

Long Term Maintenance of IgE-Mediated Memory in Mast Cells in the Absence of Detectable Serum IgE¹

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Mast cells and basophils involved in allergic responses do not have clonotypic Ag receptors. However, they can acquire Ag specificity through binding of Ag-specific IgE to FcεRI expressed on their surface. Previous studies demonstrated that IgE binding induced the stabilization and accumulation of FcεRI on the cell surface and resulted in up-regulation of FcεRI. In this study we have further analyzed the maintenance of IgE-mediated memory in mast cells and basophils in vivo by comparing kinetics of serum IgE levels, FcεRI expression, and ability to induce systemic anaphylaxis. A single i.v. injection of trinitrophenyl-specific IgE induced 8-fold up-regulation of FcεRI expression on peritoneal mast cells in B cell-deficient ($\mu\text{m}^{-/-}$) mice. Serum IgE levels became undetectable by day 6, but the treatment of mice with anti-IgE mAb induced a significant drop in body temperature on days 14, 28, and 42. The administration of trinitrophenyl-BSA, but not BSA, in place of anti-IgE mAb gave similar results, indicating the Ag specificity of the allergic response. This long term maintenance of Ag-specific reactivity in the allergic response was also observed in normal mice passively sensitized with IgE even though the duration was shorter than that in B cell-deficient mice. The appearance of IgE with a different specificity did not interfere with the maintenance of IgE-mediated memory of mast cells and basophils. These results suggest that IgE-mediated stabilization and up-regulation of FcεRI enables mast cells and basophils not only to acquire Ag specificity, but also to maintain memory in vivo for lengthy periods of time. *The Journal of Immunology*, 2003, 170: 775–780.

Antigen specificity and memory formation are unique features of adaptive immunity (1–3). B and T cells are critical players in adaptive immunity, and their Ag specificity is determined by Ag receptors, known as B cell Ag receptor and TCR, respectively, that are clonally expressed on their surface. Ag-specific reactions are observed not only in normal immune responses, but also in allergic responses. Mast cells and basophils involved in allergic responses do not express B cell Ag receptor, TCR, or any of their counterparts. The high affinity receptor for IgE (FcεRI) expressed on these cells is a critical component in allergic responses, since this receptor allows the cells to bind IgE. Ligation of IgE-bound FcεRI by multivalent Ags results in the activation of multiple signaling pathways leading to diverse effector responses, including the release of mediators responsible for allergic inflammatory reactions. Thus, the binding of Ag-specific IgE confers specific reactivity to that Ag on mast cells and basophils (4–8).

IgE binding to FcεRI induces the up-regulation of FcεRI expression on mast cells and basophils in both humans and mice (9–16). In IgE-deficient mice, levels of FcεRI expression on mast cells are only ~20% of normal levels (12). However, FcεRI expression could be up-regulated by as much as 32-fold after in vitro incubation of mast cells with IgE or injection of IgE in vivo (12, 17). Furthermore, IgE-transgenic mice had highly elevated levels of FcεRI on mast cells, as has also been observed in patients with allergies (9, 15, 18). The IgE-mediated FcεRI up-regulation was shown to enhance the ability of mast cells and basophils to release chemical mediators such as serotonin and cytokines (12, 19). Indeed, the i.v. administration of nonanaphylactogenic anti-IgE Ab to atopic patients resulted in down-regulation of FcεRI on basophils in parallel with the reduction of mediator release from activated basophils (20, 21). These results emphasize the functional importance of FcεRI regulation by IgE.

Two different mechanisms have been proposed to explain the IgE-mediated FcεRI up-regulation (7, 8, 13, 15). One mechanism involves the suppression of loss of preformed cell surface FcεRI by protection against degradation of FcεRI. The other mechanism involves an enhancement of the synthesis and/or transport of the FcεRI complex through FcεRI-mediated signaling. We and others have recently demonstrated that the stabilization and accumulation of cell surface FcεRI through IgE binding are the major mechanism of IgE-mediated FcεRI up-regulation (17, 22). Cell surface FcεRI is unstable and is quickly removed from cell surface unless it binds IgE. FcεRI with bound IgE is stabilized and stays on the surface longer than FcεRI with no bound IgE. In the presence of excess amounts of IgE, every new FcεRI transported to the cell surface is loaded with IgE, stabilized, and accumulates on the cell surface, leading to the up-regulation of surface FcεRI expression.

