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Drastic Up-Regulation of FcεRI on Mast Cells Is Induced by IgE Binding Through Stabilization and Accumulation of FcεRI on the Cell Surface[1]

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It has been shown that IgE binding to FcεRI on mast cells results in increased FcεRI expression, which in turn enhances IgE-dependent chemical mediator release from mast cells. Therefore, prevention of the IgE-mediated FcεRI up-regulation would be a promising strategy for management of allergic disorders. However, the mechanism of IgE-mediated FcεRI up-regulation has not been fully elucidated. In this study, we analyzed kinetics of FcεRI on peritoneal mast cells and bone marrow-derived mast cells. In the presence of brefeldin A, which prevented transport of new FcεRI molecules to the cell surface, levels of IgE-free FcεRI on mast cells decreased drastically during culture, whereas those of IgE-bound FcεRI were stable. In contrast, levels of FcγRIII on the same cells were stable even in the absence of its ligand, indicating that FcεRI α-chain, but not β- and γ-chains, was responsible for the instability of IgE-free FcεRI. As far as we analyzed, there was no evidence to support the idea that IgE binding to FcεRI facilitated synthesis and/or transport of FcεRI to the cell surface. Therefore, the stabilization and accumulation of FcεRI on the cell surface through IgE binding appears to be the major mechanism of IgE-mediated FcεRI up-regulation. ***The Journal of Immunology,*** **2001, 167: 3427–3434.**

The high-affinity receptor for IgE, FcεRI, expressed on mast cells and basophils is a critical component in allergic responses. Cross-linking of IgE-bound FcεRI by allergens results in activation of these cells and release of a range of preformed and newly generated chemical mediators and cytokines responsible for allergic inflammatory reactions (1–7). Thus, the binding of IgE produced against a given Ag confers specific reactivity to that Ag on these cells. Interestingly, IgE binding to FcεRI also induces up-regulation of FcεRI expression on these cells. In the late 1970s, it was noted that there was a good correlation between the density of IgE receptors on circulating basophils and the serum IgE titer (8). It was demonstrated later that FcεRI expression on a rat mast cell line RBL-2H3 was up-regulated ~2-fold by culturing cells with IgE in vitro (9–13). This FcεRI up-regulation by IgE was found to be insensitive to cycloheximide, indicating its lack of dependence on protein synthesis (11). Therefore, it was proposed that the mechanism of this up-regulation could be the inhibition of degradation of FcεRI by IgE binding.

It was recently reported that levels of FcεRI expression on mast cells freshly isolated from IgE-deficient mice were extremely low (20% of normal level) (14). However, FcεRI expression could be up-regulated up to 32-fold by in vitro incubation of mast cells with IgE or by injection of IgE in vivo (14, 15). Therefore, IgE-mediated FcεRI up-regulation is not an artifact observed in cultured cell lines. This is also true for human mast cells (16, 17) as well as human and mouse basophils (18, 19). Importantly, the IgE-mediated FcεRI up-regulation was shown to result in critical enhancement of effector functions of those cells. Both serotonin and cytokine release were substantially enhanced in terms of the sensitivity and the intensity of the response (14, 16, 20). This could be an important mechanism in facilitating host defense against parasites, while it could accelerate allergic inflammatory responses to allergens. Therefore, prevention of the IgE-mediated FcεRI up-regulation would be a promising strategy for therapy of allergic disorders. However, the mechanism by which IgE binding up-regulates FcεRI expression on mast cells and basophils has not been fully elucidated.

A question to be addressed is whether the drastic up-regulation (up to 32-fold) of FcεRI on mast cells (14) can be explained by the inhibition of FcεRI degradation proposed for the 2-fold increase of FcεRI observed in RBL-2H3 cells (9–13). In the in vitro study with bone marrow-derived mast cells (BMMCs),[3] two components of the FcεRI up-regulation by IgE were identified: an early cycloheximide-insensitive phase as observed in RBL-2H3 cells, followed a few hours later by a more sustained component that was highly sensitive to cyclohexaminde (14). It is very difficult to clearly distinguish two mechanisms of the FcεRI up-regulation from these results: the inhibition of degradation of FcεRI vs the enhancement of synthesis and/or transport of FcεRI. In the present

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[3] Abbreviations used in this paper: BMMCs, bone marrow-derived mast cells; BFA, brefeldin A; MESF, molecules of equivalent of fluorescence intensity; TNP, trinitrophenyl.

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study, to address this issue we directly examined dynamics of IgE-free vs IgE-bound FcεRI expressed on mast cells and BMMCs by culturing cells with brefeldin A (BFA), which completely inhibited the transport of new FcεRI molecules to the cell surface. We also compared transcription of FcεRI subunits as well as the supply rate of new FcεRI to the surface of cells that expressed FcεRI at basal levels vs at highly up-regulated levels. Furthermore, kinetics of up-regulation of mouse and human FcεRI expressed on the same cell was analyzed to explore possible signal transduction through IgE-bound FcεRI. From these experiments we concluded that the stabilization and accumulation of FcεRI on the cell surface through IgE binding is the major mechanism of IgE-mediated FcεRI up-regulation. Physiological and pathological roles of IgE-mediated FcεRI up-regulation will also be discussed.

Materials and Methods

Animals

BALB/c and C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan). $\mu m^{-/-}$ mice (21) were kindly provided by Dr. K. Rajewsky (University of Cologne, Cologne, Germany) and Dr. D. Kitamura (Tokyo Science University, Tokyo, Japan), and were maintained in our own animal facility. FcγRIIB$^{-/-}$ mice were described previously (22). All the experiments in this study were performed according to the "Guidelines for Animal Use and Experimentation" as set out by our institutions.

