Risk assessment in anaphylaxis: Current and future approaches

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Risk assessment of individuals with anaphylaxis is currently hampered by lack of (1) an optimal and readily available laboratory test to confirm the clinical diagnosis of an anaphylaxis episode and (2) an optimal method of distinguishing allergen-sensitized individuals who are clinically tolerant from those at risk for anaphylaxis episodes after exposure to the relevant allergen.

Our objectives were to review the effector mechanisms involved in the pathophysiology of anaphylaxis; to explore the possibility of developing an optimal laboratory test to confirm the diagnosis of an anaphylaxis episode, and the possibility of improving methods to distinguish allergen sensitization from clinical reactivity; and to develop a research agenda for risk assessment in anaphylaxis.

Researchers from the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergology and Clinical Immunology held a PRACTALL (Practical Allergy) meeting to discuss these objectives.

New approaches being investigated to support the clinical diagnosis of anaphylaxis include serial measurements of total tryptase in serum during an anaphylaxis episode, and measurement of baseline total tryptase levels after the episode. Greater availability of the test for mature β-tryptase, a more specific mast cell activation marker for anaphylaxis than total tryptase, is needed. Measurement of chymase, mast cell carboxypeptidase A3, platelet-activating factor, and other mast cell products may prove to be useful. Consideration should be given to measuring a panel of mediators from mast cells and basophils. New approaches being investigated to help distinguish sensitized individuals at minimum or no risk from those at increased risk of developing anaphylaxis include measurement of the ratio of allergen-specific IgE to total IgE, determination of IgE directed at specific allergenic epitopes, measurement of basophil activation markers by using flow cytometry, and assessment of allergen-specific cytokine responses.

Algorithms have been developed for risk assessment of individuals with anaphylaxis, along with a research agenda for studies that could lead to an improved ability to confirm the clinical diagnosis of anaphylaxis and to identify allergen-sensitized individuals who are at increased risk of anaphylaxis. (J Allergy Clin Immunol 2007;120:S2-24.)

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Abbreviations used

ACE: Angiotensin converting enzyme
C3a, C5a: Fragments of complement C3 and C5 proteins referred to as anaphylatoxins
CCDs: Cross-reacting carbohydrate determinant
HHMC: Human heart mast cell
Kit: Transmembrane tyrosine kinase receptor for stem cell factor
LTC_4: Leukotriene C_4
PAF: Platelet-activating factor
PGD_2: Prostaglandin D_2
SCF: Stem cell factor
SPT: Skin prick test

Anaphylaxis is a serious systemic allergic reaction that is rapid in onset and may cause death.[1-4] Critically important unmet needs in anaphylaxis risk assessment currently include (1) lack of an optimal, readily available laboratory test to confirm the clinical diagnosis of an anaphylaxis episode and (2) lack of an optimal method of distinguishing between individuals who are sensitized to allergens known to trigger anaphylaxis but are not at increased risk of anaphylaxis on exposure to these allergens, and those who are not only sensitized but also at increased risk of developing symptoms and signs of anaphylaxis on exposure, and of possible fatality.[5]

Inability to confirm the clinical diagnosis of anaphylaxis likely contributes to underrecognition and undertreatment of the disease.[5,6] Many more individuals are sensitized to allergens than are actually at risk for anaphylaxis,[7,8] leading to quandaries in risk assessment that may contribute to quandaries in making recommendations for long-term risk reduction.[9] Researchers from the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergology and Clinical Immunology held a PRACTALL (Practical Allergy) meeting to review effector mechanisms in anaphylaxis (Fig 1, *A* and *B*) and to deliberate issues with regard to confirming the diagnosis of anaphylaxis (Fig 2) and confirming the anaphylaxis trigger (Fig 3).

The diagnosis of anaphylaxis is based primarily on the clinical history[1,5,10,11] (Table I; Fig 2). Clinical criteria for accurate, early identification of anaphylaxis have recently been promulgated.[1] Although the clinical diagnosis can sometimes be supported by laboratory tests—for example, measurement of histamine concentrations in plasma, or of total tryptase concentrations in serum or plasma—these currently available tests have intrinsic limitations.[12] The blood sample must be obtained within minutes (histamine) to a few hours (tryptase) after onset of symptoms (Table II). This is impossible in the many patients who experience anaphylaxis in community settings and arrive in the emergency department some time later with resolving symptoms. Also, even when blood samples are optimally timed, tryptase levels are often within normal limits,[12] particularly in individuals with food-induced anaphylaxis.[13,14] Laboratory tests with increased sensitivity and practicality are therefore urgently needed to confirm the clinical diagnosis of anaphylaxis, improve recognition of the disease, and implement long-term risk reduction measures. Ideally, a rapid diagnostic test will eventually be developed for use in healthcare settings during and after immediate treatment of anaphylaxis. Currently, this goal may not be realistic in a disease that potentially causes death within minutes and mandates prompt intervention.[13,15]

Accurate risk assessment in anaphylaxis also involves verification of the trigger factor, where possible, because avoidance of the specific trigger and/or trigger-specific immunomodulation are critical steps in long-term risk reduction[5] (Table III; Fig 3). Sensitization is readily confirmed by using allergen skin tests or measuring allergen-specific IgE concentrations; however, substantial numbers of sensitized individuals do not develop any symptoms after exposure to the relevant allergen.[7,8] This discordance is not well understood, nor is it fully understood why, rarely, individuals with negative allergen skin tests and undetectable allergen-specific IgE levels develop severe or even fatal anaphylaxis to the antigen.[16,17]

In this workshop, effector mechanisms in anaphylaxis were reviewed, with emphasis on IgE-dependent mechanisms. Algorithms for risk assessment in anaphylaxis were developed, and a research agenda was created listing studies that will lead to improved risk assessment in anaphylaxis. Two important issues were discussed in depth: (1) development of an optimal test for laboratory confirmation of the clinical diagnosis and (2) development of improved methods for identification of individuals at risk of anaphylaxis from specific allergens, focusing particularly on 2 common triggers, insect venoms and foods, as examples.

EFFECTOR MECHANISMS IN ANAPHYLAXIS

Anaphylaxis involves the activation of mast cells and/or basophils (Fig 1, *A* and *B*). It is most commonly triggered by exposure to insect venoms, foods, medications such as a β-lactam antibiotic, or natural rubber latex, through a mechanism involving IgE and the high-affinity IgE receptor on these cells. The role of IgE and IgE receptors on other cells—for example, dendritic cells—during anaphylaxis remains unexplored.[18-21]

Although effector mechanisms in anaphylaxis do not need to be distinguished with regard to clinical diagnosis and acute treatment, it remains important to understand them with regard to long-term risk reduction measures. Anaphylaxis may involve immunologic mechanisms other than IgE. For example, in some individuals in whom it is deemed to be idiopathic, it may involve aggregation of FcεRI through autoimmune mechanisms.[22] The mechanisms whereby complement anaphylatoxin activation (C5a, C3a), neuropeptide release (substance P), cytotoxic mechanisms, IgG and IgM, immune complexes, or T-cell activation result in mast cell or basophil activation of sufficient magnitude to cause anaphylaxis in human beings remain to be clarified. More than 1 mechanism may be involved concurrently.[23] Anaphylaxis may also be triggered by nonimmunologic mechanisms. For example, mast cells may be activated directly by constituents of insect venoms, or by radiocontrast media, opiates, COX-1 inhibitors, vancomycin, or nonsteroidal anti-inflammatory drugs,[11] or by physical factors such as cold exposure or exercise. Studies of the role of genetic factors in human anaphylaxis have scarcely begun.[24-26]

There are few prospective studies of induced anaphylaxis in human beings because of the potentially rapid, life-threatening course of the disease. In a classic study involving a controlled insect sting challenge to assess the efficacy of specific venom immunotherapy versus whole body extract of stinging insects, most of the challenged individuals who had received immunotherapy with whole body extract or placebo (but not those receiving venom immunotherapy) developed mild anaphylaxis involving urticaria and tachycardia.[23] Three of these individuals developed severe reactions with prolonged hypotension and impaired gas exchange, and 1 had a respiratory arrest. Hemodynamic improvement took hours. Plasma histamine levels correlated with the severity and duration of cardiopulmonary manifestations but not with urticaria. Importantly, in 2 of the individuals with severe anaphylaxis, there was evidence of intravascular coagulation characterized by consumption of factor V, factor VIII, fibrinogen, and high-molecular-weight kininogen, as well as complement activation. Subsequently, involvement of these pathways has been confirmed in other individuals with anaphylaxis.[27-29]

Of all the different immune and nonimmune mechanisms underlying anaphylaxis, the one most rigorously investigated in human beings involves IgE, the high-affinity IgE receptor on mast cells and basophils, and a common allergen trigger such as insect venom or food. This report therefore focuses on IgE, on the pivotal role of mast cells and basophils, on the human heart as an important target organ, and on chemical mediators of inflammation released primarily from mast cells and basophils. In addition, IgG-mediated anaphylaxis in murine models is discussed with regard to its relevance to human anaphylaxis.

Role of IgE

Up to 25% of individuals have detectable insect venom-specific IgE levels, and approximately 60% of individuals have detectable food-specific IgE levels. Most of these individuals are not clinically reactive; that is, they do not experience signs or symptoms when exposed to the allergen to which they are sensitized.[7,8] Therefore, although the detection of allergen-specific IgE by skin testing and *in vitro* measurements is a useful marker of sensitization, the relationship between IgE and anaphylaxis is far from clear. In fact, in individuals who develop anaphylaxis from an insect sting or food, many studies show no clear relationship between the levels of allergen-specific IgE and the presence, the absence, or the severity of the clinical response to allergen. Complicating matters further, occasional individuals who have experienced anaphylaxis have no detectable allergen-specific IgE by skin testing and/or *in vitro* measurement[16,17]; as noted previously, other immunologic mechanisms may be involved.