Up to 80% of the FcεRI on mast cells is occupied with IgE even in normal BALB/c mice with basal levels of serum IgE, and IgE-bound FcεRI molecules are stable on the cell surface (17). Therefore, only

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limited space is available for newly produced IgE in the absence of IgE-mediated FcεRI up-regulation. In the case of T and B cells, each cell only has specificity to a single Ag. Therefore, the population reactive to a particular Ag is extremely small, and clonal expansion is necessary to protect the host against the foreign Ag. In contrast, large numbers of mast cells can simultaneously acquire new Ag specificity through FcεRI up-regulation induced by newly produced IgE without expansion of cells. In the present study we asked how long mast cells and basophils that had acquired Ag specificity through IgE binding to FcεRI could maintain Ag-specific reactivity sufficient to induce allergic responses in vivo. Our previous study demonstrated that levels of IgE-bound FcεRI on cultured mast cells did not significantly decline in vitro at least for 24 h (17). Therefore, one may assume that mast cells and basophils can maintain Ag-specific reactivity through FcεRI-bound IgE for lengthy periods, not only in vitro but also in vivo. On the other hand, the turnover of mast cells and IgE could be different in vivo and in vitro, thus shortening the duration of in vivo memory. There are no reported comprehensive studies on mast cell memory in vivo. Here we show that mice passively sensitized with Ag-specific IgE keep Ag-specific reactivity that can induce allergic responses such as systemic anaphylaxis long after the serum Ag-specific IgE has become undetectable. Here we will discuss the pathophysiological roles of IgE-mediated FcεRI up-regulation as they relate to memory formation of mast cells.

Materials and Methods

Animals

C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan). C57BL/6- $\mu\text{m}^{-/-}$ mice (23) were provided by Dr. K. Rajewsky (University of Cologne, Germany) and Dr. D. Kitamura (Tokyo Science University, Tokyo, Japan) and were maintained in our own animal facility. All the experiments in this study were performed according to the Guidelines for Animal Use and Experimentation as specified by our institutions.

Antibodies

Anti-mouse IgE^a mAb (UH297, rat IgG1), FITC-conjugated rat IgG mAb (G28-5), FITC-conjugated anti-mouse IgE mAb (R35-72), biotinylated anti-mouse c-Kit mAb (2B8), and allophycocyanin-conjugated streptavidin were purchased from BD Pharmingen (San Diego, CA). Trinitrophenyl (TNP)^b-specific mouse IgE^b mAb (IGELb4), TNP-specific mouse IgE^a mAb (IGELa2), anti-mouse IgE mAb (6DH5) and anti-FcγII/III mAb (2.4G2) were described previously (24–26). A hybridoma producing phenylarsonate-specific mouse IgE^a mAb (SE1.3) was purchased from American Type Culture Collection (Manassas, CA).

Cell preparation and flow cytometry

Peritoneal cells isolated from mice were depleted of RBCs by lysis with hypotonic buffer and preincubated with 2.4G2 mAb at room temperature for 5 min to prevent nonspecific binding of other Abs. To detect IgE-bound FcεRI on the cell surface, cells were stained with FITC-anti-IgE mAb R35-72. To detect total (IgE-bound plus IgE-free) FcεRI, cells were stained with R35-72 after incubation at 4°C with excess amounts of IgE (IGELb4) to saturate FcεRI with IgE. Cells were also stained with biotinylated anti-c-Kit mAb followed by allophycocyanin-conjugated streptavidin and then analyzed by FACSCalibur (BD Biosciences, Mountain View, CA). Autofluorescent cells (primarily macrophages) were rejected to clearly identify c-Kit⁺ mast cells (12, 17). The geo mean value of fluorescence intensity was converted to the linear scale number by the number of molecules of equivalent soluble fluorochrome units (MESF) using Quantum 25 microbeads (Flow Cytometry Standards Co., San Juan, Puerto Rico). ΔMESF was calculated by subtracting MESF of control staining from MESF of sample (17). The half-life of IgE-bound FcεRI in vivo was calculated from kinetics of FcεRI levels on peritoneal mast cells in mice treated with Ag-specific IgE. Namely, after administered IgE became undetectable in sera from the treated mice, levels of injected IgE-bound FcεRI on peritoneal mast cells were determined at various time points by flow cytometry using anti-IgE mAb R35-72 in the case of $\mu\text{m}^{-/-}$ mice treated

