Mechanisms of allergic diseases

The pathophysiology of anaphylaxis

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Anaphylaxis is a severe systemic hypersensitivity reaction that is rapid in onset; characterized by life-threatening airway, breathing, and/or circulatory problems; and usually associated with skin and mucosal changes. Because it can be triggered in some persons by minute amounts of antigen (e.g., certain foods or single insect stings), anaphylaxis can be considered the most aberrant example of an imbalance between the cost and benefit of an immune response. This review will describe current understanding of the immunopathogenesis and pathophysiology of anaphylaxis, focusing on the roles of IgE and IgG antibodies, immune effector cells, and mediators thought to contribute to examples of the disorder. Evidence from studies of anaphylaxis in human subjects will be discussed, as well as insights gained from analyses of animal models, including mice genetically deficient in the antibodies, antibody receptors, effector cells, or mediators implicated in anaphylaxis and mice that have been “humanized” for some of these elements. We also review possible host factors that might influence the occurrence or severity of anaphylaxis. Finally, we will speculate about anaphylaxis from an evolutionary perspective and argue that, in the context of severe envenomation by arthropods or reptiles, anaphylaxis might even provide a survival advantage. (J Allergy Clin Immunol 2017;140:335-48.)

Key words: Anaphylaxis, basophils, cysteinyl leukotrienes, epinephrine, food allergy, histamine, IgE, mast cells, platelet-activating factor, urticaria

The recent International Consensus on Anaphylaxis described anaphylaxis as “a serious, generalized or systemic, allergic or hypersensitivity reaction that can be life-threatening or fatal.” This definition is intentionally “generic” in that it does not mention any of the specific immune elements that might be involved in particular instances of the disorder because these can vary depending on individual circumstances. In this review we will describe the key immune elements, such as antibody isotypes, effector cells, and biological mediators, that can contribute to the development and pathophysiologic manifestations of anaphylaxis. In particular, we will note the extent of evidence implicating these immune components in anaphylaxis in human subjects versus that induced in mouse models of the disorder, focusing especially on forms of anaphylaxis induced by reactions of allergens with antigen-specific antibodies. We will not extensively review forms of anaphylaxis induced by the antibody-independent activation of effector cells, such as mast cells and basophils, topics that have been reviewed elsewhere.

CLINICAL ANAPHYLAXIS

The clinical definition, classification, nomenclature, and treatment of anaphylaxis have been points of controversy, varying among different medical subspecialties and in different countries, and it became clear that an important goal for the field would be to achieve a true international consensus on these important points. Subsequently, multinational multidisciplinary symposia were convened to agree on the definition of anaphylaxis, the clinical criteria for its diagnosis, and its management. Participants agreed on a description of anaphylaxis as “a serious allergic reaction that is rapid in onset and may cause death,” as well as on 3 sets of clinical criteria to diagnose anaphylaxis. These criteria were reaffirmed in the recent International Consensus on Anaphylaxis article and are more extensively reviewed elsewhere in this issue of the Journal.

A minority of patients exhibit biphasic allergic reactions, in which signs and symptoms of anaphylaxis recur hours after the early phase of the reaction has waned, and in some patients late-phase reactions occur without initial hypotension or airway obstruction. In addition to the biphasic reactions observed in some patients with anaphylaxis induced by a variety of causes, patients who have IgE reactive with the oligosaccharide galactose-alpha-1,3-galactose, which is present in mammalian meat and in some therapeutic antibodies, can exhibit anaphylaxis
after a delay of several hours during which no signs or symptoms are apparent.\textsuperscript{9}

Although there is broad consensus on many aspects of the treatment of anaphylaxis,\textsuperscript{6,10-12} such recommendations are based largely on observational studies, extrapolation from retrospective case reviews, and a few clinical trials.\textsuperscript{10,11} Injectable epinephrine is universally agreed upon as the first-line therapy for anaphylaxis\textsuperscript{10,12} and can counteract many pathophysiologic changes in patients with anaphylaxis by acting through α\textsubscript{1}-adrenergic receptors to induce vasoconstriction, which prevents or diminishes tissue/airway edema, hypotension, and distributive shock; β\textsubscript{2}-adrenergic receptors to increase heart rate and cardiac contractility; and β\textsubscript{2}-adrenergic receptors to dilate the airways.\textsuperscript{11}

In addition, epinephrine’s action on β\textsubscript{2}-adrenergic receptors might potentially block further release of mediators (histamine and eicosanoids) by mast cells and perhaps other effector cells.\textsuperscript{13,14}

Other therapies should be considered second-line therapies and not a substitute for epinephrine. Guidelines generally agree that patients should be placed in a supine position and given crystalloid to maintain perfusion, and oxygen.\textsuperscript{10,12} H\textsubscript{1}- and H\textsubscript{2}-antihistamines might be helpful in treating cutaneous and upper respiratory signs and symptoms, and corticosteroids might help prevent biphasic reactions but neither prevent nor treat airway obstruction or circulatory collapse and therefore cannot be considered alternatives to epinephrine.\textsuperscript{10-12} Development of novel therapies for anaphylaxis is likely to be guided mainly by limited data from human subjects and by observations made using animal models.