Human FcεRI α-chain transgenic mice were established in our laboratory. A 17-kbp *Bam*HI-*Bam*HI human genomic DNA fragment covering the entire structural gene with five exons, a 2-kbp promoter region, and an 8-kbp 3′ flanking region was microinjected into fertilized eggs of BALB/c mice, followed by transfer of viable eggs into the oviducts of pseudopregnant Slc:ICR mice (Japan SLC, Hamamatsu, Japan). Two founder lines of transgenic mice were established.

Antibodies

Mouse anti-trinitrophenyl (TNP) IgE mAb (C38-2), anti-mouse IgE^a mAb (UH297, rat IgG1), anti-mouse IgE^b mAb (JKS-6, rat IgG2a), anti-mouse CD23 mAb (B3B4), FITC-conjugated anti-mouse IgE mAb (R35-72), FITC-conjugated anti-mouse IgG2b (R12-3), FITC-conjugated anti-rat IgG1/2a mAb (G28-5), FITC-conjugated anti-FcγRII/III mAb (2.4G2), FITC-conjugated rat IgG1 (R3-34), biotinylated anti-mouse c-*kit* mAb (2B8), PE-conjugated anti-mouse c-*kit* mAb (2B8), and allophycocyanin-conjugated streptavidin were purchased from BD PharMingen (San Diego, CA). Anti-human FcεRIα mAb (CRA1) was purchased from Kyokuto Pharmaceutical (Tokyo, Japan), and purified human IgE was purchased from Yamasa Shoyu (Chosin, Japan). Mouse anti-TNP IgE^a mAb (IGELa2), mouse anti-TNP IgE^b mAb (IGELb4), and anti-Fcγ mAb (2.4G2) were described previously (23, 24).

Cell preparation and flow cytometry

Peritoneal cells were isolated from mice and were depleted of RBCs by using hypotonic lysis buffer for culture and staining. BMMCs were generated by culturing femoral bone marrow cells in medium containing rIL-3 as described previously (25). Peritoneal cells and BMMCs were cultured in RPMI 1640 (Iwaki, Funabashi, Japan) with 10% FCS (JRH Bioscience, Lenexa, KS) with or without IgE in the presence or absence of BFA (Epicentre Technologies, Madison, WI). During culture of peritoneal mast cells, no stimulators such as stem cell factor were added. For flow cytometric analysis, freshly isolated or cultured cells were preincubated with 2.4G2 mAb (rat IgG2b) at 4°C for 15 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 FITC-anti-IgE mAb after incubation at 4°C with excess amounts of IgE (IGELb4 or C38-2) to saturate FcεRI with IgE. To detect IgE^a- and IgE^b-bound FcεRI, cells were stained with anti-IgE^a mAb and anti-IgE^b mAb, respectively, followed by FITC-conjugated anti-rat IgG1/2a mAb. To determine levels of human FcεRI α-chain expression, cells were stained with anti-human FcεRI α-chain mAb CRA1 followed by FITC-conjugated anti-mouse IgG2b mAb R12-3. To detect FcγRIII, BMMCs derived from FcγRIIB$^{-/-}$ mice were stained with FITC-conjugated 2.4G2. For cultured cells, cells were also stained with propidium iodide and biotinylated anti-c-*kit* mAb followed by allophycocyanin-streptavidin. Propidium iodide$^-$c-*kit*$^+$ cells were analyzed as live mast cells for the expression of FcεRI by using FACSCalibur (BD Biosciences, Mountain View, CA). For freshly prepared peritoneal cells, autofluorescent cells (primarily macrophages) were rejected to clearly identify c-*kit*$^+$ mast cells (14). 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, San Juan, PR), as per the specifications of the manufacturer. ΔMESF was calculated by subtracting MESF of control staining from MESF of sample.

Northern blot analysis

Total cellular RNA was isolated from BMMCs cultured with or without IgE by using Isogen (Nippon Gene, Toyama, Japan) separated on a formaldehyde gel and transferred to nylon membranes (Hybond-N+, Amersham-Pharmacia Biotech, Piscataway, NJ). Transcripts of mouse FcεRIα, β, γ, and β actin were detected by specific probes. The radioactive bands were visualized by the phosphor imager Fuji BAS2000 (Fuji Photo Film, Tokyo, Japan).

Results

Drastic change of FcεRI expression on mast cells in vivo in correlation with serum IgE levels

Levels of FcεRI expression on c-*kit*$^+$ peritoneal mast cells were examined in three different mouse strains, B cell-deficient $\mu m^{-/-}$ mice (21), normal BALB/c mice, and IgE-transgenic BALB/c mice (26). Their serum IgE levels were undetectable at 1.3 and 30 μg/ml, respectively. Representative results of flow cytometry analyzing IgE-bound and total (IgE-bound plus IgE-free) FcεRI on peritoneal mast cells are shown in Fig. 1*A*. To compare levels of FcεRI expression accurately, the geo mean values of fluorescence intensity were converted to the numbers of MESF, as shown in Fig. 1*B*. Although no IgE-bound FcεRI was detected on mast cells from B cell-deficient $\mu m^{-/-}$ mice as expected, total FcεRI (IgE-free FcεRI) was detectable on their surface. However, its expression levels were ~20% of those on mast cells from normal BALB/c mice, which is consistent with the previous observation in mast cells from IgE-deficient mice (14). In contrast, levels of total FcεRI on peritoneal mast cells from IgE-transgenic BALB/c mice were 5–6 times as high as those on mast cells from normal BALB/c mice. Furthermore, eventually all the FcεRI molecules on mast cells from IgE-transgenic mice were occupied by IgE. Even in normal BALB/c mice, ~80% of FcεRI molecules on mast cells were occupied by IgE. Thus, levels of FcεRI on mast cells can be altered in vivo at least by 25-fold in correlation with serum IgE levels and IgE binding to FcεRI.

