

Molecular mechanisms in allergy and clinical immunology

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Molecular mechanisms of anaphylaxis: Lessons from studies with murine models

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Studies with murine models demonstrate 2 pathways of systemic anaphylaxis: one mediated by IgE, FcεRI, mast cells, histamine, and platelet-activating factor (PAF), and the other mediated by IgG, FcγRIII, macrophages, and PAF. The former pathway requires much less antibody and antigen than the latter. As a result, IgG antibody can block IgE-mediated anaphylaxis induced by small quantities of antigen without mediating FcγRIII-dependent anaphylaxis. The IgE pathway is most likely responsible for most human anaphylaxis, which generally involves small amounts of antibody and antigen; similarities in the murine and human immune systems suggest that the IgG pathway might mediate disease in persons repeatedly exposed to large quantities of antigen. Mice, like human subjects, can experience IgE/FcεRI/mast cell-mediated gastrointestinal and systemic anaphylaxis in response to ingested antigen. Gastrointestinal symptoms depend on serotonin and PAF; mediator dependence of systemic symptoms has not been determined. Both local and systemic anaphylaxis induced by ingested antigens might be blocked by IgA and IgG antibodies. IL-4 and IL-13 signaling through the IL-4 receptor α chain, in addition to promoting the mastocytosis and IgE antibody production that mediate most human anaphylaxis, exacerbates the effector phase of anaphylaxis by increasing target cell responsiveness to vasoactive mediators. As a result, IL-4 receptor α chain antagonists might be particularly effective suppressors of anaphylaxis. (*J Allergy Clin Immunol* 2005;115:449-57.)

Key words: Blocking antibody, FcεRI, FcγRIIb, FcγRIII, IgE, IL-4 receptor α chain

Compared with other allergic disorders, such as asthma, allergic dermatitis, and allergic rhinitis, the pathophysiology of anaphylaxis seems simple. Antigen cross-links antibody molecules, activating immunoglobulin receptors on inflammatory cells and causing them to release mediators that increase vascular permeability and cause smooth muscle contractions that produce urticaria, hypotension, dyspnea, abdominal cramping, and diarrhea.¹ The apparent simplicity of this process, however, is belied by observations made with rodent models of anaphylaxis that demonstrate 2 distinct pathways of systemic anaphylaxis with complex interactions between IgE and other immunoglobulin isotypes, induction of distinct symptoms by different mediators, and interactions between cytokines and mediators that synergistically increase the severity of anaphylaxis. These observations have important implications for the prevention and treatment of anaphylaxis.

PATHWAYS OF SYSTEMIC ANAPHYLAXIS IN THE MOUSE

Two distinct mechanisms can rapidly induce anaphylaxis in the mouse (*Fig 1*).²⁻⁵ The first is the classical mechanism associated with human allergy: antigen cross-linking of antigen-specific IgE bound to mast cell FcεRI stimulates mast cell degranulation, with the rapid release of histamine and serotonin and the synthesis and secretion of platelet-activating factor (PAF) and leukotrienes.^{5,6} These mediators act on target cells to increase vascular permeability, which causes hemoconcentration and depletes intravascular volume.^{5,7,8} The resulting decrease in vital organ perfusion is the primary cause of the symptoms that characterize murine anaphylaxis.⁸ Antigen-induced IgE-mediated anaphylaxis can be modeled in mice by means of injection with a monoclonal anti-IgE antibody or with an antigen-specific IgE antibody, followed by the appropriate antigen.^{5,6,9} As would be expected, active IgE-mediated anaphylaxis is IL-4 and IL-4 receptor dependent (it does

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Abbreviations used

IL-4R α : IL-4 receptor α chain
 OVA: Ovalbumin
 PAF: Platelet-activating factor
 TNP: Trinitrophenyl

not develop in IL-4-deficient or IL-4 receptor α chain [IL-4R α]-deficient mice, which fail to produce IgE), mast cell dependent (it does not occur in mast cell-deficient W/W^v mice or in normal mice treated with anti-c-kit mAb), Fc ϵ RI dependent (it does not occur in Fc ϵ RI-deficient mice), IgE dependent (it does not occur in IgE-deficient mice, unless they have been treated with exogenous IgE), and macrophage independent (it occurs in macrophage-depleted mice).⁵ It is also blocked by treatment with histamine receptor 1 antagonists and, to a lesser extent, by PAF antagonists but is not inhibited by serotonin receptor or leukotriene antagonists through depletion of complement with cobra venom factor or through blocking the activating low-affinity IgG receptor Fc γ RIII.⁵

