IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and FcγRIIb cross-linking

Richard T. Strait,1,2 Suzanne C. Morris,3,4 and Fred D. Finkelman3,5,6

1Division of Emergency Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA. 2Department of Pediatrics, Division of Emergency Medicine, and 3Department of Internal Medicine, Division of Immunology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA. 4Research Service, Cincinnati Veterans Affairs Medical Center, Cincinnati, Ohio, USA. 5Division of Immunobiology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA. 6Medicine Service, Cincinnati Veterans Affairs Medical Center, Cincinnati, Ohio, USA.

Although it has long been hypothesized that allergen immunotherapy inhibits allergy, in part, by inducing production of IgG Abs that intercept allergens before they can cross-link mast cell FcεRI-associated IgE, this blocking Ab hypothesis has never been tested in vivo. In addition, evidence that IgG-allergen interactions can induce anaphylaxis by activating macrophages through FcεRIII suggested that IgG Ab might not be able to inhibit IgE-mediated anaphylaxis without inducing anaphylaxis through this alternative pathway. We have studied active and passive immunization models in mice to approach these issues and to determine whether any inhibition of anaphylaxis observed was a direct effect of allergen neutralization by IgG Ab or an indirect effect of cross-linking of FcεRI to the inhibitory IgG receptor FcγRIIb. We demonstrate that IgG Ab produced during the course of an immune response or administered passively can completely suppress IgE-mediated anaphylaxis; that these IgG blocking Abs inhibit IgE-mediated anaphylaxis without inducing FcγRIII-mediated anaphylaxis only when IgG Ab concentration is high and challenge allergen dose is low; that allergen epitope density correlates inversely with the allergen dose required to induce both IgE- and FcγRIII-mediated anaphylaxis; and that both allergen interception and FcγRIIb-dependent inhibition contribute to in vivo blocking Ab activity.

Introduction

The rationale for allergen immunotherapy for atopic disorders has changed with time. Initially, “allergy vaccines” were thought to induce the production of IgG blocking antibody (BA), which might neutralize allergen molecules before they could interact with what were later discovered to be IgE Abs bound to FcεRI on mast cells and basophils (1, 2). More recently, this BA concept has been supplemented by evidence that IgG Ab–allergen complexes may inhibit mast cell signaling by cross-linking the immunoreceptor tyrosine activation motif–containing activating receptor FcεRI to the immunoreceptor tyrosine inhibition motif–containing inhibitory receptor FcγRIIb (3), and that immunotherapy may instead inhibit allergy by immunomodulation: decreasing Th2 cytokine production, increasing Th1 cytokine production, and/or activating regulatory T cells (4–7). Surprisingly, despite the long history of allergen immunotherapy, positive correlations between IgG Ab levels and protection against allergen-induced disease in some but not all studies (8–12), and in vitro experiments that demonstrated IgG Ab inhibition of antigen-induced (Ag-induced) mast cell/basophil degranulation and other IgE-mediated effects (5, 13, 14), there has been no in vivo proof of the BA concept.

Nonstandard abbreviations used: Ag, antigen; Asm, antiseraum; BA, blocking antibody; GIGG, goat IgG; GIGG Asm, heat-inactivated mouse anti-GIgG antiserum; GmMD, goat anti-mouse IgD antiseraum; IgGotNTP; IgE antit-TNP mAb; IgGoGtGIGG, IgG anti-GIgG; IgGoGtNTP; purified IgG fraction of αTNP Asm; IVCCA, in vivo cytokine capture assay; MMCP-1, mouse mast cell protease-1; NIP, 3-nitro-4-hydroxy-5-iodophenylacetyl; PAF, platelet-activating factor; TNP, trinitrophenyl; αTNP Asm, heat-inactivated mouse anti-TNP antiseraum; TNP-GmMD, TNP conjugated to GmMD; TNP-OVA, TNP conjugated to OVA; TNP-OVA-NIP, conjugated to TNP-OVA.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: J. Clin. Invest. doi:10.1172/JCI25575.

