Modulation of Immunoglobulin (Ig)E-mediated Systemic Anaphylaxis by Low-Affinity Fc Receptors for IgG

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Summary

It is widely accepted that immunoglobulin (Ig)E triggers immediate hypersensitivity responses by activating a cognate high-affinity receptor, FcɛRI, leading to mast cell degranulation with release of vasoactive and proinflammatory mediators. This apparent specificity, however, is complicated by the ability of IgE to bind with low affinity to Fc receptors for IgG, FcγRII and III. We have addressed the in vivo significance of this interaction by studying IgE-mediated passive systemic anaphylaxis in FcγRII-deficient mice. Mice deficient in the inhibitory receptor for IgG, FcγRIIB, display enhanced IgE-mediated anaphylactic responses, whereas mice deficient in an IgG activation receptor, FcγRIII, display a corresponding attenuation of IgE-mediated responses. Thus, in addition to modulating IgG-triggered hypersensitivity responses, FcγRII and III on mast cells are potent regulators of IgE-mediated responses and reveal the existence of a regulatory pathway for IgE triggering of effector cells through IgG Fc receptors that could contribute to the etiology of the atopic response.

Key words: systemic anaphylaxis • Fc receptor • immunoglobulin E • mast cell • gene targeting

The anaphylaxis reaction in mice has been considered to be a typical immediate hypersensitivity response determined primarily by the activation of mast cells via antigen-induced aggregation of an IgE-sensitized high-affinity receptor for IgE (FcɛRI), causing the release of potent systemic mediators (1, 2). The central role of FcɛRI in mediating the response was demonstrated by observations that mice deficient in this receptor fail to undergo IgE-dependent, passive cutaneous (3) and passive systemic anaphylaxis (4). These results were interpreted as indicating a necessary and sufficient role for FcɛRI in mediating the IgE-dependent anaphylactic response, excluding the possibility for involvement of other potential receptors for IgE (5). However, earlier observations indicated that the low-affinity Fc receptors for IgG (FcγRIIB and FcγRIII) on mouse mast cells, macrophages, and the rat mucosal type mast cell RBL-2H3 can bind IgE immune complexes in vitro (6, 7), and the engagement of FcγRIIB/III with IgE immune complexes triggers C57.1 mast cells to release serotonin (6), suggesting a greater potential complexity to the IgE-mediated anaphylactic response.

Studies on active anaphylaxis in gene-targeted mice further challenged the simple model of IgE and FcɛRI as the sole initiators of anaphylaxis and revealed a critical role for IgG and FcγR in this response. Induction of active anaphylaxis in mice deficient in IgE indicated that IgE antibodies were not essential for the expression of systemic anaphylaxis (8). In addition, mice deficient in FcεRI mounted an undiminished active systemic anaphylactic response, whereas active sensitization and challenge of animals deficient in the common γ chain (FcRγ−/−) resulted in protection (9, 10). Further support for the conclusion that type I immediate hypersensitivity has a significant dependence on IgG1 and FcγRs came from studies demonstrating that FcγRIIB-deficient (FcγRIIB−−/−) mice exhibited an enhanced reaction in IgG1-mediated passive cutaneous anaphylaxis, thereby

1 Abbreviations used in this paper: BMMC, bone marrow-derived cultured mast cells; FcɛRI, high-affinity receptor for IgE; FcγR, Fc receptor for IgG; FcRγ, Fc receptor γ subunit; FcγRIIB and FcγRIII, type IIIB and type III low-affinity receptors for IgG, respectively.
establishing the importance of FcγRIIB as an inhibitory receptor under physiologic conditions (11), as suggested previously in extensive in vitro studies by Däron and colleagues (12, 13; for review see reference 14).

Although the evidence supporting a direct role for IgG and FcγRs in the anaphylaxis reaction is compelling, the contribution of these receptors to the canonical IgE-mediated response is generally considered to be minimal. To directly analyze the roles of FcγRIIB and FcγRIII in the IgE-dependent component of the systemic anaphylaxis reaction, we compared the responses elicited in FcγRIIB–/– and FcγRIII–/– mice upon passive transfer of either anti-TNP IgE or IgG followed by intravenous challenge with TNP-OVA. As expected, FcγRIIB–/– and FcγRIII–/– mice displayed enhanced or attenuated systemic anaphylaxis to IgG1 sensitization, respectively. However, contrary to the accepted dogma, intense modulation of IgE-dependent systemic anaphylaxis was also observed in these FcγRI–/– mice as a result of the low-affinity interactions of IgE–antigen complexes with these receptors. These studies demonstrate the in vivo physiological significance of low-affinity IgE interactions with FcγRs and represent a novel regulatory pathway for classical type I hypersensitivity responses.

