

Minimal Requirements for IgE-Mediated Regulation of Surface FcεRI¹

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The IgE-FcεRI network plays a central role in allergic inflammation. IgE levels control cell surface levels of FcεRI and, in turn, FcεRI levels modulate the intensity of effector responses. Treatment of allergic patients with anti-IgE Abs has been shown to induce a decrease in FcεRI expression on basophils and a decrease in Ag-triggered histamine release. However, the mechanisms underlying IgE-mediated regulation of FcεRI expression remain unclear. Here, we designed an in vitro model system to establish the minimal cellular requirements for regulation of FcεRI by IgE. Using this system, we demonstrate that transcriptional regulation, hemopoietic-specific factors, and signaling are not required for IgE-mediated increases in FcεRI expression. IgE binding to the α-chain is the minimal requirement for the induction of FcεRI up-regulation. The rate of up-regulation is independent of the baseline level of expression. The mechanism of this up-regulation is the result of a combination of three factors: 1) stabilization of the receptor at the cell surface, which prevents receptor internalization and degradation; 2) use of a preformed pool of receptor comprising recycled and recently synthesized receptors; and 3) continued basal level of protein synthesis. It is possible that in vivo additional factors contribute to modulate the basic regulatory mechanism described here. *The Journal of Immunology*, 2001, 167: 1290–1296.

The FcεRI complex forms a high affinity cell surface receptor for IgE that is expressed in the tetrameric form (αβγ₂) on mast cells and basophils and in the trimeric form (αγ₂) on eosinophils (1–3) and APCs (4–7). The α-chain binds IgE, and the γ-chain transduces signals (8). The β-chain is an amplifier of signal strength (9, 10) and enhances the expression of unoccupied receptor at the cell surface (11). Ligation of the receptor by IgE and multivalent Ag results in the activation of multiple signaling pathways leading to diverse effector responses.

FcεRI is variably expressed on the cell surface. Long ago, before FcεRI had been characterized, the IgE binding capacity of human basophils was shown to correlate with serum IgE concentrations (12, 13). A number of studies have since confirmed that IgE levels in vitro and in vivo regulate the cell surface expression of FcεRI (14–25). Clinical trials treating allergic patients with humanized Ab to IgE show recipients to exhibit decreased levels of serum IgE with a concomitant decrease in surface expression of FcεRI on basophils (26, 27). More importantly, these changes result in decreased histamine release in response to antigenic challenge (26, 27), emphasizing the functional importance of FcεRI regulation by IgE. However, much is unknown about the mechanisms that control this process. One study has shown the process of up-regulation to be insensitive to cycloheximide, suggesting that regulation does not involve protein synthesis (14). This study proposed that inhibition of degradation of surface FcεRI by IgE could

contribute to IgE-mediated up-regulation. However, the possible contribution of other mechanisms such as recycling, was not investigated. Later studies by Galli's group (17) in collaboration with us suggested a more complex situation: an early cycloheximide-insensitive phase followed by a later cycloheximide-sensitive phase, implying a requirement for protein synthesis in the process. However, these studies do not differentiate between a requirement for basal levels of protein synthesis vs a requirement for increased protein synthesis. In addition, the possibility of IgE-mediated variations in transcription, translation, or message stability has not been investigated, and the relative contribution from the proposed inhibition of degradation remains unclear. Finally, a known amplifier of unoccupied receptor expression is the β-chain (11). However, its role in up-regulating the expression of FcεRI has not been studied.

To dissect the process of IgE-mediated regulation of FcεRI, we have created an in vitro cellular system that has allowed us to determine the minimal cellular requirements necessary for IgE-mediated regulation of FcεRI. We have found that the minimum requirement to induce up-regulation is binding of IgE to FcεRIα and that the mechanism of this up-regulation involves receptor stabilization at the membrane, recruitment of a preformed pool of receptors (recycled and recently synthesized), and continued basal synthesis of receptors.

Materials and Methods

Cell culture and assessment of FcεRI surface expression

U937 and NIH3T3 transfected cell lines were generated and maintained as described previously (9). Experiments were conducted in 96-well plates in triplicate at a density of 0.5×10^6 cells/ml. Chimeric human IgE (Serotec, Oxford, U.K.), human myeloma IgE (BioDesign International, Saco, ME), cycloheximide (1 μg/ml), or brefeldin A (BFA³; 5 μg/ml; Sigma, St. Louis, MO) were added at the initiation of culture for the indicated times. For assessment of the cell surface levels of FcεRI, cells were incubated in saturating concentrations of human IgE followed by FITC-conjugated goat

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³ Abbreviations used in this paper: BFA, brefeldin A; Endo H, endo-β-N-acetylglucosamidase; MFI, mean fluorescence intensity.

anti-human IgE (BioSource International, Camarillo, CA). Cells were analyzed by flow cytometry with FACScan software (BD Biosciences, Franklin Lakes, NJ). Nonviable cells were excluded by the addition of propidium iodide.