A second pathway of murine anaphylaxis, which is IgE independent, has been demonstrated in most studies in which mice are primed by immunization with antigen and then challenged weeks later with the same antigen.^{2,3,5} In contrast to the classical pathway, antigen-challenged mice have anaphylaxis despite the absence of mast cells, Fc ϵ RI, and IgE. This second pathway is also complement independent (not inhibited by cobra venom factor) but requires IgG antibody, macrophages (inhibited by macrophage depletion with gadolinium), Fc γ RIII (absent in mice pretreated with the anti-Fc γ RII/RIII mAb 24G2 and in Fc γ RIII-deficient mice) and PAF (but not histamine, serotonin, or leukotriene).⁵ Although platelets and neutrophils have been implicated in IgE-independent anaphylaxis in some murine studies,^{10,11} this has not been apparent in our studies. Instead, we find that techniques used to deplete platelets and neutrophils can inhibit IgE-independent anaphylaxis by producing immune complexes that desensitize macrophages,⁵ making it appear that these cells contribute to Fc γ RIII-dependent anaphylaxis. IgE-independent anaphylaxis can be modeled in mice by injecting them with 24G2, which first stimulates macrophage release of PAF by cross-linking Fc γ RIII on these cells and then inhibits IgE-independent anaphylactic responses to antigen by blocking immune complex activation of macrophages through Fc γ RIII.^{5,12}

Murine anaphylaxis induced through either pathway develops and resolves with the same short time course and has similar symptoms, although subtle differences in cardiac and pulmonary effects were noted in one study.^{5,13} IgE-dependent and IgE-independent systemic anaphylaxis are both induced by select antigen immunization and challenge protocols, whereas other protocols selectively induce IgE-independent anaphylaxis.^{5,14} The presence of antigen-specific IgE antibody and mast cells, although required for IgE-dependent anaphylaxis, is not

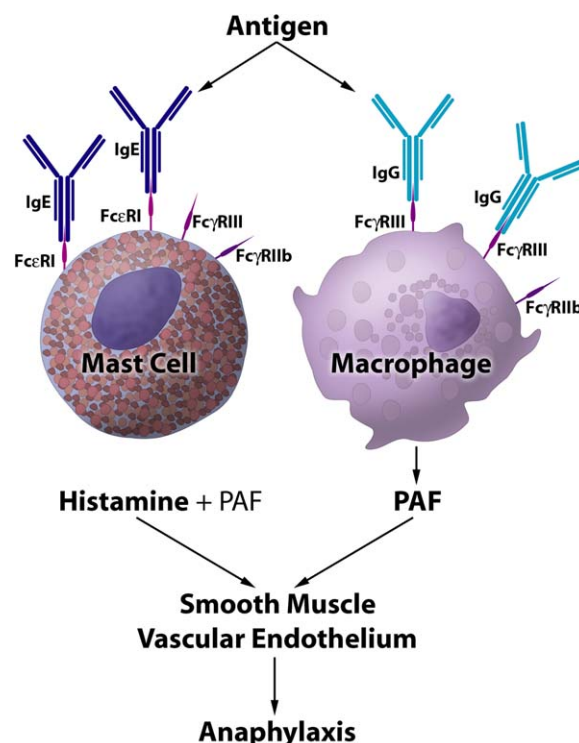


FIG 1. Mechanisms of systemic anaphylaxis. Antigen can cause systemic anaphylaxis in mice by (1) cross-linking IgE bound to mast cell Fc ϵ RI, stimulating histamine and PAF release, or (2) forming complexes with IgG that cross-link macrophage Fc γ RIII, stimulating PAF release. PAF and histamine cause the symptoms of anaphylaxis by inducing smooth muscle contraction and increased vascular permeability.

the critical factor that determines whether this type of anaphylaxis develops in mice because immunization protocols that induce the production of sufficient IgG antibody to allow the development of IgE-independent anaphylaxis also induce sufficient antigen-specific IgE antibody to mediate IgE-dependent anaphylaxis. Furthermore, the number of mast cells is sufficient, even in unimmunized mice, to mediate IgE-dependent anaphylaxis induced by anti-IgE antibody or by antigen-specific IgE antibody, followed by antigen.^{5,8} Instead, the predominant determinants that influence whether IgE-dependent anaphylaxis is induced appear to be the quantity of antigen-specific IgG antibody produced and the quantity of antigen used to challenge immunized mice (Strait et al, manuscript in preparation). This suggests that IgG antibodies, in addition to mediating IgE-independent anaphylaxis, can block IgE-dependent anaphylaxis and provided the rationale for investigating the function of blocking antibody. The characteristics of the 2 pathways of anaphylaxis are summarized in Table I.