### IMMUNOLOGIC MECHANISMS OF ANAPHYLAXIS

Only limited data on immunologic mechanisms of anaphylaxis from human subjects are available because of the life-threatening nature of anaphylaxis and obvious ethical concerns. Human studies of anaphylaxis have included inducing anaphylaxis in volunteers (most often through Hymenoptera sting challenge) and collecting samples from patients presenting for emergency management of anaphylaxis. Data obtained in such studies, as well as key findings obtained by using mouse models of anaphylaxis, are summarized in Fig 1 and Table I.\textsuperscript{15-106} The major pathophysiologic changes observed during anaphylaxis and some of the mediators that are thought to contribute to them are shown in Fig 2.

### EFFECTOR MOLECULES AND RECEPTORS

**IgE-dependent anaphylaxis**

IgE antibodies can play an important role in conferring immunologic specificity to effector cell activation in patients with anaphylaxis and other allergic diseases.\textsuperscript{15,106-109} IgE is the isotype found at by far the lowest concentrations in the circulation (50-200 ng/mL; total circulating IgE in healthy subjects vs approximately 10 mg/mL for IgG).\textsuperscript{107}; however, IgE can be found at much higher levels in patients with allergic diseases.\textsuperscript{15,16} IgE binds to the high-affinity receptor FcεRI on the surfaces of blood basophils and tissue-resident mast cells\textsuperscript{17} and (in human subjects to a greater extent than in mice) other cell types, including

### GLOSSARY

**ACTIVE SYSTEMIC ANAPHYLAXIS (ASA):** Anaphylaxis induced by means of active immunization (induction of antibodies involved in anaphylaxis through antigen sensitization of naive hosts).

**ANGIOTENSINOGEN:** Primarily synthesized in the liver, angiotensino-gen is an α\textsubscript{2}-globulin that is converted into angiotensin I by renin. Angio- tensin I is converted into angiotensin II by angiotensin-converting enzyme. Angiotensin II, in addition to promoting vasoconstriction, also mediates thirst, sodium retention, and aldosterone secretion.

**c-KIT D816V MUTATION:** c-KIT is the gene for KIT, a transmembrane tyrosine kinase receptor whose ligand is stem cell factor. The D816V mutation is the most commonly detected mutation in mastocytosis and occurs in codon 816. It consists of the substitution of valine for aspartate (Asp816Val). This activating mutation results in ligand-independent autophosphorylation of KIT.

**DISSEMINATED INTRAVASCULAR COAGULATION (DIC):** A coagulopathy that occurs as a secondary complication to many different disorders. Key features are microthrombi leading to tissue hypoxia and infarction, as well as hemorrhages caused by depletion of coagulation factors and platelets and activation of fibrinolysis. Trauma, sepsis, and malignancy are common triggers.

**MASS SPECTROMETRY:** A laboratory tool that can be used to detect and quantify the protein composition of a biological sample. Proteins are ionized, followed by separation of the ions by mass/charge ratio.

IONS then pass through a tuned field and generate an electric current that can be analyzed.

**MELOPEROXIDASE (MPO):** An enzyme that mediates the conversion of H\textsubscript{2}O\textsubscript{2} to HOCl, which can contribute to the killing of phagocytosed bacteria, fungi, and viruses, as well as to oxidative damage to host tissues.

**PASSIVE SYSTEMIC ANAPHYLAXIS (PSA):** A model in which the adoptive transfer of IgE or IgG antibodies to normal mice permits them to exhibit anaphylaxis on subsequent challenge with antigens recognized by such antibodies.

**TRYPTASE:** A serine esterase with both mature and immature forms and the most abundant serine protease of mast cells. Basophils have about 500-fold lower levels of tryptase than mast cells. The immature form is released constitutively by unstimulated mast cells, whereas the mature form is released on mast cell activation, such as in anaphylaxis. In human subjects there are 4 different tryptases (α, β, γ, and δ), of which the α and β tryptases are thought to be medically important. Tryptases can act locally, as well as remotely, on release. Tryptase’s biological functions are not entirely understood, but it is thought to have proinflammatory effects, including promotion of tissue edema and remodeling, chemokine secretion, and neutrophil recruitment. Anti-inflammatory effects include degradation of proteins, such as allergens and neuropeptides.

The Editors wish to acknowledge Daniel Searing, MS, for preparing this glossary.
neutrophils, eosinophils, monocytes and dendritic cells, and platelets.\textsuperscript{17} On exposure to a bivalent or multivalent allergen, cross-linking of Fc\textsubscript{\textepsilon}RI-bound IgE induces activation of mast cells and basophils and the immediate release of preformed mediators, such as histamine and various proteases, as well as \textit{de novo} synthesis of many inflammatory mediators, such as certain leukotrienes (LTs), prostaglandins, and cytokines.\textsuperscript{15,17} The importance of that reaction was demonstrated 50 years ago, when different groups realized that purified IgE was capable of transferring skin reactivity from sensitized human subjects to naive hosts.\textsuperscript{18-20,106} Similarly, transfer of antigen-specific IgE into naïve mice sensitizes the animals to have anaphylaxis on subsequent exposure to that allergen.\textsuperscript{21,22} Such IgE-mediated anaphylaxis is abrogated in mice lacking the high-affinity IgE receptor Fc\textsubscript{\textepsilon}RI,\textsuperscript{22} as well as in mast cell–deficient mice,\textsuperscript{23-25} highlighting the importance of IgE-mediated mast cell activation in such models of anaphylaxis.