Immunoprecipitation and Western blotting

Cells were lysed as described previously (9). In some experiments, lysates were treated with endo- β -N-acetylglucosamidase (Endo H; New England Biolabs, Beverly, MA; Ref. 28). For assessment of cell surface Fc ϵ RI α , cell surface receptors were saturated with human IgE before lysis and immunoprecipitated with mouse anti-human IgE (BD PharMingen, San Diego, CA) coupled to protein G-Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ) in serial immunoprecipitations to remove all surface receptors. Intracellular Fc ϵ RI α was immunoprecipitated using monoclonal anti-human α Ab, 15-1 (4), coupled to protein G-Sepharose. Samples were resolved on 10% (for α -chain) or 12.5% (for β - and γ -chains) SDS polyacrylamide gel in nonreducing conditions, transferred to polyvinylidene fluoride membrane, and blotted with rabbit anti-human α Ab, 997 (28), anti-human β Ab, 961 (11), or anti- γ Ab, 934 (29). Immunoreactive proteins were visualized with alkaline phosphatase coupled mouse anti-rabbit Ig, followed by enhanced chemifluorescence (Amersham), and quantitated with the Storm scanner (Molecular Dynamics, Sunnyvale, CA).

Northern blotting

Total cellular RNA was extracted using RNAzol B (Tel-Test; Friendswood, TX). RNA was electrophoresed on 1% agarose gel and then transferred to a nylon membrane. The membranes then were hybridized with ^{32} P-labeled cDNA probes for human α , β , and γ (30–32) as well as GAPDH as a positive control. Probe binding was measured with a Storm scanner and quantitated with ImageQuant software (Molecular Dynamics).

Statistical analysis

Numerical results are expressed as mean $\pm$ SD. Results were evaluated by ANOVA, which allows overall comparisons between a set of experimental data and a set of control data, for example, a time course. In some experiments, a Student's paired *t* test was used to compare experimental data and control data at the same time point (pair) in the time course.

Results and Discussion

IgE-mediated up-regulation of Fc ϵ RI expression does not require transcriptional regulation or hemopoietic-specific factors

We reasoned that IgE-mediated Fc ϵ RI up-regulation could be mediated through the following mechanisms: an increase in transcription, translation, or stability of the transcripts, improved intracel-

lular processing, or increased stability at the plasma membrane of the Fc ϵ RI subunits. Some of these mechanisms could require cell-type-specific elements to operate. Our approach was to determine the minimal requirements for Fc ϵ RI up-regulation by using a reconstitution system in which some of these potential factors could not operate. In this system, reconstitution is achieved by introducing (by transfection or infection) the cDNA for the molecule to be studied in a cell line that does not express it spontaneously. This is a general methodology that we have used successfully to dissect the signaling machinery of Fc ϵ RI (33, 34) and that of the inhibitory receptor Fc γ RIIb1 (35). We have also used it to demonstrate the signal amplification and receptor expression amplification functions of the β -chain (9, 11). In addition, we have validated this strategy by demonstrating that results obtained with this in vitro system parallel results obtained in vivo with animal models of allergic reactions (9, 10). Here we have expressed Fc ϵ RI in two different cell lines (see below) by transfecting the cDNAs for the human α -, β -, and γ -chains. To eliminate any requirement for increased transcription in IgE-mediated Fc ϵ RI up-regulation, we chose a viral promoter to drive expression of the receptor subunits in the transfected cells. Such viral promoters are stronger than eukaryotic promoters and are unlikely to be regulated by eukaryotic factors. Their use ensured that transcription of the Fc ϵ RI subunits would be high and unaffected by IgE treatment. In addition, to eliminate the contribution of mast cell-specific factors, we generated these transfectants in the monocytoid cell line U937. Cells transfected with the human α , β , and γ cDNAs or untransfected cells were cultured in the presence of saturating concentrations of monomeric IgE or vehicle and assessed for cell surface expression of Fc ϵ RI by flow cytometry. Fig. 1A shows the ratio of Fc ϵ RI expression after incubation with IgE over Fc ϵ RI expression after incubation with vehicle as a function of time. IgE treatment up-regulated Fc ϵ RI expression, whereas it had no effect on untransfected cells ($p < E-4$ vs IgE-treated untransfected U937 and vs vehicle-treated transfectants). Note that in the rat basophilic leukemia cell line RBL, which spontaneously expresses Fc ϵ RI, IgE-mediated up-regulation was comparable to that observed with transfected Fc ϵ RI (Fig. 1A). Because, in the transfection system that we used here, expression was driven by a nonregulated viral

