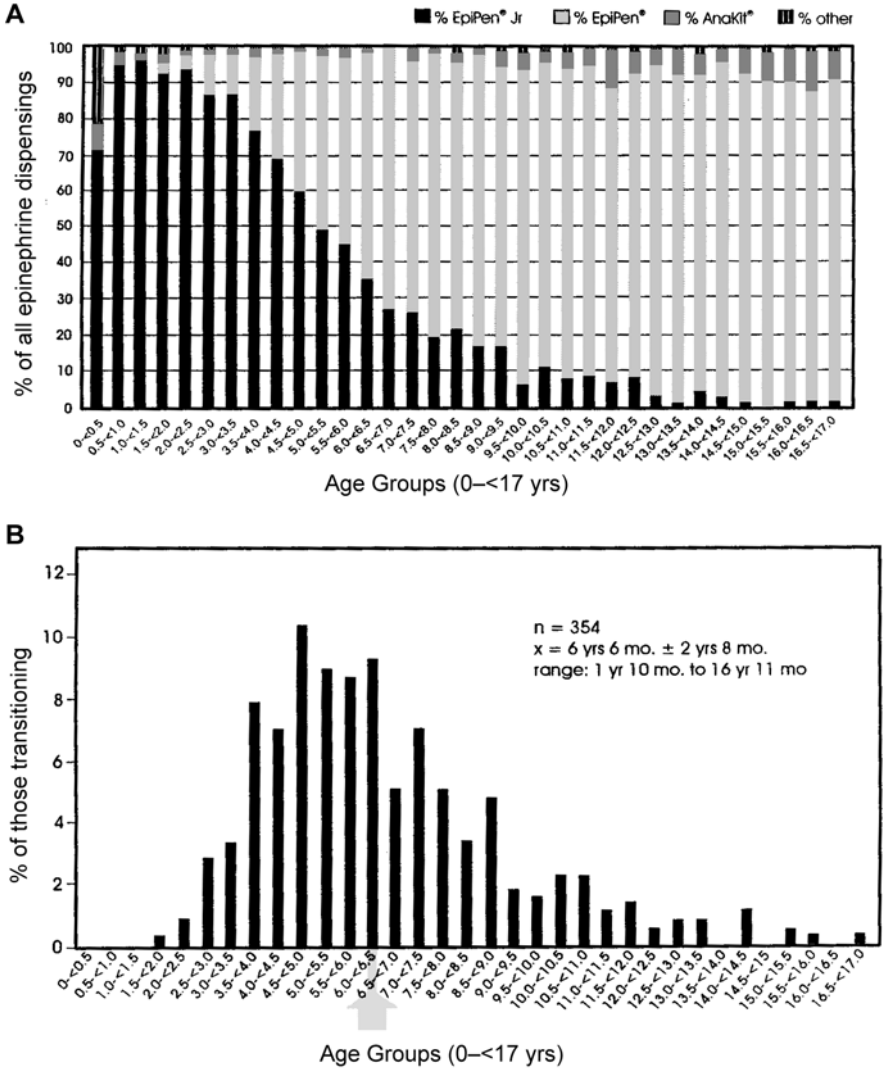
FIG. 2. (A) Epinephrine dispensing patterns were ascertained in a five year study in a defined general population of 1.2 million people. A prescription administrative claims database involving real-time links with community pharmacies was used. Epinephrine was dispensed for 0.95% of the entire population (shown as 'all ages'). There were substantial variations in epinephrine dispensing rates across subsets of the population, with rates ranging from 1.44% for individuals younger than 17 years of age, 0.9% for those 17–64 years, to 0.32% for those age 65 years or older. (B) Epinephrine dispensing rates varied with sex as well as with age, here shown by five year groupings. Until age 15 years, boys were more likely to have epinephrine dispensed than girls. From age 15 years through middle age, girls and women were more likely to have epinephrine dispensed than boys and men. In the elderly, epinephrine dispensing rates did not differ between the sexes.

Under-use of epinephrine in the community

Epinephrine is widely dispensed in the community; however, different types of studies, including retrospective studies of individuals dying from anaphylaxis, suggest that it is under-used when anaphylaxis actually occurs. Although epinephrine was used in treatment of 62% of fatal anaphylactic reactions caused



by various triggers, it was given before cardiorespiratory arrest in only 14% of reactions (Pumphrey 2000). In fatal food-induced anaphylaxis, failure to use epinephrine at all, delayed use, or inappropriate dose have been identified as contributing factors to death (Yunginger et al 1988, Sampson et al 1992). In the latter study, 12 individuals had not received epinephrine during their reaction, 10 received it too late, four died despite receiving it in a timely manner, and for six no information was available (Bock et al 2001). In other studies of anaphylaxis from all



triggers, one based on real-time reporting in a national paediatric surveillance program (Simons et al 2003) and another based on a retrospective telephone questionnaire in a paediatric allergy service (Gold & Sainsbury 2000), only about 30% of individuals who required epinephrine during an anaphylactic reaction actually received it.

Additional studies have demonstrated that many healthcare professionals and patients have inadequate knowledge about outpatient use of epinephrine. In a cross-sectional study, 76% of physicians were unaware that two EpiPen® dose formulations exist (Grouhi et al 1999). In surveys, only 55% of individuals at risk for anaphylaxis actually had in-date epinephrine on hand (Sicherer et al 2000) and only 30–40% could demonstrate how to use an auto-injector correctly (Huang 1998, Sicherer et al 2000).

Lack of response to first-aid treatment with epinephrine

Potential reasons for the lack of response to epinephrine in the first-aid treatment of anaphylaxis identified from case reports and case series include: rapid progression of the episode; failure of epinephrine to 'work' due to β blockade (Toogood 1988), α blockade (Watson 1998) or presence of an angiotensin converting enzyme (ACE) inhibitor (Yocum & Khan 1994); and adverse reaction to the metabisulfite preservative in the epinephrine solution (Luciuk 1993).

Other reasons, supported by experimental data, include delay in epinephrine treatment, administration by a suboptimal route (for example, subcutaneous injection or pressurized metered-dose inhaler, pMDI), administration of an inappropriately low dose due to the limitations currently imposed by the availability of only two fixed-dose auto-injectors (EpiPen® Jr 0.15 mg or

FIG. 3. (A) Epinephrine dispensing was studied in a defined population of 279 600 children using a prescription administrative claims database with links to community pharmacies. All out-of-hospital epinephrine dispensings for these children are shown by age group, for four consecutive fiscal years. The type of epinephrine formulation dispensed is indicated. Of a total of 6820 epinephrine prescriptions dispensed, EpiPen® Jr 0.15 mg accounted for 38.6%; EpiPen® 0.3 mg accounted for 57.4%, and all other epinephrine formulations together accounted for only 4%. EpiPen® Jr was prescribed over the age range 2 months–16 years 10 months, inclusive. EpiPen® was prescribed over the age range 1 year 8 months–16 years 8 months, inclusive. (B) In 354 children for whom EpiPen® Jr was dispensed initially followed by EpiPen® dispensed at a later date, the age of transition from EpiPen® Jr to EpiPen® is expressed as a percentage of those transitioning during the four consecutive years studied. The mean age of transition was 6 years 6 months $\pm$ 2 years 8 months (range 1 year 10 months–16 years 11 months).

EpiPen® 0.3 mg), or injection of outdated epinephrine (Simons et al 1998, 2000a,b, 2001b,c, 2002b,c).

Reasons for lack of prospective studies in humans with anaphylaxis

Not surprisingly, only a few individuals have been studied prospectively after epinephrine administration during anaphylaxis (Smith et al 1980). There are no randomized, double-blind, placebo-controlled clinical trials of epinephrine in patients actually experiencing anaphylaxis, and therefore no Level 1A evidence (Shekelle et al 1999), on which to base recommendations for its use in this disorder. Such clinical trials would be difficult to conduct because anaphylaxis occurs unexpectedly and commonly takes place in the community rather than in a healthcare setting (Simons et al 2003, Yocum et al 1999); moreover, episodes differ in severity among patients and, in the same patient, from one episode to another. Baseline measurements would therefore likely be impossible to obtain, and frequent timed measurements might be difficult to obtain. Randomized, placebo-controlled trials would also be unethical because although anaphylaxis is occasionally fatal despite epinephrine treatment, prompt injection of epinephrine is deemed to be critically important for patient survival (Pumphrey 2000, Yunginger et al 1998, Sampson et al 1992, Bock et al 2001).

Consequently, recommendations for epinephrine dosing in the first-aid, out-of-hospital treatment of anaphylaxis are based on anecdotal experience and, depending on the reference source, vary considerably with regard to maximum initial dose (0.3 mg to 0.5 mg in adults), route of injection (subcutaneous or intramuscular), and time interval between repeated doses (from 5–30 minutes).

Prospective studies of epinephrine administration in humans

Recently, prospective, randomized, blinded, pharmacokinetic/pharmacodynamic studies of epinephrine administration have been performed in individuals aged 4–35 years (Simons et al 1998, 2000a, 2001c, 2002b). Children in these studies were at risk for anaphylaxis, had received epinephrine previously, and carried epinephrine with them at all times in the form of an EpiPen® Jr or EpiPen®. None of the individuals studied had a history of severe adverse reactions to epinephrine. All of them were free from cardiovascular, central nervous system and thyroid disease, and had normal blood pressure and electrocardiograms at baseline. They had not taken α- or β-adrenergic agonists or antagonists, monoamine oxidase inhibitors or ACE inhibitors, amphetamines, methylphenidates, or cocaine before the studies. Plasma epinephrine concentrations were measured by using a HPLC technique with electrochemical detection.

Based on the observation that subcutaneous administration of epinephrine causes skin blanching at the injection site due to the powerful α_1 vasoconstrictor effect of the drug, it was hypothesized that retention at the site of subcutaneous injection might lead to a delay in epinephrine absorption into the systemic circulation. This hypothesis was initially tested in a paediatric study in which the time to peak plasma epinephrine concentration (t_{max}), 34 ± 14 minutes (range 5–120) after subcutaneous injection, was significantly longer than the t_{max} of 8 ± 2 minutes, accompanied by prompt physiological effects, after intramuscular injection (Simons et al 1998). The total amount of epinephrine absorbed was similar regardless of the route of injection, and the terminal elimination half-life was 43 minutes.

These findings were confirmed and extended in a prospective, randomized, blinded, placebo-controlled, six-way crossover study of epinephrine 0.3 mg (0.3 ml) intramuscular versus subcutaneous injection in young men (Simons et al 2001c). Peak plasma epinephrine concentrations were significantly higher ($P < 0.01$) after epinephrine injection in the vastus lateralis muscle compared with epinephrine injection in the deltoid muscle, epinephrine injection subcutaneously in the deltoid region, or placebo injection. This was attributed to the large size of the vastus lateralis and its excellent blood supply. In extremely obese individuals, intramuscular injections of epinephrine may inadvertently end up being subcutaneous injections, unless a needle at least one inch (2.5 cm) long is used to penetrate the fat pad over the vastus lateralis muscle (Chowdhury et al 2002, Simons et al 2002c). In addition to these studies in humans, studies in an animal model (Gu et al 1999) suggest that subcutaneous injection of epinephrine may result in delayed absorption compared to IM injection.

In the first-aid, out-of-hospital treatment of anaphylaxis, some physicians recommend epinephrine inhalation from a pMDI as an alternative to injection. The stated potential advantages of the epinephrine pMDI are: non-prescription availability, low cost, ease of use, relief of respiratory symptoms, ability to administer multiple doses, and absence of pain and adverse effects. In order to obtain a significant systemic effect, however, adolescents and adults require 20–30 epinephrine inhalations, and children require 10–20 inhalations, depending on their body weight. In a prospective, randomized, placebo-controlled, observer-blind study, despite careful coaching in a non-emergency situation, most children were unable to inhale the epinephrine dose required to elevate plasma epinephrine concentrations, due to the bad taste and the tingling or burning sensations attributed to vasoconstriction of oropharyngeal mucosa (Simons et al 2000a). Adverse effects included cough and dizziness. One of the few children who *did* inhale the required dose experienced anxiety, nausea, pallor, shaking and intermittent muscle twitching lasting 50 minutes. Although potentially helpful for relief of respiratory symptoms, inhaled epinephrine from currently available pMDIs should not be depended on for relief of other anaphylaxis symptoms.

Currently available epinephrine pMDIs contain chlorofluorocarbons and are no longer approved by regulatory agencies in some countries.

Unique considerations in the paediatric population: current challenges of providing an optimal and accurate epinephrine dose for infants and young children

It is impossible to give a precise epinephrine dose of 0.01 mg/kg to most children weighing *between* 15 and 30 kg, by using either the EpiPen® Jr (0.15 mg) or the EpiPen® (0.3 mg), and physicians must choose whether to potentially underdose such children with the EpiPen® Jr or overdose them with the EpiPen®. In a prospective, randomized, double-blind, parallel-group study, children age 5–8 years weighing 16–30 kg self-injected epinephrine using either an EpiPen® Jr or an EpiPen® with the aid of a physician (Simons et al 2002b). Those who received a dose of 0.01 to 0.014 mg/kg from an EpiPen® had a significantly higher mean systolic blood pressure 30 minutes after injection; however, in every child, this was accompanied by pallor, tremor, anxiety, and palpitations or other cardiovascular effects, and some children also developed headache and nausea. One child who received a precise dose of 0.01 mg/kg from the EpiPen® developed an increase in the QTc interval from 410 to 449 ms, lasting for 120 minutes after injection. In contrast, children who received 0.008 to 0.009 mg/kg from an EpiPen® Jr did not achieve a significant increase in blood pressure and had fewer adverse effects, limited to transient pallor, tremor or anxiety. The investigators concluded that the beneficial and adverse pharmacological effects of epinephrine could not be separated and that additional premeasured fixed doses of epinephrine are needed to facilitate more precise first-aid dosing in young children requiring treatment of anaphylaxis out-of-hospital. In this study, the mean t_{max} for epinephrine was 16 ± 3 (EpiPen® Jr) and 15 ± 3 minutes (EpiPen®), compared with 8 ± 2 minutes in a previous study in which a nurse gave the injections with the EpiPen® (Simons et al 1998). The investigators therefore recommended that preferably an adult should give an epinephrine injection to a young child, because although a child can learn how to self-inject the medication, his/her technique may not be optimal.