BLOCKING ANTIBODIES AND ANAPHYLAXIS

The concept that antibodies that cannot induce anaphylaxis might suppress anaphylaxis developed before IgE was identified as the reagenic antibody and provided an

important theoretical justification for allergen desensitization therapy.^{15,16} It is currently easy to envision antigen neutralization by a high concentration of circulating IgG antibody before it can cross-link mast cell-bound IgE, a mechanism that has been demonstrated *in vitro* in studies with IgG antibodies and IgE-pulsed mast cells.¹⁷ Surprisingly, however, IgG suppression of IgE-mediated anaphylaxis has not, until now, been demonstrated *in vivo*. This might reflect the problem that the induction of FcγRIII-mediated anaphylaxis can mask clinical suppression of IgE-dependent anaphylaxis, unless *in vivo* mast cell degranulation is quantitated by evaluating increases in serum levels of histamine or mouse mast cell protease 1 or FcγRIII-dependent anaphylaxis is blocked (eg, by studying FcγRIII-deficient mice or treating mice with 24G2 before antigen challenge). Using these techniques, we have demonstrated that mice primed by means of immunization with goat anti-mouse IgD antibody, which stimulates intestinal mastocytosis, a relatively large IgE anti-goat IgG antibody response, and an approximately 1,000-fold larger IgG anti-goat IgG antibody response,¹⁸ have only IgE-independent anaphylaxis when challenged 2 weeks after priming with 100 μg of goat IgG.⁵ The same goat anti-mouse IgD-primed mice, however, have IgE-dependent anaphylaxis if challenged with 10 mg of goat IgG (Strait et al, manuscript in preparation). This suggests that the ability of IgG antibody to block IgE-mediated anaphylaxis is saturable; it blocks antigen binding to mast cell-associated IgE when the challenge dose of antigen is limiting, but a large dose of challenge antigen overcomes the block and induces mast cells to degranulate. This observation also suggests that IgG antibodies block IgE-mediated anaphylaxis by physically inhibiting the binding of antigen to IgE rather than by cross-linking mast cell FcεRI to an inhibitory receptor, FcγRIIb, that suppresses FcεRI signaling.¹² This interpretation is supported by studies that show that goat anti-mouse IgD antibody-immunized mice fail to experience IgE-dependent anaphylaxis in response to challenge with 100 μg of goat IgG, even when FcγRIIb and FcγRIII have been blocked by pretreatment with 24G2 (Strait et al, manuscript in preparation).

Passive transfer studies have been performed in which mice were primed through injection of varying quantities of a monoclonal mouse IgE antibody to the hapten trinitrophenyl (TNP), a polyclonal mouse IgG anti-TNP antibody, or both and then challenged with TNP conjugated to ovalbumin (TNP-OVA) to confirm these interpretations. Results of these studies demonstrate that both IgG anti-TNP antibody and IgE anti-TNP antibody prime mice to experience an anaphylactic response to TNP-OVA. However, the quantity of TNP-OVA required to induce anaphylaxis in mice primed with IgG anti-TNP antibody is approximately 100-fold greater than that required to induce anaphylaxis in mice primed with IgE anti-TNP antibody. Increasing the molar ratio of TNP to OVA in the TNP-OVA conjugate decreases the amount of TNP-OVA required to induce either IgE-dependent or IgE-independent anaphylaxis but does not appreciably

TABLE I. Comparison of IgE-dependent and IgE-independent anaphylaxis

Characteristic	IgE-dependent anaphylaxis	IgE-independent anaphylaxis
Requirement for IgE	+	—
Requirement for IgG	—	+
Requirement for FcεRI	+	—
Requirement for FcγRIII	—	+
Mediated by histamine	+	—
Mediated by PAF	+	+
Mediated by mast cells	+	—
Mediated by macrophages	—	+
Requires high concentrations of antibody	—	+
Requires large amounts of antigen	—	+
Inhibited by blocking antibody	+	—

change the 100:1 ratio of the quantity of antigen required to induce IgE-dependent versus IgE-independent anaphylaxis (Strait et al, manuscript in preparation). As a result, IgG anti-TNP antibody can inhibit IgE-dependent anaphylaxis by acting as a blocking antibody, as long as the challenge dose of TNP-OVA is kept at less than that required to induce IgE-independent anaphylaxis. Stated differently, IgG antibody blocks IgE-dependent anaphylaxis without mediating IgE-independent anaphylaxis when anaphylaxis is induced by a low dose of allergen but not when it is induced by a high dose of allergen.