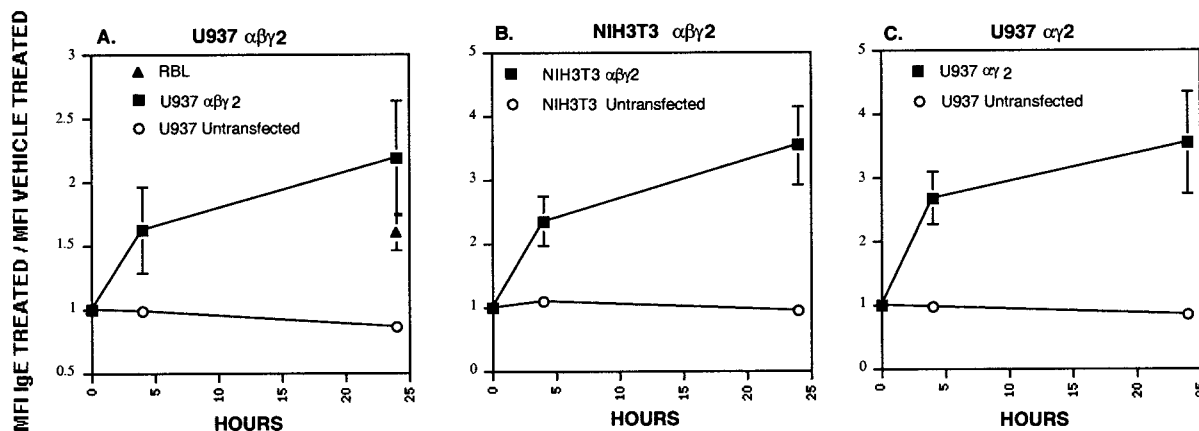


FIGURE 1. IgE can mediate up-regulation of Fc ϵ RI expression in U937 and NIH3T3 transfectants. A, U937 cells expressing the tetrameric ($\alpha\beta\gamma 2$) receptor (eight clones; ■) or untransfected U937 cells (○) were cultured in triplicate in the presence of saturating concentrations of monomeric IgE or vehicle for the indicated times. Cell surface expression of Fc ϵ RI was determined by flow cytometric analysis and expressed as the ratio of the MFI of IgE-treated cells over the MFI of vehicle-treated cells as a function of time. The data presented are the mean $\pm$ SD of eight independent experiments. Each clone was analyzed two to four times. Data for RBL cells (mean $\pm$ SD of two experiments) are also shown (▲). B, NIH3T3 cells transfected with the $\alpha\beta\gamma 2$ receptor (six clones; ■) or untransfected NIH3T3 cells (○) were cultured and analyzed as in A. The data presented are the mean $\pm$ SD of five independent experiments. Each clone was analyzed one to three times. C, U937 cells expressing the trimeric ($\alpha\gamma 2$) receptor (six clones; ■) or untransfected U937 cells (○) were cultured and analyzed as in A. Data presented are the mean $\pm$ SD of six independent experiments. Each clone was analyzed two to four times.

promoter, these studies demonstrate that IgE-mediated up-regulation does not require increased transcription. In addition, the ability to up-regulate FcεRI in a non-FcεRI expressing, monocytoid line demonstrates that IgE-mediated up-regulation does not require factors specific to mast cells or basophils.

We then tested whether hemopoietic-specific factors were required by similarly transfecting the fibroblast cell line NIH3T3 with the human α , β , and γ cDNAs. Here, too, IgE induced up-regulation of FcεRI expression ($p < E-4$ vs IgE-treated untransfected NIH3T3 and vs vehicle-treated transfectants; Fig. 1B). These experiments demonstrate that IgE-mediated regulation does not require hemopoietic specific factors.

The β -chain does not amplify IgE-mediated FcεRI up-regulation

In humans, the β -chain is not required for cell surface expression, and an alternative trimeric ($\alpha\gamma_2$) form exists. Although not required for expression, the human β -chain amplifies empty receptor expression. By associating with FcεRI α early in the endoplasmic reticulum, the β -chain enhances processing of α , and trafficking of unoccupied receptor to the cell surface (11). In addition, the β -chain may enhance the stability of the receptor, as suggested by increased resistance of $\alpha\beta\gamma_2$ receptors to dissociation in the presence of detergent (11). Because the β -chain favors receptor trafficking to the plasma membrane, it was possible that cells expressing the trimeric form of FcεRI would be less capable of increasing cell surface expression of FcεRI in response to IgE than $\alpha\beta\gamma_2$ receptors. A correlation has been shown between serum IgE levels and surface expression of FcεRI on blood cells expressing the trimeric receptor, such as monocytes (6), suggesting that cells expressing the trimeric receptor are indeed capable of up-regulating FcεRI. However, whether the β -chain would amplify IgE-mediated up-regulation has not been investigated. To examine the capacity of $\alpha\gamma_2$ receptors to up-regulate in response to IgE, we stably transfected U937 with the human α - and γ -chain cDNAs alone and measured FcεRI expression in these $\alpha\gamma_2$ transfectants in response to IgE. Our data showed that $\alpha\gamma_2$ receptors were up-regulated as efficiently as $\alpha\beta\gamma_2$ receptors and might be even more sensitive to IgE-mediated up-regulation than $\alpha\beta\gamma_2$ receptors, although further investigation would be needed to confirm this observation (Fig. 1C). Thus, we conclude that the β -chain does not amplify IgE-mediated FcεRI up-regulation.

