

A Second Amplifier Function for the Allergy-Associated FcεRI-β Subunit

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Summary

Genetics studies have identified the gene for the high-affinity IgE receptor (FcεRI) β subunit as a candidate gene for atopy. We have shown that β is an intrinsic signaling amplifier leading to enhanced allergic responses in vivo. Here we report that β has a second amplification function: the amplification of FcεRI cell surface expression. This function is due to an early association of β with α, resulting in improved trafficking and maturation of α and receptor complexes. These data provide a possible molecular explanation for the large difference in FcεRI density between β⁻ cells such as monocytes, dendritic cells, and β⁺ effector cells (mast cells, basophils). In β⁺ cells, the combined signaling and expression amplification results in an estimated 12- to 30-fold amplification of downstream events.

Introduction

The FcεRI complex forms a high-affinity cell surface receptor for the Fc region of immunoglobulin E (IgE) molecules (Kinet, 1999; Turner and Kinet, 1999). FcεRI is a member of a family of related antigen/Fc receptors that have conserved structural features and play similar roles in initiating intracellular signaling cascades. FcεRI controls the activation of mast cells and basophils (Daeron, 1997; Kinet, 1999; Turner and Kinet, 1999) and participates in IgE-mediated antigen presentation in monocytes and dendritic cells (Maurer et al., 1994, 1995, 1998, 1996). Multivalent antigens bind and cross-link IgE molecules held at the cell surface by FcεRI. Receptor aggregation induces multiple signaling pathways that control diverse effector responses, including secretion of allergic mediators and induction of cytokine gene transcription (including genes for IL-4, IL-6, TNFα, and GM-CSF) (Galli and Costa, 1995). FcεRI is therefore central to the induction and maintenance of an allergic response and, physiologically, may confer protection in parasitic infections (Jankovic et al., 1997; Kinet, 1999).

It is a conserved feature of antigen receptors that they are multimeric and devolve different functions to their individual subunits. In the case of FcεRI, the receptor is composed of three distinct protein species. The α

chain binds the Fc portion of IgE with high affinity (Hakimi et al., 1990; Blank et al., 1991; Robertson, 1993; Sutton and Gould, 1993; Letourneur et al., 1995a). The β chain has four transmembrane domains between amino- and carboxyl-terminal cytoplasmic tails (Kinet et al., 1988; Ra et al., 1989; Kuster et al., 1992). A homodimer of disulfide-linked γ chains completes the tetrameric structure (Blank et al., 1989; Ra et al., 1989; Kuster et al., 1990). In humans, the tetrameric structure is not obligatory; an alternate form is present, comprising an αγ₂ trimer (Miller et al., 1989; Kuster et al., 1990).

In terms of devolution of function, the α chain contains, in its extracellular part, two Ig-like domains that mediate binding to IgE Fc (Hakimi et al., 1990; Blank et al., 1991; Mallamaci et al., 1993; Nissim et al., 1993; Robertson, 1993; Sutton and Gould, 1993; Presta et al., 1994; Cook et al., 1997; Garman et al., 1999; Hulett et al., 1999), but its short cytoplasmic tail does not mediate signal transduction (Alber et al., 1991). The β and γ chains contain conserved immunoreceptor tyrosine-based activation motifs (ITAM) in their cytoplasmic tails that are phosphoacceptors and through which β and γ interact with signaling proteins (Reth, 1989; Letourneur and Klausner, 1991; Romeo and Seed, 1991; Cambier, 1995). However, β is unique in its function. In contrast to other ITAM-containing chains, β does not appear to possess any signaling capacity of its own but acts as an amplifier of signals generated by the other ITAM-containing chains of FcεRI, the γ chains (Jouvin et al., 1994; Lin et al., 1996). At the same receptor density, cells expressing the tetrameric FcεRI αβγ₂ generate signals that are 3- to 5-fold stronger than those from cells lacking β (Lin et al., 1996). Similarly, systemic anaphylactic responses are amplified in mice expressing the β chain compared to mice expressing similar surface FcεRI levels but lacking the β chain (Dombrowicz et al., 1998).