There are several possible explanations for the lack of a direct relationship between allergen-specific IgE and clinical reactivity. One hypothesis currently being tested is that a combination of allergen-specific IgE levels and the total IgE level, especially the ratio of these 2 measurements, determines the threshold and likelihood for cellular and clinical reactivity by influencing high-affinity receptors for IgE (FcεRI) occupancy and density, and that multiallergen sensitization might be an important issue.[30] Another hypothesis being investigated suggests that the greater the number of IgE binding epitopes recognized (epitope diversity) by an individual, the more likely he or she is to experience a severe allergic reaction.[31] A third hypothesis being tested is that some episodes of anaphylaxis, especially those induced by foods, involve a basophil-dependent response.[30] This hypothesis arises in part because, in contrast with plasma histamine levels, serum tryptase levels are seldom elevated during anaphylaxis to food,[13] or even when symptoms occur during physician-supervised food challenges in which blood samples for tryptase measurement are obtained promptly at the onset of symptoms.[14] This discordance is being explored by *in vitro* studies of the activation and releasability of mast cells and basophils, as well as differential downregulation of these cells using anti-IgE antibody or activation of inhibitory receptors.[32-35]

Beyond traditionally implicated target organs and tissues such as the skin, airways, gastrointestinal tract, and blood vessels, other organs such as the heart may play an important role. In addition, there may be different degrees

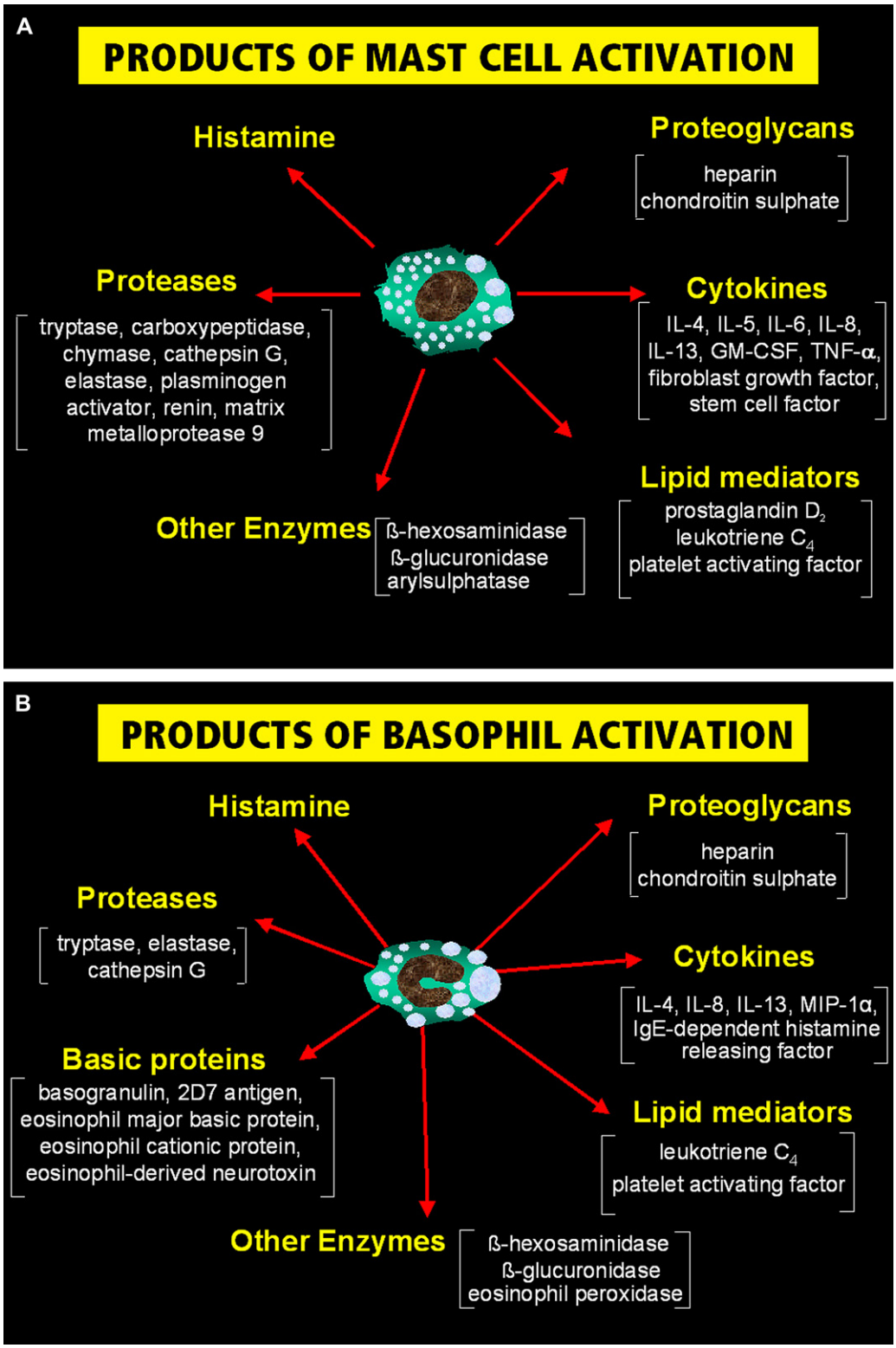

FIG 1. A, Mast cell with its activation products. **B,** Basophil with its activation products. Note that currently only 2 products of mast cell activation (histamine and total tryptase) and 1 product of basophil activation (histamine) can be measured in clinical laboratories as markers of acute anaphylaxis events. Figure courtesy of Dr A. F. Walls. *MIP,* Macrophage inflammatory protein.

of end-organ sensitivity, although to date, there is no direct evidence for this in human beings with anaphylaxis.

Mast cells: pivotal role in anaphylaxis

Mast cells have long been associated with anaphylaxis (Fig 1, *A*).[18,36] On average, individuals with recurrent anaphylaxis have more mast cells than those without anaphylaxis. Intrinsic differences in mast cell activation pathways have been suggested to predispose some individuals to anaphylaxis.

Activation of Kit, a transmembrane tyrosine kinase receptor for stem cell factor (SCF), the expression of which is increased significantly on mast cells, is critical for the growth, differentiation, and survival of normal mast cells. Moreover, there are common signal transduction elements after Kit activation and after FcεRI aggregation.[37-39]

Genetic polymorphisms and activating mutations in c-Kit such as D816V are strongly associated with mastocytosis, which is characterized by a pathologic accumulation of clonal mast cells in tissues.[40,41] Mastocytosis is associated with spontaneous episodes of hypotension and with increased risks of IgE-dependent and non–IgE-dependent anaphylaxis. It therefore offers a unique opportunity to study the contribution of mast cells to anaphylaxis.

In one study, 5 of 12 patients with recurrent anaphylaxis to an unidentified trigger who lacked major bone marrow and skin features of systemic mastocytosis but had 1 or more minor criteria of mastocytosis, were found to have aberrant expression of CD25 (IL-2Rα) on the surface of their mast cells.[42] Three of the 5 underwent mutational analysis of bone marrow fraction CD25 and were found to have the D816V (activating) mutation in the c-Kit gene. In some individuals, polymorphisms and mutations in c-Kit and other mast cell receptor genes may account for anaphylaxis that is currently described as idiopathic.

The expression of IgG receptors on human mast cells may also be relevant. Indeed, functional FcγRI receptors are transiently induced by IFN-γ, and constitutive production of FcγRIIa has been detected on skin-derived mast cells.[43] Both of these are activating receptors, suggesting that, like murine mast cells expressing activating FcγRIII, human mast cells might be activated by immune complexes. Another activating receptor is CD88, the receptor for C5a, which is expressed on the subset of mast cells that also express chymase.[44] Complement activation by IgG immune complexes might further activate such mast cells, which are the principal type found in the skin, around blood vessels, in the heart, and in the bronchial smooth muscle of patients with asthma. In this regard, it might be worthwhile to study the expression of anaphylatoxin receptors in fatal anaphylaxis, because in fatal asthma, C3aR expression is increased on submucosal and parenchymal blood vessels, and C5aR expression is increased on airway epithelium.[45]

The human heart: effector organ and target organ in anaphylaxis

Mast cells in the human heart may be important effector cells in anaphylaxis, and activation of human heart mast cells (HHMCs) may play a critical role in the development of cardiopulmonary dysfunction and fatality in anaphylaxis.[46,47] HHMCs are located between myocardial fibers, around blood vessels, and in the arterial intima. Purified HHMCs isolated from tissue obtained from patients undergoing cardiac transplantation express the FcεRI and C5a receptors.[48-50]

In vitro and possibly *in vivo*, the release of vasoactive mediators from HHMCs is initiated by cross-linking the FcεRI α-chain with anti-FcεRI or anti-IgE antibodies, and by exposure to eosinophilic cationic protein, substance P, C3a, or C5a.