The rate of IgE-mediated FcεRI up-regulation is independent of the baseline level of FcεRI expression

FcεRI expression levels vary substantially among our transfected cells with the mean fluorescence intensity (MFI) varying from 27 to 350 in U937 transfectants. To assess how the basal level of FcεRI expression influences IgE-mediated up-regulation, we plotted FcεRI expression after 24H of IgE treatment as a function of FcεRI expression after 24H of vehicle treatment for all the U937 clones ($n = 14$) used in this study. Because the plot could be fit to a straight line, it shows that FcεRI up-regulation is directly proportional to the basal level of FcεRI expression (Fig. 2; slope = 2.912; $r = 0.932$). This experiment demonstrates that the rate of FcεRI up-regulation by IgE is independent of the basal level of FcεRI expression in this reconstitution system.

Up-regulation is independent of an increase in message and intracellular protein levels and does not require signaling

The signaling pathway of FcεRI that results in degranulation and liberation of allergic mediators is initiated by phosphorylation of the β - and γ -chains, which results in the generation of calcium flux (reviewed in Refs. 8, 36, and 37). Aggregation of FcεRI expressed in NIH3T3 cells results in only minimal receptor phosphorylation

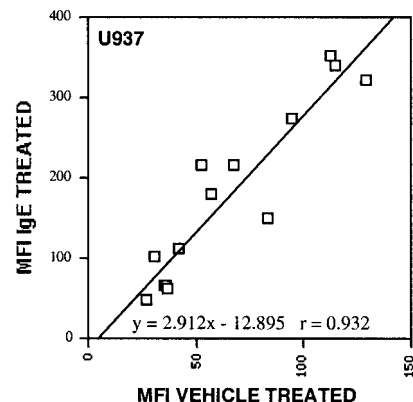


FIGURE 2. IgE-mediated up-regulation of FcεRI is proportional to basal expression level of FcεRI. Data from the U937 cells expressing the $\alpha\beta\gamma_2$ or $\alpha\gamma_2$ receptor (14 clones) shown in Fig. 1, A and C, are plotted as the ratio of MFI of IgE-treated cells at 24 h vs vehicle-treated cells at the same time point. The equation is that of the curve fitting to a straight line.

and does not induce calcium mobilization due to the lack of the hemopoietic-specific signaling apparatus involved in FcεRI signal transduction (33). This inability to signal combined with the ability to up-regulate in response to IgE in NIH3T3 transfectants (Fig. 1B) suggests that classical signaling (i.e., involving activation of the Src family kinase lyn) is not required for IgE-mediated up-regulation of FcεRI. To confirm that abrogation of the classical signaling pathway did not prevent IgE-mediated up-regulation, we assessed FcεRI transfectants made with a tyrosine-mutated form of Fcγ instead of wild-type γ . Mutation of the tyrosine residues in the immunoreceptor tyrosine activation motif in the γ -chain renders it incapable of signaling (38–41). When these cells were cultured in the presence of IgE, they showed increases in surface expression of FcεRI (Fig. 3), demonstrating that classical signaling is not required for up-regulation of FcεRI by IgE.

Some data suggest that monomeric IgE binding may in fact induce a weak signal (42). Although these observations may be secondary to contaminating aggregates of IgE, they raise the possibility that FcεRI is capable of another type of signaling that has yet to be described and that could be responsible for IgE-mediated up-regulation by increasing transcription, message stability, or protein synthesis. To evaluate this possibility, we examined total

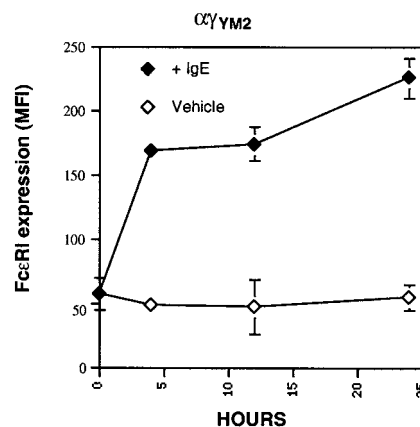


FIGURE 3. IgE-mediated FcεRI up-regulation does not require classical signaling. Cells transfected with a tyrosine-mutated γ -chain in addition to the α -chain were cultured in triplicate in the presence of IgE (◆) or vehicle (◇) and assessed for surface expression of FcεRI as in Fig. 1. The data are the mean $\pm$ the SD of triplicates and are representative of two experiments.