Materials and Methods

Antibodies. Rat anti–mouse FcγRIIB/III (2.4G2; PharMingen) and mouse anti-TNP IgE (IGELa2; American Type Culture Collection) and anti-TNP IgG1 (G1; 15) were purified from the ascites of hybridomas by ion exchange chromatography on DEAE-cellulose (Merck) (16) and by affinity isolation with protein G column (17), followed by removal of aggregated materials by ultracentrifugation at 130,000 g for 90 min at 20°C.

Animals. All experiments were performed on 6–12-wk-old mice. Male and female FcγRIIB–/– (11) or FcγRIII–/– mice (Y. Ishikawa, J.V. Ravetch, and T. Takai, unpublished results) were obtained from C57BL/6 background over six generations. FcγRIIB–/– mice were generated using RW4 embryonic stem cells (GenomSys-tems Inc.) as described previously (3, 11). Mice were housed in cages in cabinets supplied with high efficiency particulate-free air and were monitored monthly as specific pathogen free. Their tissues were removed and fixed in 10% (vol/vol) neutral buffered formalin and then embedded in paraffin. The specimens were sectioned at 3 μm and stained with toluidine blue at pH 4.0. The number of mast cell/nm² was determined under a light microscope. A “degranulated” mast cell was defined as a cell showing extrusion of >10% cell granules.

Statistical Analysis. Statistical differences were calculated using Student’s t test or Fisher’s test. P < 0.05 was considered significant.

Results and Discussion

Modulation of IgG1-mediated Systemic Anaphylaxis in FcγRIIB–/– or FcγRIII–/– Mice. Bocek et al. (7) reported that colocalization of FcγRIIB and FcγRIII on RBL-2H3 cells did not lead to stimulation of the cells, suggesting a possible inhibitory role of FcγRIIB in this process. In addition, in vitro observations by Däron et al. (12) demonstrated that mast cell secretory responses triggered by FcεRI may be controlled by FcγRIIB/III. Moreover, the regulatory role of FcγRIIB was also observed in the celluar activation process via β cell receptors (19–21) and T cell receptors (13; for review see reference 14). Our previous studies using gene-targeted mice had demonstrated the role of FcγRIIB in modulating IgG1-mediated passive cutaneous anaphylaxis (11). To establish the generality of those in vivo observations, we investigated IgG1-mediated passive systemic anaphylaxis in FcγRIIB–/– and FcγRIII–/– mice. We chose to evaluate a passive rather than active model in our studies because FcγRIIB–/– mice display enhanced humoral immune responses (11) that could complicate the comparison and interpretation of the anaphylactic responses. To elicit the anaphylactic response, mice were injected intravenously with IgG1 specific for TNP, followed by intravenous administration of Monitoring of Rectal Temperature and Heart Rate. Changes in core body temperature associated with systemic anaphylaxis were monitored by measuring changes in rectal temperature using a rectal probe coupled to a digital thermometer (Natsu-me Seisakusyo Co.) as described (4, 9, 10). Heart rate was recorded as electrocardiograms (Nihon Kohden) of mice under 2,2,2-trichloroethanol (0.25 mg/g body weight, i.p.) anesthesia.

Flow Cytometric Analysis. Bone marrow–derived cultured mast cells (BM MC) were prepared as described previously (3). For monitoring of upregulation of FcεRI protein on BM MC membrane, cells were cultured in the presence of 0.1 or 5 μg/ml biotinylated IgE or 5 μg/ml biotinylated 2.4G2 for 4 d before final staining with biotinylated IgE (5 μg/ml) plus PE-conjugated streptavidin. Peritoneal resident cells were collected by washing with Tyrode’s buffered solution and incubated with 5 μg/ml IgE for 20 min at 4°C to saturate IgE binding to FcεRI, followed by staining with FITC-conjugated rat anti–mouse IgE (Soro-tec Ltd.) for 20 min at 4°C. Flow cytometric analyses were performed with FACSCalibur™ (Becton Dickinson), and peritoneal mast cells were sorted as c-kit and IgE-positive cells as described (18).

ELISA Determinations for Blood Histamine. Blood was collected from subocular plexus of mice into microcentrifuge tubes containing EDTA on ice at 5 min after antigen challenge, and plasma was prepared. Histamine in the plasma samples was quantified using ELISA plates (ICN Pharmaceuticals, Inc.) according to the manufacturer’s instructions.

