

## 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, FcεRI, 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 FcγR-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 (FcεRI),<sup>1</sup> causing the release of potent systemic mediators (1, 2). The central role of FcεRI 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 FcεRI 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 FcεRI 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<sup>-/-</sup>) 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<sup>-/-</sup>) mice exhibited an enhanced reaction in IgG1-mediated passive cutaneous anaphylaxis, thereby

<sup>1</sup>Abbreviations used in this paper: BMMC, bone marrow-derived cultured mast cells; FcεRI, high-affinity receptor for IgE; FcγR, Fc receptor for IgG; FcRγ, Fc receptor γ subunit; FcγRIIB and FcγRIII, type IIB and type III low-affinity receptors for IgG, respectively.

establishing the importance of Fc $\gamma$ RIIB as an inhibitory receptor under physiologic conditions (11), as suggested previously in extensive *in vitro* studies by Daëron and colleagues (12, 13; for review see reference 14).

Although the evidence supporting a direct role for IgG and Fc $\gamma$ R $\alpha$ s 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 Fc $\gamma$ RIIB and Fc $\gamma$ RIII in the IgE-dependent component of the systemic anaphylaxis reaction, we compared the responses elicited in Fc $\gamma$ RIIB $^{-/-}$  and Fc $\gamma$ RIII $^{-/-}$  mice upon passive transfer of either anti-TNP IgE or IgG followed by intravenous challenge with TNP-OVA. As expected, Fc $\gamma$ RIIB $^{-/-}$  and Fc $\gamma$ RIII $^{-/-}$  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 Fc $\gamma$ R $\alpha$  $^{-/-}$  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 Fc $\gamma$ R $\alpha$ s and represent a novel regulatory pathway for classical type I hypersensitivity responses.

## Materials and Methods

**Antibodies.** Rat anti-mouse Fc $\gamma$ RIIB/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 Fc $\gamma$ RIIB $^{-/-}$  (11) or Fc $\gamma$ RIII $^{-/-}$  mice (Y. Ishikawa, J.V. Ravetch, and T. Takai, unpublished results) were generated by breeding the F2 offspring of crosses between chimeras and C57BL/6 mice, and the wild-type mice generated by the same breeding protocol were used as wild-type animals. Fc $\gamma$ R $\alpha$  $^{-/-}$  mice were generated as described previously (3) and back-crossed to C57BL/6 background over six generations. Fc $\gamma$ RIII $^{-/-}$  mice were generated using RW4 embryonic stem cells (GenomeSystems 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.

**Induction of Passive Systemic Anaphylaxis.** Mouse IgG1 or IgE anti-TNP mAbs were administered intravenously through the tail vein in volumes of ~200  $\mu$ l/mouse. 30 min after injection of anti-TNP IgG1 or 24 h after injection of IgE, mice were injected with 1.0 mg *i.v.* TNP $_4$ -OVA in PBS. Control mice received OVA in PBS instead. The concentration of IgG1 and IgE mAbs used for passive sensitization and the amount of TNP-OVA used for challenge was determined based on preliminary dose–response experiments required to produce significant drops in body temperature in wild-type and Fc $\gamma$ RIIB $^{-/-}$  or Fc $\gamma$ RIII $^{-/-}$  mice. Alternatively, systemic anaphylaxis was induced by the intravenous injection of 10  $\mu$ g 2.4G2 in 200  $\mu$ l PBS. The amount was determined based on the preliminary dose–response experiment in the same way described above. In a blocking experiment in Fc $\gamma$ RIII $^{-/-}$  mice, 100  $\mu$ g 2.4G2 was administered.

**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 (Natsume Seisakusyo Co.) as described (4, 9, 10). Heart rate was recorded as electrocardiograms (Nihon Kohden) of mice under 2,2,2-tribromoethanol (0.25 mg/g body weight, *i.p.*) anesthesia.