with IGELb4 or anti-IgE^a mAb UH297 in the case of C57BL/6 mice treated with IGELa2 (IgE^a).

Sensitization of mice with IgE and induction of systemic analysis

Mice were treated with an i.v. injection of 300 μg of TNP-specific IgE. Total IgE levels in serum were measured by ELISA as described previously (18). At various time points after IgE injection, mice were challenged with i.v. administration of 10 μg of anti-IgE mAb (6DH5) or 30 μg of BSA in PBS, either with or without conjugation to TNP. The body temperatures of the mice were monitored with a rectal probe (Shibaura Electronics, Tokyo, Japan).

Results

Kinetics of serum IgE levels and FcεRI expression on mast cells in B cell-deficient mice passively sensitized with IgE

C57BL/6- $\mu\text{m}^{-/-}$ mice, which possess no mature B cells and hence no endogenous IgE, were treated with i.v. injection of 300 μg of monoclonal IgE specific for hapten TNP. One day after the injection, serum IgE levels were ~20 μg/ml (Fig. 1, bar graph), comparable to those in mice infected with parasites such as *Nippostrongylus brasiliensis* (16, 27). Serum IgE levels declined quickly thereafter and became undetectable by day 6. Before IgE injection, the expression of FcεRI at low levels was detected on peritoneal mast cells even in the absence of IgE (Fig. 1, line graph). IgE injection induced marked up-regulation of FcεRI on peritoneal mast cells. While serum IgE levels quickly dropped from day 2, levels of FcεRI expression continuously increased up to 8-fold by day 4 and then decreased gradually (Fig. 1). On day 7 even though serum IgE levels became undetectable, FcεRI expression was still 2–3 times higher than that before injection. After 28 days FcεRI expression had returned to basal levels.

Induction of IgE-mediated systemic anaphylaxis even in the absence of detectable serum IgE in sensitized mice

We next questioned how long C57BL/6- $\mu\text{m}^{-/-}$ mice passively sensitized with IgE maintained the ability to induce IgE-mediated allergic reactions. The mice passively sensitized with IgE were treated at various times with i.v. injection of 10 μg of rat anti-mouse IgE mAb to cross-link IgE-bound FcεRI on mast cells, and

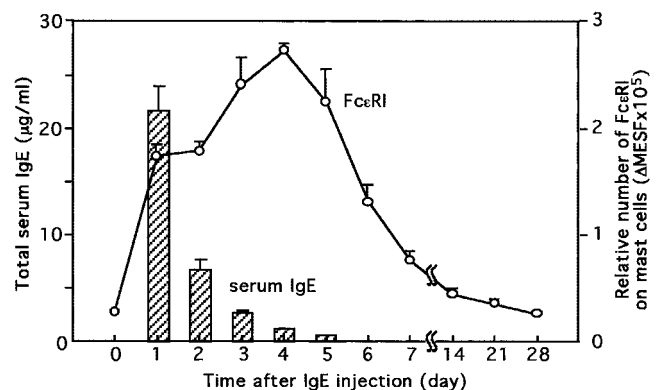


FIGURE 1. Kinetics of serum IgE levels and FcεRI expression on mast cells in C57BL/6- $\mu\text{m}^{-/-}$ mice passively sensitized with IgE. C57BL/6- $\mu\text{m}^{-/-}$ mice were treated with i.v. injection of 300 μg of TNP-specific IgE (IGELb4). At the indicated time points after the injection, serum IgE levels, measured by ELISA, were determined, as shown in the bar graph. In parallel, surface levels of FcεRI on peritoneal mast cells were determined by flow cytometry. The geo mean values of fluorescence intensity were converted to MESF. ΔMESF was calculated in each case, and its kinetics are shown in the line graph. Data are shown as the mean ± SEM ($n = 4/\text{group}$) and are representative of three repeated experiments.