Histological Study. Mice were killed by cervical dislocation. Their tissues were removed and fixed in 10% (vol/vol) neutral buffered formalin and then embedded in paraffin. The specimens were sectioned at 3 μm and stained with toluidine blue at pH 4.0. The number of mast cells/mm² was determined under a light microscope. A “degranulated” mast cell was defined as a cell showing extrusion of >10% cell granules.

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TNPOVA 30 min later. Fig. 1 A shows that FcγRIIB−/− mice developed an enhanced IgG1-dependent passive systemic anaphylactic response as compared with passively sensitized wild-type controls challenged with TNPOVA. In wild-type mice, the decrease in core temperature was also transient, reaching a nadir ~15 min after induction, whereas the drop in temperature of FcγRIIB−/− mice persisted for more than 30 min without returning to baseline.

The mAb 2.4G2 is specific for the extracellular domains of murine FcγRIIB and FcγRIII (22). 2.4G2 induces a degranulative response in BMMC, which is enhanced in cells derived from FcγRIIB−/− mice (11). This enhancement is apparent in vivo as well as shown in Fig. 1 B, where the decrease in core temperature after administration of 2.4G2 was more pronounced in FcγRIIB−/− mice than in control mice. These results indicate that FcγRIIB on effector cells, such as mast cells, inhibits the systemic anaphylaxis elicited via FcγRIII.

In contrast to the enhanced responses in FcγRIIB−/− mice described above (Fig. 1, A and B), both FcγRIII−/− mice and Fcγγ−/− mice failed to develop IgG1-mediated passive systemic anaphylaxis (Fig. 1 C), directly establishing that IgG1-mediated anaphylaxis is triggered through FcγRIII, as was indirectly suggested by others (9, 10).

Enhancement of IgE-mediated Anaphylaxis in FcγRIIB−/− Mice. As IgE immune complexes can bind with low affinity to FcγRII and III in vitro, we next induced passive systemic anaphylaxis upon anti-TNP IgE adoptive transfer and TNPOVA administration into FcγRIIB−/− mice. IgE-mediated systemic anaphylaxis was significantly enhanced in FcγRIIB−/− mice, as assessed by changes in core temperature (Fig. 2 A), heart rate (Fig. 2 B), and augmented hemorrhage in the ileum villi (Fig. 2 C). These results indicate that IgE/FcεRI-mediated anaphylaxis is facilitated by the deletion of FcγRIIB in vivo without any apparent involvement of IgG-immune complexes.

Systemic anaphylaxis can result in a fatal outcome. In

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**Figure 1.** IgG1-mediated or 2.4G2-induced systemic anaphylaxis in FcγRIIB−/− or FcγRIII−/− mice. (A) Changes in rectal temperature of mice during IgG1-induced systemic anaphylaxis. 10 wild-type (□) and 8 FcγRIIB−/− animals (■) received 200 μg i.v. anti-TNP IgG1. All of the animals received 1.0 mg i.v. TNPOVA 30 min later. Six additional wild-type (○) as well as FcγRIIB−/− mice (●) received 200 μg IgG1 and then 1.0 mg OVA as controls. The monitoring of rectal temperature was started at the time of antigen injection. Data are shown as mean ± SD. **P < 0.01. (B) Changes in rectal temperature in response to intravenous injection of 10 μg rat mAb 2.4G2 in 14 wild-type mice (□) and 8 FcγRIIB−/− mice (■). As controls, five wild-type (○) as well as four FcγRIIB−/− mice (●) received 200 μg normal rat IgG. Data are shown as mean ± SD. **P < 0.01. (C) Changes in rectal temperature during IgG1-induced systemic anaphylaxis in three Fcγγ−/− (●), five FcγRIII−/− (■), or three wild-type mice (□). For the induction, mice received 400 μg i.v. anti-TNP IgG1 and then received 4.0 mg i.v. TNPOVA 30 min later. Data are shown as mean ± SD. *P < 0.05; **P < 0.01, compared with wild-type mice.
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mice, this mortality has been shown to be associated with IgG1 and FcγRIII (9). As shown in Table I, we observed mortality as a consequence of the anaphylactic response only in FcγRIIB2/2 mice upon administration of either IgG1 or IgE and the corresponding antigen, or 2.4G2. These results confirm that either IgE- or IgG-induced systemic anaphylaxis is indeed augmented in FcγRIIB2/2 mice, as assessed by mortality during anaphylaxis.