**Flow Cytometric Analysis.** Bone marrow-derived cultured mast cells (BMMC) were prepared as described previously (3). For monitoring of upregulation of Fc $\epsilon$ RI protein on BMMC membrane, cells were cultured in the presence of 0.1 or 5  $\mu$ g/ml biotinylated IgE or 5  $\mu$ g/ml biotinylated 2.4G2 for 4 d before final staining with biotinylated IgE (5  $\mu$ g/ml) plus PE-conjugated streptavidin. Peritoneal resident cells were collected by washing with Tyrode's buffered solution and incubated with 5  $\mu$ g/ml IgE for 20 min at 4°C to saturate IgE binding to Fc $\epsilon$ RI, followed by staining with FITC-conjugated rat anti-mouse IgE (Serotec Ltd.) for 20 min at 4°C. Flow cytometric analyses were performed with FACSCalibur<sup>TM</sup> (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  $\mu$ m and stained with toluidine blue at pH 4.0. The number of mast cells/mm<sup>2</sup> 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 Fc $\gamma$ RIIB $^{-/-}$  or Fc $\gamma$ RIII $^{-/-}$  Mice.** Bocek et al. (7) reported that coclustering of Fc $\gamma$ RIIB and Fc $\gamma$ RIII on RBL-2H3 cells did not lead to stimulation of the cells, suggesting a possible inhibitory role of Fc $\gamma$ RIIB in this process. In addition, *in vitro* observations by Daëron et al. (12) demonstrated that mast cell secretory responses triggered by Fc $\epsilon$ RI may be controlled by Fc $\gamma$ RIIB/III. Moreover, the regulatory role of Fc $\gamma$ RIIB was also observed in the cellular activation process via B 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 Fc $\gamma$ RIIB 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 Fc $\gamma$ RIIB $^{-/-}$  and Fc $\gamma$ RIII $^{-/-}$  mice. We chose to evaluate a passive rather than active model in our studies because Fc $\gamma$ RIIB $^{-/-}$  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

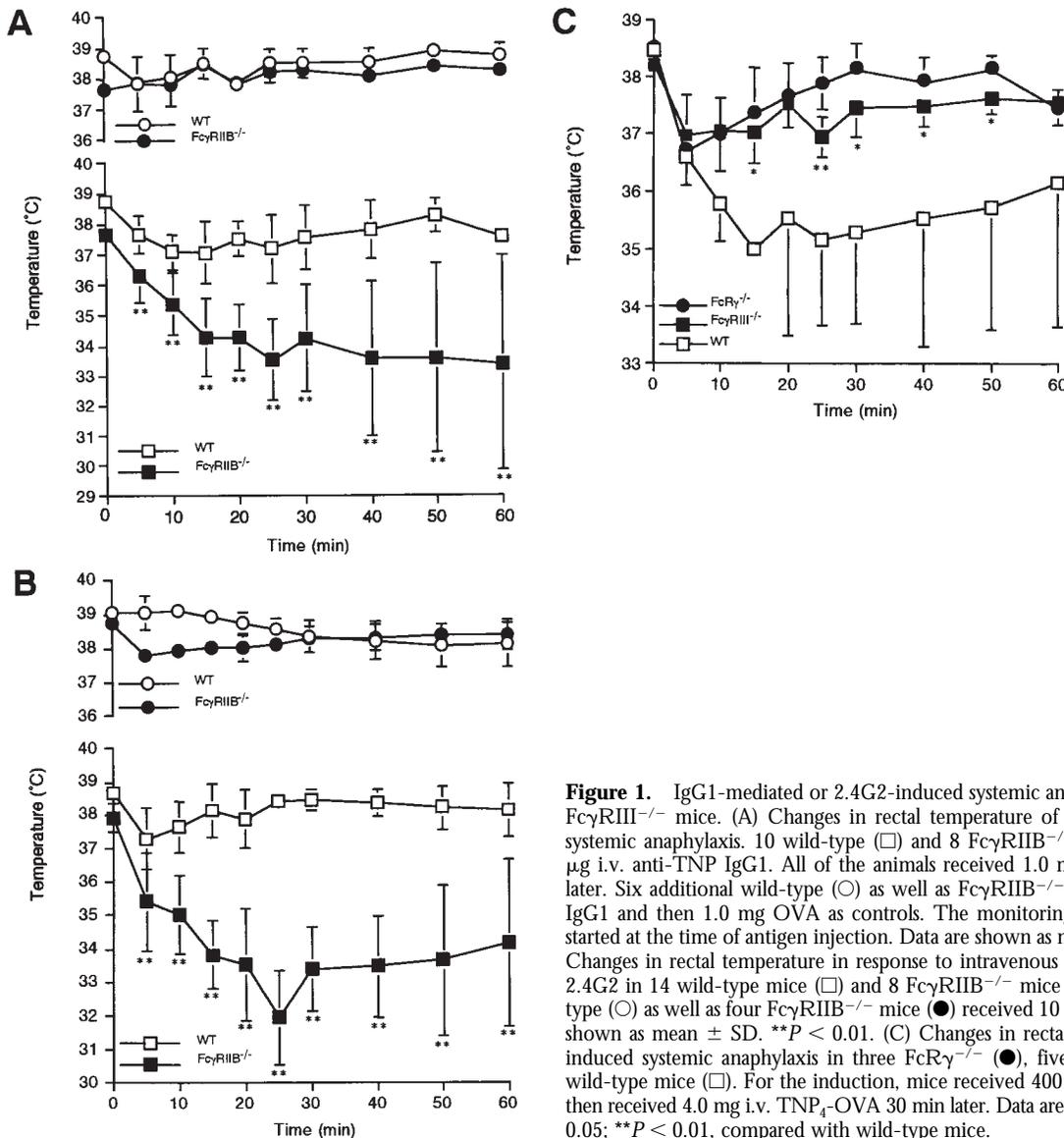
TNP-OVA 30 min later. Fig. 1 A shows that  $Fc\gamma RIIB^{-/-}$  mice developed an enhanced IgG1-dependent passive systemic anaphylactic response as compared with passively sensitized wild-type controls challenged with TNP-OVA. In wild-type mice, the decrease in core temperature was also transient, reaching a nadir  $\sim 15$  min after induction, whereas the drop in temperature of  $Fc\gamma RIIB^{-/-}$  mice persisted for more than 30 min without returning to baseline.