IgE-bound FcεRI stays on the cell surface for a much longer time than IgE-free FcεRI

To clarify the mechanism by which FcεRI expression on mast cells is drastically up-regulated upon association with IgE, we first analyzed dynamics of FcεRI on BMMCs. When BMMCs were cultured in vitro with 1 μg/ml IgE for 24 h, a 5- to 6-fold increase of FcεRI expression was observed (Fig. 2*A*). An inhibitor of intracellular protein transport, BFA, added in culture inhibited this up-regulation of FcεRI expression in a dose-dependent manner. Because addition of 1–10 μg/ml BFA resulted in complete inhibition, 3 μg/ml BFA was used for additional experiments. In the next experiment BMMCs were first preincubated with excess amounts of IgE at 4°C to saturate all FcεRI molecules on the cell surface with IgE. After unbound IgE was washed away, BMMCs were cultured without adding IgE at 37°C for 16 h in the presence or absence of BFA. In the absence of BFA, levels of IgE-bound FcεRI did not change during the culture, whereas levels of total FcεRI increased ~2-fold (Fig. 2*B*). Therefore, the increase of total FcεRI appears to be due to the addition of IgE-free FcεRI, namely the transport of new FcεRI molecules from the cytoplasm to the cell surface. Indeed, BFA completely inhibited the increase of total FcεRI. These results indicated that the half-life of IgE-bound and IgE-free

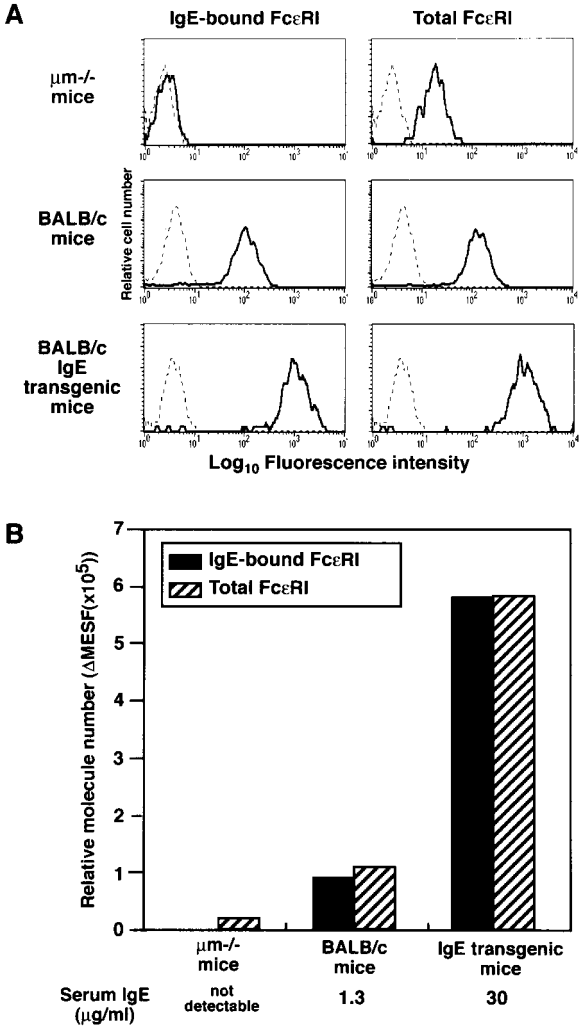

FIGURE 1. Flow cytometric analysis of FcεRI expression on peritoneal mast cells in three mice strains with different serum IgE levels. Peritoneal cells were isolated from $\mu m^{-/-}$, normal BALB/c, and IgE transgenic mice and pretreated in vitro with anti-FcγRII/III mAb 2.4G2 to prevent nonspecific binding of Abs. Cells were then stained with FITC-anti-IgE mAb R35-72 to detect IgE-bound FcεRI on the cell surface. To detect total (IgE-bound plus IgE-free) FcεRI, cells were stained with FITC-anti-IgE mAb after incubation at 4°C with excess amounts of IgE to saturate FcεRI with IgE. In both cases, cells were also stained with PE-anti-c-*kit* mAb, and c-*kit*$^+$ mast cells were analyzed for the expression of FcεRI. Representative results are shown in *A* as histograms overlayed with control staining (dotted lines). *B*, To compare levels of FcεRI expression accurately, the geo mean values of fluorescence intensity in *A* were converted to MESF, and ΔMESF was calculated in each case as detailed in *Materials and Methods*. ΔMESF values of IgE-bound FcεRI (■) and total FcεRI (▨) are shown. Data shown are representative of five repeated analyses.