³ Abbreviations used in this paper: TNP, trinitrophenyl; MESF, molecules of fluorescence intensity; PA, phenylarsonate.

change in body temperature was monitored (Fig. 2A). The treatment with anti-IgE mAb caused no significant change in body temperature in unsensitized mice (Fig. 2A, $\diamond$). In contrast, severe temperature drops of -6°C were observed within 35 min in sensitized mice, followed by their death, even as late as day 14 after sensitization when serum IgE levels were undetectable and levels of Fc ϵ RI were 7 times lower than on day 4 (Fig. 2A, $\square$). Treatment with control rat IgG mAb produced no significant change in body temperature (data not shown). The significant drop in body temperature was still induced by anti-IgE treatment on days 28 and 42, even though Fc ϵ RI expression had returned nearly to the basal level (Fig. 2A, $\triangle$, $\circ$). On day 56, three of four sensitized mice examined in one experiment still showed drops in body temperature (-1 , -1.5 , and -6°C respectively) when treated with anti-IgE mAb (data not shown).

To elicit IgE-mediated allergic responses in a more pathophysiological manner, C57BL/6- $\mu\text{m}^{-/-}$ mice passively sensitized with 300 μg of monoclonal IgE specific for hapten TNP were challenged with i.v. injection of 30 μg of TNP-coupled BSA in place of anti-IgE mAb. As shown in Fig. 2B, the injection of TNP-BSA induced a drop in body temperature on day 28 after sensitization as in the case of treatment with anti-IgE mAb. The drop in body

temperature on day 28 was more intense in TNP-BSA-treated mice (-6°C) than in anti-IgE-treated mice (-3.5°C) under these experimental conditions. The treatment with uncoupled BSA did not cause any significant change in body temperature (data not shown). On day 42, all three sensitized mice examined in one experiment still showed drops in body temperature (-2 to -3°C) upon treatment with TNP-BSA (data not shown).

IgE-mediated memory formation of mast cells in normal mice

In contrast to the C57BL/6- $\mu\text{m}^{-/-}$ mice used in the above experiments, normal C57BL/6 mice have low basal levels of serum IgE, and $\sim 50\%$ of the Fc ϵ RI on peritoneal mast cells were occupied with IgE. Therefore, we tested whether IgE-mediated memory can be formed in the presence of such low basal levels of IgE. Normal C57BL/6 mice were passively sensitized with 300 μg of TNP-specific IgE, followed by challenge with either TNP-BSA or control BSA at various time points. Serum IgE levels of <0.4 $\mu\text{g}/\text{ml}$ before sensitization increased to 25 $\mu\text{g}/\text{ml}$ on day 1, but returned to the basal level (<0.4 $\mu\text{g}/\text{ml}$) by day 5 (Fig. 3A, bar graph). Levels of Fc ϵ RI expression on peritoneal mast cells increased 5-fold on day 1, then decreased gradually (Fig. 3A, line graph). The i.v. injection of TNP-BSA, but not control BSA, induced body