Ever since the discovery that IgE can transfer allergen reactivity, antigen-specific IgE antibodies have been regarded as a key risk factor for the development of allergy, anaphylaxis, or both on subsequent antigen exposure. Indeed, quantification of specific IgE levels is used as part of the diagnostic evaluation of those thought to have allergic diseases and to identify potential triggers of anaphylaxis in patients with a history of anaphylaxis.\textsuperscript{109} Several trials have concluded that the use of the anti-IgE therapeutic antibody omalizumab as an adjunctive treatment during food or venom immunotherapy can decrease the risks of severe allergic reactions, including anaphylaxis, and in some but not all trials has been reported to improve the rapidity and efficacy of immunotherapy in achieving desensitization.\textsuperscript{26-30} In addition, limited clinical data also suggest that omalizumab might prevent spontaneous episodes of anaphylaxis in patients with systemic mastocytosis, a disease characterized by marked increases in mast cell numbers and activity\textsuperscript{31} (also see the review by Akin\textsuperscript{110} in this issue of the \textit{Journal}).

Clearly, however, IgE levels alone do not explain a subject’s susceptibility to anaphylaxis. Some patients can experience near-fatal anaphylaxis despite having low or undetectable levels of circulating allergen-specific IgE.\textsuperscript{111} Conversely, allergen-specific IgE can be detected in the plasma of many subjects who do not have clinical symptoms when exposed to that allergen.\textsuperscript{112} This is particularly true for Hymenoptera venom, with the vast majority (approximately 80%) of patients with IgE antibodies specific for Hymenoptera venoms having no history of systemic reactions to such venoms.\textsuperscript{113-116} Therefore, taken in isolation, the presence of antigen-specific IgE antibodies does not indicate that the person necessarily will exhibit any, let alone severe, clinical reactivity to the recognized antigens.\textsuperscript{117-123}
### TABLE I. Roles (or potential roles) of various antibodies, effector cells, and mediators in anaphylaxis in human subjects and mice