Results

We initiated such in vivo studies because of unexpected results that were obtained in an animal model of anaphylaxis in which mice were immunized with a goat Ab against mouse IgD (GmMD), which stimulates large IgG1, IgE, IL-4, and mast cell responses and a small IgG2a response, but little or no IgG3 or IgG2b production (refs. 15–19 and F.D. Finkelman, unpublished data) and challenged with 100 µg of the relevant Ag, goat IgG (GIGG) (20). Although GIGG challenge induced severe anaphylaxis, anaphylaxis was mediated by IgG, FcγRIIb, macrophages, and platelet-activating factor (PAF), rather than by IgE, FcεRI, mast cells, and histamine (20). In view of the strong IgE, IL-4, and mast cell responses that develop in GmMD-treated mice, it seemed unlikely that the failure of GIGG challenge to induce IgE-mediated anaphylaxis resulted from a lack of IgE or mast cells. Instead, the strong IgG anti-GIGG (IgGotGIGG) response that develops in these mice raised the possibility that IgGotGIGG blocked IgE-mediated anaphylaxis, either by intercepting GIGG before it could bind to IgE/FcεRII on mast cells or by cross-linking FcεRI to FcγRIIb. We have now performed in vivo studies to evaluate these possibilities. Our results show that allergen-specific IgG can block IgE-mediated anaphylaxis in vivo; define conditions under which blocking occurs without inducing FcγRIII-mediated anaphylaxis; and demonstrate the importance of both Ag interception and FcγRIIb-mediated inhibition as mechanisms of BA function.
GlG, induces anaphylaxis that is independent of IgE, FcεRI, and mast cells but requires IgG, FcγRIII, and macrophages (20). Three mechanisms might inhibit IgE-mediated anaphylaxis in this system: (a) IgG Ab might intercept GlG before it could be bound by mast cell–associated IgE; (b) mouse IgG–anti-GlG complexes might inhibit mast cell FcεRII signaling by cross-linking FcεRI to FcγRIIb; and (c) “nonspecific” IgE produced by GlG-immune mice might displace IgE anti-GlG Ab from mast cell FcεRI.

We attempted to distinguish among these possibilities by increasing the dose of GlG used to challenge GtoMD-immunized mice from 0.1 to 10 mg (Figure 1). Some GtoMD-immunized mice were pretreated with anti–FcγRII/RIII mAb 1 day before GlG challenge to block IgG-mediated anaphylaxis and FcγRIIb-associated inhibition of IgE-mediated anaphylaxis. Challenge with 0.1 or 10 mg of GlG induced anaphylaxis of similar severity, as measured by hypothermia (which reflects the development and degree of shock) and hemoconcentration (which reflects vascular leak), when mice were not pretreated with anti–FcγRII/RIII mAb. However, only the 10-mg dose of GlG induced anaphylaxis in anti–FcγRII/RIII mAb–treated mice (Figure 1A and B). Increasing the dose of challenge Ag should saturate BA and allow Ag to cross-link mast cell–associated FcεRI but should not affect FcγRIIb-mediated inhibition of mast cell degranulation or competition between GlG-specific and non–specific IgE for mast cell FcεRI. Thus, our observation supports the hypothesis that IgE-mediated anaphylaxis in GtoMD-immunized mice is inhibited by IgG BA interception of the challenge Ag.

These results did not eliminate the possibility that IgG BA suppresses IgE-mediated anaphylaxis in GtoMD-immunized mice by both intercepting Ag and cross-linking FcεRI to FcγRIIb. Anti–FcγRII/RIII mAb blocks both the FcγRIIb-dependent, macrophage-dependent pathway of anaphylaxis and FcγRIIb-dependent inhibition of mast cell–mediated anaphylaxis, which makes it impossible to isolate FcγRIIb-dependent inhibition in WT mice. To isolate FcγRIIb inhibition, we compared the effects of anti–FcγRII/RIII mAb on anaphylaxis induced by high-dose (10 mg) Ag challenge in GtoMD-immunized WT and FcγRII- or FcγRII-deficient mice. Anti–FcγRII/RIII mAb had its expected inhibitory effect on anaphylaxis in WT mice, but little, if any, inhibitory or stimulatory effect in FcγRII-deficient mice (Figure 1C). Thus, Ag interception, rather than the cross-linking of FcεRI to FcγRIIb, accounts for most of the inhibition of IgE-mediated anaphylaxis in GtoMD-immunized mice.

If IgG BA in GtoMD-immunized mice inhibits IgE-mediated anaphylaxis by intercepting Ag, it should be possible to demonstrate IgG-Ag complexes in the blood of immunized, Ag-challenged mice and to directly show that serum IgG Ab blocks Ag binding to IgE. Experiments were performed to test each of these predictions. Because it is difficult to assay for the mouse IgG–GlG complexes that should be formed in GtoMD-immune mice challenged with GlG, we instead used a system that takes advantage of the strong Ab response generated to molecules conjugated to GtoMD but allows more sensitive and precise detection of the Ag-Ab complex. Mice primed with a conjugate of trinitrophenyl-GlGMD (TNP-GlGMD) develop a large IgG1 anti-TNP Ab response (21). TNP-OVA–mouse IgG complexes were easily detected in serum 5 minutes after TNP-GlGMD–immunized mice were challenged with 1 mg of TNP-OVA (Figure 1D).
Figure 2
IgE/FcεRI/mast cell–dependent anaphylaxis in GpαMD-primed mice requires challenge with a high dose of Ag. Mice (4–5 per group) were primed s.c. with 0.2 ml of GpαMD, then challenged i.v. 14 days later with GlgG. Temperature was followed for 2 hours after challenge, and the maximum temperature decrease was calculated. (A) WT mice and mice deficient in FcγRIII, IgG, or both were challenged as shown. (B) WT (+) and mast cell–deficient W/Wv (−) mice were treated as shown. (C) BALB/c mice were injected 15–30 minutes before challenge with 66 µg of CV6209 (PAF antagonist), 0.2 mg of both triprolidine and cimetidine (H1 and H2 antagonists), all 3 antagonists, or no antagonist and challenged as shown. (D) BALB/c mice were injected i.v. with 1 mg of gadolinium (macrophage inhibitor) or saline 1 day before GlgG challenge. (E) BALB/c mice were injected s.c. with saline or 500 µg of anti–FcγRII/RIII mAb 1 day before GlgG challenge. Blood was drawn 2 hours after GlgG challenge, and MMCP-1 levels were determined. (F) BALB/c mice were injected s.c. with saline or 500 µg of anti–FcγRII/RIII mAb 1 day before GlgG challenge. Anticoagulated blood was obtained for histamine measurement 5 minutes after challenge. (G) BALB/c mice were bled 4 hours after challenge with the indicated dose of GlgG, and IL-4 secretion was evaluated by in vivo cytokine capture assay (IVCCA) (51). *P < 0.05.