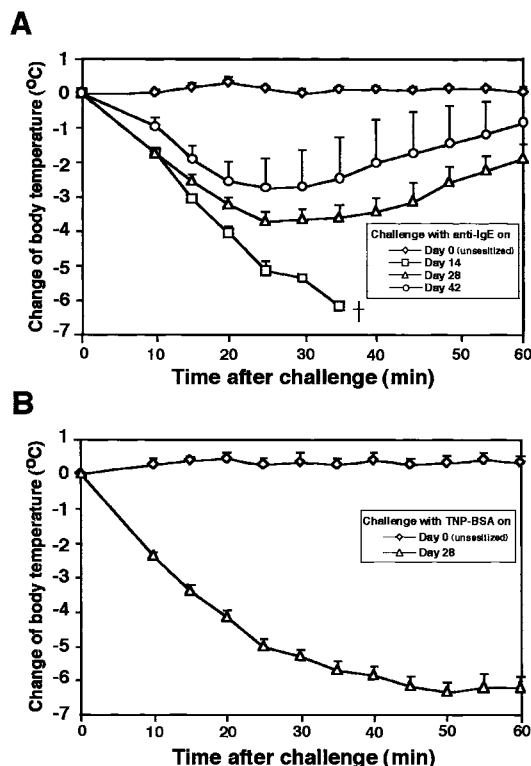


FIGURE 2. Induction of IgE-mediated systemic anaphylaxis after the sensitization of C57BL/6- $\mu\text{m}^{-/-}$ mice with IgE. C57BL/6- $\mu\text{m}^{-/-}$ mice were treated with TNP-specific IgE as described in Fig. 1. A, At the indicated time points after the injection, the mice were i.v. challenged with 10 μg of anti-IgE mAb 6DH5, and their rectal temperatures were monitored. The kinetics of temperature change are shown. The challenge on day 14 resulted in the death of all mice tested 35 min after the challenge. The challenge with irrelevant control rat IgG mAb did not produce any significant change in rectal temperature at any time point (data not shown). B, At the indicated time points after the injection of TNP-specific IgE, the mice were i.v. challenged with 30 μg of TNP-BSA, and their rectal temperatures were monitored. The challenge with BSA caused no significant change in rectal temperature at any of the time points studied (data not shown). Data are shown as the mean $\pm$ SEM ($n = 4/\text{group}$) and are representative of three repeated experiments.

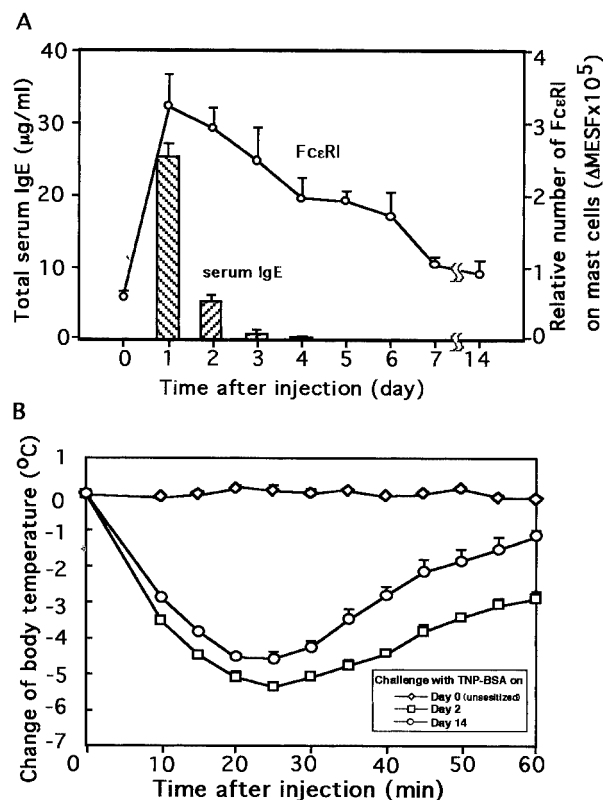
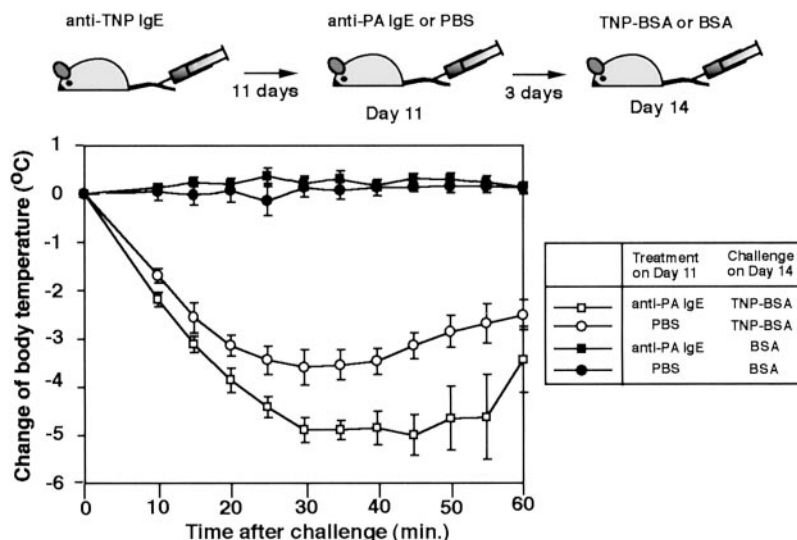