RNA and intracellular protein levels for the α -, β -, and γ -chains in cells during IgE-mediated up-regulation. U937 cells transfected with Fc ϵ RI were cultured in the presence of IgE or vehicle. At various time points, cell surface expression was measured by flow cytometric analysis and by immunoprecipitation of surface receptors. These levels were correlated to the levels of intracellular proteins detected by immunoprecipitation and to message levels detected by northern blot (Fig. 4). IgE treatment induced an increase in surface α , as assessed by immunoprecipitation (Fig. 4C), which correlated with the increase detected by flow cytometry (Fig. 4A) for both the tetrameric and trimeric receptor. Intracellular Fc ϵ RI α , β , and γ revealed no increase over the 8-h time period in response to IgE (Fig. 4D and data not shown). Northern blot showed no increase in message for α (Fig. 4B), β and γ (data not shown) at 4 or 8 h. These studies demonstrate that surface levels of Fc ϵ RI can be increased without increasing the rate of transcription or translation and without increasing message stability.

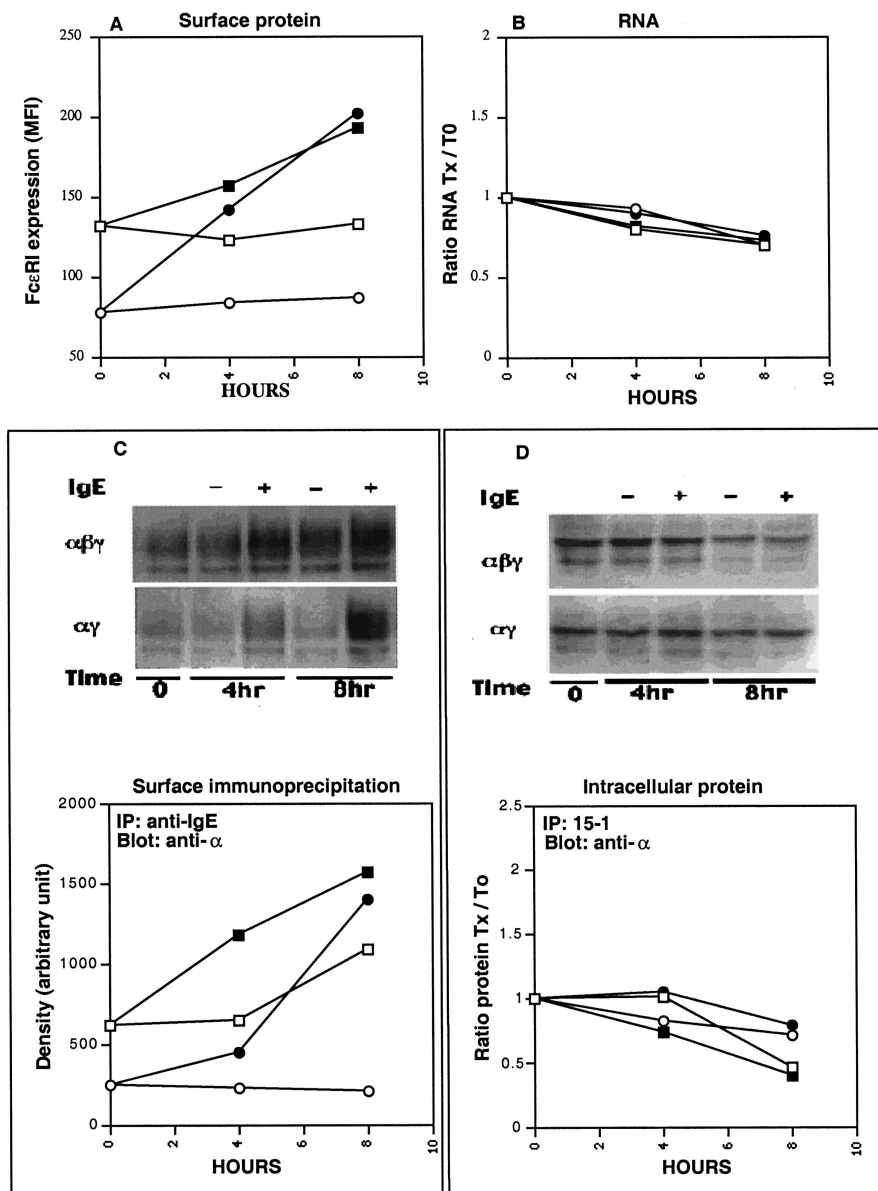
Decreased sensitivity to cycloheximide reflects use of a preformed pool of receptor