The two human isoforms of FcεRI, the tetramer αβγ₂ and the trimer αγ₂, have different cell distributions. Mast cells and basophils transcribe β and express the tetramer, whereas monocytes, cutaneous Langerhans cells, and dermal and peripheral blood dendritic cells lack β transcripts and express only the trimer (Bieber et al., 1992; Wang et al., 1992; Maurer et al., 1994; Osterhoff et al., 1994). It seems that eosinophils may express transcripts for β, but it is unclear whether the protein is translated (Gounni et al., 1994; Smith et al., 2000). Another feature that separates these two groups of cell types is the fact that FcεRI surface expression is 10- to 100-fold lower on cells that express the trimer (monocytes, dendritic cells, and eosinophils) than on cells that express the tetrameric receptor (mast cells and basophils) (Gounni et al., 1994; Sihra et al., 1997; Kita et al., 1999). The reason for this difference in receptor density is unknown. However, it is important to understand how the difference is generated, since the receptors in the FcεRI β⁻ context are primarily thought to mediate antigen presentation to T cells, while the FcεRI β⁺ receptors mediate mast cell activation and subsequent release of allergic mediators. It is conceivable that the β chain, in

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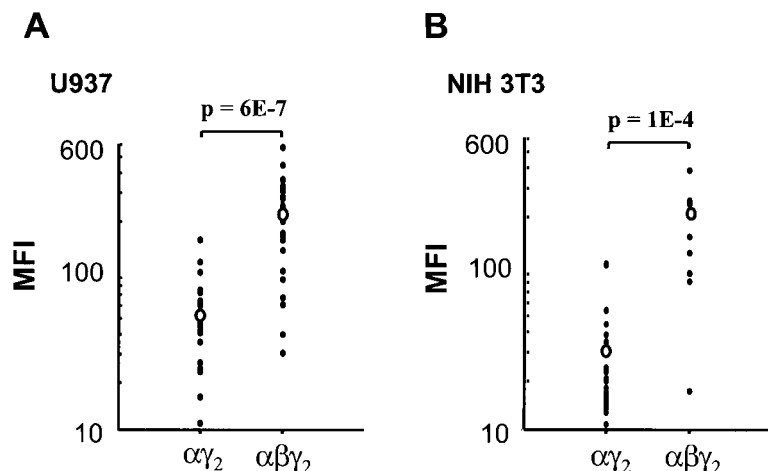


Figure 1. Distribution of FcεRI Expression in U937 and NIH 3T3 Stable Transfectants

U937 (A) and NIH 3T3 (B) cell lines were stably transfected with the α and γ cDNAs or the α , β , and γ cDNAs. FcεRI expression was analyzed on all the G418-resistant clones by flow cytometry with biotinylated human IgE followed by PE-conjugated streptavidin. Results are expressed as the mean fluorescence intensity (MFI) for each clone. The mean for each group is indicated by a white dot. This is one experiment representative of three (U937) and two (NIH 3T3) independent transfections. The mean $\pm$ SD for each group was calculated. In each cell type the means for $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ transfectants were compared with an unpaired t test.

addition to its role as a signal amplifier, could also play a role in setting these different FcεRI surface levels. This raises the possibility that β may play more than one role in defining the threshold and hence outcome of FcεRI-mediated responses. However, the question of a role for β in regulating FcεRI expression has not been investigated.

The current study addresses the question of a possible role for β in receptor surface expression. We have generated multiple $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ transfectants for our study of signaling amplification by β . Previously, we had selected $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ transfectants expressing the same FcεRI density in order to assess the intrinsic signaling capacity of both forms of receptor. Here, we use this system to examine the role of β in setting receptor surface levels. We show that the β chain facilitates FcεRI surface expression by promoting the processing and export of the α chain to the cell surface and by enhancing stability of receptor protein complexes. This represents a second amplifier function for β , where the presence or absence of β profoundly changes FcεRI surface levels.

Results

$\alpha\beta\gamma_2$ FcεRI Receptors Are More Highly Expressed at the Cell Surface Than $\alpha\gamma_2$

Human FcεRI is expressed as two isoforms, $\alpha\beta\gamma_2$ and $\alpha\gamma_2$, which have different cell type distributions. Basophils and mast cells, which transcribe the β chain, express 10–100 more receptors at the cell surface than monocytes, eosinophils, and dendritic cells, which do not express β (Gounni et al., 1994; Sihra et al., 1997; Kita et al., 1999). In an effort to understand the receptor density difference between $\alpha\beta\gamma_2$ - and $\alpha\gamma_2$ -expressing cells, we have tested the hypothesis that the β chain could be responsible for increased FcεRI at the cell surface.