For infants weighing less than 15 kg, no appropriate fixed-dose of epinephrine is available in an auto-injector and physicians often recommend that parents or caregivers draw up a dose of 0.01 mg/kg from an ampoule of epinephrine and inject it using a 1 ml syringe with attached needle. Indeed, in the dispensing study described earlier (Simons et al 2001a), epinephrine was dispensed in ampoule formulation for 20% of the infants. In a practical demonstration, 18 parents with no healthcare training were asked to draw up an epinephrine dose from an ampoule in a simulated emergency situation (Simons et al 2001b). They took significantly longer ($P < 0.05$) than the 'controls' (18 physicians, 18 general

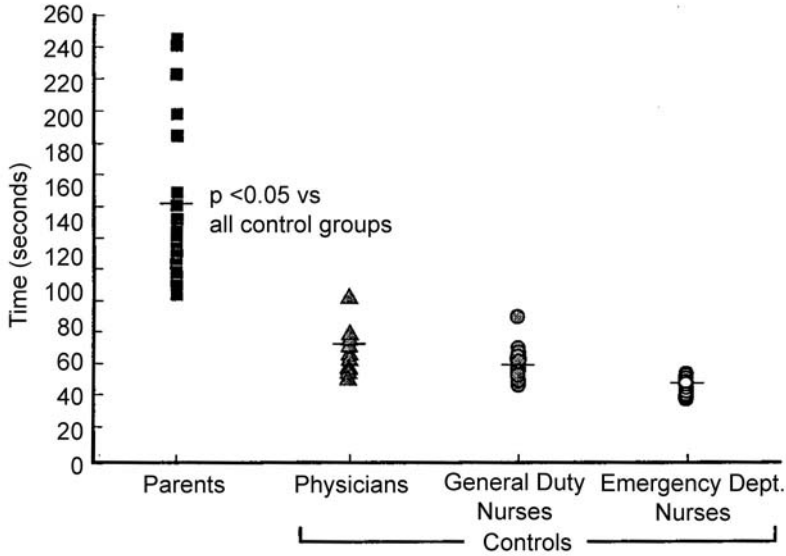


FIG. 4. A prospective, controlled study was performed with regard to the time needed by parents to draw up an infant epinephrine dose from an ampoule, and the accuracy of the dose. Eighteen parents were given written instructions and asked to draw up epinephrine 0.09 ml. Controls included 18 resident physicians, 18 general duty nurses, and 18 emergency department nurses. The parents took significantly longer ($P < 0.05$) than the controls to draw up the dose.

duty nurses, and 18 Emergency Department nurses) to draw up the dose, requiring a mean of 142 ± 13 seconds (range 83–248) versus 29 ± 0.09 seconds (range 27–33) for Emergency Department nurses, who were fastest; the control groups did not differ significantly from each other in speed (Fig. 4). The epinephrine content of the doses drawn up by the parents, as measured by using HPLC-UV, ranged 40-fold in contrast to the physicians' doses (7–8-fold); general duty nurses' doses (threefold), and Emergency Department nurses' doses (twofold). There was no correlation between speed and accuracy.

Should epinephrine be injected if it is past the expiry date?

Epinephrine is an inherently unstable chemical. In aqueous solution, it is susceptible to oxidation and inactivation by partial racemization to the dextroisomer. On exposure to air or light, it degrades rapidly, turning pink from oxidation to adrenochrome, and brown from the formation of melanin; leading to the recommendation that it should be protected from light and stored at room temperature (15–30 °C). Compendial limits for the epinephrine content of

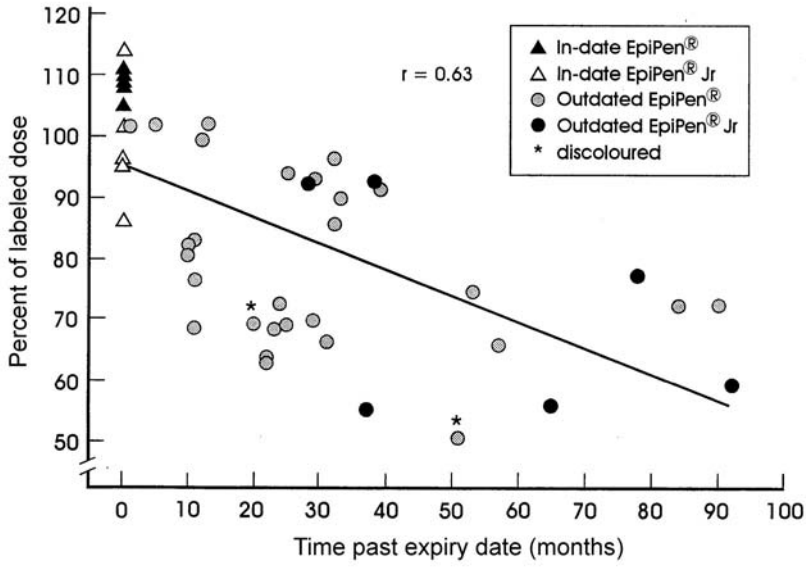


FIG. 5. Outdated, unused EpiPen® and EpiPen® Jr auto-injectors were obtained from patients at risk for anaphylaxis for the purpose of evaluating epinephrine bioavailability (not shown) and content. 28 EpiPen® and 6 EpiPen® Jr auto-injectors were studied 1–90 months after the stated expiration date. Controls included 6 EpiPen® and 6 EpiPen® Jr auto-injectors that were well within the stated expiration date. The inverse correlation between the decreased epinephrine content of the outdated EpiPen® and EpiPen® Jr auto-injectors and the number of months past the expiration date was 0.63.

commercially available formulations are 90–115% of labelled strength. In the EpiPen® and EpiPen® Jr auto-injectors, epinephrine is supplied in light-resistant packaging and contains an anti-oxidant to prevent decomposition. The total volume in each auto-injector is 2 ml, of which 0.3 ml is injected intramuscularly when the device is used correctly. Each 0.3 ml in the EpiPen® contains 0.3 mg of epinephrine, 1.8 mg of sodium chloride, 0.5 mg of sodium metabisulfite, and hydrochloric acid to adjust the pH from 2.2–5.0 in water for injection. Each 0.3 ml in the EpiPen® Jr contains 0.15 mg of epinephrine and the same non-medicinal ingredients in the same amounts as listed for the EpiPen® (*Physicians' Desk Reference* 2003).

In a prospective investigation, 28 EpiPen® and 6 EpiPen® Jrs which had never been used were studied up to 90 months after their stated expiration date. Most of the auto-injectors were not discoloured and none contained precipitates. Epinephrine bioavailability from the out-of-date auto-injectors was significantly reduced ($P < 0.05$) compared with bioavailability from the in-date auto-injectors. When the outdated auto-injectors were opened and their epinephrine content was

measured by using a HPLC-UV technique, although the auto-injectors that were long past the expiry date contained the lowest concentrations of epinephrine ($r=0.63$) (Fig. 5), all auto-injectors contained some epinephrine. The investigators concluded that if no in-date auto-injector is available, the potential benefit of giving a suboptimal epinephrine dose from an out-of-date auto-injector outweighs the potential risk of failing to give epinephrine at all (Simons et al 2000b).

Future directions

Many patients with anaphylaxis and many caregivers of children with anaphylaxis are reluctant to inject epinephrine due to anxiety about using a needle. Oral epinephrine administration is ineffective because of metabolism by catechol-*O*-methyltransferase in the wall of the gastrointestinal tract and by monoamine oxidase in the wall of the gastrointestinal tract and in the liver. Administration of epinephrine through chlorofluorocarbon-containing pMDIs, in countries where such are still approved for use, may relieve respiratory symptoms but is impractical for inhaling the high doses required to achieve other systemic effects. Based on the precedent of using sublingual nitroglycerin for treatment of angina, the feasibility of sublingual epinephrine administration for the first-aid treatment of anaphylaxis was explored in a prospective, randomized, four-way crossover study of epinephrine absorption from various dosage formulations in an animal model. In this proof-of-concept study (Gu et al 2002), administration of epinephrine as a sublingual tablet formulation resulted in rapid achievement of peak plasma epinephrine concentrations similar to those achieved after intramuscular injection.

In the foreseeable future, epinephrine will continue to be the medication of choice in the first-aid, out-of-hospital treatment of anaphylaxis, despite concerns about occasional lack of efficacy of epinephrine injection, intrinsic limitations in dosing due to its narrow toxic-therapeutic index, and current practical issues regarding the lack of easy-to-use fixed-dose formulations for out-of-hospital use. Healthcare professionals should have an up-to-date knowledge of the potential risks and benefits of epinephrine in anaphylaxis, and the reasons for failure of response to this life-saving drug when it is used in an out-of-hospital setting. New formulations that will facilitate optimal dosing in patients of all ages and sizes are needed. Recent progress in epinephrine research is encouraging; however, many important, clinically relevant questions remain to be answered about the first-aid use of this life-saving drug in anaphylaxis.

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DISCUSSION

Metzger: A fundamental question is whether the receptors that one would like to act on for therapeutic effect can be distinguished from those that are producing the toxic effect.

Simons: Very little is mentioned or discussed in the literature about this.

Metzger: This seems to be the fundamental issue and something which one should try to clarify.

Simons: The receptors on the vasculature would be the same in the various organs.

Sampson: What do you do with the 8 kg child who needs epinephrine? What weight do you use to switch over from the EpiPen Jr (0.15 mg) to the regular EpiPen (0.3 mg)?

Simons: Reluctant as I am to give a twofold or threefold overdose to an infant or young child weighing 8 kg, we are recommending EpiPen® Jr for those patients. I am concerned that some of the parents in our demonstration study could not get any epinephrine into the vial, even under conditions of optimal instruction and an

anxiety-free scenario (Simons et al 2001). On the basis of another study (Simons et al 2002) I now wait until a child weighs 23–25 kg before prescribing an EpiPen® Regular. I will make an exception if the child is asthmatic or lives or vacations in a remote rural area. This is sometimes a difficult clinical judgement to make. Without a doubt, we need a broader range of fixed epinephrine doses in user-friendly injectable formulations.

Sampson: Do you feel the data from the dog model looking at the administration of epinephrine with hypotension can be extrapolated to humans? Should this then be a message in giving epinephrine earlier rather than later?

Simons: We use this study to reinforce the message that epinephrine should be injected early, before shock is established. I always have reservations about extrapolating from an animal model to humans, and I note that one of the main outcomes in our animal model is mean arterial pressure, which may be a less sensitive measure of improvement than systolic pressure (Bautista et al 2002), but we will never have a placebo-controlled study in humans.

Leung: Along those lines, are there any situations where epinephrine doesn't work clinically? And have you had any situations where there are differences in sensitivity to epinephrine? Given your concerns about potentially paradoxical effects, have you seen situations where epinephrine may have worked adversely in anaphylaxis?

Simons: Yes. There are situations in which the patient's anaphylaxis appears to worsen despite epinephrine. In one such patient with anaphylaxis to allergen immunotherapy whom I encountered during Fellowship training, we gave subcutaneous injections of epinephrine as was standard of care in that era. At the injection sites there was obvious blanching of the skin lasting 20–30 minutes. I recall wondering why we were injecting epinephrine subcutaneously every five minutes when very evident and prolonged skin blanching suggested that it was 'sitting in the skin' at the site of the previous injections. We know we have to use epinephrine in anaphylaxis, but it is not an easy drug to use.

Vercelli: Do you think that if epinephrine is toxic once, it will be permanently toxic for a certain individual?

Simons: That's a good question but I don't have an answer to it. The literature suggests that patients are reluctant to self-inject epinephrine (Gold & Sainsbury 2000). Although it is possible that they are more reluctant to self-inject it if they have had a bad reaction to it previously, this has not documented.

Lee: What do you tell your patients to do if the first injection doesn't work?

Simons: We recommend that patients have two auto-injectors available. If a second injection is given, the time interval between the injections should be based on clinical response. Currently, an interval of 5–10 minutes is recommended in textbooks, based on anecdotal experience.

Lee: We tell them 10 minutes.

Simons: We say 10–15 minutes unless the clinical picture suggests otherwise.

Schwartz: You mentioned that the serum elimination half-life of epinephrine is 43 min. How prolonged is that compared with i.v. administration, and does it change between adults and children?

Simons: We would expect it to be shorter in children than adults. We would not expect it to change among dosing formulations. It is drug related, not formulation related. The time to peak may change.

Schwartz: Do the dogs get better if you give epinephrine early?

Simons: This experiment has been performed (Mink et al, unpublished results) and epinephrine does not work as well in this model as we would have liked it to.

Fisher: John Moss assayed the ampoules of epinephrine randomly collected from around the hospital, finding tremendous variation. Sometimes when we find that the epinephrine doesn't work, if we add a few more ampoules it does. In patients who get reactions to i.v. drugs in anaesthesia often the presenting sign is no bleeding or no pulse. They are fairly shocked when we notice, but they do respond to epinephrine. One of the things I wonder about when there is no response to epinephrine is whether the blood volume is satisfactory. These people may lose half their blood or plasma volume. You can give all the vasoconstrictors you like but if the bucket is empty you are not going to get much response. Were the dogs given fluid?