Recent studies have shown that IgE-mediated Fc ϵ RI up-regulation is sensitive to cycloheximide, reflecting a requirement for protein

synthesis (17). However, it has not been established whether this sensitivity reflects a requirement for an increase in protein synthesis or for the maintenance of the basal level of synthesis. Our results showing up-regulation in the absence of increased transcription suggest that the requirement for protein synthesis would be for maintenance of basal levels of protein synthesis rather than for increased protein synthesis. In addition, other studies have attributed the effect of IgE to inhibition of receptor degradation (14, 15, 43). In view of our results showing up-regulation in the absence of increased transcription and of classical signaling, the roles of receptor synthesis and receptor degradation were re-examined.

Studies of other cell surface receptors have examined the effects of cycloheximide and BFA to estimate the relative impact of synthesis and recycling on receptor expression. Whereas cycloheximide inhibits translation, BFA prevents anterograde movement of newly synthesized protein from the endoplasmic reticulum to the Golgi (44, 45). It also causes early endosomes to fuse with the trans-Golgi membrane and inhibits receptor recycling. Therefore, comparison of the effect of cycloheximide and that of BFA on receptor expression allows one to determine the relative contribution of synthesis and recycling to receptor expression at the plasma membrane. Expression of receptors that are long-lived and exhibit

FIGURE 4. IgE-mediated Fc ϵ RI up-regulation does not require an increase in message or intracellular protein level. U937 cells expressing either the $\alpha\beta\gamma 2$ (squares) or $\alpha\gamma 2$ (circles) receptor were cultured in the presence of IgE (filled symbols) or vehicle (open symbols) and harvested at the indicated times. The data are representative of two independent experiments. **A**, Cell surface expression of Fc ϵ RI was determined by flow cytometric analysis and expressed as MFI over time. **B**, RNA extracted from cells was probed with cDNA for Fc ϵ RI α and for GAPDH. At each time point, the α -specific signal was normalized with the GAPDH signal. Results are expressed as ratios of the normalized α signal at time x over that at time 0. **C**, Cells were incubated with IgE to saturate surface receptors. Cells were then lysed and surface receptors were immunoprecipitated with anti-IgE Ab and blotted with rabbit polyclonal anti- α Ab (top). The density of the α bands in the upper panel was measured by scanner and plotted as a function of time (bottom). **D**, To immunoprecipitate intracellular Fc ϵ RI α , the lysates from **C** (depleted of surface Fc ϵ RI α) were immunoprecipitated with an anti- α mAb and blotted with polyclonal rabbit anti- α Ab (top). The density of the α bands in the upper panel was measured by scanner and expressed as the ratio of the density of the bands at time x (Tx) over density at time 0 (To) and plotted as a function of time.



a high degree of recycling is sensitive to BFA and much less to cycloheximide. Expression of receptors that recycle very little and do not depend on the availability of intracellular pools but require protein synthesis is equally sensitive to cycloheximide and BFA. And expression of receptors that rely on protein synthesis and intracellular pools is more sensitive to BFA than cycloheximide (46).

We first investigated the effect of cycloheximide and BFA on FcεRI over time in the absence of IgE. U937 cells transfected with the tetrameric receptor were cultured in the absence of IgE with or without cycloheximide or BFA and harvested at various time points (Fig. 5A). FcεRI is expressed as the ratio of MFI of treated samples to MFI of untreated samples at the same time point. This study shows that receptor expression is sensitive both to cycloheximide ($p < E-4$) and to BFA ($p < E-4$). In addition, sensitivity to BFA is ~1.5-fold that to cycloheximide ($p < 5.5E-3$), an indication that receptor expression at steady state depends not only on protein synthesis but also on receptor recycling and the availability of an intracellular pool.