Experiments with this system also provide a way to evaluate whether FcεRI-FcγRIIb interactions contribute at all to the blocking of IgE-dependent anaphylaxis by IgG antibody. Studies were performed in which FcγRIII-deficient mice were primed with IgE anti-TNP antibody, treated with 24G2 (to selectively block FcγRIIb) or a control antibody, and protected with a variable dose of IgG anti-TNP antibody. The dose of IgG anti-TNP antibody required to inhibit IgE-dependent anaphylaxis was determined when FcγRIIb was functional and when it was blocked by 24G2. Approximately 1.5- to 2-fold more IgG anti-TNP antibody was required to block IgE-mediated anaphylaxis in the absence of 24G2 than in its presence (Strait et al, manuscript in preparation). Thus an IgG-mediated FcγRIIb-FcεRI interaction contributes to, but is not required for, inhibition of IgE-dependent anaphylaxis by IgG blocking antibody.

MURINE MODELS OF FOOD ALLERGY

Anaphylaxis can be induced by ingested, as well as injected, allergen. Most frequently, allergic responses to ingested allergen are manifested by local symptoms, such as abdominal cramping and diarrhea, but some patients experience urticaria, bronchospasm, and/or shock.^{19,20} These differences in symptoms might reflect differences in the extent of antigen-induced mediator release in the gut, differences in the extent to which ingested antigen is

absorbed, differences in the distribution of mast cells that have bound antigen-specific IgE, and/or differences in amounts of IgA and IgG antibodies that might neutralize antigen before it can cross-link mast cell-associated IgE.

Two murine models of intestinal allergy address some of these issues. In one model repeated oral inoculation with cholera toxin plus peanut allergens causes mice to experience both diarrhea and systemic symptoms, including decreased mobility, wheezing, and hypothermia.²¹ Anaphylaxis in this model is associated with an IgE antibody response to peanut allergens, low levels of IgG antibody to peanut allergens, and increased serum histamine levels, an indication of mast cell degranulation. In a second model, in which mice are primed intraperitoneally with OVA adsorbed to alum adjuvant, followed by repeated oral OVA challenge, mice experience diarrhea after 5 or more challenges.²² Diarrhea in this model is mast cell, FcεRI, and IgE dependent, although FcεRI-deficient mice eventually have diarrhea after multiple challenges. Unlike systemic IgE-dependent anaphylaxis, the development of diarrhea is histamine independent and serotonin and PAF dependent. Unlike the peanut allergen–cholera toxin model, intestinal symptoms in the OVA-alum model are not accompanied by systemic symptoms. The reason for this difference is not obvious. One possibility is that orally administered peanut allergens might be systemically absorbed with their antigenic determinants (epitopes bound by IgE) intact to a greater extent than OVA. Another possibility, which seems more plausible, is that more IgG and IgA antibody, which block IgE-mediated anaphylaxis, is produced in the OVA-alum system than in the peanut allergen–cholera toxin system. If so, OVA that is systemically absorbed would likely be neutralized by serum IgG before it could cross-link mast cell-bound IgE, whereas serum antibodies would not likely interfere with mast cell IgE cross-linking by systemically absorbed peanut allergens. The eventual induction of diarrhea in FcεRI-deficient mice in this model is consistent with the development of a large IgG response, which might both block IgE-mediated diarrhea and stimulate intestinal disease through an IgE-independent mechanism. It might also be relevant that mice deficient in J chain, which have only 10% of normal intestinal IgA, have allergic diarrhea in response to fewer and smaller oral OVA inoculations than normal mice (Strait et al, manuscript in preparation). This suggests that non-IgE antibodies can inhibit gut-based anaphylaxis by acting as blocking antibodies and supports the possibility that increasing non-IgE antibody levels in the gut and the serum would inhibit ingested allergen induction of both local and systemic anaphylaxis.