Activation of HHMCs with anti-IgE or anti-FcεRI induces the release of preformed mediators such as histamine, tryptase, and chymase, and the *de novo* synthesis of leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2), platelet-activating factor (PAF), and cytokines, including TNF-α. Generation of angiotensin II and endothelin may occur secondary to the effects of chymase on the precursors to these mediators. In addition, HHMCs can be activated by radiocontrast media and by some general anesthetics, triggering non–IgE-mediated anaphylaxis.[51]

Administration of low concentrations of histamine or cysteinyl leukotrienes to individuals undergoing diagnostic cardiac catheterization causes significant systemic and coronary hemodynamic effects.[52] When immunologically released by HHMCs, mediators such as histamine, LTC_4, and PGD_2 may lead to coronary artery spasm or myocardial injury, and the downstream generation of vasoconstrictive mediators such as angiotensin II and endothelin may result in development of cardiac arrhythmias. Moreover, there is increasing evidence that mast cells and mast cell mediators play a role in cardiac disease as such.[53]

Mediators

Histamine, tryptase, and a much broader array of preformed and newly generated mast cell and basophil mediators of inflammation are associated with anaphylaxis in human beings (Table II; Fig 1, *A and B*).[54] These include proteases in addition to tryptase (carboxypeptidase A3, chymase, cathepsin G, and matrix metalloprotease 9), proteoglycans such as heparin and chondroitin sulfate, lipid mediators such as PGD_2, LTC_4, PAF and acid hydrolases (β-hexosaminidase), and other enzymes. In addition, a variety of cytokines such as TNF-α, ILs-4, -5, -6, -13, -16, and GM-CSF, and chemokines, including IL-8, may be involved. The ability of mast cells to release these mediators might be affected by intracellular levels of Syk cytosolic protein, a member of the Syk/ZAP-70 family of tyrosine kinases. Secretion might also be reduced by engagement of surface receptors with immunoreceptor tyrosine-based inhibitory motifs, such as CD32 or sialic acid–binding Ig-like lectin 8. Recent evidence suggests that once activated, the mast cell response is further regulated by the balance of both positive and negative intracellular and molecular events that extend well beyond the traditional role of kinases and phosphatases.[55] The activities of these mediators might be affected by their turnover—for example, individuals with low levels of

PAF acetylhydrolase may inactivate PAF more slowly, allowing a prolonged presence of this vasoactive mediator.[56] In addition, the tissue responses to mast cell mediators may vary from individual to individual, perhaps governed in part by local cytokine levels (see murine models of anaphylaxis).

In human beings, infusion of histamine, the best studied mediator to date, leads to an increased heart rate, increased skin temperature, flushing, itching, bronchospasm, headache, and a drop in blood pressure. These signs and symptoms involve H_1-receptor stimulation (itching, increased heart rate), or concurrent H_1-receptor and H_2-receptor stimulation (flushing, headache, and hypotension).[57]

To date, few mediators beyond histamine and tryptase have been explored for their potential usefulness in supporting the clinical diagnosis of anaphylaxis.[12] Recent studies of chymase, mast cell carboxypeptidase A3, and PAF as potential markers of anaphylaxis are important steps forward in this area.[56,58-62]

Relevance of murine anaphylaxis mechanisms to human anaphylaxis

Systemic anaphylaxis occurs in mice through the classic pathway in which antigen cross-linking of IgE bound to mast cell or basophil FcεRI causes degranulation, and through an alternative pathway in which IgG-antigen complexes activate macrophages by cross-linking FcγRIII. In the classic pathway, anaphylaxis is mediated by histamine and, to a lesser extent, by PAF. In the IgG pathway, it is almost entirely mediated by PAF. Additionally, intestinal anaphylaxis, manifested primarily as diarrhea, is mediated chiefly by the classic pathway, but depends on release of serotonin and PAF rather than histamine.[63,64]

Although considerably less antigen is generally required to trigger IgE-mediated anaphylaxis in comparison with IgG-mediated anaphylaxis, IgG antibodies can block IgE-mediated anaphylaxis by antigen interception and lead to FcεRI-FcγRIIb coaggregation on murine mast cells. This, in turn, activates inhibitor immunoreceptor tyrosine-based inhibitory motifs on FcγRIIb that inactivate FcεRI-mediated signal transduction. In general, IgG antibodies can protect mice against anaphylaxis when antigen concentration is low, but mediate anaphylaxis when antigen concentration is high.[65] IgE antibodies, but not IgG antibodies, also probably exacerbate anaphylaxis by stimulating FcεRI-dependent basophil IL-4 and IL-13 secretion in the absence of antigen.[66] Secretion of these cytokines, which sensitize target cells such as endothelial cells in blood vessel walls to the mediators released by activated mast cells and macrophages,[67] is stimulated by antigen concentrations 1/10 those required to trigger mast cell degranulation, and thus is less easily blocked than mast cell degranulation. IL-4 also enhances expression of FcγRIII on mast cells, facilitating activation by IgG immune complexes. Consequently, IgE-dependent and IgG-dependent mechanisms can synergistically induce systemic anaphylaxis even in the absence of mast cell degranulation. The relevance of the IgG/macrophage–dependent murine pathway to human anaphylaxis is unknown at this time but should serve as a stimulus to further investigation of mechanisms beyond those involving IgE, mast cells, and basophils in human anaphylaxis.[68]

RISK ASSESSMENT: CONFIRMING THE CLINICAL DIAGNOSIS OF ANAPHYLAXIS

In making the diagnosis of anaphylaxis, the clinical history is the most important instrument available[1,5,10,11] (Table I; Fig 2).

The supreme importance of the history

Diagnosis is based on pattern recognition (identification of symptoms and signs) and on context and probability. Some antecedent events and exposures within a plausible time frame of onset and resolution are more likely to trigger anaphylaxis than others. Anaphylaxis is not always easy to recognize clinically. It may be mild and may disappear spontaneously as a result of endogenous production of epinephrine, angiotensin II, or endothelin[69]; or it may be severe and progress within minutes to respiratory or cardiovascular compromise and death.[13,15] Anaphylaxis may be difficult to recognize if it is triggered by a novel agent, if it is an individual's first episode, or if it occurs in an infant or young child, or in an aphonic, dyspneic, or unconscious individual. It may also be hard to recognize in an individual with atypical, resolving, or partially treated symptoms, as when skin signs such as urticaria are absent or masked by medications.[5] Moreover, it may be difficult to recognize in certain specific clinical situations—for example, during hemodialysis, surgery, or childbirth.[70-72]

Supporting the clinical diagnosis by use of laboratory tests can therefore be extremely helpful (Table II). Currently, measurement of plasma histamine, 24-hour urine histamine or histamine metabolites, and more commonly, serum total tryptase (pro, pro′, and mature forms of α and β tryptases) are used for this purpose. These tests are available in many clinical laboratories.[12,18,73-75]

Histamine

Plasma histamine levels typically peak within 5 to 10 minutes of onset of anaphylaxis symptoms, then decline to baseline within 60 minutes as a result of rapid metabolism by N-methyltransferase and diamine oxidase. Elevated plasma histamine levels correlate with anaphylaxis symptoms and are more likely to be increased than serum total tryptase levels. They need to be obtained at the onset of the episode, and this test is therefore impractical in many clinical circumstances; for example, histamine levels have typically returned to baseline by the time most individuals experiencing anaphylaxis in the community arrive in the emergency department. Measurement of histamine or the histamine metabolite N-methylhistamine in a 24-hour urine collection may be helpful.[12] Hyperhistaminemia may be a risk factor for recurrent anaphylaxis.[76]

Total tryptase

Currently, the most widely used laboratory test to confirm anaphylaxis is measurement of total tryptase concentrations in serum or plasma (Table II). It is optimally obtained within 3 hours of onset of symptoms, and levels, at least in insect sting-induced anaphylaxis, correlate well with the degree of hypotension.[73] Although an elevated total tryptase level (normal values, 1-11.4 ng/mL; Phadia AB, Uppsala, Sweden) supports the diagnosis of anaphylaxis, failure to document an elevation in total tryptase cannot by itself be used to refute the diagnosis, even if the blood sample has been obtained within a few hours of the onset of symptoms.

Serial measurements of total serum tryptase in serum or plasma may increase the sensitivity and the specificity of the test.[74] Further investigation of the optimal frequency of measurements is needed. Also, measurement of baseline serum tryptase levels obtained either before the anaphylaxis event in question or at least 24 hours after resolution of the clinical signs and symptoms may be helpful in ascertaining whether or not anaphylaxis occurred.[75] These 2 approaches, which need to be validated further, are currently underused in the diagnostic work-up of individuals with suspected anaphylaxis. Measurement of mature β-tryptase might also improve sensitivity.[77-80]

Somewhat puzzling to date is the fact that even when blood samples are optimally timed, elevated total tryptase levels are uncommonly found in individuals with food-induced anaphylaxis[13] or in those with positive food challenge tests in which anaphylaxis symptoms are observed.[14] There are several possible reasons for this finding. In some individuals—for example, those whose primary symptom is laryngeal edema—localized rather than generalized mast cell degranulation may predominate, and the amount of tryptase entering the circulation may be too small to raise serum levels. Tryptase released by mucosal mast cells may be further from the circulation than tryptase released by perivascular mast cells; moreover, if carried to the mucosal surface, it may enter the circulation less efficiently. Mast cells with less tryptase (MC_T in respiratory epithelium, alveolar wall, and small intestinal mucosa) versus those with more tryptase (MC_{TC} in skin, conjunctivae, heart, perivascular tissue, and intestinal submucosa) may be involved. The anaphylaxis episode may primarily involve basophils rather than mast cells, and the late phase response involving basophils and eosinophils may predominate over the early phase response involving mast cells. Also, tryptase may be eliminated very rapidly in some individuals.