We then determined the relative dependency of the up-regulation process on protein synthesis and receptor recycling/use of a preformed pool. The transfectants were cultured with IgE or vehicle and with or without cycloheximide or BFA (Fig. 5B). FcεRI is expressed as the ratio of MFI of IgE-treated samples to MFI of vehicle-treated samples at the same time point. Cells treated with IgE and cycloheximide increased their cell surface expression of FcεRI for the first 4 h, but not after ($p < E-4$ vs vehicle treated). However, the IgE-mediated increase in FcεRI expression in the presence of cycloheximide is less than the increase in the absence

of cycloheximide ($p < 5E-4$ vs IgE-treated). This experiment demonstrates the existence of a pool of preformed receptors that proceed to the plasma membrane, bind IgE, and contribute to FcεRI up-regulation even in the presence of cycloheximide. Once this pool is exhausted (after 4 h) FcεRI up-regulation cannot be maintained in the presence of cycloheximide, demonstrating the complete dependence of FcεRI up-regulation on basal receptor synthesis once the pool of preformed receptors is exhausted. In contrast, BFA inhibited IgE-mediated up-regulation completely ($p < E-4$ vs IgE treated, analyzed by ANOVA). Inhibition also is immediate, as it is present already at 2 h ($p < 3E-2$, analyzed by *t* test) and it is maintained up to 8 h ($p < 3E-2$ at 4 and 6 h, $p < 3E-3$ at 8 h; analyzed by *t* test). Thus, the effect of BFA on IgE-mediated up-regulation is greater than that of cycloheximide ($p < 1E-2$), suggesting that, in addition to protein synthesis, use of a preformed intracellular pool and recycling also are involved in the IgE-mediated up-regulation. Together with our other data (Fig. 4) that show no increase in protein synthesis during the IgE-mediated up-regulation process, these data demonstrate that in this reconstituted system, maintenance of the basal level of synthesis, use of an intracellular pool and recycling are sufficient to assure IgE-mediated up-regulation.

IgE acts by stabilizing receptors at the plasma membrane

We reasoned that, under the conditions of basal level protein synthesis, once the preformed pool of receptor is depleted, the only way to increase surface expression of receptor is to decrease surface receptor internalization and degradation.

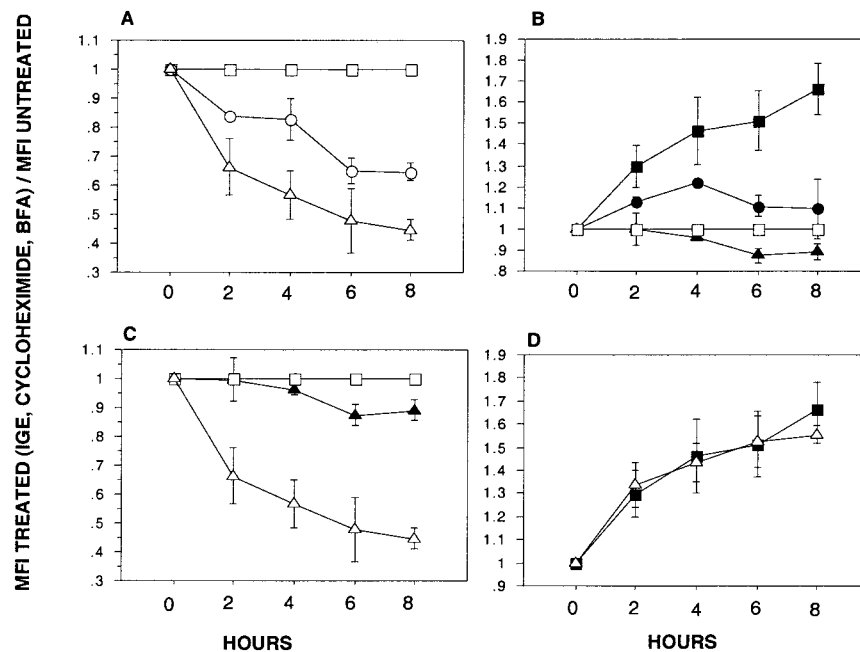


FIGURE 5. IgE mediates up-regulation of FcεRI via inhibition of receptor degradation, use of a preformed pool of receptor, and maintenance of basal levels of receptor synthesis. U937 cells expressing the $\alpha\beta\gamma 2$ receptor were cultured in triplicate in the presence of IgE or vehicle, and cycloheximide, BFA, or vehicle for the indicated times. FcεRI expression was assessed by flow cytometry and expressed as the MFI of treated cells to the MFI of vehicle-treated cells as a function of time. All data are the mean $\pm$ SD of four experiments. A, Steady-state surface expression of FcεRI is dependent on receptor synthesis and recycling. U937 cells cultured in the absence of IgE and in the presence of cycloheximide (○), BFA (△), or vehicle (□) for the indicated times. B, IgE-mediated up-regulation uses a pool of preformed receptors and is dependent on the maintenance of basal levels of receptor synthesis. U937 cells were cultured in the presence of IgE alone (■), IgE and cycloheximide (●), IgE and BFA (▲), or vehicle (□) for the indicated times. C, IgE binding to FcεRI inhibits receptor degradation. Data for U937 cells cultured in the presence of BFA alone (△), BFA plus IgE (▲), or vehicle (□) from A and B are replotted. D, IgE-mediated inhibition of receptor degradation is the mirror image of receptor degradation. Data for U937 cells cultured in the presence of IgE (■) and for cells cultured in the presence of BFA (△) from A and B are replotted. For cells treated with BFA (△), the numerical values (a) in A were recalculated as follows: $1 + (1 - a)$.