We have generated stable transfectants expressing either form of FcεRI and analyzed their spectrum of receptor expression. U937 cells were stably transfected with either the α , β , and γ cDNAs or the α and γ cDNAs, and all the selection-resistant clones were expanded and analyzed in order to assess the whole range of receptor expression. Surface FcεRI expression was analyzed by flow cytometry after staining with IgE. Figure 1A

shows the distribution of surface FcεRI levels in clones expressing $\alpha\gamma_2$ and $\alpha\beta\gamma_2$. The $\alpha\gamma_2$ transfectants express 51.3 ± 34.3 mean fluorescence intensity (MFI) units (mean $\pm$ SD) ($n = 23$ clones), whereas $\alpha\beta\gamma_2$ transfectants express 217.4 ± 136.2 MFI ($n = 31$). This difference in FcεRI surface levels is highly significant ($p = 6E-7$). A total of three transfections were performed independently and yielded similar results (data not shown).

We then investigated whether this effect was specific to hematopoietic cells or could also be observed in a nonhematopoietic cell line, the fibroblast cell line NIH 3T3. Figure 1B shows that β also upregulates receptor expression in NIH 3T3. In this cell type, $\alpha\gamma_2$ transfectants express 30.2 ± 24.2 MFI units ($n = 37$), whereas $\alpha\beta\gamma_2$ transfectants express 208.7 ± 207.8 MFI ($n = 13$) ($p = 1E-4$). Another transfection performed independently yielded similar results (data not shown).

These data establish a strong correlation between the presence of the β chain and the increased expression of the IgE binding α chain at the cell surface (4- to 6-fold increase) and show that no additional hematopoietic specific factor seems to be necessary for this enhanced expression.

Increased Cell Surface Expression of IgE Binding α Chain Is Due to the β Chain

The easiest interpretation of the above results is that β itself facilitates the surface expression of α chain. In this case, one would predict that $\alpha\gamma_2$ -expressing cells that otherwise express stable levels of cell surface receptors should upregulate their receptor expression levels upon simple introduction of β . In order to test this hypothesis, we first generated $\alpha\gamma_2$ stable transfectants in KU812, a basophilic cell line with no endogenous expression of FcεRI at the cell surface. We then transfected transiently one of these stable $\alpha\gamma_2$ clones with the β cDNA or with an irrelevant DNA and with a construct encoding the green fluorescent protein (GFP) to control for transfection efficiency. Surface levels of FcεRI were measured 8–48 hr after retransfection (Figure 2). Indeed, receptor expression is higher after β transfection compared to control transfection at all time points. At 48 hr, β induces a FcεRI expression level of 164 MFI units compared to 92 for the control cDNA ($p = 3.3E-5$). Thus, the β chain

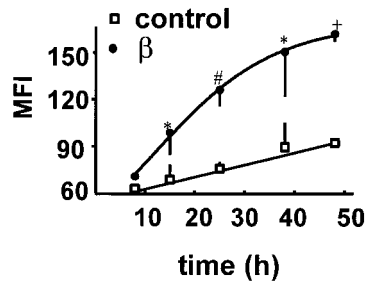


Figure 2. FcεRI Levels in $\alpha\gamma_2$ Cells after Retransfection with β KU812 clone stably expressing $\alpha\gamma_2$ was transiently cotransfected with the β cDNA (closed circles) or a control cDNA (open squares) and with a GFP construct. Cells were stained as in Figure 1. Results from two to three experiments for each time point are expressed as MFI of GFP-positive cells at different times after transfection. The means $\pm$ SD for each group were calculated and compared between β and control retransfections at each time point (asterisk, NS; number sign, $p = 2.1 \times 10^{-3}$; plus, $p = 3.3 \times 10^{-5}$).

is specifically responsible for increasing α chain expression. Note, however, that the lower intensity of the expression amplifying effect when β is expressed transiently (less than 2-fold increase) compared to that when β is stably expressed (4- to 6-fold increase) is likely due to the presence of non- β expressing cells in the GFP-positive population and of dividing cells, which do not replicate the nonintegrated transfected β construct. In spite of this caveat, this experiment establishes qualitatively that the β chain is specifically responsible for increasing the surface expression of IgE binding α chain.