To directly determine whether Ag immunization can inhibit Ag binding to IgE, we immunized mice with GpαMD or TNP-GpαMD and evaluated the ability of their serum to block TNP-OVA binding by IgE anti-TNP mAb (IgE/TNP). This was done by mixture of immune or nonimmune serum with a doubly haptenated Ag (TNP-OVA–3-nitro-4-hydroxy-5-iodophenylacetyl [TNP-OVA-NIP]), capture of this Ag onto microtiter plate wells with anti-NIP mAb, and evaluation of the ability of their serum to block TNP-OVA binding to IgE, we immunized mice with GpαMD or TNP-GpαMD and evaluated the ability of their serum to block TNP-OVA binding by IgE anti-TNP mAb (IgE/TNP). This was done by mixture of immune or nonimmune serum with a doubly haptenated Ag (TNP-OVA–3-nitro-4-hydroxy-5-iodophenylacetyl [TNP-OVA-NIP]), capture of this Ag onto microtiter plate wells with anti-NIP mAb, and evaluation of the ability of their serum to block TNP-OVA binding to IgE, we immunized mice with GpαMD or TNP-GpαMD and evaluated the ability of their serum to block TNP-OVA binding by IgE anti-TNP mAb (IgE/TNP). This was done by mixture of immune or nonimmune serum with a doubly haptenated Ag (TNP-OVA–3-nitro-4-hydroxy-5-iodophenylacetyl [TNP-OVA-NIP]), capture of this Ag onto microtiter plate wells with anti-NIP mAb, and evaluation of the ability of their serum to block TNP-OVA binding.

Characterization of anaphylaxis induced by low and high doses of challenge Ag in GpαMD-immunized mice. To provide additional evidence that induction of IgE-mediated anaphylaxis in GpαMD-immune mice requires high-dose Ag challenge, we characterized IgE, FcR, cell type, and mediator requirements for anaphylaxis in GpαMD-immunized mice challenged with either low-dose (0.1–0.25 mg) or high-dose (10 mg) GlgG. FcγRIII-deficient, IgE-deficient, and FcγRII/γE–double-deficient mice were used to evaluate the importance of the IgG/FcγRII and IgE/FcεRI anaphylaxis pathways in these experiments. With low-dose Ag challenge, anaphylaxis was FcγRIII-dependent and IgE-independent, while high-dose challenge induced anaphylaxis through both pathways (Figure 2A). Double-deficient mice failed to develop anaphylaxis when challenged with either a high or a low Ag dose. Consistent results were observed when neither anaphylaxis pathway was operative because FcγRIII-deficient mice were pretreated with anti-IgE mAb to neutralize IgE and desensitize mast cells, or IgE-deficient mice were treated with the anti–FcγRII/RIII mAb to block FcγRII/RIII and desensitize macrophages (not shown). Studies with mast cell–deficient, W/Wv mice were also consistent. Although blocking FcγRII with anti–FcγRII/RIII mAb abolished the anaphylactic response to low-dose, but not high-dose, Ag challenge in WT mice, anti–FcγRII/RIII mAb blocked this response to both low- and high-dose Ag challenge in W/Wv mice (Figure 2B). Furthermore, consistent with observations that FcγRII-mediated anaphylaxis is predominantly PAF-dependent while IgE-mediated anaphylaxis is predominantly histamine-dependent (20), responses to low-dose Ag challenge were inhibited more by a PAF antagonist than by antihistamine, while the opposite sensitivity to mediator antagonists was seen for high-dose Ag challenge (Figure 2C). Similarly, gadolinium, which inhibits macrophage, but not mast cell, function (22–24), suppressed the response to low-dose, but not high-dose, Ag challenge (Figure 2D). Finally, studies performed to directly evaluate IgE-mediated mast cell activation revealed 50-fold higher serum levels of mouse mast cell protease-1 (MMCP-1) and 10-fold higher serum levels of histamine (both markers of mast cell degranulation) in mice challenged with high-rather than low-dose Ag (Figure 2, E and F), and these responses were not substantially inhibited by anti–FcγRII/RIII mAb. In con-
To test this possibility, unprimed or IgE-TNP-primed mice were injected with saline, αTNP Asm, or, as a control, heat-inactivated mouse anti-GIgG antisemur (αGIgG Asm; produced by mice immunized with GαMD), then challenged with 1 µg of TNP-OVA.
Significant hypothermia developed in mice that initially received IgeTNP with or without αGlgG Asm but did not develop in mice that initially received both IgeTNP and αTNP Asm (Figure 3C). Thus, a constituent of serum from TNP-GtMD-immunized, but not GtMD-immunized, mice can block IgE-mediated anaphylaxis in vivo without mediating FcyRIII-dependent anaphylaxis when mice are challenged with a relatively low dose of Ag.