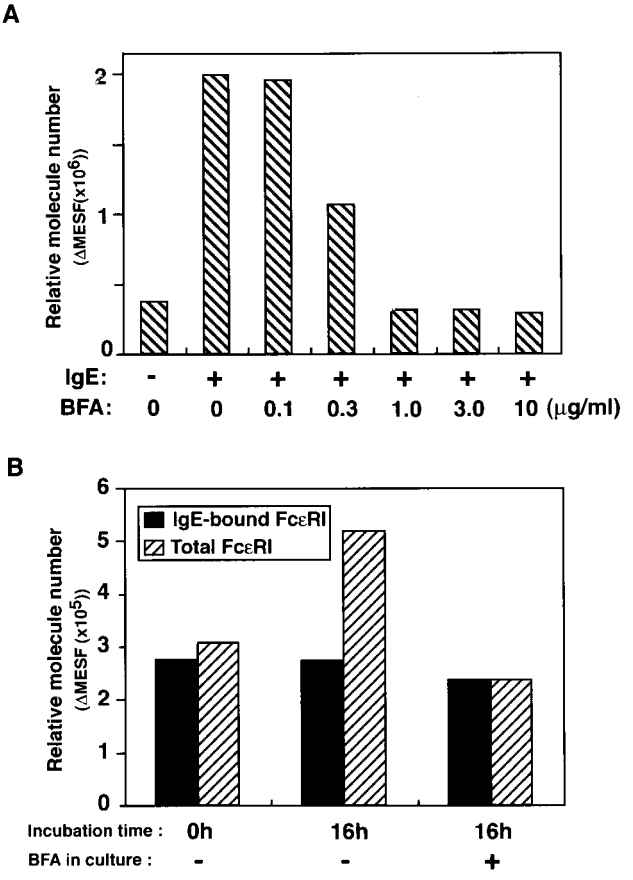

FIGURE 2. BFA inhibits IgE-mediated FcεRI up-regulation by preventing appearance of new FcεRI molecules to the cell surface. *A*, BMMCs prepared from BALB/c mice were cultured in vitro with or without 1 μg/ml mouse IgE at 37°C for 24 h. In some cases BFA was also included in culture at various concentrations as indicated. After 24 h culture, levels of total FcεRI on their surface were determined as in Fig. 1. *B*, BMMCs prepared from BALB/c mice were first incubated with IgE at 4°C to saturate FcεRI, and then unbound IgE was washed away. Subsequently, cells were cultured without adding IgE at 37°C in the presence or absence of 3 μg/ml BFA for 16 h. Levels of IgE-bound and total FcεRI were determined and are displayed as in Fig. 1. Data shown are representative of three repeated analyses.

FcεRI expressed on the cell surface can be estimated by monitoring levels of FcεRI during the culture in the presence of BFA, which blocks new supply of FcεRI to the cell surface.

During the 24-h culture in the presence of BFA, levels of IgE-free FcεRI on BMMCs went down very quickly and reached nearly zero by 12 h later (Fig. 3*A*). In contrast, levels of IgE-bound FcεRI did not change as much, and most of the FcεRI remained on the cell surface even after 12 h. Kinetics of IgE-bound vs IgE-free FcεRI on the surface of peritoneal mast cells freshly isolated from mice was also examined in the similar way (Fig. 3*B*). IgE-free FcεRI on mast cells from $\mu m^{-/-}$ mice disappeared from the cell surface very quickly during culture in the presence of BFA, and only 4% of them remained 16 h later. In contrast, IgE-bound FcεRI on $\mu m^{-/-}$ mast cells pretreated with IgE was very stable, and no significant change in its surface expression levels was observed during the 16-h culture. This was also the case in IgE-bound FcεRI on peritoneal mast cells prepared from normal C57BL/6 mice (Fig. 3*B, right*). These results indicated that FcεRI was stabilized by IgE binding and stayed for a longer time on the surface of mast cells.

Kinetics of IgE-bound FcεRI on the cell surface was further examined in BMMCs in which levels of FcεRI had been up-regulated to 7- and 12-fold by preculture with IgE for 24 and 48 h, respectively (Fig. 3*C*). As in BMMCs not precultured with IgE, levels of IgE-bound FcεRI showed little or no change during the 16-h culture in the presence of BFA. Thus, the stable expression of IgE-bound FcεRI was observed regardless of the levels of FcεRI expression on BMMCs.

New FcεRI molecules are supplied to the cell surface independent of levels of FcεRI expression

We next examined whether the appearance of new FcεRI molecules to the cell surface was altered during IgE-mediated FcεRI up-regulation. BMMCs were cultured for 24 h with or without the