The mAb 2.4G2 is specific for the extracellular domains of murine  $Fc\gamma RIIB$  and  $Fc\gamma RIIB$  (22). 2.4G2 induces a degranulative response in BMDC, which is enhanced in cells derived from  $Fc\gamma RIIB^{-/-}$  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  $Fc\gamma RIIB^{-/-}$  mice than in control mice. These results indicate that  $Fc\gamma RIIB$  on effector cells, such as mast cells, inhibits the systemic anaphylaxis elicited via  $Fc\gamma RIIB$ . In contrast to the enhanced responses in  $Fc\gamma RIIB^{-/-}$  mice

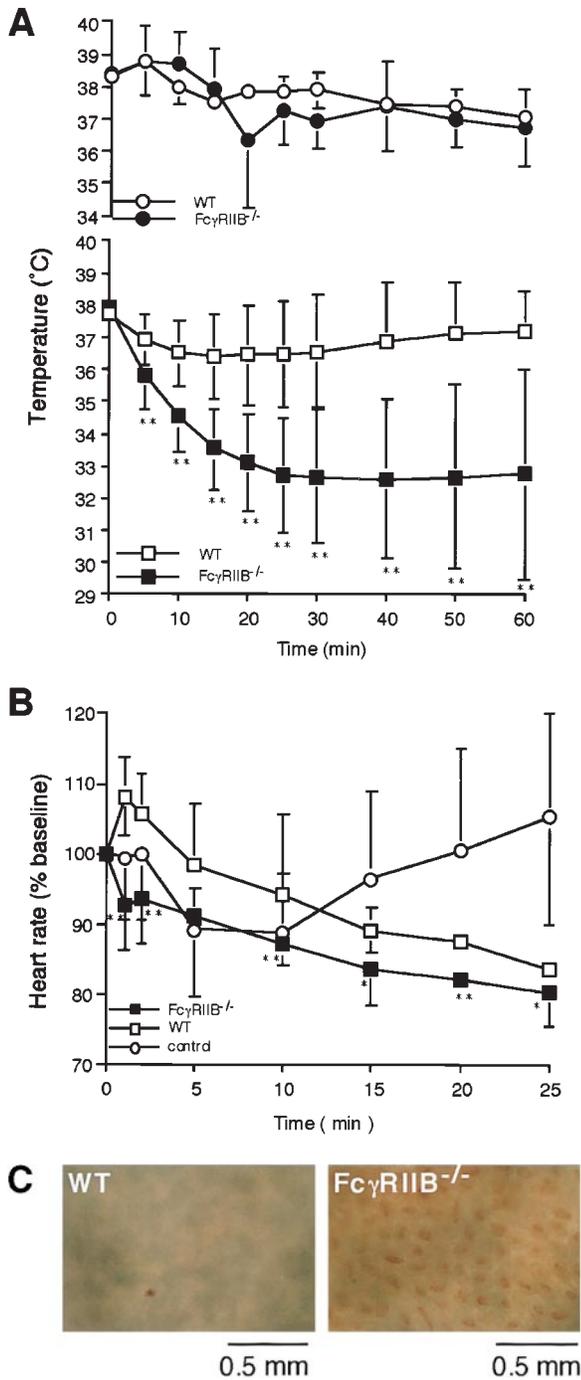
described above (Fig. 1, A and B), both  $Fc\gamma RIIB^{-/-}$  mice and  $FcR\gamma^{-/-}$  mice failed to develop IgG1-mediated passive systemic anaphylaxis (Fig. 1 C), directly establishing that IgG1-mediated anaphylaxis is triggered through  $Fc\gamma RIIB$ , as was indirectly suggested by others (9, 10).