<table>
<thead>
<tr>
<th>Effector mechanisms</th>
<th>Human subjects</th>
<th>Mice</th>
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<tr>
<td><strong>Antibody isotypes</strong></td>
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<tr>
<td>IgE</td>
<td>● Increased IgE levels are present in patients with allergic diseases. 15,16</td>
<td>● PCA and PSA are induced by transfer of antigen-specific IgE into naive mice and antigen challenge. 13,14</td>
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<td>● Purified IgE can transfer skin reactivity from a sensitized human subject to a naive host. 18-20,106</td>
<td>● IgE-mediated PCA and PSA are abrogated in mice lacking the high-affinity IgE receptor FcεRI. 22</td>
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<td></td>
<td>● The anti-IgE antibody omalizumab can decrease the risks of anaphylaxis. 26,31</td>
<td>● ASA is reduced partially in IgE-deficient or FcεRI/−/− mice in some models but not in others. 35,56,60,66,90,91</td>
</tr>
<tr>
<td>IgG</td>
<td>● No definitive evidence is present to date.</td>
<td>● IgG1, IgG2a, and IgG2b (but not IgG3) can induce PSA. 32-42</td>
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<td>● Cases of anaphylaxis were reported after treatment with therapeutic mAbs without detectable levels of anti-drug IgE. 40,92-94</td>
<td>● IgG-PSA is reduced in FcγRIII/−/− mice. 33,34</td>
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<td></td>
<td></td>
<td>● IgG1 and IgG2b (but not IgG2a) PSA is enhanced in FcγRIIb/−/− mice. 34</td>
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<td></td>
<td>● Mice deficient in FcεRI can exhibit enhanced systemic anaphylaxis on challenge with 2.4G2 anti-FcγRII/III antibodies. 17</td>
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<tr>
<td></td>
<td></td>
<td>● Mice deficient for IgG1 or FcγRII are largely protected in several ASA models. 56,63,66</td>
</tr>
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<td></td>
<td></td>
<td>● Humanized mice expressing human FcεRI or FcγRII can have IgG-mediated anaphylaxis. 73-87</td>
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<tr>
<td><strong>Complement</strong></td>
<td>● Injection of low doses of C3a, C4a, or C5a in the skin of healthy volunteers induces immediate wheal-and-flare reactions. 7,43-47</td>
<td>● Reduced peanut-induced anaphylaxis is seen in C4−/− mice. 93</td>
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<td></td>
<td>● Blood levels of C3a, C4a, and C5a correlate with the severity of anaphylaxis in human subjects. 7</td>
<td>● Reduced IgE PCA is seen in mice in which mast cells lack C3aR or C5aR. 78</td>
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<tr>
<td></td>
<td></td>
<td>● Anaphylaxis was induced by direct activation of complement by peanut extract in one model. 88</td>
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<td></td>
<td></td>
<td>● C3−/−/− mice can fully develop the IgG-PSA model. 97</td>
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<tr>
<td></td>
<td></td>
<td>● ASA is not affected in C2−, C5−, and C5αR-deficient mice or after depletion of complement by using cobra venom factor. 30,98</td>
</tr>
<tr>
<td><strong>Effector cells</strong></td>
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<tr>
<td>Mast cells</td>
<td>● Increased tryptase levels have been detected during acute anaphylaxis in human subjects. 33,48-51</td>
<td>● IgE PCA and PSA were reduced markedly in various strains of mast cell–deficient mice. 23,24,40,55</td>
</tr>
<tr>
<td></td>
<td>● There is a high occurrence of anaphylaxis in patients with mastocytosis. 2-25</td>
<td>● ASA is reduced in mast cell–deficient mice in some studies but not in others. 33,36,56,60,66,90,99</td>
</tr>
<tr>
<td>Basophils</td>
<td>● There is no definitive evidence to date.</td>
<td>● Controversial: some reports indicate a contribution of basophils to IgG PSA 36,37 or ASA, 35,56,58 whereas others found no significant role for basophils. 34,59,66,97,100</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>● Basophil activation tests were used to diagnose or confirm allergen sensitization. 61-64</td>
<td>● Antibody-mediated neutrophil depletion reduces IgG PSA and ASA in some. 34,35,38 but not all. 36,66 models.</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td>● MPO levels are increased in patients with anaphylaxis compared with healthy donors. 67</td>
<td>● Depletion of monocytes/macrophages using clodronate liposomes can reduce IgG PSA and ASA. 34,56,59,65,66</td>
</tr>
<tr>
<td>Platelets</td>
<td>● There is no definitive evidence to date.</td>
<td>● There is no definitive evidence to date.</td>
</tr>
<tr>
<td></td>
<td>● Anaphylaxis in human subjects is associated with platelet activation. 68</td>
<td>● Depletion of platelets with anti-platelet antibodies (daily for 3 d) or neuraminidase does not reduce ASA. 63</td>
</tr>
<tr>
<td><strong>Mediators</strong></td>
<td></td>
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<tr>
<td>Histamine</td>
<td>● Aerosol administration of histamine induces bronchoconstriction in healthy volunteers. 69,70</td>
<td>● Histamine injection induces anaphylaxis. 101,102</td>
</tr>
<tr>
<td></td>
<td>● Intravenous administration of histamine in volunteers can reproduce many of the symptoms of anaphylaxis. 71,72</td>
<td>● H1-antihistamines reduce IgE PSA. 101</td>
</tr>
<tr>
<td></td>
<td>● H1-antihistamines are commonly used as adjunctive therapy for acute anaphylaxis and anaphylactoid reactions. 73</td>
<td>● IgG PSA and ASA are reduced in mice pretreated with H1-antihistamine in some models. 14,66,103 but not in others. 35,65</td>
</tr>
<tr>
<td></td>
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<td>● Mice deficient for the histidine decarboxylase gene are protected from IgE PSA. 101</td>
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<td>● H1 and H2 receptor–deficient mice are partially protected from IgE PSA 102</td>
</tr>
<tr>
<td>CysLTs</td>
<td>● Levels of some CysLTs are increased during anaphylaxis onset. 70,81</td>
<td>● There is reduced IgE PSA in mice deficient for LTC4 synthase. 83</td>
</tr>
<tr>
<td></td>
<td>● Intradermal injection of LTC4, LTD4, and LTE4 induces a wheal-and-flare reaction in healthy volunteers. 52</td>
<td>● Mice deficient for CysLT receptor type 1 also have significantly reduced IgE PCA. 84</td>
</tr>
<tr>
<td></td>
<td>● Aerosol administration of LTC4 and LTD4 in healthy subjects induces bronchoconstriction. 89,70,78</td>
<td>● There is reduced IgE PCA in mice deficient for LTC4 synthase. 83</td>
</tr>
</tbody>
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(Continued)
TABLE I. (Continued)

<table>
<thead>
<tr>
<th>Effector mechanisms</th>
<th>Human subjects</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAF</td>
<td>Injection of PAF in the skin of healthy volunteers induces wheal-and-flare reactions.</td>
<td>PAF is released during IgG PSA and ASA.</td>
</tr>
<tr>
<td></td>
<td>Circulating PAF levels increase and circulating PAF-AH activity decreases in proportion to the severity of anaphylaxis.</td>
<td>Injection of PAF induces anaphylaxis.</td>
</tr>
<tr>
<td>Others</td>
<td>Anaphylaxis induces increases in levels of many mediators that could contribute (positively or negatively) to the clinical signs and symptoms. This includes various cytokines and chemokines, prostanoids, tryptase, bradykinin, and serotonin, for example.</td>
<td>Mast cell-derived prostaglandin D2 can limit IgE PCA and IgE PSA.</td>
</tr>
</tbody>
</table>

PCA, Passive cutaneous anaphylaxis.

IgE-independent anaphylaxis

The fact that some patients experience anaphylaxis despite having undetectable levels of circulating allergen-specific IgE suggests the existence of IgE-independent pathways of anaphylaxis. However, it should be noted that a lack of detection of free IgE does not mean that such patients do not have enough FcεRI-bound IgE to experience IgE-mediated anaphylaxis. More definitive evidence for IgE-independent anaphylaxis has been obtained by using mouse models (Table I).