PULMONARY INVOLVEMENT IN ANAPHYLAXIS

Dyspnea and wheezing are common symptoms in anaphylaxis and are reproduced in some, but not all, murine models of this disorder.²¹ We find no evidence for the development of respiratory distress in unprimed

mice or goat anti-mouse IgD antibody-primed mice that are challenged with anti-IgE antibody, even though these mice experience severe hypotension. In contrast, anti-IgE antibody induces dyspnea, as measured by means of barometric plethysmography, in mice that have been inoculated intratracheally several times with a strong allergen, *Ascaris* pseudocoelomic fluid, before anti-IgE antibody challenge (Strait et al, manuscript in preparation). It is not clear whether the pulmonary response reflects bronchospasm, an increase in pulmonary interstitial fluid cause by an increase in pulmonary vascular permeability, or a combination of the two. In contrast, respiratory distress has been described in the peanut allergen–cholera toxin model, even though mice in this model are never purposefully inoculated intratracheally or intranasally with allergen.²¹ Because intratracheal allergen inoculation induces allergic inflammation in the gastrointestinal tract,²³ it seems possible that a similar phenomenon occurs in reverse when mice are inoculated orally with allergen. Alternatively, it is possible that oral inoculation is followed by some degree of aspiration of the allergen inoculum. Regardless of mechanism, the development of asthma-like symptoms during anaphylaxis appears to require pre-existing pulmonary inflammation in addition to systemic mediator release.

CYTOKINE MODULATION OF ANAPHYLAXIS

The development and severity of anaphylaxis depend not only on the presence of the required IgE or IgG antibodies, inflammatory cells that express receptors for these antibodies, and mediators that are released by these cells but also on the responsiveness of cells that are targeted by these mediators. This last factor is influenced by IL-4 and IL-13, cytokines that are also important in the initial generation of the antibody and inflammatory cell responses that mediate anaphylaxis, to a considerable extent. Treatment of mice with IL-4 or IL-13 rapidly and substantially increases the severity of anaphylaxis that is mediated by either the IgE-dependent or the IgE-independent pathway.⁸ These effects depend on IL-4Rα-dependent IL-4/IL-13 activation of the transcription factor signal transducer and activator of transcription 6 and thus likely depend on new gene expression and protein synthesis, even though they develop within 1 to 2 hours after mice are treated with either cytokine.⁸ The effects are dose dependent but are substantial, even when mice are treated with low doses of IL-4 and are observed in mice infected with a helminthic parasite that induces IL-4 and IL-13 production.⁸ Although long-term exposure to IL-4 stimulates mastocytosis and can enhance mediator release,^{24–28} the most dramatic and rapid effect of IL-4 on anaphylaxis is a 3- to 6-fold enhancement of responsiveness of targeted cells to vasoactive mediators, including histamine, serotonin, PAF, and cysteinyl leukotrienes (Strait et al, unpublished data).⁸ These effects most likely exacerbate anaphylaxis by acting on vascular endothelial cells because they greatly enhance the ability of vasoactive

mediators to increase vascular permeability.⁸ Increased vascular permeability is not observed in IL-4-treated mice that are not also exposed to vasoactive mediators and might result from an increase in mediator receptor expression.^{29,30} IL-4/IL-13 exacerbation of anaphylaxis is not mimicked by IL-2, IL-5, IL-9, or IL-15 and is blocked by IFN- γ , a cytokine that inhibits many effects of T_H2 cytokines.⁸

THE BIOLOGIC JUSTIFICATION FOR ANAPHYLAXIS

The life-threatening nature of anaphylaxis suggests that the biologic processes responsible for this disorder would only have evolved if they are host protective when activated in an appropriately limited and focused fashion. Surprisingly, although there is plentiful evidence for the importance of mast cells and macrophages in host protection, there is relatively little evidence for host-protective effects of Fc γ RIII-mediated macrophage production of PAF or Fc ϵ RI-mediated mast cell mediator release. PAF promotes eosinophil, neutrophil, and macrophage chemotaxis and phagocytosis and contributes, in murine models, to host protection against *Trypanosoma cruzi*, *Klebsiella pneumoniae*, *Leishmania amazonensis*, and *Candida albicans*³¹⁻³⁴; however, it is not known whether Fc γ RIII-mediated PAF production is important for host protection against any of these pathogens. Localized cutaneous anaphylaxis, which is mediated by IgE and mast cells, promotes host protection against some ectoparasites, specifically certain tick species,³⁵ probably through a rapid local increase in vascular permeability that interferes with the parasite's access to blood vessels. Mast cell degranulation is also essential for rapid expulsion of some gastrointestinal nematode parasites, such as *Trichinella spiralis*, during a second infection; however, IgE is not absolutely essential for this process and appears to play a largely redundant role.^{36,37} In contrast, IL-4 enhancement of target cell sensitivity to mediators appears to be critical for host protection against this parasite: *T spiralis* is not expelled by mice that express IL-4R α only on bone marrow-derived cells, even though these mice produce large IL-4, IgE, and mast cell responses.³⁸ Interestingly, the cross-linking of IgE bound to Fc ϵ RI on basophils leads to the rapid secretion of large quantities of IL-4 and IL-13 that could increase the sensitivity of intestinal cells to the effects of mediators released by mast cells or macrophages. Furthermore, the basophil cytokine response requires considerably less IgE cross-linking than the induction of mast cell mediator release.³⁹ This makes it likely that IL-4/IL-13 secretion by basophils occurs earlier in infection than mast cell mediator release, allowing targeted cells to develop maximal sensitivity to mediators by the time they are produced and released. Thus at least some of the mechanisms that contribute to the pathogenesis of anaphylaxis appear to also contribute to host protection against worm infection; however, these mechanisms might have a net deleterious effect in the developed