**Enhancement of IgE-mediated Anaphylaxis in  $Fc\gamma RIIB^{-/-}$  Mice.** As IgE immune complexes can bind with low affinity to  $Fc\gamma RIIB$  and III in vitro, we next induced passive systemic anaphylaxis upon anti-TNP IgE adoptive transfer and TNP-OVA administration into  $Fc\gamma RIIB^{-/-}$  mice. IgE-mediated systemic anaphylaxis was significantly enhanced in  $Fc\gamma RIIB^{-/-}$  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/ $Fc\epsilon RI$ -mediated anaphylaxis is facilitated by the deletion of  $Fc\gamma RIIB$  in vivo without any apparent involvement of IgG-immune complexes.

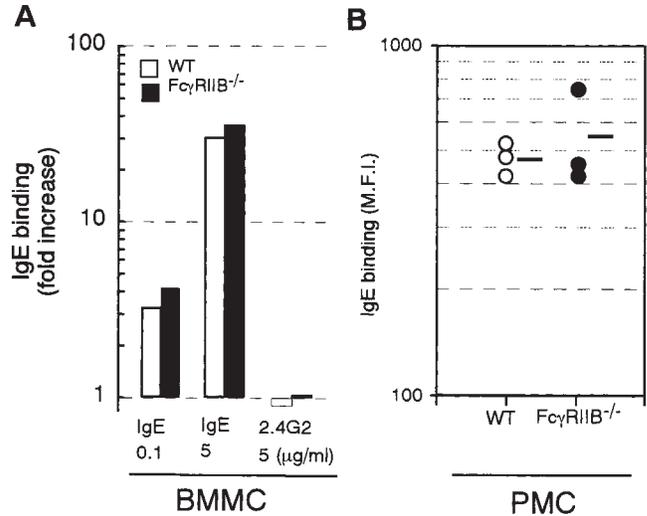
Systemic anaphylaxis can result in a fatal outcome. In