To demonstrate that IgG is the TNP-GtMD immune serum constituent that blocks IgE-mediated anaphylaxis, we purified the IgG fraction of αTNP Asm (IgGtTNP) from this serum and tested its ability to block IgE-mediated anaphylaxis. Concentrations of the αTNP Asm and its IgG fraction were adjusted to similar anti-TNP Ab titers, as determined by ELISA (not shown). Anaphylaxis was inhibited by the IgG fraction at least as well as by the unfractioned antiserum (Figure 3D). To determine whether IgGtTNP Ab could also mediate anaphylaxis, presumably through the FcyRIII-dependent mechanism, in mice challenged with a higher dose of Ag, mice primed with purified IgGtTNP were challenged with 70 ng or 500 µg of TNP-OVA. Anaphylaxis developed in mice challenged with the high, but not the low, TNP-OVA dose (Figure 3E). Finally, to prove the FcyRIII-dependence of anaphylaxis in mice primed with αTNP Asm and challenged with Ag and demonstrate the ability of high-dose Ag to overcome IgG blocking of IgE-mediated anaphylaxis, as in our active anaphylaxis model, we primed mice with IgeTNP, αTNP Asm, or both, blocked FcyRII/RIII-mediated anaphylaxis with anti–FcyRII/RIII mAb in some mice, and challenged mice with 1 or 500 µg of TNP-OVA. IgE-dependent anaphylaxis was induced by challenge with 1 µg of TNP-OVA in mice primed only with IgeTNP but blocked in mice that also received αTNP Asm. This blocking was overcome when the dose of challenge Ag was increased to 500 µg (Figure 3F). The 500-µg dose of Ag also induced FcyRIII-mediated anaphylaxis (it induced anaphylaxis in mice pretreated with only αTNP Asm but not in mice pretreated with both αTNP Asm and anti–FcyRII/RIII mAb). Taken together, these results demonstrate that (a) IgE-dependent anaphylaxis requires less Ag than FcyRIII-dependent anaphylaxis in the absence of IgG BA; (b) Ag-specific IgG BA increases the dose of Ag required to induce IgE-mediated anaphylaxis and, if the Ag dose is sufficiently high, allows the development of FcyRIII-dependent anaphylaxis; and (c) the inhibitory effect of IgG BA on IgE-mediated anaphylaxis can be overcome by an increase in the dose of challenge Ag. These results are consistent with observations in our active immunization anaphylaxis model, in which the high concentrations of mouse IgGtGlgG induced by GtMD immunization support FcyRIII-mediated anaphylaxis when mice are challenged with 100 µg of GlgG but block IgE-mediated anaphylaxis unless the dose of challenge Ag is increased substantially.

Influence of Ag epitope density on the inhibition of anaphylaxis by blocking Ab. Our conclusions about BA function were drawn from studies in which anti-TNP Ab–primed mice were challenged with a TNP-OVA preparation that averaged 10.4 TNP moieties per OVA molecule (TNP10.4-OVA). Because not all allergens have so many identical determinants (epitopes) on a single Ag molecule and high epitope density should increase the ability of an allergen to cross-link IgE/FcERI on mast cells and make it more difficult to block IgE/FcERI cross-linking with an IgG BA, we investigated the influence of Ag epitope density on IgE- and FcyRIII-mediated anaphylaxis and on IgG BA inhibition of IgE-mediated anaphylaxis (Figure 4). As expected, the quantity of TNP-OVA required to induce anaphylaxis in mice primed with a fixed dose of IgeTNP or αTNP Asm increased as the molar TNP/OVA ratio decreased, although the increase was less marked for IgE-mediated anaphylaxis than for IgG-mediated anaphylaxis (Figure 4A, left and right panels, respectively).