TABLE II. Anaphylaxis-relevant similarities and differences between the mouse and human immune systems

Characteristic	Mouse	Human
Presence of IgE	+	+
Presence of Fc ϵ RI on mast cells and basophils	+	+
Presence of Fc ϵ RI on macrophages and Langerhans cells	—	+
Presence of Fc γ RIIb on mast cells	+	+
Presence of Fc γ RIII on macrophages	+	+
Presence of Fc γ RIIA, Fc γ RIIIA, and Fc γ RIIIC	—	+
Mast cell production of histamine	+	+
Macrophage production of PAF	+	+
Activation of mast cells by IgG	+	—
IgE binding to Fc γ RIIb and Fc γ RIII	+	+

world, in which protozoan and helminthic infections are uncommon.

DIFFERENCES BETWEEN MURINE AND HUMAN ANAPHYLAXIS

Mice are not people, and their immune systems are not identical. Consequently, the results of studies with murine models of anaphylaxis suggest, but do not prove, how the process develops in human subjects. Although no definite conclusions can be drawn about possible interspecies differences in anaphylaxis without directly studying the process in human subjects, it might be revealing to examine established similarities and differences between the murine and human immune systems and consider how they might affect anaphylaxis (summarized in Table II).

Some critical similarities are that (1) both species produce IgE that binds with high affinity to Fc ϵ RI and IgG that binds with low affinity to Fc γ RIII^{40,41}; (2) both species express Fc ϵ RI on mast cells and basophils and Fc γ RIII on macrophages^{40,41}; (3) activated mast cells in both species secrete histamine and PAF, and activated macrophages in both species secrete PAF⁴²⁻⁴⁴; and (4) histamine and PAF increase vascular permeability in both species.⁴⁵⁻⁴⁸ Some potentially important differences are that (1) mouse IgG1 has some ability to activate mast cells, an effect that is not shared by any human IgG isotype⁴⁹; (2) IgE binds weakly to murine, but not human, low-affinity Fc γ Rs⁵⁰; (3) human, but not mouse, macrophages, Langerhans cells, and dendritic cells can express Fc ϵ RI⁵¹⁻⁵³; and (4) human platelets, B cells and natural killer cells, and neutrophils express low-affinity IgG receptors (Fc γ RIIA, Fc γ RIIIC, and Fc γ RIIIB, respectively) that are not expressed in the mouse.⁵⁴ On the basis of these considerations, there is little reason to expect that there will be substantial differences between human and murine anaphylaxis.

Although IgG-mediated anaphylaxis has not been described in human subjects, human anaphylaxis has

TABLE III. Key concepts

1. Anaphylaxis in the mouse can occur through 2 independent pathways: one that involves IgE, FcεRI, mast cells, PAF, and histamine and one that involves IgG, FcγRIII, macrophages, and PAF
2. Considerably less antibody and antigen are required to trigger IgE-dependent anaphylaxis than IgG-mediated anaphylaxis.
3. Most human anaphylaxis is probably IgE dependent because it involves small quantities of antigen.
4. IgG- and IgA-blocking antibodies inhibit the ability of small quantities of antigen to induce IgE-dependent anaphylaxis by neutralizing antigen before it can cross-link mast cell-associated IgE.
5. IgG antibodies also inhibit IgE-dependent anaphylaxis by mediating an interaction between FcεRI and FcγRIIb on mast cells.
6. Gastrointestinal anaphylaxis is induced by an IgE/FcεRI/mast cell/PAF plus serotonin pathway and can cause systemic symptoms if levels of blocking antibodies are low.
7. Pulmonary involvement in anaphylaxis requires pre-existing pulmonary allergic inflammation.
8. Signaling through IL-4Rα promotes anaphylaxis by stimulating IgE production and mastocytosis and increasing target organ sensitivity to vasoactive mediators.
9. Localized and limited anaphylactic responses contribute to host protection against insect infestation and worm infection.
10. The murine and human immune systems are sufficiently similar that these observations about murine anaphylaxis are likely to apply to human subjects.