Nearly all of the α/β tryptases spontaneously secreted by resting mast cells are in their pro form. Most individuals with systemic mastocytosis and some atopic individuals may have elevated total tryptase levels when no symptoms of anaphylaxis are present.[81,82] This appears to reflect an elevated mast cell burden and constitutes a significant and substantial risk factor for hypotensive anaphylaxis. Total tryptase is modestly influenced by other factors; for example, the β-α haplotype increases the total tryptase level in healthy individuals by about 0.5 ng/mL from the mean, and male sex decreases the mean levels by about 0.2 ng/mL.[79,80]

Total tryptase levels may be elevated in a variety of other clinical contexts besides anaphylaxis and

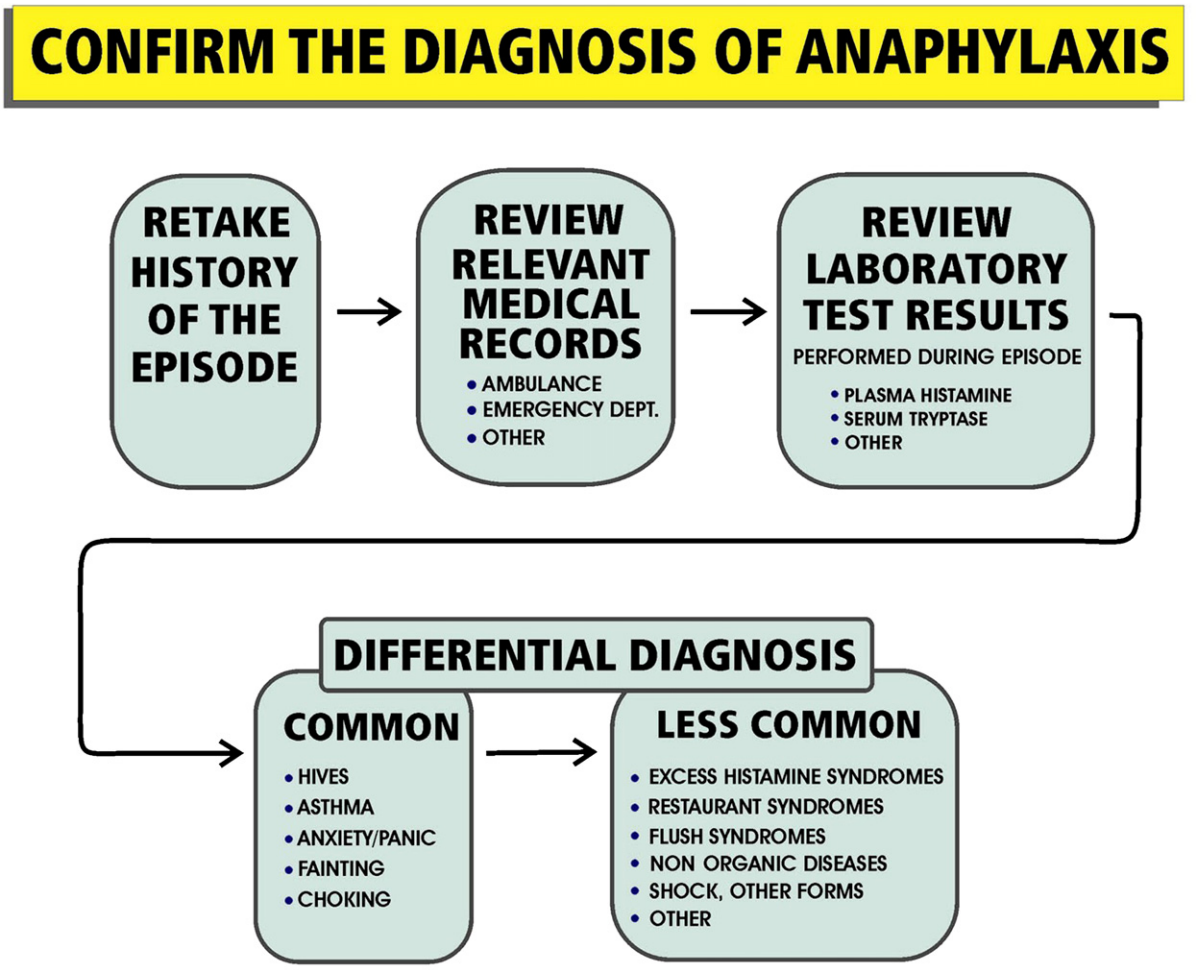

FIG 2. Algorithm for confirming the diagnosis of anaphylaxis. This involves retaking the history, obtaining and reviewing relevant medical records (ambulance, emergency department, and other hospital or clinic records, eg, hemodialysis, perioperative, and so forth), and reviewing laboratory test results, if any. In the differential diagnosis, 40 or more alternatives need to be considered, some of which are common and some of which are not. Excess histamine syndromes include systemic mastocytosis, urticaria pigmentosa, basophilic leukemia, and hydatid cyst. Restaurant syndromes include monosodium glutamate sensitivity, sulfite sensitivity, and scombroid poisoning. Flush syndromes include flushing as a result of carcinoid, menopause, and autonomic epilepsy. Nonorganic diseases include Munchausen syndrome and vocal cord dysfunction. The possibility of hemorrhagic, cardiogenic, or endotoxic shock should be considered. Other potential diagnoses include pheochromocytoma, hereditary angioedema, red man syndrome, seizure, and stroke. *DEPT.*, Department. Figure courtesy of Dr F. E. R. Simons.

mastocytosis. These include acute myelocytic leukemia, various myelodysplastic syndromes, hypereosinophilic syndrome associated with the FIP1L1-PDGFRA mutation, exogenous SCF administration, end-stage renal disease with elevated endogenous SCF concentrations, and treatment of onchocerciasis.[18]

Measurement of total serum tryptase at postmortem can be helpful in implicating anaphylaxis as the cause of death; however, in death as in life, the absence of an elevated total tryptase level does not rule out the possibility of anaphylaxis.[83] Postmortem blood samples should be collected from femoral vessels rather than from the heart, where nonspecific serum tryptase elevation is more likely to occur, as a result of passive diffusion from mast cells in the heart and lungs. In addition to tryptase elevations in anaphylaxis-related deaths, tryptase may also be elevated in trauma- or heroin-related deaths, and in myocardial infarction, sudden infant death syndrome, and unexplained deaths, raising the possibility of mast cell involvement in these clinical situations.[83]

Mature tryptase

An increase in mature β-tryptase concentrations in serum or plasma, detected by using the G5 mAb (rather than the G4 mAb used in the ELISAs available in many clinical laboratories for measurement of total tryptase), reflects mast cell activation. Mature tryptase levels are often elevated (>1 ng/mL) during hypotensive anaphylaxis episodes, particularly those triggered by parenteral exposure to an inciting agent. Levels typically peak within 1 hour of the onset of anaphylaxis symptoms, and then decrease with an elimination half-life of about 2 hours. The peak level generally correlates with severity of symptoms, specifically with the nadir in mean arterial pressure. In individuals with insect sting anaphylaxis, at any single time point after onset of symptoms, mature tryptase levels are a more sensitive measurement than total tryptase levels; however, mature tryptase can only be measured in 1 research laboratory in the world at the present time.[77-80]

Chymase

Chymase, a serine protease stored mainly in secretory granules of human mast cells, has been reported to be elevated in individuals dying from anaphylaxis, and to be below detectable levels (<3 ng/mL) in those dying from other causes.[58]

Mast cell carboxypeptidase A3

A sensitive and specific ELISA using new mAbs has been developed for identification of mast cell carboxypeptidase A3 in serum or plasma, and carboxypeptidase A3 is currently being investigated as a marker for anaphylaxis episodes. Serum carboxypeptidase A3 levels are elevated (>14 ng/mL) in individuals with a clinical diagnosis of anaphylaxis, but not in healthy blood donors or individuals with asthma or other IgE-mediated allergic diseases. In patients with anaphylaxis, mast cell carboxypeptidase A3 and tryptase seem to appear at different rates in the circulation, and the serum levels of these mediators do not necessarily correlate with each other. Mast cell carboxypeptidase A3 levels remain elevated longer than total tryptase levels do, and high serum carboxypeptidase A3 levels have been detected in individuals with clinically diagnosed anaphylaxis who did not have elevated total tryptase levels.[59-62]

Previously, poor correlation between histamine and total tryptase levels has been reported in individuals with nonhypotensive anaphylaxis, and it has been observed that measurement of both histamine and total tryptase improves sensitivity of testing and ability to confirm the clinical diagnosis of anaphylaxis.[12] Extending this observation, it might be useful to measure a panel of mast cell mediators such as histamine, tryptase, chymase, PAF, mast cell carboxypeptidase A3, and others such as basogranulin whose potential use as markers of anaphylaxis have not yet been explored. In addition, the effect of treatment—for example, with epinephrine or intravenous fluids—on levels of mediators needs to be investigated.

Platelet activating factor

PAF is secreted by other cells such as macrophages and monocytes as well as by mast cells and basophils. PAF levels are markedly elevated in individuals experiencing anaphylaxis triggered by peanut and correlate with severity of the episode.[56] Further studies involving measurement of PAF levels in anaphylaxis triggered by other agents will be of interest.

RISK ASSESSMENT: BEYOND DETECTION OF SENSITIZATION TO ALLERGENS

An individual's clinical risk for anaphylaxis is determined not only by sensitization to allergen, but also by other intrinsic risk factors; in addition, the nature of the allergen itself plays a role.

Clinical risk factors for anaphylaxis

Important clinical risk factors for anaphylaxis include age; comorbidities such as asthma, cardiovascular disease, psychiatric disease, substance abuse, mastocytosis, or benign mast cell hyperplasia; certain concurrent medications such as nonselective β-blockers; severe previous reactions; and other patient-related factors[5,84-89] (Table I; Fig 3). Individuals who are at risk because of subclinical mastocytosis or activating mutations of mast cells can be identified by measurement of total tryptase levels in serum or plasma[81,82] (Table II), which has been combined with flow-cytometric immunophenotyping of bone marrow mast cells.[42] Decreased activity of, or deficiency of, PAF acetylhydrolase, the enzyme that inactivates PAF, has been described as a risk factor for fatal anaphylaxis to peanut.[56] In some individuals, more than 1 trigger may be required to initiate an anaphylaxis episode—for example, exercise plus a cotrigger such as food, medication, or cold exposure.[10,90]

Some food allergens such as peanut, tree nuts, finned fish, shellfish, egg, and milk, and some species of stinging insects have a higher intrinsic risk for triggering anaphylaxis than others.[5,10,13,15,88]

Skin tests to detect allergen-specific IgE

Confirmation of sensitization to the allergen that is suspected of triggering anaphylaxis on the basis of clinical history is traditionally performed by using skin prick/puncture tests with appropriate positive (histamine) and negative (diluent) controls (Table III; Fig 3). Optimally, tests are performed at least 3 to 4 weeks after the anaphylaxis episode.[91] In individuals with anaphylaxis triggered by venom or β-lactam antibiotics, intradermal (intracutaneous) tests, which have increased sensitivity but decreased specificity, are often needed. Some skin test instruments and techniques have been well validated.[92] Large wheal and flare responses in the skin test do not necessarily correlate with the highest degree of clinical risk. Skin prick testing is a relatively safe procedure, although rarely, fatality has been reported.[93] Use of standardized allergens, where available, and standardized systems for recording skin test results improve risk assessment. Although use of recombinant allergens in skin tests is promising,[94] additional studies and improved precision are needed. Unvalidated techniques for the identification of sensitization to allergens remain in use.[95]