**Figure 1.** IgG1-mediated or 2.4G2-induced systemic anaphylaxis in  $Fc\gamma RIIB^{-/-}$  or  $Fc\gamma RIIB^{-/-}$  mice. (A) Changes in rectal temperature of mice during IgG1-induced systemic anaphylaxis. 10 wild-type (□) and 8  $Fc\gamma RIIB^{-/-}$  animals (■) received 200  $\mu$ g i.v. anti-TNP IgG1. All of the animals received 1.0 mg i.v. TNP<sub>4</sub>-OVA 30 min later. Six additional wild-type (○) as well as  $Fc\gamma RIIB^{-/-}$  mice (●) received 200  $\mu$ g 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  $\pm$  SD.  $^{**}P < 0.01$ . (B) Changes in rectal temperature in response to intravenous injection of 10  $\mu$ g rat mAb 2.4G2 in 14 wild-type mice (□) and 8  $Fc\gamma RIIB^{-/-}$  mice (■). As controls, five wild-type (○) as well as four  $Fc\gamma RIIB^{-/-}$  mice (●) received 10  $\mu$ g normal rat IgG. Data are shown as mean  $\pm$  SD.  $^{**}P < 0.01$ . (C) Changes in rectal temperature during IgG1-induced systemic anaphylaxis in three  $FcR\gamma^{-/-}$  (●), five  $Fc\gamma RIIB^{-/-}$  (■), or three wild-type mice (□). For the induction, mice received 400  $\mu$ g i.v. anti-TNP IgG1 and then received 4.0 mg i.v. TNP<sub>4</sub>-OVA 30 min later. Data are shown as mean  $\pm$  SD.  $^{*}P < 0.05$ ;  $^{**}P < 0.01$ , compared with wild-type mice.



**Figure 2.** IgE-mediated systemic anaphylaxis in  $Fc\gamma RIIB^{-/-}$  mice. (A) Changes in rectal temperature during IgE-mediated systemic anaphylaxis. 29 wild-type (WT;  $\square$ ) and 24  $Fc\gamma RIIB^{-/-}$  animals ( $\blacksquare$ ) received 20  $\mu$ g i.v. anti-TNP IgE. All of the animals received 1.0 mg i.v. TNP<sub>4</sub>-OVA 24 h later. Three additional wild-type ( $\circ$ ) as well as  $Fc\gamma RIIB^{-/-}$  mice ( $\bullet$ ) received IgE and then OVA. The monitoring of rectal temperature was started at the time of antigen injection. Data are shown as mean  $\pm$  SD.  $^{**}P < 0.01$ . (B) Changes in heart rate during IgE-mediated systemic anaphylaxis in three  $Fc\gamma RIIB^{-/-}$  ( $\blacksquare$ ) and three wild-type ( $\square$ ) mice. The induction protocols were the same as in A. As controls, three wild-type mice ( $\circ$ ) received OVA. Note the transient rise maximizing  $\sim 1$  min after TNP-OVA injection in wild-type mice and the gradual decrease during the 25 min after induction, in contrast to the changes in  $Fc\gamma RIIB^{-/-}$  mice, showing no transient rise and continuous decrease in heart rate.  $^{*}P <$



**Figure 3.** Expression levels of  $Fc\epsilon RI$  on BMMC and peritoneal mast cells after induction with IgE. (A)  $Fc\epsilon RI$  upregulation in vitro. BMMC were cultured for 4 d in the presence of 0.1 or 5  $\mu$ g/ml IgE or 5  $\mu$ g/ml 2.4G2. Mean values of  $Fc\epsilon RI$  expression levels were assessed by flow cytometric measurement of IgE binding. (B)  $Fc\epsilon RI$  levels in vivo mast cells.  $Fc\gamma RIIB^{-/-}$  ( $\bullet$ ) and wild-type ( $\circ$ ) mice received 20  $\mu$ g i.v. IgE, and 24 h later, mean fluorescence intensities of IgE binding on peritoneal mast cells were compared by flow cytometry as described in Materials and Methods.

mice, this mortality has been shown to be associated with IgG1 and  $Fc\gamma RIIB$  (9). As shown in Table I, we observed mortality as a consequence of the anaphylactic response only in  $Fc\gamma RIIB^{-/-}$  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  $Fc\gamma RIIB^{-/-}$  mice, as assessed by mortality during anaphylaxis.