Role of IgG and FcγRs

In addition to IgE, we now know that mouse IgG also can induce passive systemic anaphylaxis (PSA) reactions, with physiologic manifestations similar to those seen in patients with IgE-dependent PSA (mainly hypothermia, vasodilatation, and cardiopulmonary changes). Whether IgG antibodies also mediate anaphylaxis in human subjects still remains to be proved and is the topic of a recent review. As demonstrated in mice, IgG-mediated anaphylaxis typically requires a much larger dose of antigen than does IgE-mediated anaphylaxis, and systemic anaphylaxis also requires systemic absorption of ingested antigen. Such conditions could be encountered in the case of anaphylaxis occurring in response to infusion of large quantities of a drug or a therapeutic mAb (Table I).

Role of complement

Activation of the complement cascade occurs in response to many stimuli and leads to generation of the small polypeptides C3a, C4a, and C5a, also named anaphylatoxins, which are potent inflammatory mediators. Multiple lines of evidence suggest that anaphylatoxins might be involved in anaphylaxis. Depletion of complement levels and production of C3a and C5a is observed in human anaphylaxis. Anaphylatoxins can activate various myeloid cells, including mast cells and basophils. Injection of low doses of C3a, C4a, or C5a into the skin of healthy volunteers induces immediate wheal-and-flare reactions. In addition, one study showed that blood levels of C3a, C4a, and C5a correlated with the severity of anaphylaxis in human subjects. Several transgenic mouse models have been used to study the importance of the complement pathway in anaphylaxis. Data obtained by using these transgenic models are reviewed in Table I and suggest that in mice the effect of complement components on anaphylaxis can be in most cases largely redundant with that of other mediators and might depend on the specific model used.

POTENTIAL EFFECTOR CELLS OF ANAPHYLAXIS

Mast cells

Mast cells are viewed as key players in IgE-dependent allergies and anaphylaxis. Mast cells ordinarily express large numbers of the high-affinity IgE receptor FcεRI. During IgE-dependent immune responses, the antigen-dependent cross-linking of antigen-specific IgE bound to FcεRI induces aggregation of FcεRI, promoting the activation of downstream signaling events that lead to secretion of several biologically active products thought to be implicated in allergic reactions, such as histamine and various cysteinyl leukotrienes (CysLTs). The molecular mechanisms of such IgE-dependent stimulation of mast cells have been extensively reviewed. There is compelling evidence of activation of mast cells during acute anaphylaxis. Although histamine detection can be used to diagnose anaphylaxis, detection of histamine in clinical blood specimens is difficult because of its extremely short half-life, and histamine is not a mast cell–specific product because it can also be released by other cells, including basophils and neutrophils.

Tryptase is much more stable than histamine and is considered a largely mast cell–derived product. Mature β-trypetase is stored in mast cell granules and released on activation, such as in anaphylaxis, whereas α- and β-protryptases are secreted constitutively by mast cells, and therefore increased blood levels might indicate increased mast cell burden rather than anaphylaxis. Increased levels of tryptase have been detected during acute anaphylaxis in human subjects. However, the roles of tryptase or other mast cell–derived proteases in anaphylaxis remain unknown. Moreover, in some patients with anaphylaxis, such as children with food allergen–induced anaphylaxis, increased blood levels of tryptase have not been detected. Additional evidence for a role of mast cells in anaphylaxis comes from the observation that patients with mastocytosis, a disease characterized by the presence of high numbers of mast cells in various organs, have a high occurrence of anaphylaxis. In children with mastocytosis, increased serum tryptase levels, which are used as an indicator of mast cell burden, are a risk factor for anaphylaxis and for the severity of anaphylactic episodes.

Studies with various strains of mast cell–deficient mice also confirmed the key role of mast cells in IgE-mediated anaphylaxis. Several reports now demonstrate that mast cell–deficient mice also have reduced peanut-induced anaphylaxis in active systemic anaphylaxis (ASA) models. However, the role of mast cells in ASA models using other...
antigens/allergens is more controversial (Table I). Therefore it is likely that mast cells can play either dominant or largely redundant roles in anaphylaxis and that the mast cell’s role can be enhanced or masked depending on factors such as the exact model, adjuvant, and allergen used.

**Basophils**

Human basophils also express high levels of the high-affinity IgE receptor FceRI\(^{139}\) and express the activating IgG receptor FcγRIIA and inhibitory IgG receptor FcγRIIB.\(^{140}\) Several lines of evidence suggest that basophils participate in anaphylaxis.\(^ {134}\)

For example, IgE-dependent activation of human basophils is associated with increased levels of certain basophil cell-surface markers, such as CD203c or CD63, and this forms the basis of basophil activation tests, which can be used to diagnose or confirm allergen sensitization and monitor the effects of efforts to treat these conditions with immunotherapy.\(^ {111-66}\) However, it is difficult to ascertain how important a contribution basophils make to the pathology of anaphylaxis in human subjects, given the concomitant mast cell activation that occurs in this setting. Even in mice, the role of basophils in anaphylaxis is unsettled and appears to be model-dependent (Table I).