In assessment of medication-induced anaphylaxis, skin testing is only predictive for reactions involving IgE. With the exception of haptenated penicilloyl determinants and minor determinants of penicillins and cephalosporins, antigenic determinants are not well characterized or validated for medications. The immunogens relevant to anaphylaxis are not known for most medications, perhaps because they are derived from metabolites or unidentified degradation products.[96,97]

In vitro measurements: allergen-specific IgE and cellular tests

In vitro tests for measurement of allergen-specific IgE are now widely available in clinical laboratories for many allergens, including inhalants (rare triggers of anaphylaxis), foods, stinging insect venoms, natural rubber latex, and medications such as β-lactam antibiotics (Table III; Fig 3). Quantitative tests such as ImmunoCAP (Phadia AB and others) are the preferred tests for *in vitro* use. It must be cautioned that allergen-specific IgE levels measured by using different commercial assays may still not be equivalent. Although some healthcare professionals think that the likelihood of symptoms increases in direct proportion to the increased level of allergen-specific IgE, available data do not support this concept.[16,17] Recently, it has been demonstrated that using the sum of the specific IgE antibody levels in combination with the number of positive tests (elevated specific IgE levels) to food and other allergens may improve the diagnostic efficiency of *in vitro* testing for allergen-specific IgE.[30,98]

In contrast with mast cells, basophils are readily accessible, although they make up only a minor fraction, typically 0.2% or less, of peripheral blood leukocytes. Traditionally, cellular tests are based on histamine release after direct stimulation with allergen.[99-101] A cellular antigen stimulated test based on *de novo* synthesis of sulfidopeptide leukotrienes has been developed[102]; however, it is not fully validated and is reported to lack diagnostic utility.[103]

With the development of flow cytometry, changes in cell surface expression of basophil antigens are now more commonly measured than mediator release (Table III). The basophil activation test measures the change in basophil surface markers such as CD63 or CD203c after incubation with different concentrations of allergen.[99-101] Additional activation markers may be useful.[104] The test can be performed rapidly on a small volume of blood and provides objective, sensitive, precise, reproducible results that correlate well with those from the basophil histamine release test and with allergen skin tests and specific IgE levels.[105] The basophil activation test can also determine an individual's sensitivity to allergen by challenging basophils with serial dilution of the allergen. It can be used to identify responses to inciting substances in both IgE-mediated and non–IgE-mediated anaphylaxis. Allergens tested to date include pollens,[99-101] foods,[99-101] venoms,[106-109] natural rubber latex,[110] and medications such as β-lactam antibiotics,[111] neuromuscular blockers,[112,113] aspirin and other nonsteroidal anti-inflammatory drugs,[114] dexchlorpheniramine, and heparin, as well as antiseptics such as chlorhexidine. In addition to confirming sensitization to allergen, this test is being assessed for its utility to confirm allergen exposure[115]

TABLE I. Risk assessment in anaphylaxis: clinical factors that increase the risk of an anaphylaxis episode and/or fatality

- Age* (adolescents and young adults are at high risk for fatality from food anaphylaxis; elderly individuals are at high risk for fatality from insect venom anaphylaxis)
- Comorbidities*
  - Asthma
  - Cardiovascular disease
  - Psychiatric disease (may impair recognition of symptoms)
  - Mastocytosis† (symptomatic or asymptomatic)
  - Activating Kit mutations†
  - Thyroid disease (some individuals with idiopathic anaphylaxis)
  - Reduced level of PAF acetylhydrolase activity
  - Hyperhistaminemia
  - IgE-mediated allergic diseases†‡
  - Emotional stress
  - Acute infection
  - Decreased host defenses
- Concurrent chemical/medication use*
  - May affect recognition of anaphylaxis
    - Ethanol
    - Recreational drugs
    - Sedatives
    - Hypnotics
  - May increase the severity of anaphylaxis
    - β-blockers
    - ACE inhibitors
    - Angiotensin II receptor blockers
- Other relevant factors*
  - Severity and/or priming effect of previous anaphylaxis episodes
  - Strenuous exercise
  - Occupation
- Allergens with increased intrinsic risk of triggering anaphylaxis
  - Foods: peanut, tree nuts, finned fish, shellfish, egg, milk, sesame
  - Insect stings/bites: Hymenoptera (bees, vespids, ants), some biting insects (mosquitoes, kissing bugs, pigeon ticks)
  - Inhalants (cat, hamster, and horse dander; grass pollen)
  - Natural rubber latex
  - Medications (such as β-lactam antibiotics, neuromuscular blockers)

*In some individuals, several factors may need to be present concurrently for risk to be increased—for instance, elderly person plus cardiovascular disease plus medication. In others, concurrent triggers may be needed—for instance, food plus exercise.
†Suggested by elevated baseline total tryptase levels.
‡Atopy is a risk factor for anaphylaxis triggered by food, exercise, latex, and radiocontrast media, but not a risk factor for anaphylaxis triggered by insect stings, penicillin, or insulin.

TABLE II. Risk assessment in anaphylaxis: confirming the clinical diagnosis

- Tests currently available in clinical laboratories*
  - Histamine
    - Blood sample needs to be obtained within 1 hour, preferably within minutes, of symptom onset (plasma or serum must be frozen immediately)
    - 24-hour urine histamine and N-methylhistamine may also be helpful
    - Histamine levels are also elevated in scombroid poisoning (which usually affects more than 1 person eating the same fish)
  - Total tryptase (pro, pro′, and mature forms of α/β tryptases)
    - Blood sample should optimally be obtained within 3 hours of onset of anaphylaxis symptoms
    - Consider comparing the level measured during the acute event with a baseline level (obtained 24 hours after resolution of the acute event) or on stored serum, if available (levels are stable for at least 1 year in stored frozen sera)
    - If higher in acute serum than in baseline serum, the diagnosis of anaphylaxis is confirmed
    - If elevated (≥20 ng/mL) in both acute and baseline sera, the diagnosis of mastocytosis should be considered
    - If within normal limits in a blood sample taken during anaphylaxis, the diagnosis of anaphylaxis cannot be excluded
    - Total tryptase level can be measured in postmortem serum (blood samples preferably obtained from femoral vessels rather than the heart; the level needs to be correlated with the clinical history)
- Potentially useful tests currently performed in research laboratories
  - Mature tryptase
  - Chymase
  - Mast cell carboxypeptidase A3
  - PAF

*When sorting out the differential diagnosis of anaphylaxis, the detailed clinical history and physical examination may suggest the need for additional laboratory tests to confirm or rule out diseases such as mastocytosis, basophilic leukemia, hydatid cyst, carcinoid (serum serotonin level, urinary 5 hydroxyindoleacetic acid), medullary carcinoma of the thyroid/vasoactive polypeptide-secreting gastrointestinal tumor (substance P, vasointestinal polypeptide), pheochromocytoma (free metanephrine in plasma, urinary vanillylmandelic acid), and hereditary angioedema (C4, C1 esterase inhibitor).
The time course of the appearance, peak, and return to baseline levels in serum or plasma differs for various mediators released from mast cells and basophils. Mature tryptase might be a more precise marker of anaphylaxis than total tryptase. Measurement of a panel of mediators might eventually prove most helpful. Investigation of the complement cascade (C4a, C5a, C3a), the contact system (bradykinin, high-molecular-weight kininogen, kallikrein-C1-inhibitor complexes, factor XIIa-C1-inhibitor complexes), and coagulation pathway factors (V, VIII, fibrinogen), although usually not performed, may support the clinical diagnosis of anaphylaxis; however, these tests appear to lack specificity.

and might be useful in confirming the diagnosis of an anaphylaxis episode when performed on basophils collected within a few hours of onset of symptoms.

Examples of clinical situations in which *in vitro* tests for sensitization are useful

Clinical situations in which *in vitro* measurement of IgE can be helpful, and in which cellular tests for IgE-mediated reactivity are being explored, include assessment of perioperative anaphylaxis,[112,113] transfusion-related anaphylaxis,[116,117] finned fish anaphylaxis versus *Anisakis* (fish parasite)–induced anaphylaxis,[118,119] and identification of novel triggers for anaphylaxis.[120-123]

In perioperative anaphylaxis, guided by the history of the episode and the time course of exposure to potential triggers in relationship to the onset of symptoms and signs, a wide variety of allergens and agents may need to be tested. These include neuromuscular blockers, which are