FIGURE 3. IgE-mediated memory formation in mast cells of normal mice. Normal C57BL/6 mice were treated i.v. with 300 μg of TNP-specific IgE^a mAb (IGELa2). A, At the indicated time points after IgE injection, serum IgE levels were measured by ELISA, and their kinetics are shown in the bar graph. In parallel, levels of Fc ϵ RI on peritoneal mast cells were determined by flow cytometry, and their kinetics are shown in the line graph. B, At the indicated time points after IgE injection, the mice were challenged i.v. with 30 μg of TNP-BSA, and rectal temperature was monitored. The kinetics of temperature change after the Ag challenge are shown. The challenge with control BSA produced no significant change in rectal temperature at any of the time points (data not shown). Data are shown as the mean $\pm$ SEM ($n = 4/\text{group}$) and are representative of three repeated experiments.

FIGURE 4. Maintenance of IgE-mediated memory of mast cells despite the appearance of IgE with a different Ag specificity. C57BL/6- $\mu\text{m}^{-/-}$ mice were treated i.v. with 300 μg of TNP-specific IgE^b mAb (IGELb4). Eleven days after the first treatment (on day 11), the mice were injected i.v. with 300 μg of PA-specific IgE^a mAb (SE1.3) or control PBS. Three days after the second treatment (on day 14), the mice were i.v. challenged with 30 μg of TNP-BSA or control BSA, and rectal temperature was monitored. The kinetics of temperature change after the Ag challenge are shown. Data are shown as the mean $\pm$ SEM ($n = 4/\text{group}$) and are representative of three repeated experiments.



temperature drops even on day 14 when FcεRI expression on peritoneal mast cells had returned to the basal level (Fig. 3, A and B). One of four sensitized mice examined in one experiment showed significant drops of body temperature (-4°C) as late as day 21 (data not shown).

Maintenance of IgE-mediated memory of mast cells is not disturbed by the appearance of IgE with another Ag specificity

We next examined whether the appearance of IgE with a different Ag specificity influenced the maintenance of IgE-mediated memory. C57BL/6- $\mu\text{m}^{-/-}$ mice were passively sensitized with 300 μg of TNP-specific IgE (day 0). The expression of FcεRI on peritoneal mast cells increased until day 4 after treatment with anti-TNP IgE and then declined, as observed in Fig. 1. On day 11 when TNP-specific IgE had become undetectable in their sera, the mice were treated with i.v. injection of 300 μg of phenylarsonate (PA)-specific IgE or control PBS. In control mice treated with PBS, FcεRI expression continued to decline, and the relative number of FcεRI on day 14 was $0.44 \pm 0.01 \times 10^5 \Delta\text{MESF}$ ($n = 4$), close to the basal level ($0.35 \pm 0.02 \times 10^5 \Delta\text{MESF}$; $n = 4$). In contrast, in mice treated with anti-PA IgE on day 11, FcεRI expression was again up-regulated after treatment. The relative number of FcεRI on day 14 was $2.2 \pm 0.54 \times 10^5 \Delta\text{MESF}$ ($n = 4$), 5 times higher than that in control mice. In separate experiments mice treated with anti-TNP IgE, followed by anti-PA IgE or PBS in the same protocol, were challenged on day 14 with i.v. injection of 30 μg of TNP-BSA or control BSA, and body temperature was monitored (Fig. 4). A drop in body temperature was observed in a TNP-specific manner in the mice treated with PA-specific IgE as much