been described in which there was no evidence of antigen-specific IgE antibodies or mast cell degranulation products, such as histamine and tryptase.⁵⁵⁻⁵⁹ Some of these cases might reflect immunoglobulin-independent activation of inflammatory cells, but others might reflect the IgG/FcγRIII/macrophage/PAF pathology that has been well characterized in mice. Equally important, the presence of human IgG receptors that can activate macrophages to secrete PAF makes the occurrence of FcγRIII-dependent anaphylaxis likely.

Although mouse IgG1, unlike any human IgG isotype, can mediate cutaneous anaphylaxis, presumably by weakly associating with mast cell FcεRI and cross-linking this receptor,⁴⁹ this phenomenon is not involved in murine IgG-mediated systemic anaphylaxis, which is mast cell independent.⁵ Consistent with this, murine FcγRIII-dependent anaphylaxis can be mediated by IgG2a and IgG2b, neither of which mediates cutaneous anaphylaxis but both of which are bound by FcγRIII.⁶⁰

Because FcγRIIb is an inhibitory receptor, whereas FcγRIII is a stimulatory receptor,⁵⁴ the weak binding of mouse IgE to FcγRIIb on mast cells might suppress IgE-mediated, mast cell-dependent anaphylaxis, whereas the weak binding of IgE to FcγRIII on macrophages might contribute to macrophage-dependent, FcγRIII-mediated anaphylaxis. In support of the former possibility, anaphylaxis mediated by a monoclonal anti-IgE antibody is more severe in FcγRIIb-deficient mice than in wild-type mice.¹² In contrast, we find no enhanced severity of IgE antibody-

TABLE IV. Therapeutic implications

1. Induction of blocking antibodies should protect against anaphylaxis that is induced by small, but not large, quantities of antigen.
2. Therapeutic anti-IgE antibodies and IgE-IgG fusion proteins should protect against anaphylaxis that is induced by small quantities of antigen and might increase the safety of immunization required to induce blocking antibody production.
3. IL-4Rα antagonists, which suppress the pathophysiology of anaphylaxis at several points, might be a particularly effective prophylactic therapy for anaphylaxis.
4. PAF and serotonin antagonists might be more effective than histamine receptor 1 antagonists at ameliorating local symptoms of food allergy.

mediated anaphylaxis in FcγRIII-deficient mice that have been treated with 24G2 to block FcγRIIb (Strait et al, manuscript in preparation). Thus the increased severity of anaphylaxis in FcγRIIb-deficient mice might reflect developmental consequences of the permanent absence of this inhibitory receptor rather than failure of IgE antibody to cross-link mast cell FcεRI with FcγRIIb during IgE-mediated mast cell activation. IgE enhancement of mouse macrophage-mediated anaphylaxis seems farfetched. Although IgE-FcγRIII interactions might have the potential to contribute to macrophage activation, this putative contribution should be quite small relative to interactions between FcγRIII and the much more abundant IgG antibodies, and FcγRIII-dependent anaphylaxis occurs normally in IgE-deficient mice.⁵

The expression of FcεRI by human, but not murine, macrophages might allow IgE-mediated macrophage activation to contribute to human anaphylaxis but does not argue against the existence of human FcγRIII-mediated anaphylaxis. Similarly, the expression of stimulatory FcγRs on human, but not murine, platelets⁶¹ suggests an additional mechanism (platelet release of inflammatory mediators) that could potentially contribute to human FcγR-mediated anaphylaxis.