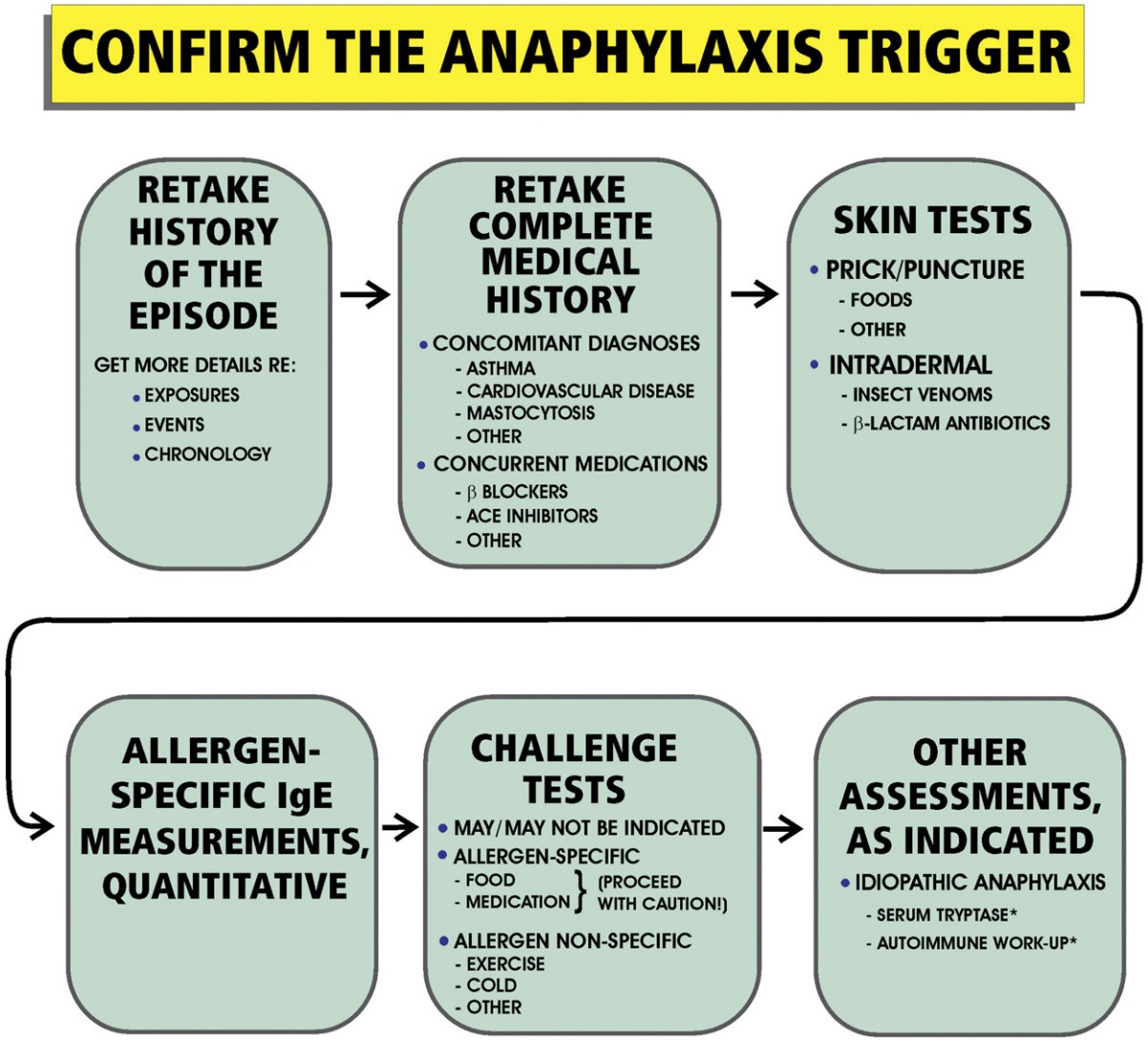

FIG 3. Algorithm for confirming the anaphylaxis trigger. This involves retaking the history of the episode, retaking the complete medical history, and, if IgE is involved in the mechanism, determining sensitization by using allergen skin tests and measuring allergen-specific IgE levels. Currently, challenge tests with a food or medication are sometimes needed in risk assessment. Insect sting challenge tests are performed only as research procedures. If basophil activation tests and other *in vitro* tests (Table III and Table IV) can be validated as risk assessment tools, this will be an important step forward. Traditionally, idiopathic anaphylaxis has been a diagnosis of exclusion, made in individuals with a negative detailed history of antecedent events and exposures, negative allergen skin tests, and allergen-specific IgE measurements that are absent or undetectable.* Consideration should be given to measuring a serum tryptase level and performing a work-up for autoimmune disease—for example, autoimmune thyroid disease—in these individuals. Figure courtesy of Dr F. E. R. Simons.

TABLE III. Risk assessment in anaphylaxis: confirmation of sensitization/likely trigger for anaphylaxis episode

- Currently available tests*
  - Allergen skin tests
    - Percutaneous (prick or puncture)
      - Use standardized extracts where available
      - Positive tests (wheal diameter 3 mm greater than negative control) are common in the general population (approximately 60% of whom are sensitized to food, and as many as 25% of whom are sensitized to venom), and indicate sensitization, not necessarily a causal relationship
      - Skin test response does not necessarily correlate with risk of future anaphylaxis episodes or severity of the episodes
      - Wait 3 to 4 weeks after an anaphylaxis episode before skin testing (to allow time for re-arming of mast cells with IgE and recovery of mast cell releasability)
      - Select the allergens for testing on the basis of history of antecedent exposures and events
      - Commercial extracts of many foods (eg, fruits and vegetables, produce false-negative tests as a result of destruction of the allergen during manufacturing and storage; therefore, consider prick-prick tests with fresh foods)
      - Assessment of individuals with pollen food syndrome (oral allergy syndrome), a minority of whom are at risk for anaphylaxis, presents unique issues
      - If skin tests are unexpectedly negative, repeat them after an interval, and measure allergen-specific IgE
    - Intradermal (intracutaneous)
      - Intradermal tests are often needed in insect venom and β-lactam antibiotic allergy
      - Intradermal tests are contraindicated in food allergy due to high likelihood of false-positive tests and the possibility of triggering anaphylaxis in at-risk individuals
  - Allergen-specific serum IgE measurements
    - Quantitative ELISAs, RASTs
    - Available for foods, insect venoms, and latex; not available for most medications or biologicals
    - Refer to predictive values, where available; for example, for foods such as peanut, tree nuts, finned fish, cow's milk and hen's egg
    - Determine correlation with, or lack of correlation with, skin test results
    - Total IgE levels and number of allergens to which the individual is sensitized may affect interpretation
  - Controlled allergen challenge tests
    - Performed with food or medication: different indications and considerations for each; stinging insect challenges are useful in research
    - Open, single-blind, or double-blind challenges, depending on clinical history and on the allergen
    - First do no harm: challenge *only* if assessment (clinical history, skin tests and/or measurement of allergen-specific IgE) indicates the individual is at low risk for anaphylaxis
    - Perform *only* under controlled conditions in a hospital or other healthcare facility equipped for cardiopulmonary resuscitation, with close monitoring by trained and experienced healthcare professionals on site
  - Challenges to confirm nonimmune mechanisms
    - Depending on the history, an exercise, cold, heat, or sunlight challenge test may be needed
    - In exercise-induced anaphylaxis, co-triggers such as a food, medication, or cold exposure usually need to be assessed
  - Basophil activation tests
    - Histamine release (traditional)
    - Measurement of activation markers, or combination of markers, CD63 or CD203c
    - More commonly used in Europe than in North America, where they remain research tools (utility, validity, and benefits still evolving)
  - Idiopathic anaphylaxis
    - Detailed history of antecedent events/exposures does not yield any clues about triggers
    - Skin tests and allergen-specific IgE measurements are negative
    - Tryptase levels should be measured
    - Autoimmune work-up should be considered
- Potentially useful tests currently performed in research laboratories
  - Investigation of novel allergen triggers (ELISAs, immunoblotting)
  - Recombinant allergens for *in vitro* testing and skin testing
  - Ratios of allergen-specific IgE levels to total IgE level (mirrors basophil allergen sensitivity)
  - Using the sum of allergen-specific IgE levels in combination with the number of positive tests to improve allergy diagnosis
  - Microarray immunoassays
  - Assessment of epitope diversity
  - *In vitro* lymphocyte activation tests (drug allergy)
  - c-Kit mutational analysis
  - Mature typtase
  - PAF-acetylhydrolase (deficiency increases the risk of fatal anaphylaxis to peanut)

*Most of the tests listed have been validated or are in the process of being validated. Some old tests that have not been validated in controlled studies remain in use, particularly for food allergy, such as food-specific IgG or IgG_4 antibody levels, food antigen-antibody complexes, lymphocyte activation tests with food, and sublingual or intracutaneous provocation tests with food.

Except for β-lactam antibiotics, no allergens are available for skin testing or for *in vitro* medication-specific IgE measurements. There are many unresolved issues in performing tests with medications, including pro-drugs, metabolites, haptens, and drug class effects.

Vaccines to prevent infectious disease seldom trigger anaphylaxis; however, SPTs with excipients such as egg (relevant for influenza and yellow fever vaccines) or gelatin (relevant for measles vaccine) may be useful in this context. SPTs for natural rubber latex sensitization may also be helpful. For other anaphylaxis triggers, such as contrast media, skin tests are not helpful, because the underlying mechanism does not generally involve IgE.

the most common triggers in this setting, and other medications such as general anesthetics, opiates, antibiotics, and nonsteroidal anti-inflammatory drugs; natural rubber latex; plasma expanders such as dextran or gelatin preparations; antiseptics such as chlorhexidine; and dyes such as fluorescein and isosulfan blue.[112,113]

Blood transfusions can cause anaphylaxis through several different mechanisms. These include cytotoxic reactions involving IgG or IgM, inadvertent transfusion of minute amounts of IgA to IgA-deficient individuals, and passive transfusion of IgE antibodies from donors with allergy with subsequent transient sensitization of basophils and mast cells in the recipients. Approximately 25% of blood donors have IgE antibodies to common allergens, and about 1/3 of these donors have high (>10 kU/L) allergen-specific IgE levels. Recipients of blood or blood products from such donors may be transiently at risk of anaphylaxis, even if they have no personal history of clinical allergy. *In vitro* measurements of IgE to food or other allergens that are recognized anaphylaxis triggers, and to which the recipient has been exposed, may help clarify the clinical picture.[116,117]

Some individuals who have a history of apparent anaphylaxis to finned fish, especially those reacting to raw or undercooked fish and/or experiencing delayed symptoms, but who have no evidence of elevated IgE antibody levels to fish may be reacting to the larva of the live sea fish nematode parasite *Anisakis simplex*. A recombinant allergen, UA3, which is a 387 amino acid secretory antigen, mainly induces IgE responses and has been used to develop a highly sensitive and specific ELISA to identify individuals with anisakiasis. An *A simplex*–free population may also have specific IgE against *A simplex* because of cross-reactivity with other parasites. As many as 20% of blood donors in some geographic areas have specific IgE and/or skin test positivity to *A simplex*.[118,119]

Novel allergen triggers for anaphylaxis continue to be described. Some allergy and immunology laboratories have the capability of developing customized, sensitive, and specific ELISAs, immunoblotting, and other *in vitro* tests to detect the presence of specific IgE to such allergens. In this situation, it is helpful if the suspected trigger allergen (leftover food, vomited food, stinging or biting insect, medication) has been saved for use as the capture allergen in the test. Recombinant allergens are being used increasingly to identify anaphylaxis triggers.[5,120-123]

Sensitization versus risk

Skin tests and measurements of allergen-specific IgE are useful in determining sensitization; however, to predict clinical reactivity, especially to a food or medication, closely monitored incremental challenges conducted in appropriately equipped and staffed healthcare facilities may be required (Table III; Fig 3). Challenges are time-consuming, costly, and not without risk. In some countries, basophil activation tests are now used to sort out clinical situations in which the history, the skin tests, and the allergen-specific IgE levels are discordant. In most countries, however, these tests remain research tools, pending additional studies to standardize the techniques with different allergens and to verify the reliability of this approach in distinguishing sensitization from risk.[30,99-101]

RISK ASSESSMENT IN INDIVIDUALS WITH HYMENOPTERA VENOM ALLERGY

Current approaches to risk assessment are reviewed, and future directions for improving risk assessment are discussed.