Simons: Yes.

Fisher: Were they given thin or thick fluid?

Simons: Thin fluid. Your second point is very well taken. I'd like to comment on your previous point. You probably noticed that in the control EpiPens, which were well within the expiry date, the epinephrine content varied from 86–114% of the labelled dose. Despite a light resistant container and the addition of sodium metabisulfite, epinephrine has a tendency to break down readily.

Lasser: I am bothered by your dog study. How did you give the epinephrine?

Simons: We gave it by various routes of injection. Intravenously, but not by the intramuscular or subcutaneous routes, it produced a significant transient immediate increase in the mean arterial pressure, cardiac output, stroke volume and pulmonary pressure ($P < 0.01$).

Lasser: In the anaphylactic responses we study in radiology we are seeing patients very close to their nadir before we decide to treat. There must be other studies like your dog study.

Simons: Some patients die despite injecting epinephrine in a timely manner (Pumphrey 2000).

Lasser: Is there another animal model showing that there is some effect when it is given at the nadir?

Simons: I'm not aware of any.

Mosbech: You mentioned that in the animal model giving adrenalin early would not be a big help.

Simons: It's better than giving it late, but it is still not perfect.

Mosbech: Could you subdivide the group of animals into those with very low and those with not-so-low blood pressure?

Simons: They are all shocked to the same level defined as a reduction in mean arterial pressure to approximately 50% of that measured at baseline before allergen challenge.

Mosbech: Have you tried looking at 30%, for instance?

Simons: No. It would be another large experiment to do.

Golden: In the studies of intramuscular versus subcutaneous injection in children, is there any reason to believe that these normal children are showing results that differ from what one would find in anaphylaxis in children?

Simons: This cannot be ruled out. However, when I realized that it would never be possible to do a prospective randomized double-blind placebo-controlled study in children actually experiencing anaphylaxis, my only alternative was to study allergic children at risk for anaphylaxis who were relatively well on the day of study.

Golden: Which way do you speculate it might go?

Simons: Anaphylaxis differs among individuals and in the same individual on different occasions. We must be guided by the clinical response.

MacGlashan: Has there been any effort by the manufacturers of the EpiPen to expand the dosing choices?

Simons: No, however, other manufacturers may be interested in this.

Dubois: I agree with Henry Metzger's point that if we want to develop better drugs we need to understand which mechanism of action we require these drugs to have. There are quite a lot of $\beta 2$ agonists with some level of specificity around. Have these ever been tried systemically?

Simons: Absolutely. Salbutamol, for example, can be administered intravenously in severe acute asthma.

Dubois: Does it work?

Simons: Yes, but it doesn't work any better than frequent high-dose inhalations of salbutamol. I would like to make the point that selective $\beta 2$ agonists such as salbutamol should not be depended upon for treatment of anaphylaxis. These drugs were developed for use in asthma (Travers et al 2001) and were designed to be free from α_1, α_2 and β_1 pharmacological effects. We need these α_1 and β_1 activities in anaphylaxis treatment. In the Canadian Pediatric Surveillance study (Simons et al 2003), one of the problems we identified is that children with asthma were given salbutamol (Ventolin) when they experienced anaphylaxis symptoms with consequent delay in epinephrine administration. The important message is that

salbutamol (Ventolin) is not a substitute for epinephrine in the treatment of anaphylaxis.

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New approaches for the treatment of anaphylaxis

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Abstract. Anaphylaxis represents the most extreme form of life-threatening allergic reactions. However, effective long-term therapies for this condition are not currently available. A number of potential approaches have proven effective in murine models of peanut-induced anaphylaxis and are currently being considered in humans, including the use of vaccines containing 'engineered' recombinant food proteins and Chinese herbal medications. TNX-901 is a humanized IgG1 anti-IgE mAb that recognizes and masks an epitope in the CH3 region responsible for binding to the high affinity Fc epsilon receptor (FcεRI) on basophils and mast cells. Recently, we conducted a double-blinded, placebo-controlled, randomized, dose escalation trial in 84 patients with a history of peanut allergy. Allergy was confirmed and the threshold dose of encapsulated peanut established by a double-blinded, placebo-controlled oral food challenge (DBPCOFC) at screening. Patients were randomized 3:1 in three dose groups to receive either TNX-901 (150, 300 and 450 mg) or placebo subcutaneously every four weeks for four doses. They underwent a final open food challenge within 2–4 weeks after the last dose of study medication. From mean baseline values of 178–436 mg in the various treatment groups, the mean increases in the open food challenge threshold were 710, 913, 1650 and 2627 mg for the placebo, 150, 300 and 450 mg for TNX-901 dose groups, respectively ($P = 0.0004$, 450 mg vs. placebo; $P = 0.0008$ for trend with dose). TNX-901 was well tolerated. TNX-901 at a dosage of 450 mg significantly increased the threshold of sensitivity to peanut by open food challenge from a level of about half a peanut (178 mg) to almost nine peanuts (2805 mg). These studies suggest that treatment of patients with anti-IgE therapy may represent an effective long-term approach for management of food-induced anaphylaxis.

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Anaphylaxis is a life-threatening allergic reaction. At present, there is no effective long-term treatment for anaphylaxis with the exception of allergen avoidance. If the allergen is unknown or ubiquitous in the patient's environment, however, this can be very challenging. Food-induced anaphylaxis affects about 6–8% of children less than 4 years of age and nearly 2% of the US population beyond the first decade of life (Sampson 1999). It is the single leading cause of anaphylaxis treated in

hospital emergency departments in the US and many 'westernized' countries (Yocum et al 1999). Peanut and tree nut allergy account for the majority of fatal and near-fatal anaphylactic reactions (Sampson et al 1992, Bock et al 2001). A national survey indicated that about 1.1%, or three million Americans, are allergic to peanuts and/or tree nuts (Sicherer et al 1999), 50–100 of whom die each year from unintended ingestion (Yocum et al 1999, Bock et al 2001). Despite an increasing public awareness of food allergy, most patients are ill-prepared to deal with anaphylactic reactions (Gold & Sainsbury 2000). In a recent series, over 80% of patients dying from food allergic reactions had not been previously provided with appropriate information or self-injectable epinephrine to avoid or manage accidental food-induced reactions (Bock et al 2001).

Unlike traditional immunotherapy for inhalant and bee sting allergy, the benefit-to-risk ratio for injections of peanut extracts is unacceptable. In a trial of standard rush immunotherapy, four of six patients were able to achieve 'maintenance' levels, but in two subjects the dose had to be reduced because of intolerable side effects (Oppenheimer et al 1992). While the four patients achieving 'maintenance' therapeutic doses were able to ingest significantly more peanut during subsequent blinded challenges compared to six placebo control subjects, this beneficial effect was lost in two patients when the allergen dose had to be decreased due to adverse side effects. Overall, the adverse reactions rate was 23% during the 'build-up' phase and 39% during the 'maintenance' phase.

Given this unfavourable benefit-to-risk ratio of traditional immunotherapy, several novel immunotherapeutic strategies are being examined as treatment modalities for food allergy. The most promising approaches include:

- humanized anti-IgE monoclonal antibody therapy
- 'engineered' (mutated) allergen protein immunotherapy, and
- Traditional Chinese medicine.

The rationale and recent results from each of these three potential therapies for treating food allergy will be reviewed in the following sections.

Anti-IgE therapy

Rationale

Allergic reactions are mediated by antigen-specific IgE bound to high affinity Fc epsilon receptors (FcεRI) on mast cells and basophils (Oettgen & Geha 2001). TNX-901 is a humanized IgG_1 anti-IgE monoclonal antibody that binds with high affinity specifically to an epitope in the CH3 domain, masking a region responsible for binding to both the FcεRI and low affinity Fc epsilon receptors

(FcεRII [CD23]) (Chang 2000). In addition to inhibiting binding of IgE to mast cells and basophils, anti-IgE treatment also reduces FcεRI expression on basophils (MacGlashan et al 1997), and may inhibit allergen-specific T cell activation through interference with IgE receptor-mediated antigen-presenting-cell processing (van Neerven et al 2001).

Study design

This was a randomized, double-blinded, placebo-controlled, dose-escalation study (Leung et al 2003) involving study subjects who were 12–60 years of age with a history of peanut allergy manifested by urticaria, angioedema, lower respiratory symptoms, and/or hypotension. Inclusion criteria required a serum IgE level between 30 and 1000 IU/ml, and a positive skin prick test to peanut. Prior to enrollment, confirmation of each study subject's peanut allergy was confirmed and the threshold for reactivity established by a randomized, double-blind, placebo controlled oral food challenge (DBPCFC). Patients were subsequently randomized in groups of 28 to each of three dose cohorts: 150 mg, 300 mg and 450 mg of TNX-901. Patients were centrally randomized 3:1 in blocks of four per site to receive either TNX-901 or placebo subcutaneously every four weeks for four doses, and underwent a final open food challenge with peanut within 2–4 weeks after administration of the last dose of study medication. Patients periodically received blood and urine tests and were evaluated for adverse events. The final evaluation occurred eight weeks after administration of the last dose of study medication (Week 20).

The primary measure of efficacy was the change from baseline in the $\log_{10}$-transformed threshold dose for hypersensitivity to peanut as assessed by oral food challenge. The peanut was de-fatted, and then varying doses (1 mg–2 g) were loaded into gel capsules. Matching placebo capsules were filled with comparable amounts of cornstarch. During the DBPCFC, patients were given an increasing dose of placebo or peanut every 40 minutes until the principal investigator at each site judged that a definite reaction was occurring. In order to maximize patient safety and prevent severe reactions, the endpoint for the open food challenge was the threshold dose for an allergic reaction. At screening, the planned escalation began at 1 mg, and proceeded to 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 mg of peanut or matching placebo capsules. Patients who could tolerate 2000 mg were considered to have a negative test. The final open food challenge (peanut only) was initiated at 1 mg or 100 mg, depending upon the screening threshold, and escalated to 4000 and 8000 mg, if tolerated. Dose escalation was terminated when an investigator felt there were clear-cut symptoms/signs of a hypersensitivity reaction, and the patient was given activated charcoal slurry.

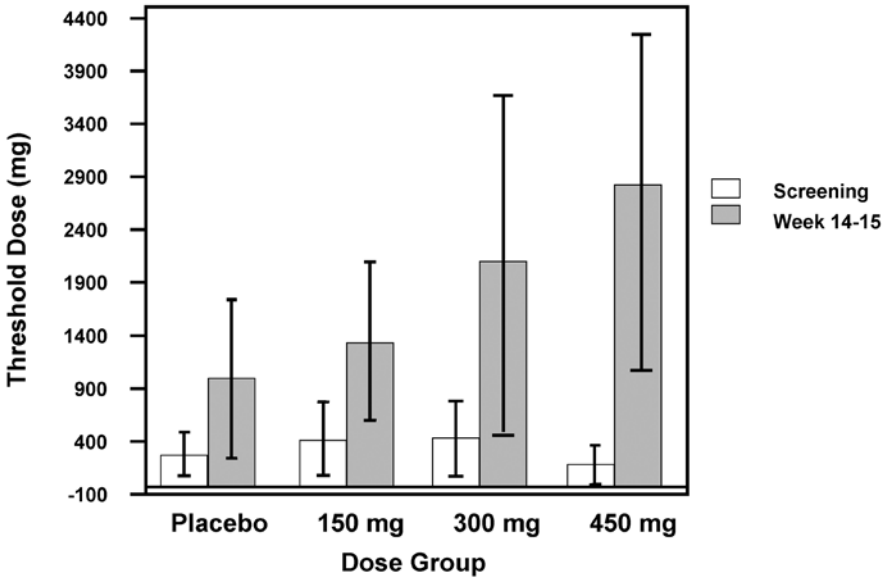

FIG. 1. The mean threshold (±95% confidence interval) dose to peanut eliciting symptoms. Compared to the placebo group, the increase in threshold for patients treated with anti-IgE reached significance for the 450 mg dose group ($P < 0.001$, $\log_{10}$-transformed data); the test for a trend with increasing dose was also highly significant ($P < 0.001$). (Published with permission from Leung et al 2003.)

Results

One hundred and sixty-four patients were screened at seven medical centres in the USA. Eighty-four patients were randomized and 81 completed the study. 23, 19, 21, and 21 patients were randomized to receive placebo, 150, 300 and 450 mg of TNX-901, respectively. The threshold sensitivity to peanut was determined by a constellation of signs and symptoms typical of food allergic reactions, at least one of which was judged to be moderate or severe in nature in all but 14 challenges. Among the most common terminating signs and symptoms during the open food challenge were nausea, abdominal pain, vomiting, throat tightness, chest tightness, wheezing, persistent cough, rhinitis, conjunctivitis, pruritus, hives and angioedema.

The mean threshold sensitivity to peanut at the final open food challenge increased from baseline in a dose-responsive manner (Fig. 1). The proportion of patients who achieved at least a 0.9 $\log_{10}$ increase was greater in all the TNX-901 groups than the placebo group, but this difference only reached statistical significance for the 450 mg group (21.7%, 52.6%, 47.4% and 76.2% for the

placebo, 150, 300 and 450 mg dose groups, respectively; $P=0.002$ for the 450 mg group vs. placebo). However, there was a strong trend with increasing dose ($P<0.001$). The proportions of patients able to achieve the highest threshold tested, 8 g, were 4.3%, 0.0%, 21.1% and 23.8%, for the placebo, 150, 300 and 450 mg groups, respectively. Statistically significant trends with dose were noted for the 4 and 8 g threshold ($P=0.02$ for both thresholds).