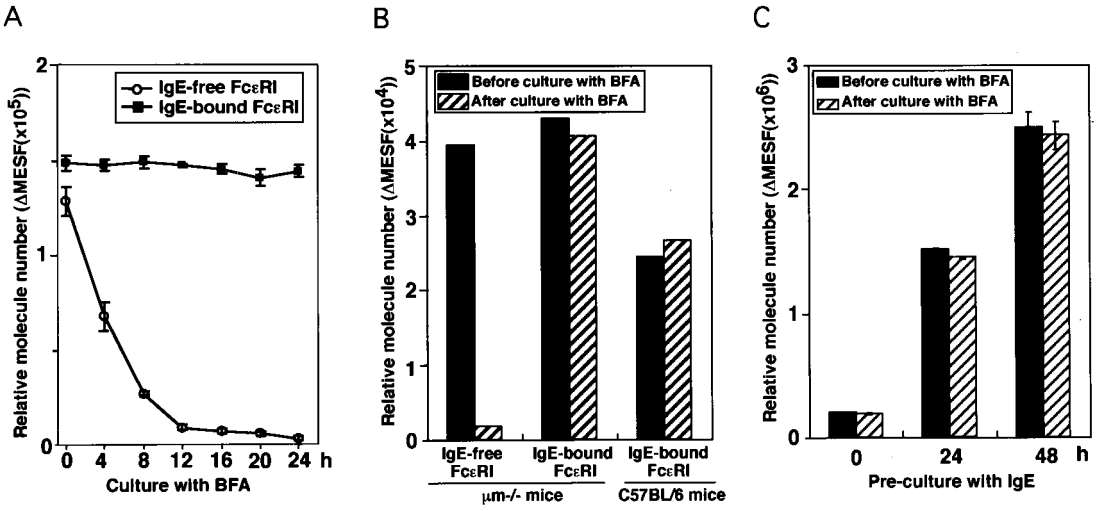

FIGURE 3. FcεRI is stabilized by IgE binding and stays for longer time on the cell surface. *A*, BMMCs prepared from BALB/c mice were incubated with excess amounts of IgE at 4°C to saturate FcεRI with IgE. After unbound IgE were washed away, the cells were cultured in the presence of 3 μg/ml BFA at 37°C for 24 h. At indicating time points during culture, the cells were harvested and stained with anti-IgE mAb to determine levels of IgE-bound FcεRI on the cell surface (■). In parallel, untreated BMMCs were cultured in the same way. Levels of FcεRI (IgE-free) at indicated time points during culture were determined by staining cells with anti-IgE mAb after incubation at 4°C with excess amounts of IgE to saturate FcεRI with IgE (○). Data are shown as ΔMESF. Mean ± SD ($n = 4$). *B*, Peritoneal mast cells freshly prepared from $\mu m^{-/-}$ mice were cultured in the presence of 3 μg/ml BFA at 37°C for 16 h with or without preincubation with excess amounts of IgE as in *A*. Levels of FcεRI (IgE-free or IgE-bound) were determined as in *A* before (filled bars) and after 16 h culture (hatched bars). In parallel, freshly prepared peritoneal mast cells from C57BL/6 mice were cultured in the same way, and levels of IgE-bound FcεRI were determined before and after the culture. Data shown are representative of three repeated analyses. *C*, BMMCs prepared from BALB/c mice were cultured in the presence of 5 μg/ml IgE at 37°C for 0, 24, or 48 h followed by incubation with excess amounts of IgE at 4°C. After unbound IgE was washed away, cells were further cultured without adding IgE in the presence of 3 μg/ml BFA at 37°C for 16 h. Levels of IgE-bound FcεRI were determined before (filled bars) and after 16 h culture (▨). Mean ± SD ($n = 3$).

b allotype of IgE (IgE^b), followed by saturation of their FcεRI with IgE^b. After unbound IgE^b was washed away, the cells were cultured with the a allotype of IgE (IgE^a) for an additional 6 h, and levels of total, IgE^b-bound and IgE^a-bound FcεRI expression were determined by flow cytometric analysis with IgE-specific and IgE allotype-specific mAbs, respectively. A 6-fold difference in levels of FcεRI expression was observed after the 24-h incubation with vs without IgE^b (Fig. 4, *upper panel*, ■). Levels of IgE^b-bound FcεRI did not change during the following 6-h culture with IgE^a, while levels of total FcεRI increased in both cases (Fig. 4, *upper* and *middle panels*, ▨). Therefore, the degree of FcεRI up-regulation during the 6-h culture was determined as the amount of IgE^a-bound FcεRI. Interestingly, that was comparable in the two cases (Fig. 4, *bottom panel*, ▨) despite the big difference in total amounts of FcεRI on the cell surface (Fig. 4, *upper panel*, ▨). These results suggested that the input rate of new FcεRI molecules to the cell surface was consistently independent of the total number of surface FcεRI, at least within the range we examined.

Levels of FcεRI transcripts in BMMCs are not altered by IgE binding to FcεRI

Northern blot analysis was performed to examine the possibility that IgE binding to FcεRI transduces signals to increase transcripts of FcεRI subunits. BMMCs prepared from BALB/c mice were cultured with or without IgE for 8 h. Although levels of FcεRI expression on the cell surface increased up to 2.5-fold by culture with IgE (Fig. 5, *upper panel*), no significant difference in levels of transcripts of FcεRI subunits (α-, β-, and γ-chains) was detected at any time point (2, 4, and 8 h) of culture between cells cultured with IgE and those without IgE (Fig. 5, *lower panel*). Thus, IgE binding to FcεRI did not alter levels of FcεRI transcripts.

Human IgE up-regulates human FcεRI, but not mouse FcεRI expressed on the same cell

To explore the possibility that IgE binding to FcεRI induces signals to accelerate synthesis and/or transport of FcεRI to the cell surface, we used BMMCs derived from human FcεRI α-chain transgenic mice. Two-color flow cytometric analysis confirmed that mouse and human FcεRI α-chains were simultaneously expressed on BMMCs (data not shown). Both mouse and human FcεRI α-chains were associated with mouse β- and γ-chains, as shown previously (27–29). The chimeric FcεRI complex composed of human α-chain and mouse β- and γ-chains was competent to transduce signals because cross-linking of human α-chains on mast cells with specific Ab in vivo resulted in systemic anaphylaxis in the transgenic mice (data not shown). BMMCs prepared from the transgenic mice were cultured with human IgE for 16 h. Human IgE binds to human FcεRI, but not mouse FcεRI (30). Levels of human FcεRI increased ~6-fold during the culture (Fig. 6). If signaling via β- and γ-chains of FcεRI is involved in this up-regulation through acceleration of synthesis and/or transport of FcεRI to the cell surface, one may expect that levels of mouse FcεRI expression on the same cells were also up-regulated. However, no significant alteration was observed in levels of mouse FcεRI expression during the culture. Therefore, it is unlikely that IgE binding to FcεRI facilitates synthesis and/or transport of FcεRI to the cell surface.

Difference of α-chain in FcεRI and FcγRIII in correlation with their different stability on the cell surface

To know which subunit of FcεRI determines the stability of FcεRI on the cell surface, we compared kinetics of surface FcεRI and FcγRIII, which carry different α-chains, but share β- and γ-chains.