By definition, at steady state, receptor accumulation on the cell surface (from synthesis and recycling) is equal to receptor disappearance from the cell surface (including degradation and recycling). As indicated above, BFA blocks receptor accumulation and recycling and therefore is ideal to reveal degradation rates (Fig. 5A). IgE-mediated up-regulation could be attributable to stabilization of the receptors at the plasma membrane and inhibition of internalization, which would also inhibit recycling and degradation. Alternatively, it could be attributable to inhibition of degradation of internalized receptors and recycling back to the plasma membrane of these protected receptors. If inhibition of internalization is the major mechanism by which IgE mediates Fc ϵ RI up-regulation, it follows that (1) IgE should almost completely prevent the decrease in Fc ϵ RI expression induced by BFA; and (2) the up-regulation rate should be equal to the degradation rate. However, if inhibition of degradation of internalized receptors is the main mechanism, then one should expect IgE to prevent the decrease in Fc ϵ RI expression induced by BFA only in part. We addressed this question by replotting in Fig. 5, C and D, some of the data of Fig. 5, A and B. Fig. 5C shows that IgE almost completely prevented the decrease in Fc ϵ RI expression induced by BFA. In Fig. 5D, comparison of the mirror image of the BFA/no IgE curve from Fig. 5A and the IgE-treated curve from Fig. 5B shows that the rate of degradation is almost identical (but opposite) to the rate of up-regulation ($p > 0.05$, no significant difference). These results demonstrate that in this in vitro system, stabilization at the cell surface accounts for the majority of the up-regulation, providing that basal synthesis is maintained.

In vivo, it is likely that various factors such as IL-4 modulate the basic mechanism described here. In a study of fetal liver-derived human mast cells, IL-4 has been reported to synergize with IgE and increase the percentage of Fc ϵ RI-positive cells (47). IL-4 has also been shown to increase IgE-mediated Fc ϵ RI expression on cord blood-derived mast cells (23). However, mechanisms for these effects have not been investigated. It is unlikely that IL-4 would act on the receptor subunits during intracellular processing, at the cell surface, or after internalization. Rather, the synergy between IL-4 and IgE is more likely to result from an effect of IL-4 on transcription of the Fc ϵ RI subunits, given the fact that numerous effects of IL-4 are mediated through activation of transcription factors. An IL-4-induced increase in transcription of the Fc ϵ RI subunits would result in an increased availability of receptors that could be used for IgE-mediated up-regulation and in an increase in efficiency of up-regulation. This effect would translate into an increase in the slope of the straight line that describes the relationship between basal Fc ϵ RI expression and expression after IgE treatment (see Fig. 2). Other cytokines could also modulate the IgE-mediated up-regulation by affecting the basal level of synthesis of the receptor subunits.

Conclusions

Our studies show that binding of IgE to the α -chain of Fc ϵ RI is the minimal requirement to induce IgE-mediated up-regulation of Fc ϵ RI on the cell surface, as this regulation does not depend on transcriptional regulation, hemopoietic-specific factors, or classical signaling. Rather, up-regulation is the consequence of receptor stabilization at the plasma membrane, use of recycled and recently synthesized receptors, and continued basal level of receptor synthesis.

We propose that the α -chain is the primary candidate for initiating the receptor stabilization that plays a critical role in the IgE-mediated regulation. First, we show here that the β -chain is not necessary for receptor up-regulation. Second, a primary role for the γ -chain in receptor stabilization is unlikely because the γ -chain

is also part of Fc γ Rs, the expression of which is not influenced by IgG levels. This is based on the observation that mice that are devoid of B cells and therefore lack Igs, express normal levels of Fc γ RI, Fc γ RII, and Fc γ RIII (J. Ravetch, unpublished observations). In contrast, IgE-knockout mice express decreased levels of Fc ϵ RI (17, 18). However, a role for the γ -chain in conjunction with α cannot be ruled out because Fc ϵ RI α cannot be expressed at the cell surface without γ . And third, recently reported crystallography data show a conformational change in Fc ϵ RI α on IgE binding (48). Although the functional significance of this conformational change is not known, it is tempting to speculate that it mediates receptor stabilization. Further studies will be needed to address this point at the molecular level.

The in vitro system used here was designed and has allowed us to dissect the minimal requirements for IgE-mediated Fc ϵ RI up-regulation. It is likely that in vivo various factors, such as cytokines, may modulate this basic mechanism.

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