Current methods of distinguishing venom-sensitized individuals at risk of anaphylaxis from those who are clinically tolerant

Positive venom skin tests and/or elevated specific IgE levels to venoms are found in as many as 25% of adults without a history of a systemic sting reaction, yet only about 3% of individuals in the general population have experienced a systemic sting reaction.[7] This discordance occurs for a number of reasons, including alteration of the immune response and transiently elevated IgE antibody level after an uneventful sting, a false-positive intradermal (intracutaneous) test with high venom concentrations, or a false-positive skin test or elevated IgE level as a result of cross-reacting carbohydrate determinants (CCDs) between venom and plant allergens.[124-126] Identification of specific IgE to bromelain and other CCDs, and inhibition tests with CCDs of plant origin, help identify positive tests that result only from CCDs. Sting challenge tests in such individuals could definitively prove the clinical irrelevance of the CCDs, but such challenges have not been systematically performed in an adequate number of individuals to date.

In untreated individuals with a history of reactions to insect stings, and in those receiving venom immunotherapy, the presence of IgE specific for Hymenoptera venoms as detected either by skin tests or quantitative serum IgE measurements is not necessarily predictive of future clinical reactivity (Table III; Fig 3). The risk of a reaction to a subsequent sting varies with the insect species. In prospective sting challenge studies in untreated Dutch patients, 52% of individuals with bee venom allergy but only 25% of individuals with vespid venom allergy developed a systemic reaction again.[127] More than 90% of individuals with a history of Hymenoptera sting anaphylaxis within the preceding year had positive venom skin tests and increased venom-specific serum IgE levels.[89] If these history-positive and diagnostic test–positive patients are subsequently restung before receiving venom immunotherapy, 30% to 75% of those with severe systemic sting reactions will have a positive sting challenge on the basis of data collected in 11 studies worldwide involving 1195 patients.[69,127-129] In comparison with the Dutch studies in untreated patients, prospective observations in other European countries, the United States, and Australia, based on sting-challenged patients in controlled studies, suggest higher reaction rates because there was no selection of patients with a history of less severe reactions.[23,128]

There are clinically relevant differences between children and adults. In the long-term follow-up of untreated children who have had allergic reactions to stinging insects and were restung, 32% of those with moderate or severe initial systemic reactions again developed systemic reactions, and 13% of those with urticaria (cutaneous systemic reactions) later developed systemic reactions.[130]

Between 5% and 30% of adults with a history of a systemic sting reaction have a negative venom skin test, although some of these individuals may have an elevated venom-specific IgE. Conversely, occasional individuals have positive intradermal (intracutaneous) tests yet have undetectable allergen-specific IgE. History-positive individuals at risk for subsequent sting reactions may have negative skin tests and absent or undetectable IgE for a variety of reasons, including underlying systemic mastocytosis.[81,82] The immune response may have altered after the sting. For unknown reasons, venom skin test positivity may vary over time. Also, a minority of systemic sting reactions are not IgE-mediated.

In as many as 60% of individuals with a history of anaphylaxis after an insect sting, double-positivity of diagnostic tests with bee venom and vespid venom is observed.[131] This may be a result of true double-sensitization, or cross-reactivity between bee and vespid venom protein allergens (hyaluronidase has about 50% sequence homology), or cross-reacting CCDs. Inhibition tests with both venoms and CCDs using RAST inhibition or immunoblot have been used to distinguish between double-sensitization and cross-reactivity,[125,126,131,132] which is important for the selection of venoms for immunotherapy.

Venom allergy: improving risk assessment

Identification of individuals at high risk for systemic sting reactions may be improved by paying close attention to details in the clinical history[85,86,88,89] and by using tests beyond traditional venom skin tests and *in vitro* measurement of venom-specific IgE (Table IV). Because of different clinical factors, some individuals with negative venom skin tests and absent or undetectable venom-specific IgE after a systemic reaction to a sting may subsequently be at risk for severe or fatal anaphylaxis from stings. These factors potentially include underlying asymptomatic systemic mastocytosis or activating mutations in mast cells, severity of a previous reaction or priming effect of a previous reaction, older age, pre-existing cardiovascular disease, and use of concurrent medications such as β-blockers or angiotensin converting enzyme (ACE) inhibitors. Acute infection, stress, altered host-defense mechanisms, occupation (eg, beekeeper), concurrent strenuous exercise, and even the body area stung may also increase an individual's risk level. Measurement of baseline serum total tryptase levels, which, if elevated, indicate increased risk, are currently underused in risk assessment.[81,82,89]

A new approach to investigation of individuals with double-positivity of allergen skin tests and allergen-specific IgE involves looking at the presence of specific IgE to recombinant species-specific nonglycosylated major venom allergens from *Apis mellifera* (phospholipase A2) and *Vespula vulgaris* (antigen 5). If this proves to be predictive of clinical reactions, it will be an important advance.[125]

In vitro tests that predict the occurrence, type, and severity of systemic sting reactions are urgently needed.

TABLE IV. Research agenda for anaphylaxis

- Mechanisms and pathogenesis of anaphylaxis
  - Determine the relative roles of mast cells, basophils, dendritic cells, and other cells in IgE-mediated and non-IgE-mediated human anaphylaxis, and the potential risks and benefits of depleting/suppressing/stabilizing these cells
  - Identify cellular signaling pathways that promote and suppress the development of anaphylaxis, and how these can be influenced
  - Determine whether IgG-mediated anaphylaxis exists in human beings and, if so, identify the mechanism
  - Further investigate the relative importance or role of the following:
    - Comorbidities (eg, asthma, cardiovascular disease, infection, stress)
    - Exercise
    - Concurrent medications used (eg, nonselective β-adrenergic blockers, ACE inhibitors, angiotensin II receptor blockers) and the route of their administration
    - Other determinants of end-organ sensitivity
    - Mast cell/basophil activation state as evidenced by elevated baseline levels of tryptase or histamine
    - Levels and polymorphisms of cytokines and cytokine receptors that increase sensitivity to mediators or act as mediators (eg, IL-4, IL-13, TNF-α)
    - Receptors for mediators: histamine (H_2-, H_3-, and H_4-, as well as H_1-), cysteinyl leukotrienes, and others
    - Adrenergic receptor expression differences or polymorphisms
    - Enzymes that catabolize mediators
  - Determine whether allergen acts at the site of exposure to generate messengers that reach the systemic circulation, or whether the allergen itself enters the systemic circulation and leads directly to symptoms and signs in target tissues such as the skin, airways, and vasculature
  - Determine the relative roles of bronchospasm and pulmonary vascular leak as causes of cough and dyspnea
  - Obtain additional information about the heart in anaphylaxis; specifically, seek to understand the role of heart mast cells in cardiovascular collapse in anaphylaxis, and the effects of mast cell/basophil mediators on cardiac contractility, relaxation, rate and rhythm, and coronary artery function
  - Determine whether the nervous system influences anaphylaxis and, if so, how
- Confirming the clinical diagnosis of an anaphylaxis episode
  - Identify more sensitive mediators or other markers
  - Develop rapid laboratory tests to confirm the diagnosis
  - Identify the importance of the following factors in respiratory/cardiovascular collapse:
    - Mediators (histamine, PAF, leukotrienes, prostaglandins)
    - Proteases produced by mast cells and other inflammatory cells
    - Cytokines
    - Chemokines
    - Growth and differentiation factors
    - Kinins, complement, and clotting factor fragments
    - Nitric oxide
- Assessment of sensitization versus risk in anaphylaxis
  - Validate a clinical instrument for risk assessment
  - Determine why allergen-specific IgE measurements correlate poorly with risk of anaphylaxis
  - Assess the value of total IgE levels
  - Confirm the value of assessing the ratio of allergen-specific IgE to total IgE
  - Evaluate the relative importance or role of the following:
    - Numbers/types of epitopes bound by IgE
    - Affinity of specific IgE antibodies
    - IgG blocking antibodies
  - Determine whether the use of recombinant allergens would improve identification of the allergen inducers of anaphylaxis and, if so, identify the appropriate allergens
  - Cellular tests
    - Basophil activation tests: role in determining risk
    - Cellular antigen stimulation test: does it have a role?
  - Other
    - Identify additional allergens that may be important in anaphylaxis
    - Develop a protocol for assessment of novel allergens
    - Identify/develop new and improved methods and instruments for assessment of risk (eg, clinical and epidemiologic instruments, including validated questionnaires, gene scan/proteomics, identification of relevant polymorphisms)

Basophil activation tests and basophil activation marker expression have high retrospective sensitivity and specificity, and the latter has been predictive of systemic reactions to venom immunotherapy.[106-109] During venom immunotherapy, however, activation of basophil markers persists, although the rate is slightly reduced from pretreatment values. A single prospective study of these tests in relation to a sting challenge during immunotherapy has been disappointing with regard to the predictive value of this test.[108] Further studies of basophil priming, and the mechanisms that regulate basophil responsiveness, including the involvement of dendritic cells and T cells, may help improve our understanding of why some sensitized individuals react to stings and others do not.[106-109]

Allergen-specific stimulation of PBMCs with venom may also provide improved ability to assess the immune response. Studies in beekeepers, and in individuals before and during venom immunotherapy, using venom stimulation of T-cell cultures show that sensitization is characterized by high proliferation of T_H2 lymphocytes and secretion of IL-4, whereas protection is characterized by low proliferation and dominant secretion of IL-10. Bee venom immunotherapy results in decreased IL-4 and IL-5 secretion in venom-stimulated T-cell cultures. This test, too, has high retrospective sensitivity and specificity. Its predictive value needs to be confirmed in prospective studies involving the reaction to a sting challenge in untreated patients.[133,134]

Currently, predictive values of venom skin tests and venom-specific IgE levels are not optimal, either in untreated individuals or in individuals on venom immunotherapy. In the future, predicting the risk of subsequent systemic reactions to insect stings may be improved by use of dialyzed venom or recombinant venoms[135] in both skin tests and *in vitro* tests, by use of basophil activation marker profile expression,[106-109] and possibly by cytokine/chemokine profiling.[133,134]

RISK ASSESSMENT IN INDIVIDUALS SENSITIZED TO FOODS

Current approaches to risk assessment are reviewed, and future directions for improving risk assessment are discussed.