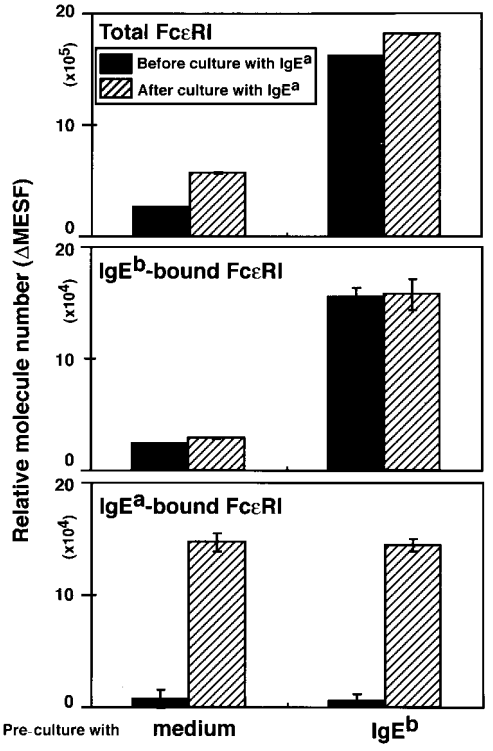

FIGURE 4. New FcεRI molecules are supplied to the cell surface independent of levels of FcεRI expression. BMMCs prepared from BALB/c mice were cultured with or without 10 μg/ml IgE^b at 37°C for 24 h followed by incubation at 4°C with excess amounts of IgE^b to saturate FcεRI with IgE. After unbound IgE^b was washed away, cells were further cultured with 10 μg/ml IgE^a at 37°C for 6 h. Before (■) and after the 6 h culture with IgE^a (▨), levels of total FcεRI were determined by staining cells with FITC-labeled anti-IgE mAb (R35-72) while those of IgE^a-bound and IgE^b-bound FcεRI were determined by staining cells with anti-IgE^a mAb (UH297, rat IgG1) and anti-IgE^b mAb (JKS-6, rat IgG2a), respectively, followed by FITC-labeled anti-rat IgG1/2a mAb. Data are shown as ΔMESF. Mean ± SD (n = 3).

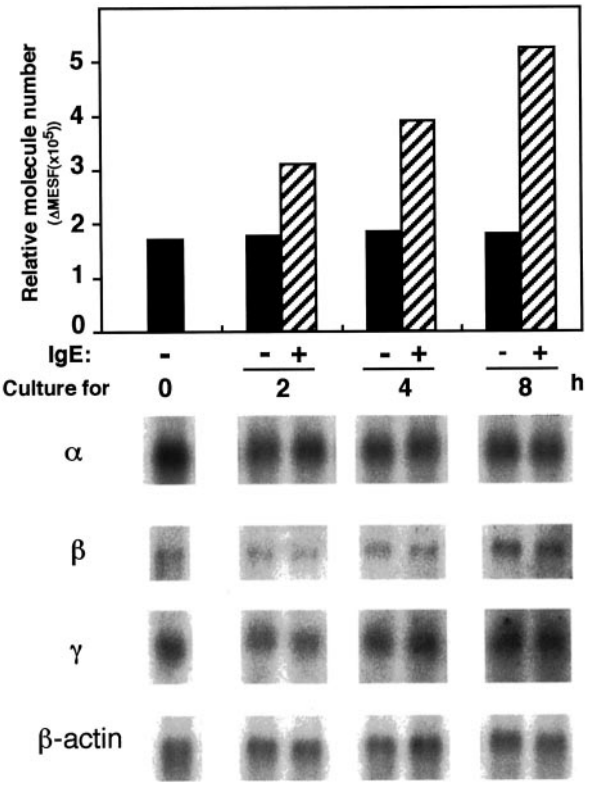

FIGURE 5. Levels of FcεRI transcripts in BMMCs are not altered by IgE binding to FcεRI. BMMCs derived from BALB/c mice were cultured with or without 10 μg/ml IgE at 37°C for 8 h. At the indicated time points during the culture, cells were harvested and analyzed for surface expression of FcεRI by flow cytometry (*upper panel*) as well as for transcripts of FcεRI subunits and β-actin by Northern blot (*lower panel*).

Because 2.4G2 mAb reacts with both FcγRIIB and FcγRIII, BMMCs derived from FcγRIIB-deficient mice were used for this purpose. Levels of FcεRI on BMMCs were reduced to 14% of normal level after a 16-h culture in the presence of BFA, whereas those of FcγRIII detected by 2.4G2 were unchanged during the culture (Fig. 7). Therefore, the difference of α-chains in these two receptors appears to account for their different stability on the cell surface in the absence of ligands.

Efficient acquisition of Ag specificity through IgE-mediated FcεRI up-regulation

To understand the physiological role of IgE-mediated FcεRI up-regulation, BALB/c mice were treated with i.v. administration of 300 μg of monoclonal IgE^b specific to hapten TNP or an equivalent volume of PBS twice at a 3-day interval. Three days after the second treatment, the expression of IgE^a-bound and IgE^b-bound FcεRI as well as IgE-free FcεRI on their peritoneal mast cells was determined by flow cytometry with IgE allotype-specific mAbs. Relative amounts of each FcεRI on mast cells prepared from IgE^b-treated and PBS-treated mice are shown in Fig. 8. In PBS-treated mice, only 20% of FcεRI on mast cells were free of IgE, and the rest of them were occupied by endogenous IgE^a. In IgE^b-treated mice, total amounts of FcεRI increased 3-fold, 80% of which were occupied by exogenous IgE^b. The rest were occupied by endogenous IgE^a. Thus, IgE-mediated FcεRI up-regulation appears to facilitate acquisition of new Ag specificity in a short period.

Discussion

The present study clearly demonstrates that the stabilization of FcεRI through IgE binding followed by the accumulation of IgE-bound FcεRI on the cell surface is the major mechanism of IgE-mediated FcεRI up-regulation in mast cells. Two different mechanisms have been proposed to explain the IgE-mediated FcεRI up-regulation (6, 7, 15, 18). One is the suppression of loss of preformed FcεRI expressed on the cell surface by protecting against the degradation of FcεRI. The other is the enhancement of synthesis and/or transport of FcεRI complex through FcεRI-mediated signaling. In the previous study using a cultured mast cell line RBL-2H3, cycloheximide could not inhibit IgE-mediated FcεRI expression (11). Furthermore, the tracing of ^{125}I-labeled FcεRI on the surface of RBL-2H3 revealed that IgE-bound FcεRI stayed on the surface much longer than IgE-free FcεRI (11–13). These results favor the former possibility. However, the FcεRI up-regulation in RBL-2H3 cells was up to 2-fold and much less than that observed in mast cells in vivo and BMMCs in vitro. Therefore, it remained to be determined what the mechanism is that underlies IgE-mediated FcεRI up-regulation in normal mast cells.

A recent study demonstrated that IgE-mediated FcεRI up-regulation on mouse BMMCs has two components: an early cycloheximide-insensitive phase, followed by a later and more sustained component that is highly sensitive to inhibition by cycloheximide (14). These findings were interpreted that IgE could up-regulate FcεRI during the first hours simply by protecting against degradation of FcεRI without being dependent on FcεRI synthesis.