To determine whether the quantity of αTNP Asm required to inhibit IgE-mediated anaphylaxis or IgE-mediated basophil IL-4 production is affected by challenge Ag epitope density, mice were primed with 10 µg of IgeTNP, then challenged with doses of TNP10.4-OVA, TNP9.7-OVA, TNP13-OVA, or TNP9.4-OVA that induce similar degrees of mast cell–dependent hypothermia and basophil-dependent IL-4 production but are too low to induce FcyRIII-dependent anaphylaxis. Results of these studies demonstrate that the quantity of αTNP Asm required to block hypothermia and IL-4 production is relatively constant when differences in challenge Ag epitope density are compensated for by adjustment of challenge Ag dose and that more αTNP Asm is required to inhibit IL-4 production than to block the development of hypothermia (Figure 4B). Because the amount of IgG Ab required to block IgE/FcERI–mediated anaphylaxis is not affected by decreas-
es in Ag epitope density that are compensated for by increases in Ag dose while decreases in Ag epitope density increase the Ag dose required to induce IgG/FcRIII-mediated anaphylaxis more than the dose required to induce IgE/FcRI-mediated anaphylaxis, the ability of IgG Ab to block IgE/FcRI-mediated anaphylaxis without permitting FcRIII-mediated anaphylaxis increases as Ag epitope density decreases.

IgG BA inhibits anaphylaxis by 2 mechanisms. Our active anaphylaxis studies suggested that IgG BA suppresses IgE-mediated anaphylaxis by Ag interception rather than by cross-linking FcεRI to FcγRIIB. It remained possible, however, that Ag interception and FcεRI/FcγRIIB cross-linking are redundant inhibitory mechanisms. If so, the inhibitory effect of FcεRI/FcγRIIB cross-linking might only become apparent when concentrations of IgG BA are limiting. To evaluate this possibility, we compared the ability of αTNP Asm to (a) inhibit IgE-mediated anaphylaxis and IgE induction of basophil IL-4 secretion in WT versus FcγRIIB-deficient mice (Figure 5A) and (b) inhibit the same phenomena in FcεRII/RII-deficient mice that had been treated with anti-FcεRII/RII mAb, to selectively block FcεRII signaling, or with an isotype-matched control mAb (Figure 5B). Inhibition of FcεRII signaling did not affect IgE-mediated anaphylaxis but substantially decreased the basophil IL-4 response, in the absence of αTNP Asm, in both sets of experiments. Addition of αTNP Asm inhibited IgE-mediated anaphylaxis and basophil IL-4 secretion in all experiments, even when FcεRII was absent or blocked. However, 2- to 4-fold more αTNP Asm was required to suppress IgE-mediated anaphylaxis, and more than 4-fold more αTNP Asm was required to suppress basophil IL-4 secretion to the same extent in mice in which FcεRIIB was absent or blocked as in mice in which FcεRII was present and functional. Thus, IgG BA inhibits IgE-mediated anaphylaxis by both intercepting Ag molecules and cross-linking FcεRI to FcγRIIB. FcεRI-FcγRIIB cross-linking is not required to inhibit IgE-mediated anaphylaxis or IL-4 production when IgG BA is present in excess, but it amplifies the inhibitory effect of limiting concentrations of IgG BA.

Discussion

Our studies provide direct in vivo evidence that allergen-specific IgG BA can protect against IgE-mediated immunopathology. This evidence was obtained in 2 in vivo systems: a relatively natural model (active immunization) and a model that is more artificial but also more precise and flexible (passive immunization). Priming in the active immunization model was achieved by immunization with GtlMD, which induces large IgG-specific IgE and IgG responses (15, 16). Using this model, IgE/FcεRI/mast cell–mediated anaphylaxis could only be induced by a high dose of Ag, while a lower Ag dose could induce IgG/FcεRIII/mast cell–dependent anaphylaxis. This combination of a large IgG response to immunization and the need for high-dose Ag challenge to induce IgE-mediated anaphylaxis suggested that the IgG was intercepting challenge Ag before it could reach the IgE. This possibility was supported by direct evidence that IgG Abs in serum form complexes with injected Ag and inhibit Ag binding to IgE.

This interpretation was confirmed in a system in which Ab transfer was used both to prime mice for IgE-mediated anaphylaxis
and to inhibit IgE-mediated anaphylaxis. Studies with this passive transfer system demonstrated that IgE-mediated anaphylaxis can be inhibited by transfer of purified Ag-specific IgG Ab. This transfer system also allowed differentiation of Ag dose requirements for IgE- versus IgG-mediated anaphylaxis and definition of the circumstances in which IgG Ab can protect against IgE-mediated anaphylaxis without inducing anaphylaxis through the IgG-FcγRII/macrophage pathway. The most critical differentiating factor for the induction of IgE- versus IgG-mediated anaphylaxis was the amount of challenge Ag. In the absence of IgG BA, IgE-mediated anaphylaxis could be induced by less than 50 ng of TNP-OVA, while induction of IgG-mediated anaphylaxis required more than 1 µg of the same Ag. In contrast, in the presence of BA, the quantity of Ag required to trigger IgE-mediated anaphylaxis increased substantially, until considerably more Ag was required to induce IgE-mediated anaphylaxis than IgG/FcγRII-meditated anaphylaxis, as seen in our active anaphylaxis system. Thus, IgG BA has a purely protective effect when the quantity of challenge Ag is less than that required to trigger IgG-mediated anaphylaxis. This protective effect is lost, however, as the amount of challenge Ag dose is increased. This results both from insufficient interception of challenge Ag before it can cross-link IgE/FcRI on mast cells and from the generation of enough Ag–IgG Ab complexes to activate FcγRII-dependent mediator production by macrophages. Thus, IgG BA should be more protective in people challenged with a low dose of allergen (for example, an insect sting) than in people challenged with a high dose of allergen (for example, infusion of an antibiotic).