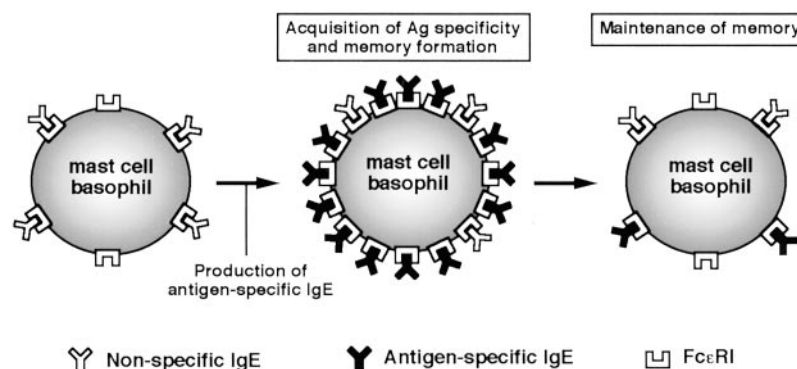
as or even more intensely than in PBS-treated control mice. Thus, the maintenance of allergic reactivity to the Ag recognized by the first IgE was not disturbed by subsequent appearance of the second IgE with a different Ag specificity.

Discussion

The present study demonstrated that IgE-mediated memory could be established in vivo by mast cells and basophils for the induction of immediate allergic reactions, such as systemic anaphylaxis. In B cell-deficient mice, a single i.v. injection of Ag-specific IgE established the memory that persisted as long as 56 days despite the finding that serum levels of injected IgE had become undetectable by day 6. This suggests that the IgE-mediated memory persists even after the production of Ag-specific IgE ceased completely in vivo. Our results also suggest that the maintenance of IgE-mediated memory is not disturbed by the subsequent production of IgE carrying a different specificity once the memory has been established.

FcεRI expressed on mast cells and basophils is most likely involved in the memory formation, since mice deficient for the α -chain of FcεRI have been shown to be resistant to IgE-dependent systemic anaphylaxis (28). In B cell-deficient mice, levels of serum IgE quickly declined soon after passive IgE sensitization and became undetectable by day 6. On the other hand, levels of FcεRI expression on peritoneal mast cells continuously increased up to 8-fold until day 4. The high affinity binding of IgE by FcεRI and the stabilization of FcεRI by IgE binding appeared to be sufficient to up-regulate FcεRI on mast cells even as serum IgE levels decreased. FcεRI expression subsided gradually after day 4 and

FIGURE 5. A model for rapid acquisition of Ag specificity and establishment of IgE-mediated memory in mast cells and basophils through IgE-mediated FcεRI up-regulation.



returned to basal levels by day 28. However, typical systemic anaphylaxis could still be elicited on days 28 and 42 upon ligation of IgE on mast cells and basophils by either multivalent Ag or anti-IgE Ab. Therefore, the elicitation of Ag/IgE-mediated systemic anaphylaxis does not seem to require increased expression of FcεRI. However, we cannot exclude the possibility that some mast cells localized outside peritoneal cavity maintained high levels of FcεRI expression.

The IgE-mediated FcεRI up-regulation and stabilization as well as the slow kinetics of IgE dissociation from FcεRI (8) appear to be essential for establishing long-lasting memory of mast cells and basophiles. Approximately 80% of FcεRI on mast cells are occupied with IgE even in normal BALB/c mice with basal levels of serum IgE, and IgE-bound FcεRI molecules are stable on the cell surface (17). Therefore, only limited space is available for newly produced IgE unless IgE-mediated FcεRI up-regulation is induced. When substantial amounts of IgE are produced in response to an Ag, every new FcεRI transported to the cell surface binds the Ag-specific IgE and becomes stabilized on the cell surface. This results in accumulation of the Ag-specific IgE/FcεRI complex on mast cells (Fig. 5). The large number of complexes and their stability on mast cells enable the IgE-mediated memory to persist even after the Ag-specific IgE disappears from the circulation.