**Monocytes/macrophages**

Monocytes and macrophages express high levels of activating FcγRs\(^ {141}\) and can also respond to anaphylatoxins.\(^ {142}\) Studies in mice have shown that depletion of monocytes/macrophages by using clodronate liposomes can reduce anaphylaxis in both IgG-mediated passive models and active models (Table I).\(^ {34,56,59,65,66}\) These data suggest that monocytes/macrophages might play an important role in anaphylaxis. However, to the best of our knowledge, the extent to which monocytes/macrophages can contribute to anaphylaxis in human subjects has not yet been determined.

**Neutrophils**

The potential functions of neutrophils in patients with anaphylaxis have been recently reviewed in detail.\(^ {143}\) Human...
and mouse neutrophils express several activating FcγRs, and can produce histamine, can release platelet activating factor (PAF; please see below for details on the role of PAF in anaphylaxis) in response to stimulation with immune complexes in vitro. Moreover, human neutrophils reportedly can express FcεRI, particularly in some patients with asthma. The major enzyme stored in neutrophils is myeloperoxidase (MPO). A recent report shows that circulating MPO levels are increased in patients with anaphylaxis compared with those in healthy donors. Consistent with this, increased MPO activity can also be detected as soon as 2 minutes after antigen challenge in an active mouse model of anaphylaxis. However, it should be noted that these results do not provide definitive proof of neutrophil activation in anaphylaxis because MPO could also be potentially released by other cell populations, including macrophages. Reduced expression of the activating IgG receptors FcγRIII and FcγRIV on mouse neutrophils occurs after IgG-mediated PSA, which suggests more definitively that neutrophils could be directly activated by IgG immune complexes during anaphylaxis. Antibody-mediated neutrophil depletion can reduce anaphylaxis in mice exhibiting IgG-mediated PSA or mast cell–independent ASA models. However, neutrophil-depleting antibodies had no effect in a mast cell–dependent ASA model induced without artificial adjuvants. This suggests that neutrophils might be particularly prominent in ASA models induced with adjuvants and that such models might not require any nonredundant contributions of mast cells.

Platelets
Anaphylaxis in human subjects is associated with platelet activation, presumably in response to PAF and/or other mechanisms, and activated platelets can release mediators, such as platelet factor 4 and serotonin, which can contribute to the pathophysiology of anaphylaxis. Moreover, human (but not mouse) platelets can express FcεRI, FcεRII, and FcγRIIA, and platelets can be activated ex vivo after incubation with serum from allergic patients and subsequent exposure to the relevant allergen. Two recent reports have shown that during basophil activation tests performed in blood specimens ex vivo, basophils (a potential source of PAF) can form associations with platelets, identifying this interaction as one that should be investigated further in the context of anaphylaxis.

Potential mediators of anaphylaxis
Histamine
Histamine has long been considered an important mediator of anaphylaxis. Weiss et al showed that aerosol administration of histamine induces bronchoconstriction in healthy volunteers, although the effect of histamine was much less potent than that of LTs. Intravenous administration of histamine in volunteers can reproduce many of the signs and symptoms of anaphylaxis, including cutaneous flushing, headache, airway obstruction, and transient hemodynamic changes, mainly represented by systemic hypotension, tachycardia, and increased left ventricular performance. There are 4 known histamine receptors, named H1 to H4. Studies with receptor antagonists suggest that some of the systemic effects of histamine, including airway obstruction and tachycardia, are mainly mediated through the H1 receptor, whereas some others, including cutaneous flushing and headaches, seem to be mediated through both H1 and H2 receptors. H3-antihistamines are commonly used as adjunctive treatment for acute anaphylaxis and anaphylactoid reactions. The contribution of histamine to anaphylaxis has also been confirmed by using mouse models (Table I). Mast cells and basophils likely represent the main sources of histamine in patients with anaphylaxis. In agreement with this, histamine release is abrogated in mast cell–deficient mice in a model of IgE-mediated PSA, and increases in plasma histamine levels are also abrogated in 2 models of ASA in mice deficient for both mast cells and basophils.

PAF
PAF is a potent phospholipid-derived mediator implicated in platelet aggregation and thought to play an important role in a variety of immune and inflammatory responses. The biology of PAF and its potential role in anaphylaxis have been recently reviewed in detail. PAF can be released by a variety of human cells, including purified lung mast cells and blood basophils after ex vivo stimulation with anti-IgE antibodies and purified neutrophils after incubation in vitro with heat-aggregated human IgG. Many of the cell populations that produce PAF can also respond to PAF, including platelets, mast cells, neutrophils, and macrophages. Injection of PAF in the skin of healthy volunteers induces wheal-and-flare reactions. Because these reactions could be blocked by H1-antihistamines, it was first proposed that PAF induced wheals through secondary histamine release by dermal mast cells. However, unlike human lung mast cells and peripheral blood–derived mast cells, skin mast cells do not degranulate in response to PAF stimulation ex vivo. In addition, Krause et al showed that intradermal injection of PAF, unlike histamine and codeine, did not cause a statistically significant increase in dermal histamine levels in healthy volunteers.