IgE-mediated anaphylaxis in mice primed with IgE or TNP and challenged with TNP-OVA was suppressed when mice were also injected with heat-inactivated serum pooled from mice immunized with TNP-GalMD, which contained IgG anti-TNP and IgG anti-GalG Ab, but not when mice were injected with heat-inactivated serum pooled from GalMD-immunized mice, which contained anti-GalG but not anti-TNP Ab. Therefore, IgG inhibition of IgE-mediated anaphylaxis is Ag-specific.

Transfer of IgE and IgG Ab allowed comparison of the effects of varying the epitope density of the challenge Ag on IgE- versus IgG-mediated anaphylaxis and on the consequent ability of IgG Ab to protect against IgE-mediated anaphylaxis without mediating FcγRII-dependent anaphylaxis. Increasing the hapten density of TNP-OVA reduced the quantity of TNP-OVA required to induce IgG-mediated anaphylaxis more than it reduced the quantity of TNP-OVA required to induce IgE-mediated anaphylaxis, and, as a result, decreased the relative ability of IgG Ab to inhibit IgE-mediated anaphylaxis without inducing FcγRII-dependent anaphylaxis. These observations suggest that immune complexes that contain several IgG molecules may be required to efficiently cross-link FcγRII (a low-affinity receptor) and activate macrophages, while more limited cross-linking of mast cell FcRI by a high-affinity interaction between Ag and FcRI-associated IgE can efficiently induce mast cell degranulation.

Finally, studies with both active and passive immunization models defined and quantitated the importance of FcεRI-FcγRIIb interactions in BA inhibition of anaphylaxis. Interactions between the stimulatory and inhibitory receptors were not required for BA suppression of IgE-mediated anaphylaxis: suppression was seen in both the active and the passive anaphylaxis models in FcγRIIb-deficient mice and in WT and FcγRII-deficient mice in which FcεRIIb function was blocked by anti-FcεRII/RII mAb. Furthermore, IgE-mediated anaphylaxis, in the absence of BA, did not differ in severity between WT and FcγRIIb-deficient mice or between anti-FcεRII/RII mAb–treated and control mAb–treated FcγRIIb-deficient mice. This suggests that a direct IgE-FcγRIIb interaction did not inhibit IgE-mediated anaphylaxis in our model, although such inhibition has been observed in another study (26). However, our data suggest inhibition of IgE-mediated basophil IL-4 production by an IgE–FcγRIIb interaction: IgE-mediated IL-4 responses were 2- to 3-fold higher in FcγRIIb-deficient mice than in WT mice, and in WT mice treated with anti–FcγRII/RII mAb than in WT mice treated with a control mAb. Furthermore, experiments in our passive anaphylaxis model confirmed the previously reported importance of IgG-FcγRIIb interactions in the regulation of anaphylaxis (26, 27). Two- to 4-fold more IgG BA was required to inhibit IgE-mediated anaphylaxis in FcγRIIb-deficient mice than in WT mice, and in anti–FcγRII/RII mAb–treated FcγRII-deficient mice than in mice of the same strain that were treated with a control mAb. Thus, IgG BA inhibits IgE-mediated anaphylaxis through 2 mechanisms: it intercepts Ag before it can cross-link mast cell FcRI-associated IgE, and it cross-links FcεRI to FcγRIIb. FcεRI–FcγRIIb cross-linking appears to contribute importantly to BA function when BA levels are limiting but is redundant when BA concentrations are high relative to concentrations of Ag. Our demonstration that FcεRI–FcγRIIb cross-linking can suppress IgE-dependent anaphylaxis is consistent with evidence that IgG-IgE Fc fusion proteins suppress mast cell degranulation (28, 29).

Because IgG BA may be present in limiting amounts in allergy patients who have received immunotherapy, the inhibitory effect of cross-linking FcεRI to FcγRIIb is likely to have an important role in controlling IgE-mediated anaphylaxis. As a result, the efficacy of immunotherapy may be affected by FcγRIIb polymorphisms: BA and immunotherapy that induces BA production may most effectively suppress IgE-mediated anaphylaxis in people who have allelic forms of the FcγRIIb gene that are associated with the most potent inhibitory FcεRIIb function (30, 31).