Change in the $\log_{10}$-transformed threshold correlated similarly with dose on an absolute, mg/kg, and mg/kg per total IgE at baseline basis, and these relationships were statistically significant. Efficacy correlated less well with dose on the basis of mg/kg per Pn-IgE at baseline and mg/kg per % of total IgE that was peanut-specific at baseline, and these correlations were not significant. Trough drug levels were roughly dose proportional and reached steady state at week 12 (means of 11.6, 32.2 and 57.5 g/mL at the 150, 300 and 450 mg dose levels, respectively). Taking the trough level at week 12 as a measure of drug exposure, there was a similar correlation with change in threshold and trough drug concentration ($r=0.392$, $P<0.001$) as for dose ($r=0.381$, $P<0.001$).

Serum free IgE levels were measured every four weeks prior to dosing with study drug, and reduced IgE levels were sustained at all three dosage levels of TNX-901. From baseline levels of 199.5, 262.0, 158.9 and 242.0 IU/ml for the placebo, 150, 300 and 450 mg dose groups, free IgE was changed to 207.4 (+3.9%), 30.4 (−88.4%), 17.0 (−89.3%), and 16.6 (−93.2%) IU/ml, respectively at the end of week 4, just prior to the second dose of study drug, and similar reductions were observed throughout the dosing period. Eight weeks after the last dose of TNX-901, free IgE was still reduced from baseline by 71.6, 79.1 and 88.7% in the 150, 300 and 450 mg dose groups, respectively.

TNX-901 was well tolerated. The incidence of adverse events was similar in the active treatment and placebo groups. The total number of systemic adverse events reported (range; 45–50 per group) and the number of patients experiencing these events (range: 15–19 per group) were similar across the four treatment groups. Of the local adverse events, injection site reactions were noted in 13–14 patients in all treatment groups, and consisted primarily of erythema, and to a lesser extent, swelling and burning. All injection site reactions were considered mild except in one patient in the 450 mg group who experienced moderate erythema/oedema on two occasions. There were no significant changes in routine laboratory variables (haematology, including platelet count, serum chemistry and urinalysis).

Conclusions

While the average dosage of peanut consumed in an accidental exposure has not been accurately quantified, it is generally believed to be one to two peanuts or fewer, or the equivalent of ∼325–650 mg of peanut. The thresholds achieved in

the 300 and 450 mg dose groups, 2083 and 2805 mg, respectively, equivalent to approximately six and eight peanuts, should therefore provide substantial protection for most peanut allergic patients. Additionally, 21% and 24% of patients at these dose levels, respectively, were able to ingest at least 8 g of peanut (~24 peanuts), the final dose in the food challenge, before experiencing a reaction. This study suggests that administering monthly injections of humanized recombinant anti-IgE antibodies appears to be effective in preventing allergic responses in peanut-sensitive subjects, at least to small amounts of peanut protein.

Mutated allergen immunotherapy

Rationale

'Engineered' major allergenic peanut proteins that eliminate IgE binding but retain T cell proliferation have been developed. It was felt that these proteins could be injected safely without eliciting an allergic response, but perhaps more importantly, they would not be taken-up by IgE-bearing antigen-presenting cells (APCs) and therefore would not augment a Th2 response. The three major peanut proteins (Ara h1, Ara h2 and Ara h3) were identified, isolated, sequenced and the sequential IgE-binding epitopes mapped. The DNA encoding these proteins was then cloned. Critical amino acids for IgE binding to allergenic epitopes was determined by single amino acid substitutions of peptides generated on a nitrocellulose matrix (Genysis SPOTs membranes) and then recombinant proteins were 'engineered' to eliminate the IgE-binding epitopes. Using site-directed mutagenesis of the allergen cDNA clones followed by recombinant production of the modified allergen, engineered DNA was developed that coded for proteins that differ by a single amino acid within each of the IgE-binding epitopes (Stanley et al 1997). Overall, 23 binding sites (epitopes) were modified in Ara h1, 10 binding sites in Ara h2 and 4 sites in Ara h3. The engineered recombinant proteins had markedly reduced IgE-antibody binding utilizing sera from peanut-allergic patients, but promoted comparable T cell proliferation as seen with the native peanut proteins.

The use of antigen combined with bacterial adjuvants, such as heat-killed *Listeria monocytogenes* (Yeung et al 1998) can induce deviation of antigen-specific Th2 responses to more Th1-like responses. Since these engineered recombinant proteins are generated in *Escherichia coli*, which itself might serve as an adjuvant to promote Th1 responses, administration of heat-killed *E. coli* containing 'engineered' r-Ara h1–3 proteins (amino acid substitutions within IgE-binding epitopes to abrogate IgE binding) were expected to be more efficacious than administering 'engineered' Ara h1–3 proteins alone. Subsequent experiments

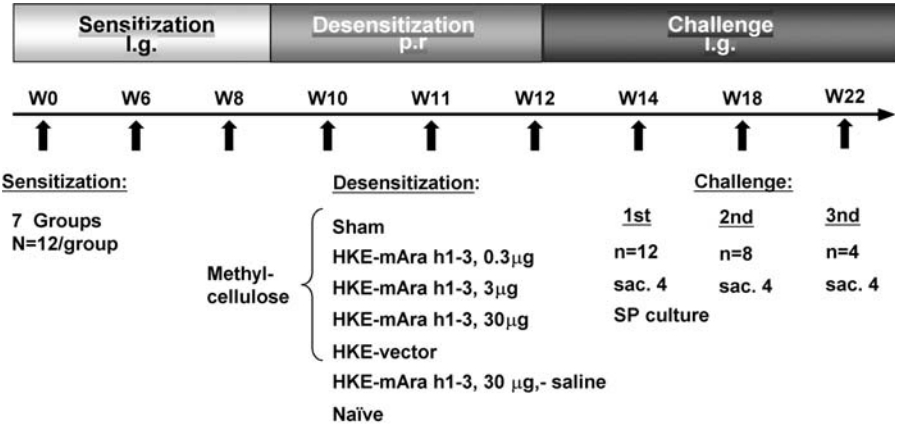

FIG. 2. Protocol comparing desensitization with HKE-mAra h1–3 administered rectally at different doses in methylcellulose or saline (Reproduced with permission from Li et al 2003).

demonstrated that administering heat-killed *E. coli* containing r-Ara h1–3 proteins (HKE-Ara h1–3) subcutaneously or per rectum was more effective at 'desensitizing' peanut-allergic mice, as determined by post-challenge clinical score, body temperature, airway response and plasma histamine compared to the PBS control (sham-treated) mice or mice receiving HKE-mAra h1–3 administered by the intragastric route. Experiments were designed to determine the efficacy of different doses of HKE-mAra h1–3 administered in a methylcellulose carrier to desensitized peanut allergic mice compared to sham-treated mice.

Study design

Five-week-old female C3H/HeJ mice were sensitized with freshly ground whole peanut in the presence of cholera toxin boosted weekly for 6 weeks and again at week 8 as previously described with modification (Li et al 2000a). Mice were then treated with HKE-mAra h1–3 or sham-treated as depicted in Fig. 2. Following treatment, mice were challenged every four weeks with peanut, and anaphylactic symptoms, body temperatures, plasma histamine and IgE levels were measured. T cell proliferative responses and cytokine production were also determined. Four mice were sacrificed following each challenge for histological and immunological studies.

Results

As depicted in Fig. 3, the rectal administration of 3 μg/ml or 30 μg/ml of HKE-mAra h1–3 led to protection of peanut-sensitized mice, with an absence of

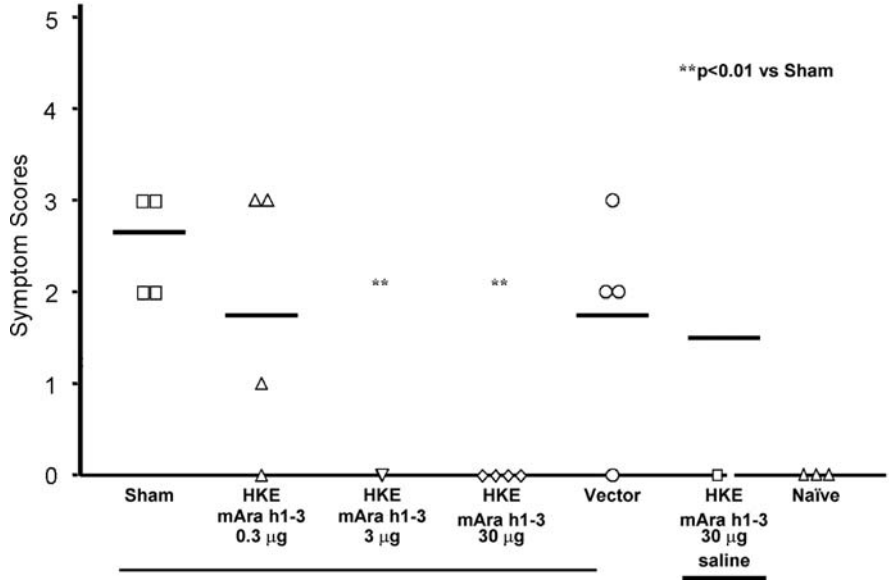

FIG. 3. Symptom scores following third oral peanut challenge three months after mAra h1–3 (Week 22) administered rectally (Reproduced with permission from Li et al 2003).

symptoms and rise in plasma histamine following the peanut challenge. In addition, this protective effect was more prolonged than that seen with the 0.3 μg/ml dose of HKE-mAra h1–3 or the purified mAra h1–3. Peanut-specific serum IgE levels were significantly reduced at the time of challenge in the 3 μg/ml and 30 μg/ml HKE-mAra h1–3-treated mice compared to the sham-treated group, and remained lower following the third and final peanut challenge. In addition, splenocytes from the 3 and 30 μg/ml HKE-mAra h1–3-treated mice secreted significantly less interleukin (IL)4, IL5 and IL13 than splenocytes from sham-treated mice following peanut stimulation (Li et al 2003). However, IL4, IL5 and IL3 secretion following Con A stimulation of splenocytes from HKE-mAra h1–3-treated and sham-treated mice was not significantly different.

Conclusions

Administration of HKE-mAra h1–3 in a methylcellulose carrier provided sustained protection in peanut-sensitized mice compared to other forms of administration. Based on these studies, it appears that the rectal administration of HKE-mAra h1–3 could provide a number of advantages: the heat-killed *E. coli*

is being administered into an environment replete with *E. coli* and other bacterial organisms which should lessen concerns about safety of such administration, the rectal route of HKE-Ara h1–3 required fewer doses of the engineered proteins than the vaccine administered by the subcutaneous route (Li et al 2003), and the protective effect appeared to be more long lasting. These studies suggest that in the future, peanut-allergic patients may be successfully 'desensitized' utilizing a suppository-like form of HKE-mAra h1–3.

Traditional Chinese medicine

Rationale

Traditional Chinese medicines (TCM) have been reported to have anti-allergic properties, which may be useful for treating peanut allergy. A recent report that an herbal formula, MSSM-02, reversed allergic airway hypersensitivity associated with reduction of Th2 responses in a mouse model of allergic asthma (Li et al 2000b) prompted an investigation into the effect of a second TCM herbal intervention for the treatment of peanut allergy. Unlike asthma and other allergic diseases, food allergy is not described in the TCM literature, and no previous research into developing herbal interventions for food allergy has been reported. In light of the gastrointestinal symptoms induced by food allergic reactions, and the Th2 dominant responses of food allergy, it was hypothesized that a TCM herbal formula, FAHF-1 (Food Allergy Herbal Formula-1) containing Ling Zhi (LZ), an herb shown to have 'anti-inflammatory' and anti-allergy properties (Perdue et al 1984, Sampson & Eigenmann 1996) and Wu Mei Wan (WMW) used to treat colic, vomiting, and chronic diarrhoea accompanying ascaris infestation might be useful for the treatment of food allergy. In this study, we tested its therapeutic effects on a murine model of peanut allergy.

Study design

Five-week-old female C3H/HeJ mice were sensitized with freshly ground whole peanut in the presence of cholera toxin and boosted one and three weeks later, as previously described (Li et al 2000a). FAHF-1 treatment was initiated one week later and continued for seven weeks. Following treatment, mice were challenged with peanut, and anaphylactic symptoms, body temperatures, plasma histamine and IgE levels were measured. T cell proliferative responses and cytokine production were also determined (Li et al 2001).