Current methods of distinguishing clinical tolerance from clinical risk of anaphylaxis in food-sensitized individuals

Among individuals who are sensitized to foods, there are no completely reliable methods for distinguishing those who are clinically tolerant from those who are at risk for food-induced anaphylaxis[136-138] (Table III; Fig 3). The instruments most frequently used include history, skin prick/puncture tests, and quantitative measurement of allergen-specific IgE to the implicated foods. Fewer than 40% of histories of food allergy are confirmed by positive skin tests or elevated allergen-specific IgE measurements. Moreover, fewer than 40% of positive skin tests or elevated allergen-specific IgE measurements are confirmed by a positive oral double-blind challenge with the relevant food allergen.[8]

The identification of allergen-specific IgE levels with greater than 95% predictive risk values of a positive food challenge test has been a useful advance, although the IgE levels do not predict the type or severity of any reaction that may occur. The values need to be established separately for each food. Currently, they are only available for cow's milk ($\geq$15 kU/L), hen's egg ($\geq$7 kU/L), peanut ($\geq$14 kU/L), tree nuts ($\geq$15 kU/L), and finned fish ($\geq$20 kU/L). Values published for soy ($\geq$30 kU/L) and wheat ($\geq$26 kU/L) are not yet validated as being 95% predictive of a positive challenge test with the relevant food. Cutoff values may vary among different populations; for example, lower values for milk ($\geq$5 kU/L) and egg ($\geq$2 kU/L) have been identified in infants.[139,140] For some allergens such as egg white and peanut, in addition to the allergen-specific IgE level, the size of the skin prick test (SPT) wheal may also provide predictive information.[141,142]

Food challenge tests

If an individual has a history consistent with anaphylaxis to the isolated ingestion of a specific food and/or an allergen-specific serum IgE level above the decision point for that food, an oral food challenge is contraindicated and is potentially dangerous because it places him or her at increased risk for anaphylaxis and fatality. If, on the other hand, the history of anaphylaxis is questionable and the specific IgE level is below the decision point for that food, a carefully conducted food challenge may be justified (Table III; Fig 3). Challenges are most often used to eliminate incriminated foods that are highly unlikely to cause symptoms, or to document whether an individual has acquired clinical tolerance to a food after experiencing food-induced anaphylaxis in the past, avoiding the food for years, and losing sensitization to it, as documented by skin testing and measurement of specific IgE. If a late-phase clinical reaction is suspected, or if the individual has only had subjectively reported symptoms, a double-blind, placebo-controlled food challenge is recommended. Rarely, individuals with negative SPT to food allergens develop symptoms on food challenge.[136-138]

Food challenges should only be conducted in well equipped hospitals or other healthcare settings with close monitoring by highly trained physicians and other healthcare professionals who have the skills and experience to treat anaphylaxis and perform cardiopulmonary resuscitation if necessary. A controlled food challenge is not equivalent to an accidental ingestion of a food in the community setting, and cannot entirely predict either the occurrence or the severity of an allergic reaction to a food in the community. There are several reasons for this. Challenges should not be performed, and indeed could be dangerous to perform, if a comorbid condition such as asthma is active, or, in individuals old enough to perform spirometry, if the FEV_1 is less than 70% predicted, and they should be deferred even if an individual has an upper respiratory tract infection. Medications such as H_1-antihistamines should be discontinued before challenge. During a controlled challenge, small amounts (5-250 mg) of lyophilized food, with the dose doubled every 15 minutes, are introduced over several hours. Except for the food being tested, other foods are not ingested during the challenge because they might potentially enhance or delay absorption. Moreover, at the earliest symptom or sign of an adverse reaction, treatment should be given promptly, and the challenge should be halted.[136-138]

Risk factors for fatal or near-fatal anaphylactic reactions to food have been identified. Patient-related factors include age (with many fatalities occurring in adolescents and young adults), associated asthma (a comorbidity in nearly all fatalities), strenuous exercise, and ingestion of medications such as nonspecific β-blockers. Other comorbidities such as acute infection or stress require validation. In addition, risk may be elevated if the previous food reaction was severe, occurred to a trace amount of food, or involved denial of symptoms.[15,87,90,143] Skin prick test size and elevated specific IgE level do not necessarily predict severity of clinical reactions.[144,145]

Some risk factors are intrinsic to the foods themselves.[136-139] Peanut and tree nuts account for more than 90% of food anaphylaxis fatalities; however, finned fish, shellfish, cow's milk, and egg have also caused fatality, and any food may do so.[13,15] In addition, risk of fatality might be affected by food characteristics such as amount ingested and amount absorbed (food matrix effect, digestion effect), by degree or type of immune response to the food, and by target organ sensitivity.[13,143]

Occult sensitization to food appears to be common. In response to a questionnaire, only 19% of 622 individuals with self-reported peanut allergy stated that they had knowingly been exposed to peanut before their first documented reaction to it, and in these individuals, the amount of sensitization to peanut did not predict the clinical severity of the reaction.[145]

The history of an individual's most recent reaction to peanut in the community may have poor ability to predict the severity of his or her reaction in double-blind, placebo-controlled peanut challenge. This was apparent in a study in which the peanut specific IgE level correlated well with the challenge score, although not with the community reaction score, and SPT with peanut did not correlate significantly with either score.[146]

The natural history of peanut allergy in children diagnosed before age 4 years suggests that, regardless of the severity of the initial reaction, more than 50% of subsequent reactions involve potentially life-threatening symptoms.[147]

Food allergy: improving risk assessment

Clinical tolerance develops with increasing age in 80% of infants and young children with cow's milk and egg allergy.[136,137] Serial measurements of allergen-specific IgE concentrations and mathematical modeling of the rate of change of these concentrations in relationship to age might be helpful in predicting this favorable outcome.[148]

Use of peptide microarray immunoassays to identify individuals with IgE directed at large numbers of epitopes or at sequential epitopes has the potential to improve risk assessment in individuals with peanut allergy or cow's milk allergy.[31,149] In a recent peanut allergy study, a set of 213 overlapping residue 20 peptides was synthesized corresponding to the primary sequences of Ara h 1, Ara h 2, and Ara h 3. These peptides were arrayed in triplicate along with corresponding recombinant proteins on glass slides and used for immunolabeling. Most children with peanut allergy in the study, with reactions of varying severity and peanut-specific IgE ranging from 1.97 to >100 kU/L (median, >100 kU/L), were found to have specific IgE to at least 1 of the recombinant allergens, and 87% of them had detectable IgE to sequential epitopes. There was heterogeneity in the number and patterns of epitope recognition. Peanut-sensitized children with IgE antibodies that recognized a greater number of epitopes had experienced more severe allergic reactions than those with limited epitope recognition, although there was no correlation between reaction severity and total IgE or peanut-specific IgE levels. Greater epitope diversity seemed to correlate with relatively more peanut-specific binding sites present on mast cells and greater releasability of histamine. Also, the individuals with IgE directed at sequential epitopes rather than at conformational epitopes seemed to be at greater risk for persistent symptoms. In the future, tests such as IgE epitope mapping might predict the severity of food-induced allergic reactions and thus improve on the predictive value of allergen skin tests, allergen-specific IgE levels, and controlled food challenges, as currently used.

Additional promising developments that might improve risk assessment in individuals with a history of anaphylaxis from food include use of standardized food allergens, fresh food allergens, or recombinant food allergens in skin tests and *in vitro* tests[94] (Table IV). Allergen-specific flow-cytometric analysis of CD63 or CD203c expression as markers of basophil activation merit further study as adjunctive tests in patients with food allergy.[99-101] Allergen-specific cytokine and chemokine production patterns have not yet been used to predict future anaphylactic responses; however, the prevalence and nature of allergen-specific T-cell–dependent cytokine and chemokine responses are being investigated, and useful individual markers or, more likely, a panel of markers may eventually be identified.[150]

The development of biomarkers that robustly distinguish between sensitized individuals at risk of food-induced anaphylaxis and sensitized individuals who can tolerate the food remains a major unmet need.

SUMMARY

Risk assessment of individuals with anaphylaxis can be improved with the development of (1) improved sensitivity and practicality of laboratory tests to confirm the clinical diagnosis of an anaphylaxis episode and (2) improved safety and practicality of tests to distinguish allergen-sensitized clinically tolerant individuals from those at increased risk for anaphylaxis symptoms after allergen exposure. Algorithms for risk assessment in anaphylaxis have been developed, and a research agenda for studies that could lead to improved, evidence-based risk assessment in anaphylaxis has been created.

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