Two reservations must be considered about the relevance of our predictions to human disease and therapy. First, FcγRII-mediated anaphylaxis, as demonstrated in our mouse model, has never been demonstrated in humans. This may result from the difficulty of detecting this phenomenon rather than from its absence. Because humans, like mice, have macrophages that express FcγRIII and that can be induced by IgG-Ag complexes to secrete inflammatory mediators (32), there is no a priori reason to believe that mice and humans differ in this regard. More likely, the quantities of allergen-specific IgG Ab and allergen that are required to induce FcεRII-dependent anaphylaxis may rarely be achieved in humans. The occurrence of Ag-mediated anaphylaxis in the absence of detectable IgE specific for the relevant Ag (33), however, suggests that IgG-mediated anaphylaxis may be a human, as well as a mouse, phenomenon. Furthermore, more aggressive allergen immunization, made possible by blocking of IgE-mediated anaphylaxis with a human IgG anti-IgE mAb (34) and potentially with other chimeric proteins (28, 35), may raise quantities of allergen-specific IgG Ab to the level required to induce IgG-mediated anaphylaxis.

Secondly, it is not clear that IgG blocking of IgE-mediated anaphylaxis, which we demonstrated in a model in which mice are challenged i.v. with allergen, will occur when allergen challenge occurs through mucosal routes. Because IgG levels are low in the gastrointestinal tract and mast cells that can bind allergen-specific IgE are located in intestinal vili, it seems doubtful that IgG Abs inhibit the induction of intestinal mast cell degranulation by...
ingested allergens. Results of preliminary studies, however, support the possibility that other isotypes, such as IgA, inhibit IgE-mediated mucosal allergy; lower doses of Ag are required to induce IgE/mast cell–mediated allergic diarrhea in I chain–deficient mice, which have approximately 10% of normal intestinal IgA levels, than in WT mice of the same background strain (R.T. Strait et al., unpublished data). It is also possible that ingested Ags only induce systemic anaphylaxis if they are absorbed from the gut and bind to mast cells associated with the circulation. If so, IgG BA would be expected to have a major role in limiting systemic anaphylaxis even when Ag is ingested. Consequently, it seems likely that immunotherapy suppresses anaphylactid and other IgE-mediated allergic disorders, including allergic disorders that predominantly affect mucosal organs, by inducing BA, as well as through distinct mechanisms that decrease IgE secretion, suppress Th2 responses, and stimulate Th1 and regulatory T cell responses (36–42).

Methods

Mice. BALB/c mice were purchased from the National Cancer Institute. Mast cell–deficient WBB6F1-Kit+/Kaβ−/α (W/W) mice and WBB6F1-Kit+/Kaβ−/α × WBB6F1-Kaβ−/αF1 (W/+ ) mice (which have a normal phenotype) (43) along with FcγRIIb-deficient (27) and C57BL/6 FcγRIIb-sufficient mice were purchased from Jackson Laboratory. IgE-deficient mice (44) were a gift from Philip Leder (Harvard University, Cambridge, Massachusetts, USA), and FcγRII-deficient mice (26) were a gift from Jeffrey Ravetch (Rockefeller University, New York, New York, USA). All experimental procedures were performed with approval from the Institutional Animal Care and Use Committees of the Cincinnati Children’s Hospital Research Foundation and the Department of Veterans Affairs Medical Center (Cincinnati, Ohio, USA).

Reagents. GentMD (15, 45); GlG; rat IgG2b anti–mouse FcγRII/RII mAb (24G2) (46) from ATCC; rat IgG2b anti–4-hydroxy-3-nitrophenylacetyl mAb (J1.2), a gift from John Abrams (DNAX Research Inc., Palo Alto, California, USA); rat IgG2a anti–mouse IgE mAb (EM-95) (47), a gift from Zelig Eshhar (Weizmann Institute, Rehovot, Israel); and mouse IgExTNP (J1.2) (48) from ATCC were prepared as described (20, 49). TNP-labeled GnMD was prepared by mixture of 20 ml of GnMD in 1 ml of 0.1 M NaHCO3 buffer, pH 9.6, with 25 mg of TNP-succinyl-Osu (Biosearch Technologies Inc.) dissolved in 1 ml of DMSO and incubation of the mixture overnight at room temperature. The incubated solution was dialyzed against changes of 0.15 M NaCl/0.01 M NaHCO3, pH 8.0. TNP-OVA was similarly produced by mixture of 50 mg of OVA in 5 ml of bicarbonate buffer with serial 4-fold dilutions of TNP-succinyl-Osu (starting concentration, 25 mg/ml) in DMSO. TNP-OVA-NIP was produced by mixture of NIP-succinyl-Osu (Biosearch Technologies Inc.) with TNP0.4-OVA at a 1:2 weight ratio in DMSO and dialyzing as above. TNP-OVA was biotinylated with E-Z Link sulfo-NHS-biotin (Pierce) at a 1:01 weight ratio in DMSO. TNP Asm was produced by injection of BALB/c mice i.p. with 0.2 ml of TNP-GnMD. Mice were bled 10–12 days after immunization, and sera were pooled. The pooled serum was heated to 56°C for 30 minutes to inactivate complement and IgE. The IgG fraction of TNP Asm was purified by ammonium sulfate fractionation (25–50% saturated cut) followed by DEAE-cellulose (DE-52; Whatman International Ltd.) ion exchange chromatography. Fractions were tested for the presence of mouse IgG1 and non-IgG proteins by gel double diffusion, and appropriate fractions were pooled. The PAF antagonist CV6209 was purchased from BIOMOL. The H1 receptor antagonist tripolidine and the macrophage inhibitor gadolinium were purchased from Sigma-Aldrich. The H2 receptor antagonist cimetidine was purchased from Tocris. Abs for measurement of in vivo IL-4 secretion were obtained from BD.