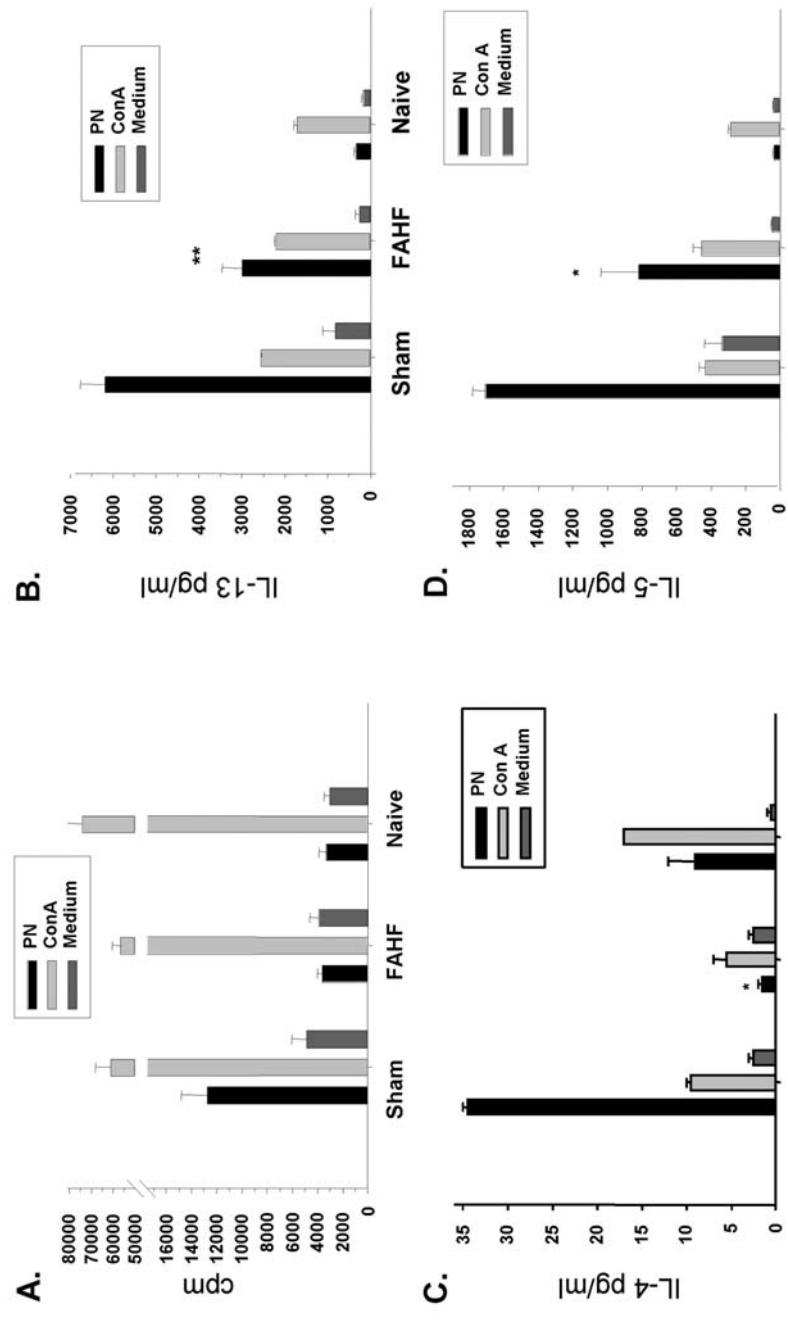

FIG. 4. *In vitro* proliferative responses and Th2 cytokine production by splenocytes from peanut-sensitized mice following peanut challenge. (Published with permission from Li et al 2000a.)

Results

FAHF-1 completely blocked peanut-induced anaphylactic symptoms and markedly reduced mast cell degranulation and histamine release. Peanut-specific serum IgE levels were significantly reduced at the time of challenge (mean ± SEM 531 ± 80 to 347 ± 98 ng/ml in FAHF-1 group vs. 544 ± 82 to 726 ± 95 ng/ml in sham group), and remained lower (343 ± 69 ng/ml) 4 weeks after discontinuation of treatment. Splenocytes from FAHF-1-treated mice secreted significantly less IL4, IL5 and IL13 than splenocytes from sham-treated mice following peanut stimulation (Fig. 4). However, IL4, IL5 and IL13 secretion following Con A stimulation of splenocytes from FAHF-1-treated and sham-treated mice was not significantly different. FAHF-1 treatment did not alter either peanut- or Con A-stimulated interferon (IFN)γ production (peanut: 1367 ± 582 ng/ml in sham and 1417 ± 1140 ng/ml in FAHF-1; Con A: 1461 ± 569 ng/ml in sham and 1761 ± 1132 ng/ml in FAHF-1). These results suggest that FAHF-1 treatment resulted in specific suppression of both peanut-induced T cell proliferation and peanut-activated Th2 cytokine secretion. No toxic effects on liver or kidney functions and no overall immune suppression was observed.

Conclusions

The herbal formula, FAHF-1, protected peanut-sensitized mice from anaphylactic reactions and significantly reversed established IgE-mediated peanut allergy. This effect was associated with a reduction in peanut-specific IgE and mast cell degranulation, and the down-regulation of Th2 cytokine production. Although animal models are not identical to human disease, and further studies regarding effects of long-term administration and/or interactions with prescription drugs are required, this study suggests that FAHF-1 may be useful for the treatment of peanut allergy, and perhaps other IgE-mediated food allergies.

Final comments

The number of severe and fatal food-allergic reactions indicate that the current methods of therapy, i.e. allergen avoidance, are not adequate for dealing with this disorder. In addition, standard immunotherapeutic approaches also are not adequate due to their unacceptably high risk-to-benefit ratio. Novel therapeutic approaches are clearly needed for the treatment of food allergy. In the past several years, several potentially effective therapeutic approaches have been identified that may be effective in the treatment of peanut allergy. Using a mouse model of peanut-induced anaphylaxis, preliminary studies indicate the efficacy of 'engineered' recombinant proteins as well as Chinese herbal medications in reducing peanut-induced allergic reactions. Most recently, clinical trials in

patients with peanut-induced anaphylaxis indicate that monthly treatment with anti-IgE is highly effective at reducing serious allergic reactions. Further studies are needed to confirm these promising results.

Acknowledgement

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DISCUSSION

Kricek: I am going to describe an approach that we are pursuing at the Novartis Research Institute in Vienna, which aims at developing anti-allergic drugs which may act against all types of IgE-mediated anaphylaxis. Our target is the α chain of the high affinity IgE receptor (FcεRIα). This connects with Dr Metzger's paper earlier in this meeting (Metzger 2004, this volume). It is one of the major nodes in the whole network. In order to study the topology of this receptor region and its involvement in IgE binding and triggering, a couple of years ago we produced a series of monoclonal antibodies against the α chain of this receptor. One of these antibodies is 5H5F8, which was found to be biologically active by shutting off IgE-mediated receptor triggering. 'Shutting off triggering' means that we don't measure any mediator release. Furthermore, we see an effect on calcium mobilization, and we can demonstrate that this antibody inhibits the phosphorylation of the γ chain which is involved in signalling. It also works on rhesus monkey α chain expressed on skin mast cells: passive cutaneous anaphylaxis reaction induced after sensitizing monkey mast cells by intracutaneous injection of human IgE and subsequent cross-linking by an anaphylactogenic anti-hIgE mAb can be completely blocked by simultaneous local administration of the 5H5 antibody. This biological effect would be expected for an antibody which recognizes an epitope involved in the binding of IgE to the receptor. However, the first surprising observation was that this antibody does not inhibit binding of IgE to the receptor. Therefore, we conclude that shutting off of the receptor must be accomplished by a novel non-IgE-mediated mechanism. We became interested in finding the epitope that this antibody binds to, because this could represent a potential novel drug target. The second surprising observation was that mAb 5H5 recognizes just a small linear epitope with the amino acid sequence KAPREKYWL. We further characterized this epitope by using a peptide array in which we replaced any of its amino acid residues by any other naturally occurring amino acid. Now we know exactly the required residues for mAb 5H5

binding. KAPREKYWL is the stalk region of the α chain. IgE binds to a different site within the α2-immunoglobulin-like domain, and KAPREKYWL is the membrane-proximal region that sticks out of the membrane. Binding of the antibody or even its much smaller Fab fragment to this region, acts in the way I have just described. Seeing that this Fab fragment is active also excludes a possible negative regulation effect induced by the interaction of the Fc part of mAb 5H5 with FcγRI2B, and thus raises the question of how 5H5 works. We don't know. One possibility would be that fixation of the stalk region may act on the cytoplasmic part of the α chain by blocking or inhibiting the exposition of a signalling motif which hasn't yet been identified. Another possibility would be that we interfere with the interaction of the stalk region with extracellular domains of the other subunits of the receptor. It might also be possible that the stalk region acts on some as yet unknown component of the cell membrane. Based on the observation that we see the inhibitory effect with a Fab fragment that recognizes a very small linear peptide epitope which can be chemically synthesized and retains antibody binding we thought that KAPREKYWL may be a potential target for screening low molecular weight compounds that inhibit signalling of the high affinity IgE receptor. We tried to verify this assumption by confocal nanoscanning. This technology was developed in Vienna in our institute. We start with synthetic KAPREKYWL peptide that is fluorescently labelled on the C-terminus. This is then mixed with a combinatorial chemistry library. Chemical compounds are attached to the surface of microbeads via a linker moiety that is also a fluorescence label. When the whole system is excited with a laser, and if the labelled peptide binds to a bead, you get an energy transfer and this bead lights up. The positive beads are then sorted automatically, and by photo cleavage we clip off the compound from the bead. Mass spectrometry is used to determine the structure of the compound. We started with about 350 000 compounds, and 15 turned out to be binders. Half of these were also able to bind to the resynthesized compound lacking the linker moiety proved to be biologically active on various systems. For example, we have demonstrated the inhibition of sulfidoleukotriene release from rat basophilic leukaemia RBL-2H3 cells transfected with the human IgE receptor α chain. If we trigger these cells via the human α chain with human IgE the substances inhibit leukotriene synthesis with an IC_{50} in the submicromolar range. If we trigger these cells non-specifically with ionomycin there is no inhibition and cell toxicity in terms of an antiproliferative effect is only seen at $>15\,\mu$M concentrations after three days exposure. The substances are also active on primary cells. In human blood basophils and human umbilical cord blood mast cells we also see inhibition of leukotriene release. In contrast, these substances are not active on murine mast cells and cell lines, but like mAb 5H5 they also work on rhesus monkey basophils and mast cells. Moreover, they are also active *in vivo* by

suppressing passive anaphylactic skin reaction in rhesus monkeys. By a chemical derivation program we currently try to get substances that we can develop into drugs. We are also using computer-assisted structural modelling. We sequenced the hypervariable regions of mAb 5H5 and used these sequences to generate a 3D computerized model of the antigen-binding site of this antibody. This site forms a cleft into which the KAPREKYWL sequence is docked mathematically in its minimal energy conformation. From this structure one can deduce points of contact with the antibody and define a model pharmacophore which is now being used to optimize our lead compounds and extract new compounds from existing libraries. Our hypothesis is that the described stalk region is not only an effector region in FcεRI, but may be a general feature of Fc-like receptors. Sequence alignment of a couple of Fc receptors from different species indicates that this might be the case.

Leung: When you down-regulate the KAPREKYWL target, if you were to attempt to activate the mast cells through non-FcεRI stimuli, would they still be activated?

Kricek: I have shown this for ionomycin, where it works fine. We get specific inhibition only by triggering through the human receptor. The substances are inactive on mouse mast cells. We have this recombinant RBL cell, which expresses both the recombinant human and the rat α chain. We can trigger this cell via the human and the rat IgE. Unfortunately, rat and mouse IgE bind both human receptor α chain and rat α chain. Therefore it is tricky to discriminate between signalling through the human or the rat α chain.

MacGlashan: We have recently found that not all mouse IgEs bind to human receptor, so you could do that experiment.

Marone: Do you have any evidence that your compounds can also inhibit the IgE-mediated release of cytokines from basophils?

Kricek: That is what we are currently investigating. The most prominent candidates are IL4, IL13 and TNFα. We also want to differentiate our active test compounds from classical antihistimines, in order to increase the spectrum of possible applications. We know that these compounds are inactive on human T cells and also other cells as far as cytokine synthesis is concerned. We see some effect on the IL10 levels in human monocytes, which is very interesting because monocytes also express the human high-affinity IgE receptor without the β chain. It has been shown that cross-linking of this type of receptor on monocytes has an influence on IL10 synthesis.

Lee: Did you measure IgE receptor expression?

Leung: No, it was a low budget study. It is something we would consider looking at in a future study.

Lee: In chronic studies of severe asthma, I understand that IgE receptor expression goes down as well. You didn't present data on specific IgE, but I presume this must have gone down?

Leung: It went down, but it was no better than monitoring total IgE as a measure. The correlations were actually better with total IgE than peanut-specific IgE.

Vercelli: I would like you to clarify whether you may plan to use the anti-IgE therapy as a way to allow you to do immunotherapy?

Leung: That would be the obvious route to go. But I didn't imply this at all in my paper. Certainly, in a future study, this is a way to go: to use anti-IgE in immunotherapy. There is a paper in the *Journal of Allergy and Clinical Immunology* suggesting fewer side effects (Kuehr et al 2002).

Mosbech: With respect to the anti-IgE, did you do some preliminary experiments looking at what would happen when you stopped the treatment? Could you expect a more permanent effect, or a rebound?

Leung: There is no rebound. Actually, before people went into the extension study they were off anti-IgE therapy for eight weeks. Interestingly, even in the 450 group there was still an 85% reduction in serum IgE. There might be evidence for a long-term down-regulatory effect. In the original studies that Paul Jardieu did in mice, there were suggestions of a negative feedback to the IgE B-cell, but with human trials this down-regulation of IgE synthesis was not seen. We don't expect to see a long-term effect. All of our patients are now on long-term therapy so we haven't really looked at what happens when they are off therapy.

Schwartz: Can you say something about the new antibody compared with the old one? Is it humanized or chimeric?

Leung: It is a humanized antibody. It is very similar to Xolair except for a few amino acid differences in the antigen-binding region.

Schwartz: Do they recognize the same epitope?

Leung: They both recognize epitopes in the CH3 region.

Schwartz: Did you look at skin-test reactivity to see whether you are really depleting mast cell-bound-IgE?

Leung: That is a good point. After the first four months we didn't see much shift in the end-point skin test titration. We are hoping that we can do the skin testing one-year-out, to see whether that has now shifted.

Schwartz: In our discussion earlier, we talked at length about how food-induced anaphylaxis may not be mast cell dependent. You are seeing a substantial change in the ability to ingest food without much change in mast cell reactivity as measured by skin tests. This suggests that different mast cell populations are involved in food-induced reactions compared to reactions induced by other routes of allergen administration.

Sampson: They saw the same things in the asthma studies: there wasn't a good correlation between skin-test reactivity early on. It may be that the off-loading of IgE in mast cells in the skin may be slower.