*Neither  $Fc\epsilon RI$  Expression Level nor Mast Cell Density Is Up-regulated in  $Fc\gamma RIIB^{-/-}$  Mice.* These unexpected observations for IgE-mediated anaphylaxis prompted us to examine whether deletion of  $Fc\gamma RIIB$  influenced  $Fc\epsilon RI$  expression levels on effector cells. We confirmed by flow cytometric analysis that the expression level of  $Fc\epsilon RI$  on BMMC from  $Fc\gamma RIIB^{-/-}$  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  $Fc\epsilon RI$  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  $Fc\gamma RIIB^{-/-}$  or wild-type mice displayed the same level of upregulation of  $Fc\epsilon RI$  in response to IgE (18). Similarly, peritoneal mast cells isolated from  $Fc\gamma RIIB^{-/-}$  and wild-type mice 24 h after intravenous administration of 20  $\mu$ g IgE had equivalent levels of  $Fc\epsilon RI$  (Fig. 3 B). Histopathological examinations indicated that the density and morphology of mast cells in ear, abdominal skin, and trachea from the mutant

0.05;  $^{**}P < 0.01$ , compared with wild-type mice. (C) Increased hemorrhage in ileum villi during IgE-mediated systemic anaphylaxis in  $Fc\gamma RIIB^{-/-}$  mice. 1 h after the anaphylaxis induction, mice were killed, and ileum samples were observed under light microscopy. Hemorrhage in tips of microvilli was evident in  $Fc\gamma RIIB^{-/-}$  mice. Magnification 40.

**Table I.** Mortality During Systemic Anaphylaxis

Induction <sup>a</sup>	Death rates <sup>b</sup>		Times until death (min)	
	Wild type	Fc $\gamma$ RIIB <sup>-/-</sup>	Wild type	Fc $\gamma$ RIIB <sup>-/-</sup>
IgE	0/29 (0%)	5/29 (17%)*	N.A.	5, 20, 25, 40, 40
IgG1	0/10 (0%)	2/10 (20%) <sup>§</sup>	N.A.	20, 30
2.4G2	0/14 (0%)	6/14 (43%) <sup>‡</sup>	N.A.	10, 25, 25, 30, 30, 30

<sup>a</sup>Systemic anaphylaxis was induced with anti-TNP IgG1 transfer and TNP-OVA injection, 2.4G2 administration, or anti-TNP IgE transfer and TNP-OVA injection.

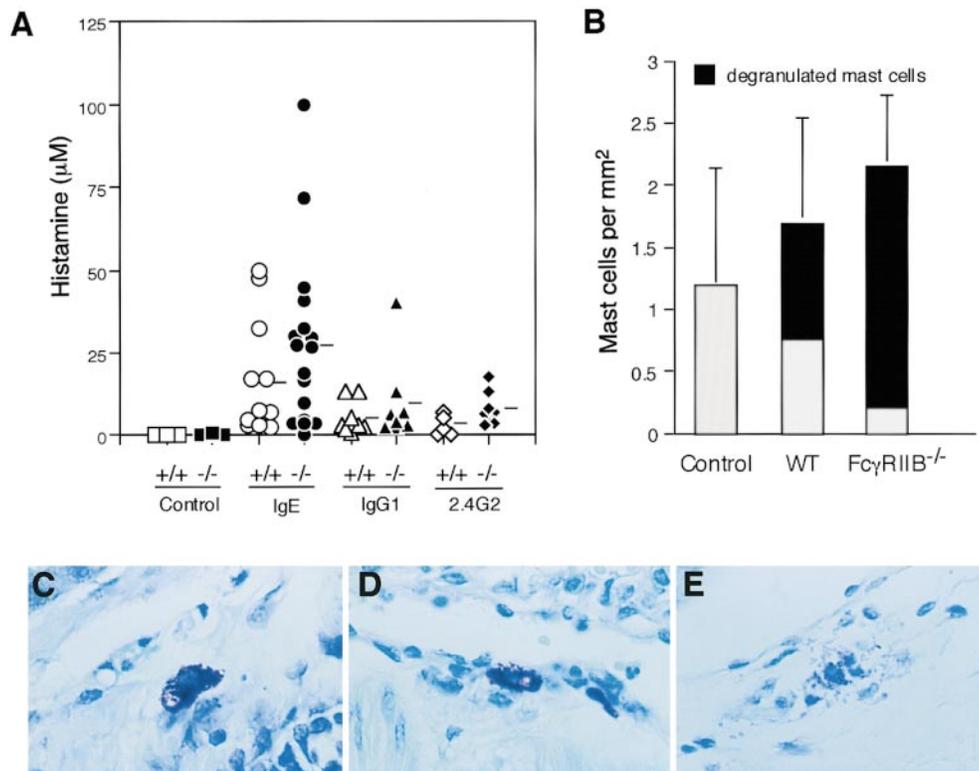
<sup>b</sup>Statistical analyses were performed between wild-type and Fc $\gamma$ RIIB<sup>-/-</sup> mice using Fisher's test: \* $P < 0.05$ ; <sup>‡</sup> $P < 0.01$ ; <sup>§</sup>NS. N.A., not applicable.

mice were not significantly different from those in wild-type mice (data not shown).