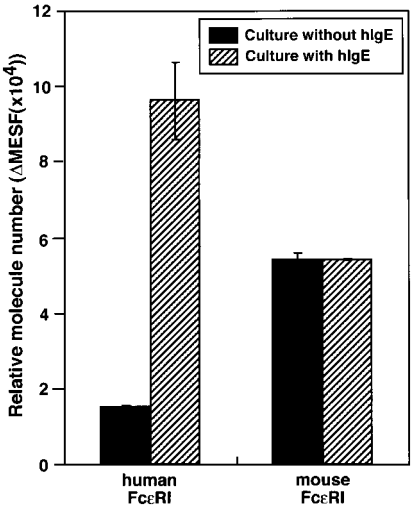

FIGURE 6. Human IgE up-regulates human FcεRI but not mouse FcεRI expressed on BMMCs derived from human FcεRI α-chain transgenic mice. BMMCs prepared from human FcεRI α-chain transgenic mice were cultured with 1 μg/ml human IgE at 37°C for 16 h, and levels of human and mouse FcεRI α-chains were compared before and after the culture. To determine levels of human FcεRI α-chain expression, cells were stained with anti-human FcεRI α-chain mAb. To determine levels of mouse FcεRI α-chain expression, cells were first incubated at 4°C with human IgE to prevent mouse IgE for binding to human FcεRI α-chains and then incubated with mouse IgE followed by staining with anti-mouse IgE mAb. Data are shown as ΔMESF. Mean ± SD (n = 3).

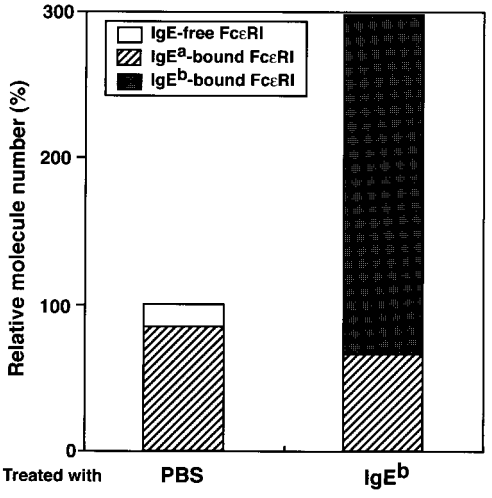

FIGURE 8. Efficient acquisition of Ag specificity through IgE-mediated FcεRI up-regulation. BALB/c mice were treated i.v. twice with 300 μg of IgE^b or PBS each at 3 days interval. Three days after the second treatment, the expression of total, IgE-bound, IgE^a-bound, and IgE^b-bound FcεRI on their peritoneal mast cells was determined as in Fig. 4. Relative amounts of IgE-free, IgE^a-bound, and IgE^b-bound FcεRI on mast cells were calculated, and levels of total FcεRI on peritoneal mast cells from PBS-treated mice are set as 100%. Data shown are calculated on basis of four independent experiments.

Later, when the pool of available FcεRI was fully used, further accumulation of FcεRI by the same mechanism became dependent on protein synthesis (6). Though the data were not inconsistent with the interpretation, they could not rule out other mechanisms of FcεRI up-regulation such as enhancement of synthesis and transport of FcεRI complex. In the present study we first used BFA, an inhibitor of intracellular protein transport, to study kinetics of FcεRI on mast cells. The up-regulation of FcεRI expression on BMMCs cultured with IgE was completely inhibited by BFA. As expected, BFA inhibited the supply of new FcεRI molecules to the cell surface, while it had no significant effect on the expression of pre-existing IgE-bound FcεRI on the cell surface. Therefore, the fate of surface FcεRI molecules can be investigated simply by monitoring levels of FcεRI expression during culture in the presence of BFA. Under such culture conditions, levels of IgE-free FcεRI on BMMCs decreased very quickly, whereas those of IgE-bound FcεRI were fairly stable. This was also true for FcεRI on peritoneal mast cells. These results clearly indicate that surface FcεRI is unstable and quickly removed from cell surface unless IgE binds to FcεRI. Upon association with IgE, FcεRI is stabilized and stays on the surface for a much longer time than IgE-free FcεRI. With excess amounts of IgE in culture without BFA, every new FcεRI supplied to the cell surface is loaded with IgE, stabilized, and accumulated on the cell surface, leading to the up-regulation of surface FcεRI expression.