Kricek: You have shown data with the Tanox antibody, and you get a dramatic decrease in serum levels of free IgE. Still, there are 16 units or so present. A couple

of receptors occupied by IgE seem to be efficient for triggering the cells and inducing anaphylactic reactions. So the fact that removal of IgE will also down regulate receptor expression may be one explanation. Has anyone thought about the possibility that the treatment with this antibody might generate an anti-idiotypic antibody against the Tanox antibody, which would then mimic the receptor binding site of IgE? The anti-idiotypic antibodies might bind to the receptor and act as a vaccine that inhibits the binding of IgE to the receptor. This could cause a long-term effect of passive immunotherapy.

Leung: That is a good point. We haven't explored this. The only thing that goes against what you just said is that the skin test didn't change very much at the end of four months.

Kricek: The antibody is not formulated as a vaccine. It is given in a way that induces tolerance in such high doses. One would have to make experiments where one formulates low amounts of this antibody as a vaccine and look at what happens.

Finkelman: I wanted to go back to the work at the beginning of Donald's paper about the transfected *E. coli* approach. How did it affect IgG and IgE antibodies?

Sampson: IgG1 came down and IgG2a went up. IgE came down.

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Patient's perspective and public policy regarding anaphylaxis

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Abstract. It is estimated that close to 7 million Americans have food allergy. The incidence of food allergy, particularly peanut allergy, is believed to be on the rise. Several studies have shown that in spite of a patient's best efforts to avoid ingesting the allergy-causing food, reactions will occur. These reactions occur from incorrect ingredient information in food service or restaurant settings, incorrect product labels, or mistakes in label reading. In the hospital setting, patients are sometimes treated for an anaphylactic reaction in the emergency room but are not given instructions to see a specialist to determine the cause of their reaction, nor are they given a prescription for epinephrine to arm them to treat future allergic emergencies. Two studies of fatal and near fatal allergic reactions concluded that a delay in administration of epinephrine could have been a factor in the fatal outcomes. However, schools often do not have written emergency action plans in place for children with documented food allergy, and patients and caregivers often report not knowing when to use the epinephrine kit or how to use it. Until there is a cure for food allergy and anaphylaxis, avoidance of the allergen is key. There is much work to be done in education and public policy regarding anaphylaxis.

2004 Anaphylaxis. Wiley, Chichester (Novartis Foundation Symposium 257) p 265–275

In this modern age, few childhood disorders have no cure or treatment. Parents are accustomed to receiving a diagnosis followed by prescriptions for treatment and prevention of a number of childhood maladies including infections, allergies and asthma. For food allergy, however, there is no cure or medication to prevent a reaction (Sicherer et al 2001a). Parents are given the diagnosis and simply told to avoid the allergen. The medications they may be prescribed are to be used only when avoidance has failed and their child is experiencing an allergic reaction (Sicherer et al 2001a). Parents are also told that quick administration of the medication is critical for a positive outcome.

Avoidance of the allergen becomes a full-time, year-round task. Every decision the family makes, every day of the week, includes a consideration about food and avoiding a reaction (Sicherer et al 2001a).

Using the Children's Health Questionnaire (CHQ-PF50) to measure the physical and psychosocial impact of childhood food allergy on quality of life, investigators found that parents whose children have more than two food allergies had significantly lower scores in 7 of 12 established norms than families whose child had only one or two food restrictions. Families with only one or two food allergies also scored significantly lower than established norms on a number of scales. Families showed a reduced perception of their child's general health, an increased level of stress and worry by the parents, greater family tension and limitations on the usual family activities due to the child's food allergy (Sicherer et al 2001a).

The stress of raising a child with food allergies affects each family differently, depending, in large part, on how the physician initially presents the information and the family's coping style.

Based on how the information is presented to the family, they can leave the doctor's office terrified and unsure whether they will be able to prevent the next reaction, or concerned but confident that they can keep their child safe. Parents who have been told that each reaction will be worse than the previous one, and that the next one may be fatal, or that this is 'the most allergic child' the doctor has seen in all his/her years of practice, become so fearful that they take their precautions and vigilance to the extreme (Mandell et al 2002). Some may elect to home-school their child rather than risk a reaction in the school setting. Afraid of the risks when eating outside the home, many retreat and minimize or eliminate their child's contact with others.

Concerned that wait staff will be unable to provide them with accurate information about ingredients used to prepare their food (Furlong et al 2001), some elect not to take their child to restaurants. Visits to the homes of friends and relatives who don't understand the allergy are eliminated.

On the other hand, parents who have been told the facts about anaphylaxis, who have been trained regarding when and how to use epinephrine but encouraged to 'live a normal life while taking precautions', appear to adjust and allow the child to participate in the usual childhood activities both during and after school, including attending camp, dining in restaurants, and visiting friends' homes. Decisions about special occasions are viewed as challenges that can be met. Their 'can do' attitude is contagious, making the child feel empowered and confident.

The diagnosis of food allergy has a profound effect on the patient and the family, and in spite of their best efforts to prevent a reaction, studies indicate that reactions will occur (Vander Leek et al 2000). A series of 32 fatal food allergy-induced deaths showed that reactions occurred in college dorms, restaurants, school, camp, childcare and dance class. The cause of the reactions was varied as well, ranging from incorrect ingredient statements, incorrect information at a restaurant,

first-time reactions, hidden or undeclared ingredients and cross contact (Bock et al 2001).

For patients with food allergy, any place that has food can be a potential source of trouble. Patients must always be on alert for a reaction and be ready to treat it as quickly as possible. Unfortunately, in the USA, the public policy for schools, food labelling, restaurants, emergency medical services and airlines has a number of gaps which can leave these patients at risk.

Schools

In the United States, individuals with life-threatening food allergy are protected by various disability rights laws. This is especially apparent in the school setting.

Two of these laws, the Individuals with Disabilities Education Act and the Rehabilitation Act of 1973, Section 504, apply to institutions that receive federal funds, and are designed to protect children in the school or childcare setting. In order to comply with these laws, schools must develop a protocol to keep these children safe and allow them full and equal participation in all school activities.

The Americans with Disabilities Act (ADA) is a relatively new law (1990), and hence largely untested in the area of food allergy. The ADA requires *public accommodations* (privately operated entities that cater to the public, i.e. restaurants, retail stores, theatres, hotels, private schools, etc.) to make reasonable modifications to their policies, practices and procedures so that disabled individuals can enjoy the full benefits of their services.

Problematic, however, is the interpretation of *reasonable modification* as it pertains to individuals with food allergy. To illustrate, the ADA requires private childcare facility staff to be trained and prepared to treat an allergic reaction. On the other hand, restaurants are not required to divulge ingredient information or to prepare an allergy-safe meal.

Indeed, the amount of protection afforded food-allergic individuals by the ADA and Section 504 is an evolving topic, primarily because food allergy is still an emerging issue, and is not yet 'settled' in the law. The current dilemma related to students with severe allergies is not so much whether they should be accommodated, but rather 'exactly how far should a school go?' (Rosenfeld 1998).

In spite of these federal laws, there are still instances where a child is turned away from a school, camp, or childcare centre because of the child's food allergy and the potential liability if a reaction occurs (LaPetite Settlement Agreement 1997). The reticence on the part of these professional institutions has come, in part, from the parent's presentation of the food allergy history.

A number of parents, in their attempt to educate the school staff and others about the seriousness of their child's food allergy, state that their child could die within minutes of breathing in the fumes from an open jar of peanut butter, or that the

child will suffer a reaction from simply walking into a building where peanut butter is being served. Others, so afraid of a reaction occurring, demand that schools ban certain food from the premises in order to keep their children safe.

A review of reactions to peanuts and tree nuts showed that reactions occurred in spite of a peanut ban (Sicherer et al 2001b). Additionally, in schools where a ban has been instituted, it is not uncommon for the parents of students who do not have allergies to complain that the rights of the majority are being infringed upon to protect one or two children (Brown 2002).

In their attempt to protect their allergic children, parents occasionally inadvertently alarm caregivers to the point where they doubt their ability to keep the child safe, leading some school administrators or childcare providers to discharge the student from their care, or require that the child's parents sign a liability waiver in case of a reaction. In spite of their concern for keeping students safe, and their attempt to protect themselves from legal liability, several studies have shown that schools often don't have written plans in place for systematically handling an allergic emergency (Rhim & McMorris 2001, Nowak-Wegrzyn et al 2001).

Over the past 5–10 years, awareness of day-to-day management strategies for food allergies has increased. The American Academy of Allergy, Asthma & Immunology and the American Academy of Pediatrics have published position papers, and the Food Allergy & Anaphylaxis Network (FAAN) has issued 'School Guidelines for Managing Students With Food Allergies'. These documents, along with training seminars and educational materials, have helped ease the tensions in the school setting, but clearly there is much work to be done.

Medication policies in schools

Generally, there are no state laws specifically pertaining to the use of epinephrine in the school setting. State agencies and/or departments often develop guidelines and model policies on school health issues; however, it is incumbent upon local school boards/districts to develop and implement specific policies. Generally, local policies allow other school personnel (teachers, coaches, bus drivers, etc.) to be trained to administer an epinephrine auto-injector in the nurse's absence, because many schools do not have a full-time nurse (FAAN Staff, personal communication 2002).

Several localities allow a student to carry his or her epinephrine auto-injector throughout the school day (with parental and physician authorization), while other localities allow the epinephrine auto-injector to be 'passed' from teacher to teacher as the child changes locations during the school day (FAAN Staff, personal communication 2002).

Unfortunately, many local school policies require that all medicines, including epinephrine, be kept in a secure location, such as the nurse's or principal's office. Such a policy may be problematic, because delay in getting medications quickly during an allergic reaction is considered a factor in a number of deaths in the school setting. Currently there is a movement to pass legislation that will enable children to carry their own medications including asthma inhalers and epinephrine kits.

Labelling

Reading labels is the cornerstone of avoiding a reaction. While the USA is considered by many to have some of the best labelling regulations, there are a number of policies in place that are problematic for patients with food allergies.

Ingredient labelling for packaged foods is regulated by the Food and Drug Administration (FDA). The Food, Drug and Cosmetic Act requires a complete listing of all the ingredients of a food. There are two exemptions: (1) spices, flavourings and colourings may be declared collectively without naming each one; (2) incidental additives do not have to be declared if they are not functional in the finished product and are present in insignificant amounts. 'Insignificant' is not defined; however, industry practice is in the parts per million range.

Ingredients in flavours or spices are not required to be labelled, yet even at these low levels, reactions have occurred from common allergens, particularly milk. In 1996, the FDA's Center for Food Safety and Applied Nutrition issued an 'FDA Allergy Warning Letter' to the food industry after several allergic reactions were reported from allergens in low levels found in incidental additives or flavours. The FDA stated, 'an amount of a substance that may cause an adverse reaction is not insignificant' and called for 'the voluntary declaration of an allergenic ingredient of a colour, flavour or spice.'

The large companies are, for the most part, following this suggestion. Regulation or legislation may be needed to ensure that all food manufacturers list allergens when present, regardless of the low levels.

Additionally, current food labels use scientific or technical terms for common food allergens (Table 1). A study conducted at the Mt. Sinai School of Medicine showed that only 7% of parents of children with milk allergy, 22% of soy allergy families and 54% of those avoiding peanuts were able to correctly identify these foods on the ingredient labels (Joshi et al 2002).

The increasing use of precautionary allergen statements has added further confusion and frustration. Patients are advised to avoid products with precautionary allergen statements; however, they find it harder and harder to do so given the increasing number of food products that now contain these statements. The variety of statements, with no explanation of the conditions

TABLE 1 Label reading, not as simple as it seems

Milk words	*Egg words*	*Wheat words*	*Peanut words*
Casein	Albumin	Cracker meal	Ground nuts
non-dairy	Lysozyme	Semolina	Monkey nuts
Calcium caseinate	Ovalbumin	Spelt	Valencias
Whey			

Partial list of synonyms for common allergens that may appear on an ingredient label in the USA.

under which one or another is used, has also added to the confusion. A recent review of the products in one store in Virginia yielded 25 variations of these statements.

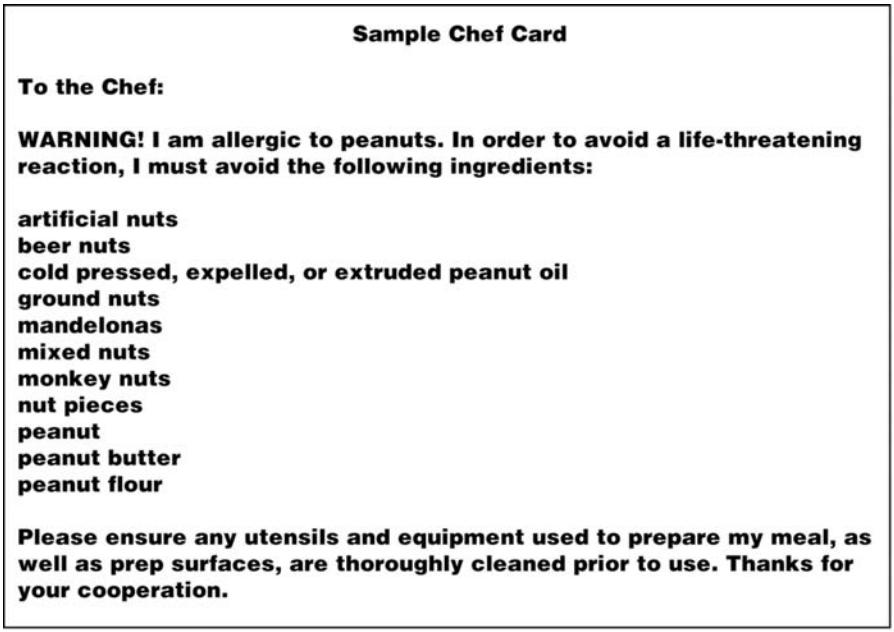
A survey of 550 FAAN members conducted by FAAN in 2000, regarding precautionary allergen statements, indicated that 92% of the individuals avoid products with 'May contain . . .(allergen).' However, only 66% avoid products stating 'Manufactured in a plant that also produces . . . (allergen).' (FAAN unpublished work 2001). The food industry is using both of these statements to convey potential risk. However one-third of the individuals in this survey group are ignoring the 'Manufactured in a plant that also produces . . . (allergen).' warning.