A limited number of reports have assessed concentrations of PAF or platelet-activating factor acetylhydrolase (PAF-AH), an enzyme responsible for the rapid degradation of PAF, after anaphylaxis in human subjects. In these reports circulating PAF levels were increased, and circulating PAF-AH activity was inversely correlated with the severity of anaphylaxis. The contribution of PAF to anaphylaxis has been studied in more detail using pharmacologic and genetic approaches in mouse models (Table I). In most models combined inhibition of histamine and PAF almost entirely blocked anaphylaxis, suggesting additive or synergistic effects of histamine and PAF. The main cellular source of PAF in these reports likely depends on the exact anaphylaxis model used. Using an adjuvant-free active anaphylaxis model, we recently reported that the PAF receptor antagonist CV-6209 can reduce anaphylaxis in wild-type mice but has no effect on the residual anaphylaxis observed in monocyte/macrophage-depleted mice, suggesting that monocytes/macrophages represent the major source of PAF in this model.

CysLTs
A third class of potential mediators of anaphylaxis was originally termed slow-reacting substance of anaphylaxis and
consists of 3 bioactive CysLTs: LTB₄, LTC₄, and LTD₄. CysLTs are synthesized from arachidonic acid by a variety of cells, including mast cells, basophils, and macrophages. CysLTs and their metabolites can be measured by using mass spectrometry, and several reports show that levels of some of these products, namely LTE₄, 2,3-dinor-9α,11β-PGF₂α, and 9α,11β-PGF₂α, are increased during the onset of anaphylaxis. Although these reports indicate that CysLTs and their metabolites might be good biomarkers of anaphylaxis, they do not prove that these compounds make an important contribution to the clinical manifestations of anaphylaxis. However, multiple observations suggest that CysLTs can promote acute allergic reactions. When injected intradermally in healthy volunteers, each of the 3 CysLTs elicited a wheal-and-flare reaction. In addition, aerosol administration of LTC₄ and LTD₄ in healthy subjects induced bronchoconstriction with 1,000-fold more potency than histamine (Table I).

More definitive evidence for a role of CysLTs in anaphylaxis comes from studies in mice. Mice deficient for LTC₄ synthase (an enzyme responsible for biosynthesis of LTC₄) or for the CysLT receptor CysLT receptor type 1 have markedly reduced IgE-mediated passive cutaneous anaphylaxis. CysLTs are synthesized from arachidonic acid by a variety of cells, including mast cells, basophils, and macrophages. CysLTs and their metabolites can be measured by using mass spectrometry, and several reports show that levels of some of these products, namely LTE₄, 2,3-dinor-9α,11β-PGF₂α, and 9α,11β-PGF₂α, are increased during the onset of anaphylaxis. Although these reports indicate that CysLTs and their metabolites might be good biomarkers of anaphylaxis, they do not prove that these compounds make an important contribution to the clinical manifestations of anaphylaxis. However, multiple observations suggest that CysLTs can promote acute allergic reactions. When injected intradermally in healthy volunteers, each of the 3 CysLTs elicited a wheal-and-flare reaction. In addition, aerosol administration of LTC₄ and LTD₄ in healthy subjects induced bronchoconstriction with 1,000-fold more potency than histamine (Table I).

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Other potential mediators
Anaphylaxis induces changes in levels of many other mediators, which could contribute (positively or negatively) to the clinical signs and symptoms (Table I). This includes tryptase, prostaglandins, and cytokines/chemokines. Depletion of the bradykinin precursor high-molecular-weight kininogen has been observed in patients with anaphylaxis, likely through activation of the plasma contact system and kallikrein. Patients with anaphylaxis can also experience depletion of clotting factors, including Factors V and VIII, and in extreme cases experience disseminated intravascular coagulation. Although most patients promptly treated for anaphylaxis recover without obvious sequelae, some have recurrent signs and symptoms that require continued treatment with epinephrine and for which corticosteroids are administered. Such sequelae are thought to reflect the “late” consequences of some of the mediators released by effectors of anaphylaxis, such as CysLTs, cytokines, and chemokines, or by structural cells activated in this setting. Finally, mast cells can release adenosine on IgE-dependent activation, and adenosine can have complex effects mediated through various adenosine receptors with distinct functions, which have the potential to influence the pathophysiology of anaphylaxis. However, more work is needed to define the importance of most of these mediators in anaphylaxis, particularly in human subjects.

INSIGHTS FROM HUMANIZED MODELS OF ANAPHYLAXIS
Several humanized mouse models of anaphylaxis have been developed to investigate the functions of human antibodies, Fc receptors, and effector cells in anaphylaxis. Transgenic mice expressing human FcεRI instead of the mouse protein (hFcεRIIATg mice) were generated, and the expression profile of the human FcεRI transgene is very similar to that found in humans. Transgenic mice expressing human FcεRI instead of the mouse protein (hFcεRIIATg mice) were generated, and the expression profile of the human FcεRI transgene is very similar to that found in humans. Transgenic mice expressing human FcεRI instead of the mouse protein (hFcεRIIATg mice) were generated, and the expression profile of the human FcεRI transgene is very similar to that found in humans.
Although mice can exhibit both IgE- and IgG-dependent anaphylaxis, the existence of IgG-mediated anaphylaxis in human subjects has not been conclusively demonstrated.

In addition to mast cells and basophils, macrophages, neutrophils, and perhaps other leukocytes and platelets also might produce a diverse array of inflammatory mediators during anaphylaxis, and such products have the potential to contribute to reactions that might be difficult to treat, protracted in nature, or biphasic.