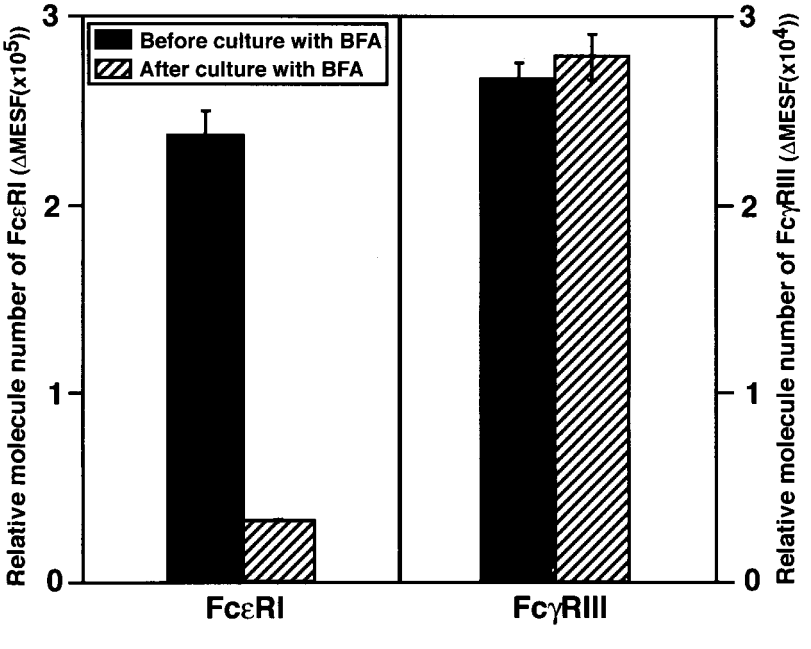

FIGURE 7. Comparison of FcεRI and FcγRIII in their stability on the cell surface. BMMCs derived from FcγRIIB$^{-/-}$ mice were cultured in the presence of 3 μg/ml BFA at 37°C for 16 h, and levels of FcεRI and FcγRIII were compared before and after the culture. Levels of FcεRI was determined as in Fig. 3 while those of FcγRIII were determined by staining cells with FcγRIIB/FcγRIII-specific mAb 2.4G2. Data are shown as ΔMESF. Mean ± SD (n = 3).

Is the prevention of FcεRI degradation on the cell surface only the mechanism of IgE-mediated up-regulation? One may assume that the binding of monomeric IgE to FcεRI could trigger the transducing of signals for enhancement of synthesis and/or transport of FcεRI. Because BFA inhibits the appearance of new FcεRI molecules to the cell surface, it is impossible to rule out this possibility from the results of experiments using BFA. To address this issue we first examined levels of FcεRI transcripts in BMMCs when cultured with or without IgE. Northern blot analysis revealed that IgE binding to FcεRI did not increase transcripts of any subunit composed of FcεRI even though it increased levels of surface FcεRI. We next examined kinetics of FcεRI on BMMCs derived from human FcεRI α-chain transgenic mice in which both mouse and human FcεRI α-chains are expressed on the cell surface. Mouse β- and γ-chains are associated as signal transducing subunits with both mouse and human FcεRI α-chains (27–29). Therefore, the outcome of signal transduction via mouse FcεRI and human FcεRI is most likely the same. Indeed, both receptors were competent for inducing allergic reactions in vivo when cross-linked with specific mAbs (our unpublished observations). If IgE binding to FcεRI triggers the transduction of signals, leading to acceleration of synthesis and/or transport of FcεRI, it is expected that both mouse and human FcεRI are up-regulated regardless of which receptor is fired by IgE binding. Culturing BMMCs with human IgE induced up-regulation of human FcεRI as expected, but the up-regulation of mouse FcεRI expressed on the same cell was not observed. Therefore, it seems unlikely that IgE binding to FcεRI facilitates synthesis and/or transport of FcεRI to the cell surface. Furthermore, the rate of appearance of new FcεRI to the cell surface was found to be comparable between cells expressing the basal level of FcεRI and cells expressing highly up-regulated FcεRI. This favors the idea that IgE binding does not accelerate the transport of new FcεRI to the cell surface. The level of FcεRI expression on peritoneal mast cells is relatively low in normal mice, even though ~80% of FcεRI are occupied with IgE. This also supports the above idea. Taken together, we would like to conclude that the stabilization of FcεRI through IgE binding followed by accumulation of IgE-bound FcεRI on the cell surface is the major mechanism of IgE-mediated FcεRI up-regulation in mast cells.

The in vivo experiments using different allotypes of IgE indicated that mast cells could easily acquire new Ag specificity through IgE-mediated FcεRI up-regulation in a short period. Up to 80% of FcεRI on mast cells are occupied with IgE even in normal mice with basal level of serum IgE, and IgE-bound FcεRI molecules are stable on the cell surface. Therefore, only limited space is available for newly produced IgE if IgE-mediated FcεRI up-regulation is not induced. In case of T and B cells, each cell has only one specificity to a given Ag. Therefore, the population reactive to a particular Ag is extremely small, and each clone needs to expand to protect host against foreign Ag. In contrast, large numbers of mast cells can simultaneously acquire new Ag specificity without expansion of cells through FcεRI up-regulation induced by newly produced IgE. Mast cells can also acquire multiple Ag specificity by binding large numbers of different IgE species with distinct Ag specificities. Furthermore, the stable expression of large amounts of Ag-specific IgE on mast cells enables mast cells to keep their immunological memory for a fairly long period (memory of mast cells). This should help with protection from repeated reinfections of pathogens (31). Thus, IgE-mediated FcεRI up-regulation appears to benefit the host in the environment where infection of pathogens such as parasites prevails.

It has been shown that IgE-dependent up-regulation of FcεRI expression significantly enhances the ability of mouse mast cells to release serotonin, IL-6, IL-4, and vascular permeability factor/vascular endothelial cell growth factor in response to challenge with specific Ag (14, 20). Similar enhancement was also observed in human basophils (16). The augmentation of the sensitivity and the intensity of those responses would be a critical mechanism to facilitate host defense. Unfortunately, the same mechanism could also increase the severity of allergic disorders. In other words, the prevention of IgE-mediated FcεRI up-regulation can be a promising strategy to manage the disorders. 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 (32, 33). In this context, it is essential to know the exact mechanism by which IgE binding stabilizes FcεRI. In humans, it has been reported that the FcεRI expression on monocytes was higher in atopic subjects when compared with normal subjects (34). FcεRI on human monocytes is composed of only α- and γ-chains in contrast to FcεRI ($\alpha\beta\gamma_2$ tetramer) on mast cells and basophils (35–40). Therefore, β-chains of FcεRI appear to be nonessential for IgE-mediated FcεRI up-regulation. The comparison of FcεRI and FcγRIII stability in the present study suggested that FcεRI α-chains, but not β- and γ-chains, are responsible for the instability of IgE-free FcεRI. IgE binding to FcεRI α-chain might induce some conformational change of α-chain to make FcεRI resistant to degradation. Elucidation of this switching mechanism should facilitate the development of a new type of therapy for allergic disorders.

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