The need for improvements in food labelling is a hot topic in the United States from the perspective of patients, food industry, regulators and legislators. Unfortunately, while many are talking about it, progress is very slow.

Restaurants

Eating in a restaurant is one of the toughest challenges for families with food allergies. Restaurant meals are not regulated by the FDA; therefore, restaurant guests must trust that the wait staff is taking their food allergy queries seriously, will follow through in the kitchen, and will speak up if a mistake is made in meal preparation or presentation. Most restaurant staff receive virtually no training in food allergy, according to an industry spokesperson. High staff turnover in this industry is cited as the primary barrier to food allergy training programmes.

Studies have shown that reactions, some resulting in death, commonly occur in these settings. In a recent review of 32 deaths, 47% were caused by restaurant or food service food (Bock et al 2001). Common causes of allergic reactions include staff not knowing ingredients in menu items, cross-contact with allergy-containing foods in the kitchen, ingredient substitutes (such as pistachios instead of pine nuts



Sample Chef Card

To the Chef:

WARNING! I am allergic to peanuts. In order to avoid a life-threatening reaction, I must avoid the following ingredients:

artificial nuts
beer nuts
cold pressed, expelled, or extruded peanut oil
ground nuts
mandelonas
mixed nuts
monkey nuts
nut pieces
peanut
peanut butter
peanut flour

Please ensure any utensils and equipment used to prepare my meal, as well as prep surfaces, are thoroughly cleaned prior to use. Thanks for your cooperation.

FIG. 1. Sample chef card.

in pesto), allergens in reused cooking oil and surprise ingredients (e.g. crushed nuts in pie crust or peanut butter in chilli) (Furlong et al 2001).

Individuals with peanut or tree nut allergy should be advised to avoid restaurants with Asian cookery because of the high risk for cross contact during meal preparation and cooking. Mexican foods are also reportedly causing a growing number of reactions due to the use of peanut butter in sauces. Caution is advised when eating at these restaurants or any food establishment where the wait staff are not fluent in English and the chances for effective communication are jeopardized (Furlong et al 2001).

To ensure that the wait staff convey the allergy information to the kitchen, some teens rely on a personalized 'chef card' (Fig. 1). These cards are customized to the teen's allergy and other critical information. They are printed on bright paper in a variety of sizes, from business card to 4×6-laminated cards, to suit the individual's tastes. A downloadable template is available at *www.fanteen.org*.

Fast food restaurants, whose menus are primarily set by one corporate office, are often a favourite for families with young children. These establishments commonly have pre-printed ingredient information booklets, thereby allowing the patient to make an informed decision about what is safe to eat.

Emergency medical services

Patients are often instructed to call the rescue squad at the first sign of a reaction for transport to the hospital. A 1998 FAAN study of epinephrine availability on ambulances revealed that only 7 of the 50 states fully authorized all potential emergency personnel (EMTs) responding to an anaphylactic patient to carry and administer epinephrine. Thanks in part to FAAN members, in collaboration with medical professionals and state lawmakers, many states have changed either their laws or regulations to allow more EMTs to carry and administer epinephrine. A number of other states are soon expected to follow suit.

Once at the hospital, patients should be given a referral to an allergy specialist or a prescription for epinephrine. Currently, this is not consistent from hospital to hospital. Indeed, in several cases of fatal anaphylaxis, the patient reportedly had a number of previous visits to the hospital for anaphylactic reactions, but was never told the reactions were caused by a potential allergy to a food nor advised to seek follow-up care (Bock et al 2001, Sampson 2002).

Airlines

Air travel is another difficult challenge for some families, particularly those with peanut allergy. Their fear of an inhalation reaction to peanut particles as 300 passengers consume peanut snacks in an enclosed cabin with no access to an ambulance and a hospital, leads some individuals to request that airlines 'guarantee' them a peanut-free flight. This is something the airlines cannot do.

In order to protect the health of peanut-allergic passengers, some airlines have stopped serving peanuts entirely; other airlines will provide non-peanut snacks to all passengers upon request, while others will provide a peanut-free 'buffer zone' two or three rows in front of and behind the peanut-allergic passenger.

However, air travel may still present a risky proposition to those with peanut allergy. One study, looking at ventilation system filters in commercial airliners, found measurable quantities of peanut allergens (Jones et al 1996). In a subsequent study, investigators found that 42 passengers had experienced allergic reactions during commercial flights; the reactions were caused by ingestion (20 subjects), skin contact (8 subjects), and inhalation (14 subjects), and the causal food was generally served by the airline (37 of 42 subjects) (Sicherer et al 1999).

In preparation for a reaction, airlines are required to carry one dosage of epinephrine in their emergency medical kits (as of February 2004, the requirement will be two dosages), and passengers may bring their prescribed epinephrine onto the plane with them provided that it is accompanied by written medical authorization from their physician (T. Furlong, personal communication 2002).

Conclusion

The instant a physician makes a diagnosis of food-induced anaphylaxis in a paediatric patient, that patient and the patient's family faces an abrupt lifestyle change. Food allergies affect every minute of every day all year long for patients, their families, and other caregivers. While it may seem simple to advise someone to avoid eating a food, studies have shown, and families have reported, that it is easier said than done. Patients and their families must be directed toward educational resources to learn how to successfully manage their food allergy, and they must be given medications and written instructions for handling emergency situations (Bock et al 2001).

Public policy regarding management of students with food allergies in schools and other childcare settings, restaurant staff training, food product labelling, and availability of epinephrine on emergency vehicles, must change to meet the needs of the individuals at risk for a potentially-life threatening allergic reaction; their health and well-being depends on it.

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DISCUSSION

Lee: I want to raise a general point. One of the big difficulties for patients is the accessibility of the internet and the information that can be found on the internet. It is difficult to control all this information. Even helplines and reputable charitable bodies provide information that is factually incorrect and professionally confusing. There is very little anyone can do about this. As far as public policy is concerned this is an important issue.

Golden: One issue that needs to be addressed is what emergency departments do or don't tell people. If someone leaves the emergency department with a head injury they get a computer-generated printout of advice. Have you explored the possibility of creating a system like this for anaphylaxis?

Muñoz-Furlong: There is a sheet that is distributed for anaphylaxis. It could use some improvement. We have tried unsuccessfully to find a company to work with, to make the suggestions you mentioned.

Galli: I heard one disturbing anecdote of a child with a peanut allergy. There had been a lot of education done at the child's school to inform all the children and the teachers about the problem. Sadly, one of the child's classmates hid some peanut inside his sandwich as a 'joke' and a very serious reaction ensued. Is this a common problem?

Muñoz-Furlong: Yes, and when we look into this sort of problem we see it is based on ignorance and curiosity. We have created programmes to help educate the classmates so they don't put the allergic children at risk. In some schools, where there has been repeated harassment like this, we have advised the school staff to have a police officer come in and explain the seriousness of the situation and that anyone who harms someone can be arrested. Whenever this has been tried, it has quickly ended the harassment.

Galli: With regard to the labelling problems, do you deal with a single manufacturing group, or is it a matter of finding and then working with multiple organizations that have overlapping responsibility?

Muñoz-Furlong: We helped put together an allergy issues alliance a few years ago, involving the food industry, trade associations and companies, so we could all discuss our various perspectives on labelling and have the messages and decisions go out to a broad segment of the food industry.

This has been successful, but again the labelling guidelines that have come out of this group are voluntary. The large companies are on board and are taking these policies to their plants around the world, but the little companies that are

getting people into trouble. The small companies typically don't belong to the trade associations and so we can't reach them with allergen information and guidance. Therefore, the message we give to parents is to stick with the big companies, in order to avoid having a reaction from a poorly-labelled product.

Final discussion

Galli: I would like to begin this final discussion by summarizing some points that have struck me during this meeting. Nomenclature was raised at the beginning by Johannes Ring and was extensively discussed. There seemed to be two main approaches. The first, which would be used by the splitters (those most mechanistically inclined), would be to use the nomenclature actually to attempt to classify the reactions according to the mechanisms by which anaphylaxis occurs. This approach of course must depend on our understanding of those mechanisms, and this itself is a bit of a moving target. The second group, the lumpers, would attempt to designate together in one group those clinically similar problems that need similar treatment, regardless of the underlying mechanism. Why should we care about our nomenclature? It is clear that we ideally should agree on a useful nomenclature in order to communicate effectively not only with each other, but also with specialists such as paediatricians, radiologists and emergency-room physicians. We also need a useful and widely accepted classification so that we can communicate effectively with patients, parents and the public. I don't see a clear path ahead here. Those who would like to use nomenclature to address the underlying pathophysiology will probably stick to their guns, and those who would like to call things that are treated in a similar way the same thing will probably continue to do so.

Golden: As I illustrated in my paper, I would argue from the investigator's point of view that we need a standard nomenclature for severity of reactions, so we can compare different studies that have been done properly. I don't know how this is going to be accomplished, but I would like to see it happen.

Galli: So in addition to a useful nomenclature for the phenomena themselves, you'd like to see a uniform severity scale.

Pumphrey: There are systematic differences in the course of reactions to different causes. If you start off with a classification of severity that is based on dextran or sting reactions, these appear to me to be quite inappropriate for classifying food reactions. It will be difficult to come up with a generalized severity index.

Schwartz: Because this is a systemic disease that affects multiple organ systems, it is not as simple as coming up with a severity score for a single organ system. I don't see why one can't separate the gastrointestinal, pulmonary, cardiovascular and cutaneous manifestations, and within each of those have mild to severe. Then it would be up to each study to define what their target(s) might be.

Metzger: For those of you who are involved in clinical situations, is it true that all things that lead to clinically similar problems respond to similar treatments? I thought part of the concern with the 'lumper' position was that not all things that lead to hypotension and respiratory distress should be treated the same way regardless of whether or not they are immunologically caused?

Galli: It's a circular point. In other words, those clinically similar problems that need similar treatment should have the same name. Certainly one doesn't want to treat something that is not anaphylaxis like anaphylaxis. If one were to agree that epinephrine is the first-line treatment of choice for severe reactions, then under what circumstances should one split up the designation of those disorders that are properly treated with epinephrine?

Sampson: When I started in the area of food allergy, everyone seemed to be a 'lumper'. This made the whole field of food allergy more or less a joke. I wouldn't even tell people I worked in the field for the first 10 years. The problem with lumping is that you suddenly get everyone talking about all kinds of bizarre reactions, which are really not relevant, and then people start to ignore it. If you do surveys, 20% of those asked will say they have a food allergy, and 2% do have a genuine allergy and this group has a big problem. This is largely ignored, and I think that a lot of inertia from regulatory agencies, restaurants and so on comes from the fact that people have been desensitized to the issues because we are not precise about what we are talking about.

Fisher: The problem with the nomenclature is that anaphylaxis means two things. It implies a clinical syndrome, and it implies severity. Then it also means an IgE-mediated allergic reaction as opposed to an anaphylactoid reaction where there is no mechanism detected, or there is no mechanism. We have got round this by talking about clinical anaphylaxis when we are lumping, and anaphylaxis and anaphylactoid when we are splitting. There are times one needs to lump and times one needs to split. It is good to lump on the warning bracelet.

Finkelman: This problem may take care of itself. Terminology acceptance tends to be determined by practical issues. Right now if IgE-mediated and other anaphylactic-like reactions are treated similarly, most people are not going to care too much about it except for those who are doing research in the area. If, however, there are very specific therapies for IgE-mediated anaphylaxis that differ from those for other types of anaphylaxis, then we will have a better way to define the process, and we'll have more of a need for distinguishing terminologically different types of clinically similar reactions.

Lee: I agree. It is only useful to start splitting, if by splitting we understand the process better or treat it differently, or the prognosis is different. We are coming to a stage where we do have effective treatments for IgE-mediated anaphylaxis. For this reason I would be supportive of taking the IgE-mediated reactions under the classical name of anaphylaxis, and calling others anaphylactoid.

Galli: With respect to this point, the penetration of the mechanistic information and its implications for treatment will first be among experts in this area. It still then has to be communicated to others, such as emergency-room physicians, family practitioners, parents and schools. In these cases what they need to know is how they will deal differently with this problem as opposed to that problem.

Moving on to pathophysiology, three general areas occurred to me. First, the immunological and biological roles of antibodies other than IgE. In a number of different settings there is the choice that the immune system makes with respect to the balance between IgG4 and IgE. There is the issue of blocking antibodies as conventionally defined. In addition, something that hasn't been discussed extensively at this meeting is the role of IgG antibodies and negative signalling in effector cells. Second, there are the mechanisms and mediators. Those that are responsible for the late and protracted consequences of anaphylaxis remain fully to be understood, even if we feel that we have a good understanding of those responsible for the early events. Although the mouse offers the promise of the manipulation of the genome in order to manipulate selectively individual mediators, this information in some cases may not apply directly to humans. Finally, genetic factors have not been dealt with extensively in the presentations here. More specifically, we are talking about the interaction between the genetic factors and the environment. This has to do with, for example, the production in a Th2 response of the proportion of IgE and IgG4 antibodies, the responsiveness of the effector cells themselves (including the so-called 'releasability' phenomenon), the influence of asthma and atopy on the intensity and occurrence of anaphylactic reactions, target-organ effects, and the influence of genetics on the individual's responses to immunotherapy or the drugs used to manage the acute or late events.