Genetic modifiers and other host factors, as well as gene-environment interactions, can influence the development of anaphylactic reactivity, as well as the presentation, severity, or both, of anaphylaxis.

Although the potential evolutionary benefit of anaphylaxis remains uncertain, recent evidence in mice suggests that anaphylaxis can have effects that can reduce the toxic effects of certain arthropod or reptile venoms.

### RECOVERY FROM ANAPHYLAXIS

Many of those who have experienced anaphylaxis and were not treated have survived the episode, particularly those with less severe presentations. What is the basis of such recovery? Variations in metabolism of mediators, including PAF and bradykinin, can influence manifestations of anaphylaxis and theoretically the ability to recover from these manifestations. In animal models of anaphylaxis and in human subjects undergoing insect sting challenge, levels of substances with endogenous vasopressor activity, including epinephrine, norepinephrine, and angiotensin II, are increased within minutes after development of anaphylaxis, which is likely to compensate for the vasodilation and fluid extravasation occurring during anaphylaxis. Observations that β-adrenergic blockade can exacerbate systemic anaphylaxis in mouse and rat models and in patients with severe anaphylaxis caused by multiple causes, particularly when combined with angiotensin-converting enzyme inhibitors, support a role for endogenous vasopressors in limiting the severity of pathophysiologic changes in the setting of anaphylaxis. Mast cell degranulation releases chymase, which can convert angiotensin I to angiotensin II and thereby directly contribute to increased angiotensin II levels observed after anaphylaxis. In a recent article Nakamura et al showed that mice in which mast cells cannot produce prostaglandin D2 have enhanced...
manifestations of IgE-mediated anaphylaxis. Therefore it appears that mast cells also can secrete antianaphylactic mediators, which might help to limit anaphylactic responses. 89 Evidence in mice indicates that sphingosine-1-phosphate can both enhance features of anaphylaxis via effects on mast cells 202 and also, via effects on other cell types, enhance histamine clearance, thus contributing to recovery from anaphylaxis. 203 Finally, it is possible that genetically determined or other differences in mast cell activation or mediator release profiles, or in the tissue responses to such mediators, might contribute to differences in the manifestations of or recovery from anaphylaxis.

CAN ANAPHYLAXIS BE BENEFICIAL?

Using mouse models, we recently reported that the development of a type 2 immune response to honeybee venom (BV) could increase the survival of mice challenged with whole BV. 204 Also, others have shown in mice that a type 2 immune response to BV phospholipase A2 (which is considered to be the major BV allergen in human subjects) could diminish the decrease in body temperature induced by challenge with a “near-lethal” dose of BV phospholipase A2. 204,205 Importantly, these effects were dependent on IgE 204 and on the high-affinity IgE receptor FceRI. 204,206 In a follow-up study we also provided evidence that IgE, FceRI, and mast cells can enhance the survival of mice injected with Russell’s viper venom. 206

One of the mechanisms by which innate activation of mouse mast cells can enhance the survival of naive mice on their first exposure to various arthropods 207-208 or reptile 206-208 venoms is the proteolytic reduction of the toxicity of venom components by mast cell–derived carboxypeptidase 3A 208-209 or mouse mast cell protease 4 (chymase). 207 Given that snake (or arthropod) envenomation in the field can result in systemic distribution of the venom, one could argue that systemic IgE-dependent mast cell activation in this setting could both produce the clinical picture of anaphylaxis and also result in the systemic release of mediators (ie, mast cell proteases) that can degrade toxic components of the venom. In such settings anaphylaxis could be beneficial if it prevents death by envenomation and the unfortunate subject also survives the anaphylaxis. Although we do not know whether human IgE also can enhance resistance to venoms (and we imagine that we would have some trouble enlisting volunteers for such a study), it is tempting to speculate that anaphylaxis induced by small amounts of venom (eg, a single or wasp bee sting) represents only the most extreme and maladaptive end of a spectrum of acquired IgE-mediated immune responses to venom that includes, at the other end of the spectrum, appropriately regulated immune responses that can enhance resistance to such venoms.

CONCLUDING REMARKS

Anaphylaxis represents one of the most urgent of medical emergencies, in which rapid diagnosis and prompt and appropriate treatment can mean the difference between life and death. Although there has been steady progress in our understanding of the antibodies, effector cells, and mediators that can contribute to the development and manifestations of anaphylaxis, especially in the context of mouse models of the disorder, the basic clinical management of anaphylaxis has changed little in decades (see Castells 6 in this issue of the Journal and Table II). In a report published in 2005, Sampson et al2 identified as major research needs both the development of “universally accepted diagnostic criteria” and the importance of identifying “reliable laboratory biomarkers to confirm the clinical impression.” As noted in our introduction, the first need largely has been addressed by international and interdisciplinary efforts to forge consensus. However, the second need remains essentially unfulfilled. It is our hope that further progress in understanding the immunopathogenesis and pathophysiology of anaphylaxis in all of its various forms will help to guide efforts to devise more effective strategies for preventing this disorder and also to provide more effective options for rapidly diagnosing and effectively treating anaphylaxis when it occurs.

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