Neither FcεRI Expression Level nor Mast Cell Density Is Upregulated in FcγRIIB2/2 Mice. These unexpected observations for IgE-mediated anaphylaxis prompted us to examine whether deletion of FcγRIIB influenced FcεRI expression levels on effector cells. We confirmed by flow cytometric analysis that the expression level of FcεRI on BMMC from FcγRIIB2/2 mice was comparable to the level on wild-type BMMC (data not shown). In addition, we could not demonstrate any significant difference in the expression levels of FcεRI on mast cells after IgE-induced upregulation in vitro or in vivo (Fig. 3, A and B). As shown in Fig. 3 A, BMMC derived from either from FcγRIIB2/2 or wild-type mice displayed the same level of upregulation of FcεRI in response to IgE (18). Similarly, peritoneal mast cells isolated from FcγRIIB2/2 and wild-type mice 24 h after intravenous administration of 20 μg i.v. IgE had equivalent levels of FcεRI (Fig. 3 B). Histopathological examinations indicated that the density and morphology of mast cells in ear, abdominal skin, and trachea from the mutant mice.
mice were not significantly different from those in wild-type mice (data not shown).

Increases in the Number of Degranulated Mast Cells and in Blood Histamine Levels after IgE-mediated Anaphylaxis Induction. The mechanism by which FcγRIIB−/− mice augmented IgE-mediated anaphylaxis was examined by determining the activation of effector cells in these animals as compared with their wild-type counterparts. Blood histamine levels were measured after the induction of anaphylaxis in FcγRIIB−/− and wild-type mice. As shown in Fig. 4 A, blood obtained both from wild-type or FcγRIIB−/−-sensitized animals 5 min after challenge with antigen or 2.4G2 revealed increased histamine concentrations. The histamine levels seen in FcγRIIB−/−-challenged mice were consistently higher in response to IgE, IgG1, or 2.4G2 stimulation than in control mice, suggesting that the enhanced anaphylaxis in FcγRIIB−/− mice could be interpreted in part by accelerated activation of mast cells in the mutant animals. To directly demonstrate enhanced degranulation, lung samples from FcγRIIB−/− or wild-type mice were removed before and 30 min after the induction of IgG-mediated passive systemic anaphylaxis and examined histopathologically. As shown in Fig. 4 B and E, mast cells around bronchi in FcγRIIB−/− mice displayed quantitatively more degranulation than comparable samples taken from wild-type mice subjected to similar treatment.

Conclusions. Although Takizawa et al. (6) demonstrated that FcγRIIB and FcγRIII act as low-affinity receptors for

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**Table I. Mortality During Systemic Anaphylaxis**

| Induction | Death rates | Times until death (min) |
|-----------|-------------|-------------------------|
|            | Wild type   | FcγRIIB−/−               | Wild type   | FcγRIIB−/−               |
| IgE        | 0/29 (0%)   | 5/29 (17%)*              | N.A.        | 5, 20, 25, 40, 40         |
| IgG1       | 0/10 (0%)   | 2/10 (20%)*              | N.A.        | 20, 30                   |
| 2.4G2      | 0/14 (0%)   | 6/14 (43%)*              | N.A.        | 10, 25, 30, 30, 30       |

*Statistical analyses were performed between wild-type and FcγRIIB−/− mice using Fisher’s test: *P < 0.05; ‡ P < 0.01; § NS.

N.A., not applicable.