Why then has there been so little evidence for FcγRIII-mediated anaphylaxis in human subjects? Our guess is that it reflects the large amounts of IgG antibody and antigen that are required for this phenomenon relative to IgE-dependent anaphylaxis. Most anaphylaxis models in the mouse feature purposeful immunization with potent immunogens that stimulate large, antigen-specific IgG antibody responses and challenge mice with relatively large quantities of antigen.⁵ These features ensure the presence of the relatively large quantities of IgG-antigen complexes that are required to trigger FcγRIII-dependent anaphylaxis and IgG antibody that are required to inhibit IgE-dependent anaphylaxis. In contrast, immunization is accidental for most cases of human anaphylaxis and generates serum concentrations of allergen-specific IgG much lower than those seen in most murine models. The amounts of challenge allergen in most cases of human anaphylaxis are also much lower than those used in most murine models, particularly when the approximately 3500-fold difference in mouse and human weight is taken

into account. Thus human anaphylaxis usually involves low concentrations of antibody and small amounts of allergen, amounts that meet requirements for IgE-mediated, but not Fc γ RIII-mediated, anaphylaxis. Anaphylaxis that occurs in persons who have been repeatedly inoculated with large amounts of foreign and potentially immunogenic molecules (eg, antibiotics or partially humanized antibodies) might be an exception.

CLINICAL IMPLICATIONS

If the same mechanisms are involved in murine and human anaphylaxis, as we suggest, observations made with murine models of anaphylaxis (Table III) have important implications for the treatment of human subjects with this disorder (Table IV).

Human anaphylaxis that is induced by small quantities of allergen (eg, insect sting or peanut ingestion) can be assumed to be mediated by IgE, Fc ϵ RI, and mast cells, whereas human anaphylaxis that is induced by a large quantity of allergen might be mediated by IgG, Fc γ RIII, and macrophages. Anti-IgE antibodies that block IgE binding to Fc ϵ RI and do not bind Fc ϵ RI-associated IgE should be efficacious inhibitors of IgE-mediated anaphylaxis; indeed, this has been observed in the first published study with anti-IgE therapy for food allergy.⁶² In contrast, these therapeutic antibodies would not be expected to inhibit IgG-mediated anaphylaxis. Consistent with this, rare individuals have experienced anaphylactic responses to therapeutic anti-IgE antibodies.⁶³

Blocking antibodies can be expected to inhibit human anaphylaxis that is provoked by small doses of allergen. Although it is dangerous to treat patients prone to food-induced anaphylaxis with immunization therapy, prior treatment with antibodies that block IgE-Fc ϵ RI interactions might substantially decrease the risk of such therapy, making it a practical alternative. Clinical trials are needed to test this possibility.

In atopic patients who produce large amounts of IL-4 and IL-13, treatment with anti-IL-4R α antibodies might inhibit anaphylaxis through 3 mechanisms: suppressing IL-4/IL-13-dependent IgE responses, decreasing mast cell numbers, and decreasing sensitivity to mediators released by mast cells.

Pharmaceuticals, such as IgE-IgG fusion proteins, that inhibit mast cell activation by cross-linking Fc ϵ RI to Fc γ RIIb^{64,65} are more likely to be efficacious inhibitors of anaphylaxis that is induced by small quantities of allergen (IgE-dependent anaphylaxis) than anaphylaxis that requires large quantities of allergen (Fc γ RIII-dependent anaphylaxis).

If Fc γ RIII-mediated anaphylaxis occurs in human subjects and involves the same mediator as in mice, PAF antagonists might be effective prophylactics.

If the same mediators are involved in human as in murine intestinal allergy, PAF and serotonin antagonists might provide considerable relief from local symptoms, such as cramping and diarrhea.

FUTURE DIRECTIONS

Advances made during the past several years already promise more effective treatment of anaphylaxis. Additional experiments are required, however, to address several questions.

1. Are human and murine anaphylaxis as similar as we suggest? Studies in human subjects or with mouse-human chimeric models (eg, mice that express human Fc ϵ RI)⁶⁶ are needed to test our view and determine whether the clinical implications that we suggest have merit.
2. Do ingested allergens cause systemic disease by activating mast cells in the gut or by activating vasculature-associated mast cells after systemic absorption? In the former case induction of allergen-specific IgA would be more likely than induction of IgG to block the relevant mast cell activation. In the latter case IgG antibody should be at least equally protective.
3. What is the best way to induce antigen-specific IgG or IgA antibody without augmenting IgE production? The use of specific adjuvants, particularly antigen-coupled immunostimulatory DNA sequences (CpGs), might be particularly promising in this regard.⁶⁷
4. Are human memory IgE responses IL-4R α dependent, as is the case in the mouse?⁶⁸ If so, IL-4R α should be a particularly good therapeutic target for the reasons noted above.
5. Can a single antibody block both IL-4 and IL-13 stimulation of IL-4R α with high potency? If not, a combination of reagents or separate IL-4 and IL-13 antagonists might be required.

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