Measurement of IL-4, histamine, and MMC-P-1. Mice were injected with biotinylated anti–IL-4 mAb (BVD4-1D11) (50) at the time of TNP-OVA challenge. Serum was collected 2 hours later, and IL-4 was measured by in vivo cytokine capture assay (IVCCA) (51). Blood drawn 5 minutes after Ag challenge and placed immediately on ice had histamine content measured by ELISA with a kit purchased from IBL. Serum levels of MMC-P-1 were measured in blood drawn 2 hours after Ag challenge with an ELISA kit purchased from Moredun.

ELISAs. IgG1 anti-TNP activity was quantitated with ELISA plate wells coated with TNP0.4-OVA and blocked with SuperBlock (Pierce). Serial dilutions of sera and serum fractions were added to wells, followed sequentially by affinity-purified rabbit anti–mouse y1 Ab (15), alkaline phosphatase–labeled goat anti-rabbit Ab (15), and Tris-based buffer with p-nitrophenol phosphate substrate (Calbiochem). IgG1–TNP-OVA-biotin complexes in mouse serum were captured onto ELISA plate wells coated with streptavidin and were detected with rabbit anti–mouse IgG1 Ab (Zymed Laboratories Inc.), followed by alkaline phosphatase–labeled goat anti-rabbit Ig (15) and substrate (p-nitrophenol phosphate; Calbiochem). The ability of IgExTNP to bind to TNP in the presence of IgG anti-TNP was determined by addition of serum containing TNP-OVA-NIP with or without IgG anti-TNP Ab to ELISA plate wells coated with J1.2, a rat IgG2b anti–4-hydroxy-3-nitrophenylacetyl mAb that cross-reacts with NIP, and then addition of biotin-labeled IgExTNP, followed by HRP-streptavidin and SuperSignal ELISA substrate (Pierce Biotechnology). ELISA plates were read for absorbance with a Multiskan MCC/340 ELISA reader (Thermo Electron Corp.) or for luminescence with a Fluoroskan Ascent FL reader (Thermo Electron Corp.).

Active anaphylaxis model. Mice (5 per group except where noted otherwise) were primed with 0.2 ml GnMD or TNP-GnMD s.c., then challenged 14 days later i.v. with GlGG or TNP-OVA. All experiments were repeated at least once.

Passive anaphylaxis model. Mice were primed i.v. with different combinations of 10 μg of IgExTNP and variable amounts of oGlGG Asm, oTNP Asm, or IgGGeG, then challenged i.v. 24 hours later with TNP-OVA or OVA.

Anaphylaxis. The severity of the anaphylactic shock was assessed by change in temperature, activity level, and/or hematocrit, as previously described (20, 52).

Treatment with inhibitors. FcγRIIb/RIII, histamine, PAF, and macrophage function was inhibited as described (20, 53).

Evaluation of TNP-OVA molar ratio. The absorbance of TNP-OVA conjugates was measured at wavelengths of 280 and 340 μM with a Spectronic GENESYS Spectrophotometer (Spectronic Instruments), and TNP/OVA molar ratio was determined as described (54).

Statistics. Differences in temperature, hematocrit, and concentrations of histamine, MMC-P, and IL-4 between groups of mice were compared using the Mann-Whitney t test (GraphPad Prism 4.0; GraphPad software). A P value less than 0.05 was considered significant.

Acknowledgments

This work was supported by NIH/National Institute of Allergy and Infectious Diseases grant K08 AI50006 (to R.T. Strait), a Veterans Affairs Merit Award (to F.D. Finkelman), a grant from the Food Allergy and Anaphylaxis Network, and a grant from the Asthma and Allergy Foundation of America (to R.T. Strait). The authors thank Steve Dennis, Cathy Griffith, and Lucy Voegele for their technical assistance.

Received for publication May 6, 2005, and accepted in revised form January 3, 2006.

Address correspondence to: Fred D. Finkelman, Division of Immunology, University of Cincinnati, College of Medicine, 231 Albert Sabin Way, Cincinnati, Ohio 45267-0563, USA. Phone: (513) 558-4701; Fax: (513) 558-3799; E-mail: ffinkelman@pol.net.

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