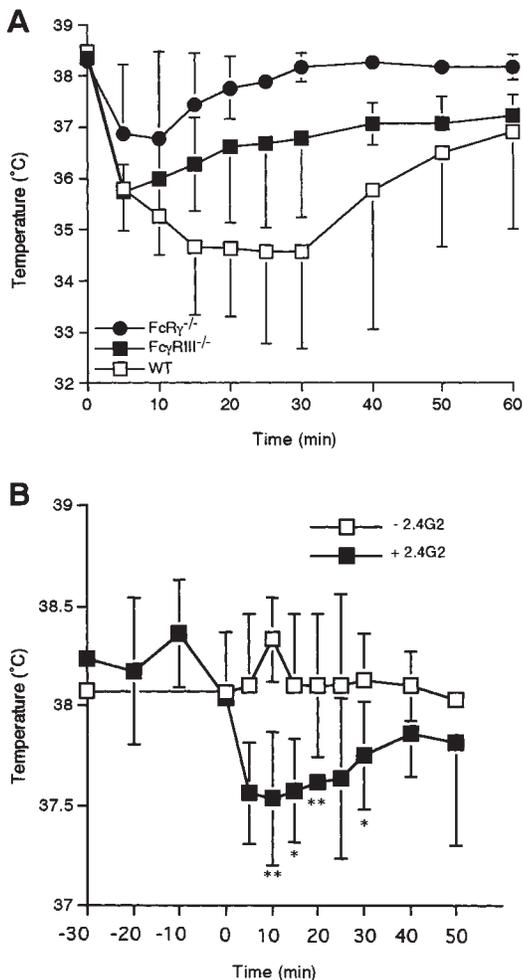
**Increases in the Number of Degranulated Mast Cells and in Blood Histamine Levels after IgE-mediated Anaphylaxis Induction.** The mechanism by which Fc $\gamma$ RIIB<sup>-/-</sup> mice augmented IgE-mediated anaphylaxis was examined by determining the activation of effector cells in these animals as compared with their wild-type counterparts. Blood histamine levels were measured after the induction of anaphylaxis in Fc $\gamma$ RIIB<sup>-/-</sup> and wild-type mice. As shown in Fig. 4 A, blood obtained both from wild-type or Fc $\gamma$ RIIB<sup>-/-</sup>-sensitized animals 5 min after challenge with antigen or 2.4G2 revealed increased histamine concentrations. The histamine levels seen in Fc $\gamma$ RIIB<sup>-/-</sup>-challenged mice were

consistently higher in response to IgE, IgG1, or 2.4G2 stimulation than in control mice, suggesting that the enhanced anaphylaxis in Fc $\gamma$ RIIB<sup>-/-</sup> mice could be interpreted in part by accelerated activation of mast cells in the mutant animals. To directly demonstrate enhanced degranulation, lung samples from Fc $\gamma$ RIIB<sup>-/-</sup> 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 Fc $\gamma$ RIIB<sup>-/-</sup> 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 Fc $\gamma$ RIIB and Fc $\gamma$ RIII act as low-affinity receptors for



**Figure 4.** Enhanced mast cell activation in Fc $\gamma$ RIIB<sup>-/-</sup> mice during systemic anaphylaxis. (A) Elevated plasma histamine in Fc $\gamma$ RIIB<sup>-/-</sup> 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  $\mu$ M. Horizontal bars, mean values. (B) Enhanced degranulation of lung mast cells in Fc $\gamma$ RIIB<sup>-/-</sup> 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  $\pm$  SD. The densities of control (before induction), wild-type (WT), and Fc $\gamma$ RIIB<sup>-/-</sup> mice were not significantly different. However, the number of degranulated mast cells (closed columns) was significantly higher in Fc $\gamma$ RIIB<sup>-/-</sup> 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 Fc $\gamma$ RIIB<sup>-/-</sup> (E) mice after induction. Toluidine blue staining. Magnification 1,000.