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**Figure 4. Enhanced mast cell activation in FcγRIIB−/− mice during systemic anaphylaxis.** (A) Elevated plasma histamine in FcγRIIB−/− mice during IgE- or IgG1-mediated or 2.4G2-induced systemic anaphylaxis. Plasma histamine 5 min after antigen challenge in each wild-type (+/+) and mutant (−/−) mouse is presented as μM. Horizontal bars, mean values. (B) Enhanced degranulation of lung mast cells in FcγRIIB−/− mice during IgE-mediated systemic anaphylaxis. Densities of lung mast cells were calculated by counting the cells in four different sections derived from two mice under light microscopy. The results are expressed as mean ± SD. The densities of control (before induction), wild-type (WT), and FcγRIIB−/− mice were not significantly different. However, the number of degranulated mast cells (closed columns) was significantly higher in FcγRIIB−/− mice (P < 0.005, Fisher’s test). (C–E) Photographs of lung mast cells in wild-type mice before anaphylaxis induction (C), and in wild-type (D) or FcγRIIB−/− (E) mice after induction. Toluidine blue staining. Magnification 1,000.
IgE on cultured mast cells and macrophages in vitro, the physiological significance of this interaction between IgE and FcγRs in vivo would result in IgE immune complexes binding not only to FcεRI but also to FcγR II/III on those cells and potentially modulating mediator release. Dombrowicz et al. (4) have shown that although BM MC from FcεRI−/− mice can bind IgE immune complexes via FcγR II/III in vitro, the abrogation of IgE-mediated systemic anaphylaxis in vivo by deletion of FcεRI would indicate that the interaction of IgE with FcγR is not significant. However, an alternative explanation for their data is suggested by the present studies, as the FcεRI−/− strain retains FcγR IIIB as well as FcγR III on its mast cells (4). Based on our data, we propose that the IgE immune complex–mediated response would represent the sum of three components, i.e., an FcεRI-mediated major positive factor, an FcγR IIIB negative response, and an FcγR III-mediated positive component, respectively. When the FcεRI component had been lost, the sum of the remaining FcγR IIIB and FcγR III components would be negligible. Our present results predict that a sum of the components of FcεRI and FcγR IIIB would be a positive, although diminished, response. This prediction is supported by the IgE-mediated anaphylactic response in FcγR III−/− mice. As shown in Fig. 5 A, FcγR III−/− mice indeed show a decreased response in IgE-mediated systemic anaphylaxis. Moreover, we found that blocking of FcγR IIIB by preadministration of 2.4G2 resulted in an enhanced response in IgE-mediated systemic anaphylaxis in FcγR III−/− mice (Fig. 5 B). Taken together, these results support the conclusion that FcγR IIIB attenuates IgE-mediated anaphylactic responses triggered by FcεRI or FcγR III.

Further support for the role of FcγR IIIB in modulating the IgE-mediated response comes from studies in Src homology 2-containing inositol phosphatase (SHIP)-deficient mice (23). This inositol polyphosphate phosphatase is recruited to FcγR IIIB upon cross-linking with an immunoreceptor tyrosine-based activation motif (ITAM)-containing activation receptor through its SH2 (Src homology 2) domain and leads to the hydrolysis of phosphatidylinositol 3,4,5-trisphosphate, with release of Bruton’s tyrosine kinase and phospholipase Cγ from the inner leaflet of the cell membrane (24). The net result of this pathway is the termination of calcium influx, with subsequent inhibition of activation responses (20, 21, 25). Mast cells derived from SHIP-deficient mice display a hyperresponsive IgE phenotype similar to the response seen in FcγR IIIB−/− mice (26). Thus, functional uncoupling of FcγR IIIB from its signaling pathway results in similar phenotype deletion of the receptor itself.

The observations presented here support the hypothesis that IgE-mediated activation is modulated by inhibitory receptors like FcγR IIIB. Perturbation of an inhibitory pathway would be predicted to render mast cells more sensitive to IgE activation and could account for some atopic phenotypes. Upregulation of FcγR IIIB or its constitutive engagement would result in desensitization of mast cells to IgE triggering and reversal of the atopic state.

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References

1. Ando, A., Martin, T.R., and S.J. Galli. 1993. Effects of chronic treatment with the c-kit ligand, stem cell factor, on immunoglobulin E-dependent anaphylaxis in mice. J. Clin. Invest. 92:1639–1649.

2. Martin, T.R., S.J. Galli, I.M. Katona, and J.M. Drazen. 1989. Role of mast cell anaphylaxis. Evidence for the importance of mast cells in cardiopulmonary alterations and death induced by anti-IgE in mice. J. Clin. Invest. 83:1375–1383.

3. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J.V. Ravetch. 1994. FcR-γ chain deletion results in pleiotropic effector cell defects. Cell. 76:519–529.

4. Dombrowicz, D., V. Flamand, K.K. Brigman, B.H. Koller, and J.-P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α chain gene. Cell. 75:969–975.

5. Segal, D.M., S.O. Sharrow, J.F. Jones, and R.P. Siragandian. 1981. Fc (IgG) receptors on rat basophilic leukemia cells. J. Immunol. 126:138–145.

6. Takizawa, F., M. Adamczewski, and J.-P. Kinet. 1992. Iden-


The text appears to be a continuation of the references section from a scientific paper, discussing various studies related to immunology and anaphylaxis. The references cited cover a range of topics including the role of mast cells, the effects of chronic treatment with c-kit ligand, and the role of specific receptors in the immune system. The detailed citations and references are not translated into the natural language.