Our previous study demonstrated that levels of IgE-bound FcεRI on cultured bone marrow-derived mast cells persisted in vitro at least for 24 h. Levels of IgE-bound FcεRI on freshly prepared peritoneal mast cells also showed no significant change during 16-h culture ex vivo. In contrast, the half-life of injected IgE-bound FcεRI on mast cells in vivo calculated as described in *Materials and Methods* was ~24 h in both μm^{-1} and normal C57BL/6 mice, which is much shorter than in vitro. This could be attributed to the quicker turnover of mast cells in vivo due to the elimination of old mast cells, a new supply of mast cells from bone marrow, and the proliferation of mast cells in response to growth factors such as stem cell factor, IL-3, and IL-9 (29, 30). Nevertheless, the 24-h half-life of IgE-bound FcεRI in vivo is still much longer than the 14-h half-life of free IgE in serum in the present study. Thus, IgE-mediated stabilization and up-regulation of FcεRI enable mast cells and basophils not only to acquire Ag specificity but also to maintain memory in vivo for long periods of time. The IgE-mediated memory formation of mast cells and basophils was observed in both B cell-deficient and normal mice. However, the duration of the memory was shorter in normal than in B cell-deficient mice. This difference could be explained in part by the presence of the basal level of IgE in normal mice. Due to the presence of endogenous IgE, the basal level of FcεRI on mast cells is 2- to 3-fold higher in normal C57BL/6 than in B cell-deficient mice. Approximately 50 and 80% of FcεRI molecules on mast cells in normal C57BL/6 and BALB/c mice, respectively, are already occupied with endogenous IgE, whereas all FcεRI molecules in B cell-deficient mice are free of IgE. Consequently, the occupancy of FcεRI by exogenous IgE cannot reach 100% in normal mice even after IgE-mediated FcεRI up-regulation. This appears to explain at least in part why the duration of the memory was shorter in normal than in B cell-deficient mice. Infection with parasites sometimes results in elevation of serum IgE to levels much higher than those observed in our experiments (27). In such cases, the IgE-mediated mast cell memory could be maintained for a much longer time in normal mice.

Analysis of B cell-deficient mice sensitized sequentially with two different IgE revealed that once the memory had been established, the maintenance of IgE-mediated memory was not disturbed by the appearance of IgE carrying the other specificity. The treatment of mice with the second IgE induced the second wave of

FcεRI up-regulation. On day 3 after the treatment, FcεRI expression became 5 times higher than that in control mice treated with PBS, and the vast majority of IgE bound to FcεRI was the second IgE. Nevertheless, mast cells kept the reactivity to the Ag recognized by the first IgE to induce systemic anaphylaxis. This appears to be attributed to IgE-mediated FcεRI stabilization and slow kinetics of IgE dissociation from FcεRI. Interestingly, the Ag-specific systemic anaphylaxis was more severe in mice treated with the second IgE than in control mice treated with PBS. It has been shown that IgE-mediated up-regulation of FcεRI expression significantly enhances the ability of mast cells to release chemical mediators such as serotonin and cytokines (12, 19). In mice treated with the second IgE, FcεRI expression at the time of Ag challenge was 5 times higher than that in control mice treated with PBS. This could be the reason why systemic anaphylaxis was more severe in mice treated with the second IgE.

In the present study we demonstrated that IgE-mediated memory of mast cells and basophils could be established and maintained even after Ag-specific IgE disappears from the circulation. This mechanism of memory formation and maintenance is totally different from the immunological memory of B and T cells. In the case of T and B cells, a small proportion of cells that expanded clonally in response to an Ag remained as memory cells. In contrast, large numbers of mast cells can simultaneously acquire new Ag specificity and establish memory. This mechanism might be beneficial in the protection against repeated infection with pathogens such as *Shistosoma hematobium* (31), but deleterious for allergic patients. IgE-mediated stabilization and up-regulation of FcεRI are the driving forces for both acquisition of Ag specificity and memory formation in mast cells and basophils. Therefore, it is important to understand the mechanism of IgE-mediated FcεRI stabilization to develop new types of therapies for allergic disorders. It would be intriguing to explore the possibility of memory formation through other Ig isotypes, such as IgG and IgA.

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