**Figure 5.** IgE-mediated systemic anaphylaxis in Fc $\gamma$ RIII<sup>-/-</sup> mice. (A) Changes in rectal temperature of mice during IgE-induced systemic anaphylaxis. Three wild-type (WT; □) and three Fc $\gamma$ RIII<sup>-/-</sup> (■) animals as well as three Fc $\gamma$ RIIIB<sup>-/-</sup> mice (●) received 20  $\mu$ g i.v. anti-TNP IgE. All of the animals received 1.0 mg i.v. TNP-OVA 24 h later. Data are shown as mean  $\pm$  SD. (B) Effect of preadministration of 2.4G2 on changes in rectal temperature in IgE-mediated systemic anaphylaxis. At time -24 h, Fc $\gamma$ RIII<sup>-/-</sup> mice received 20  $\mu$ g IgE; they were administered 100  $\mu$ g 2.4G2 (■) or vehicle alone (□) at time -30 min and then received TNP-OVA at time 0. Data are shown as mean  $\pm$  SD. \* $P$  < 0.05; \*\* $P$  < 0.01.

IgE on cultured mast cells and macrophages in vitro, the physiological significance of this interaction between IgE and Fc $\gamma$ RIIIB/III has not been established. The consequence of a low-affinity interaction between IgE and Fc $\gamma$ R $\alpha$ s in vivo would result in IgE immune complexes binding not only to Fc $\epsilon$ RI but also to Fc $\gamma$ RIIIB/III on those cells and potentially modulating mediator release. Dombrowicz et al. (4) have shown that although BMDC from Fc $\epsilon$ RI<sup>-/-</sup>

mice can bind IgE immune complexes via Fc $\gamma$ RIIIB/III in vitro, the abrogation of IgE-mediated systemic anaphylaxis in vivo by deletion of Fc $\epsilon$ RI would indicate that the interaction of IgE with Fc $\gamma$ R $\alpha$ s is not significant. However, an alternative explanation for their data is suggested by the present studies, as the Fc $\epsilon$ RI<sup>-/-</sup> strain retains Fc $\gamma$ RIIIB as well as Fc $\gamma$ RIII 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 Fc $\epsilon$ RI-mediated major positive factor, an Fc $\gamma$ RIIIB negative response, and an Fc $\gamma$ RIII-mediated positive component, respectively. When the Fc $\epsilon$ RI component had been lost, the sum of the remaining Fc $\gamma$ RIIIB and Fc $\gamma$ RIII components would be negligible. Our present results predict that a sum of the components of Fc $\epsilon$ RI and Fc $\gamma$ RIIIB would be a positive, although diminished, response. This prediction is supported by the IgE-mediated anaphylactic response in Fc $\gamma$ RIII<sup>-/-</sup> mice. As shown in Fig. 5 A, Fc $\gamma$ RIII<sup>-/-</sup> mice indeed show a decreased response in IgE-mediated systemic anaphylaxis. Moreover, we found that blocking of Fc $\gamma$ RIIIB by preadministration of 2.4G2 resulted in an enhanced response in IgE-mediated systemic anaphylaxis in Fc $\gamma$ RIII<sup>-/-</sup> mice (Fig. 5 B). Taken together, these results support the conclusion that Fc $\gamma$ RIIIB attenuates IgE-mediated anaphylactic responses triggered by Fc $\epsilon$ RI or Fc $\gamma$ RIII.

Further support for the role of Fc $\gamma$ RIIIB 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 Fc $\gamma$ RIIIB 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 C $\gamma$  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 Fc $\gamma$ RIIIB<sup>-/-</sup> mice (26). Thus, functional uncoupling of Fc $\gamma$ RIIIB 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 Fc $\gamma$ RIIIB. 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 Fc $\gamma$ RIIIB or its constitutive engagement would result in desensitization of mast cells to IgE triggering and reversal of the atopic state.

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