Vercelli: Personally, I am happy with the way you are summarizing this. It would have been nice to discuss these genetic factors a little more. From what we have heard it is already quite clear that there is enough heterogeneity in the responses to indicate that this is where we should start looking in these complex pathways.

Marone: There is one important area which was covered briefly in Fred Finkelman's presentation, which is the importance of cytokines in systemic anaphylaxis. This entire area should be expanded, because there is compelling evidence that several cytokines and chemokines can be produced by human mast cells and basophils (de Paulis et al 1999, Genovese et al 2003).

Galli: I wouldn't argue that the mast cell is the only source of important mediators, including cytokines and chemokines, but it is clearly a source of some of the initial mediators which get the reaction going. We have done a cDNA microarray analysis of populations of cord-blood-derived human mast cells. They were stimulated through the IgE receptor and analysed for mRNA expression at 1 and 2 hours post-stimulation (Sayama et al 2002). The results

obtained from three mast cell populations from unrelated donors are quite similar although not identical. Of the 14 000 genes examined, about 2500 exhibited changes in expression of two- to 200-fold compared with baseline. Half of these went up and half went down. Of all of these genes, about 50% of them are of unknown function. Among those which have been identified are many of our favourite cytokines and chemokines, including interleukin (IL)3, IL4, IL5, IL9 and tumour necrosis factor (TNF)α, as well as IL8, MCP-1 and MIP-1α. However, the study also demonstrated increased expression of IL11 and many other cytokines and chemokines (or molecules that influence interactions with T cells, B cells or dendritic cells) that had not previously been identified in mast cells (Sayama et al 2002). Many of these cytokines and chemokines influence multiple aspects of immune responses and tissue responses. These results of course do not indicate that the mast cell is the only source of such mediators, or even the most important source, but when we consider what sort of 'markers' and mediators we are currently using or thinking about in the context of anaphylaxis, we may essentially be looking under the lampposts — that is, we have been looking at the mediators which have already been studied, and there are now many additional candidates that have not yet been evaluated. There is a lot of work to do here.

Factors contributing to fatality and/or severity have been emphasized in a number of the presentations. These include asthma and existing cardiovascular disease. It would appear that the risk of death due to anaphylaxis is greater in some settings than others, such as in reactions to foods and drugs. Again, genetic factors probably also are involved.

Fisher: You can add β blockers to your comorbidity risk.

Galli: There seems to be a consensus that if one is going to have an anaphylactic reaction, the presence of asthma is a bad thing. This seems to be true not only in food allergy, but also in other reactions.

Müller: Perhaps mastocytosis should be added.

Galli: Interestingly, people invited to comment on mastocytosis at this meeting have so far said that they didn't have a lot of experience with it.

Müller: Insect sting allergy in people with pre-existing mastocytosis is more severe.

Golden: Yes, there are more adverse effects, more relapse after discontinuation and a higher frequency than in the general population.

Galli: The observation of an increased severity of anaphylaxis in subjects with mastocytosis of course could be used to support the argument that mast cells are important effector cells in anaphylaxis. Although patients with mastocytosis are not generally thought to have elevated basophil levels (Galli et al 2001), I'm not sure how thoroughly this has been investigated. Indeed, because absolute blood basophil counts are so rarely performed, I think that it will be difficult to identify a large enough group of patients with significantly different levels of blood

basophils to evaluate whether differences in basophil levels might also influence the severity of anaphylaxis.

Sampson: I only have limited experience with mastocytosis patients with food allergy. But at least in the food allergic patients, they get a lot more in the way of GI symptoms and cutaneous symptoms. Is it the same with insect venom allergies?

Müller: Almost all have cardiovascular reactions.

Schwartz: I take care of half a dozen mastocytosis patients. At least three or four have recurrent anaphylactic episodes where they become hypotensive and their tryptase levels go way up. There is no obvious precipitating event.

Galli: Although we have focused on the IgE antibody and its receptor as targets, there are other approaches being taken to target the mast cell itself. The idea is that, since this is the major effector cell, it would be beneficial to eliminate this cell entirely. This brings up the whole question of what benefits mast cells may provide to the host. In mice, in which these cells can be manipulated, mast cells appear to be necessary for optimal innate immune responses to certain types of bacterial infection, and for optimal acquired immunity to certain parasites (Echtenacher et al 1996, Malaviya et al 1996, Matsuda et al 1990, Galli et al 1999, Malaviya & Abraham 2001, Mekori & Metcalfe 2000, Wedemeyer & Galli 2000). Although data from humans obviously are quite limited, one would expect that some of the same general principles are likely to apply. However, protective roles for mast cells in host defence in humans certainly have not yet been proven.

Finkelman: Would you say that we don't know, though, that in any of those settings that IgE-mediated activation is essential?

Galli: That is a fair generalization. However, a study of primary infection with *Schistosoma mansoni* showed that, compared to wild-type mice, $IgE^{-/-}$ mice exhibited a significantly increased worm burden (by $\sim$50%) and an $\sim$40% reduction in liver granulomas at 8 weeks of infection (King et al 1997). This work indicates that IgE-independent mechanisms contribute to host resistance in this setting. The only case in which it has been clearly shown that both mast cells and IgE can contribute to optimal host defence is acquired immunity to the cutaneous feeding of larval Ixodid ticks of the species *Haemaphysalis longicornis* (Matsuda et al 1990). Matsuda et al (1990) showed that immunity to the feeding of larval *H. longicornis* ticks required both mast cells and IgE. Ixodid ticks, by the way, are economically and medically important.

However, mast cells apparently are not absolutely required for acquired immune responses to all Ixodid ticks. Steeves & Allen (1990) showed that acquired immunity to larval *Dermacentor variabilis* ticks *was* expressed in mast cell-deficient W/W^v mice. In this case, basophils may have provided functions similar to those of mast cells, since the tick feeding sites contained large numbers of basophils, both in the W/W^v mice and in the wild type mice. In guinea pigs, an anti-basophil serum was shown to markedly reduce blood basophils, and basophils infiltrating tick

feeding sites, and to markedly reduce acquired immunity to the cutaneous feeding of larval *Amblyomma americanum* ticks (Brown et al 1982).

In summary, immunity to ectoparasites is the area in which there is the strongest evidence for a real benefit in host defence for the mast cell/basophil-IgE system. However, the relative importance of mast cells vs. basophils in these responses may vary according to the species of tick and the host species.

Finkelman: It is likely, although not proven, that the IgE system has a benefit in certain worm infections. For more typical infections in the western world, such as peritonitis, where mast cells are known to be important, it isn't known whether IgE is also important.

Gould: There is both epidemiological and experimental evidence to support the notion that atopy is protective against certain types of cancers, such as ovarian cancer. We have demonstrated the superior protection conferred by an IgE antibody compared to an IgG1 antibody of the same tumour antigen specificity in animal models of ovarian carcinoma (Gould et al 1999, Karagiannis et al 2003).

Galli: It would be interesting to get beyond the epidemiological evidence to mechanistic evidence. I am often thankful that I don't do epidemiological studies.

Lee: I'd like to make a point about co-morbidity and I was interested in the case history presented by Ulrich Müller on the use of β blocker and immunotherapy. Reading through some of the lists of risk factors for comorbidity, one might be tempted to say, for example, that we shouldn't give immunotherapy to people with asthma because they are at greater risk of problems. In fact, a few years ago some immunotherapy guidelines were produced which proposed that immunotherapy should not be given to anyone with rhinitis if there was accompanying asthma. Many people would now accept that each case has to be considered on its merit.

Galli: Gianni Marone might want to comment on this case. The clinical data suggest that some of the findings discussed in his presentation may be very relevant for interpreting what might have happened to the patient.

Marone: It was clear that this patient had dilated cardiomyopathy, which is not an acute event. This is an example of the copathology that is important to exaggerate the cardiovascular effects of the endogenous release of vasoactive mediators released from mast cells and basophils. This is a beautiful example of how a small dose of antigen can cause the release of small amounts of vasoactive mediators which can then have profound cardiovascular effects. It is clear from my side that this patient had a number of cardiovascular risk factors: previous evidence of arrhythmias, arterial hypertension, dilated cardiomyopathy and β blockers withdrawn.

Galli: And probably also increased numbers of mast cells in the heart.

Marone: If you believe my data, yes.

Galli: Let's turn to diagnosis and management. As Richard Pumphrey has emphasized, it is critical first to identify the problem as anaphylaxis, and then to

attempt to identify: (1) whether it is likely to be an IgE-associated problem, (2) the possible allergens, and (3) other provoking or augmenting factors (the summation effect). We then can look for levels of specific IgE and then consider searching for markers of effector cell activation, such as tryptase. This raises the question of whether we have the right markers, or enough markers. Tryptase is an extremely useful one, and my guess is that there will be others as well. We also can attempt to identify predictors of the severity of the reactions. We know that asthma is a predictor, and the question is what other criteria might be useful clinically. Is there anything on the horizon that might permit us to make a guess as to which patients who are at risk for developing anaphylactic reactions may develop truly serious ones?

Golden: The potential problem with identifying predictors is deciding what level of certainty we have with these. One of the comments I made yesterday was about the level of sensitivity. The strength of the skin test or the level of IgE is statistically correlated with severity, but not absolutely. We have to avoid the pitfall of believing too much in our predictors and ignoring the outliers. As long as we keep this in mind predictors can be very useful.

Metzger: Just to connect your point about identifying allergens and other provoking and augmenting factors with your previous points, I would like to ask those of you who are following patients with anaphylaxis whether there is an attempt to collect clinical specimens that could be used to look at genes that are turned on during the attack, or generally in patients who are subject to attacks.

Galli: That is a good point. It may be possible to analyse material that already exists. Mass spectrometry-based approaches for identifying patterns of proteins in biological specimens are quite well developed and are currently being used to search for useful 'markers' in other clinical settings.

Metzger: Is there an attempt by any group to collect material from the appropriate clinical background? Is this an appropriate time to start collecting such specimens?

Galli: Our group and others are starting to do this in animal models, and it can be extended to humans quite readily. Since the analysis can be done rapidly it could even be done during the course of a prolonged episode. However, I am not aware that this is going on at the moment.

There are two more points I wanted to make. First, concerning treatment and later, regarding public policy. We have heard about new approaches to treatment, such as targeting IgE or its receptors. We have also discussed a form of treatment that has been used for a very long time, epinephrine. As a pathologist who does not often use epinephrine (actually, I never use it), I was startled about the amount of controversy as to whether to use epinephrine in patients with anaphylaxis, when to use it and how to administer it. I am still confused about how best to make this decision in an individual patient. We didn't get a chance to talk very much about

the effects of corticosteroids and what they might be doing in patients with anaphylaxis. In this setting, can we even contemplate not using corticosteroids in a controlled study of serious cases of anaphylaxis, in order to study what would happen if they were omitted?

Golden: That is an important study to do. I don't know how the ethics committees would view this. My understanding is that there is not any evidence that corticosteroids prevent biphasic reactions.

Sampson: That is our experience. They don't.

Galli: Have you ever contemplated not using them?

Sampson: Yes, but I have never not done it.

Fisher: One thing about epinephrine which hasn't been discussed is that acutely administered in high doses, it can produce a cardiomyopathy. It is quite a dangerous drug when it is given for a long time in high doses. We tried hard with steroids, manipulating the databases, but it is very difficult. There is evidence that if you get someone who gets anaphylaxis with asthma they are going to be there for an hour. There is less good evidence if it is just hypotension. This study is impossible to do. There is no evidence corticosteroids hurt, so you might as well give them.

Lasser: I don't see the risk in using corticosteroids in a questionable case. They haven't resulted in any adverse reactions in our experience. I'm not clear about why you ask about only using it in the case of biphasic reactions.

Golden: I said this because one of the main arguments for using it is to prevent prolonged biphasic, protracted anaphylaxis.

Lasser: What about the severity of the initial reaction, or ablating the initial reaction?

Golden: I would have to ask the same question: is there any evidence that it is doing anything?

Lasser: Yes.

Golden: In what forms of anaphylaxis?

Lasser: I am referring to anaphylaxis in my own field.

Sampson: Is this administering corticosteroids before the contrast agent is given?

Lasser: Yes.

Sampson: This is a very different situation. There are data that anaphylaxis can be altered by steroids in intentional provocation situations where it is possible to give them before the allergen is encountered. But in insect sting or food anaphylaxis, we don't know when the reactions will occur. There is not much evidence that steroids administered after anaphylaxis have an effect.

Finkelman: I have become very confused about the meaning of the term 'biphasic reaction'. In asthma I understand it to be two different sequential pathological processes. In anaphylaxis I am unsure as to whether this is also true, or whether it just represents the initial treatment wearing off.

Galli: In closing this meeting, I wish to comment on aspects of nomenclature and public policy. I certainly hope that it will be possible to achieve a true international consensus about the nomenclature and treatment of anaphylaxis. Is this likely? We have an expression in the USA that unfortunately may apply here: 'Dream on'. However, even if such an international consensus is not soon achieved, one thing is clear: a close coordination of advocacy, scientific and medical groups is essential to advance understanding of effective approaches for the prevention and treatment of anaphylaxis, both among physicians and the public. This is clearly not happening optimally at the moment, particularly on the Internet. We have heard at this meeting of an excellent effort by the advocacy group FANN, that has built a strong alliance with scientists and physicians interested in this topic. More efforts of this sort clearly are needed, and, as illustrated by FANN's work, one key to success is effective communication with and education of those who produce, label and serve foods, as well as those who train and supervise medical care givers.

In closing, I would like to thank the many who have helped make this meeting one that has been extremely educational and interesting.

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