The β Chain Amplification of α Chain Surface Expression Is Posttranslational

We then investigated whether the β chain could increase the expression of IgE binding α chain at the level of synthesis and/or processing of the α chain in a pulse-chase experiment. We compared the rate of accumulation of nascent α chain in $\alpha\beta\gamma_2$ and in $\alpha\gamma_2$ clones using an $\alpha\beta\gamma_2$ clone with a higher level of cell surface α chain than the $\alpha\gamma_2$ clone (Figure 3A). The cells were pulsed for 15 min with a [^{35} S]methionine/cysteine labeling cocktail and chased for 0, 45, or 90 min with a cold amino acid mixture. The lysates were then immunoprecipitated with an anti- α monoclonal antibody, 15-1, and the immunocomplexes were resolved by SDS-PAGE (Figure 3B). The intensity of the bands corresponding to nascent α chain was quantified and plotted as a function of time (Figure 3C). If the increased cell surface expression of α chain seen in $\alpha\beta\gamma_2$ cells is due to a positive effect of the β chain on α chain synthesis, one should expect the rate of accumulation of nascent α chain to be higher in $\alpha\beta\gamma_2$ cells than in $\alpha\gamma_2$ cells. However, these data show that at time 0, there is more nascent α chain accumulated in the $\alpha\gamma_2$ than in the $\alpha\beta\gamma_2$ clone. Moreover, the accumulation of nascent α chain in the $\alpha\gamma_2$ clone increases substantially at 45 min and remains stable after 90 min of chase. In contrast, the accumulation seen in the $\alpha\beta\gamma_2$ clone after 45 min is lower than in the $\alpha\gamma_2$ clone and decreases substantially after 90 min. Another such experiment (data not shown) confirmed that there is less nascent α chain accumulated in an $\alpha\beta\gamma_2$ than in an $\alpha\gamma_2$

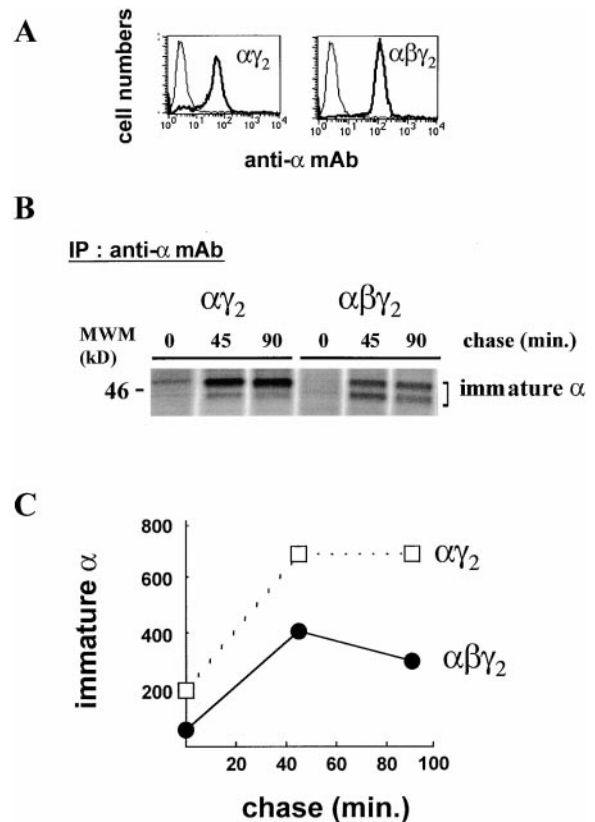


Figure 3. Immature α Is More Stable in $\alpha\gamma_2$ Clones Than in $\alpha\beta\gamma_2$ Clones

One $\alpha\gamma_2$ and one $\alpha\beta\gamma_2$ clone with comparable surface FcεRI expression levels were pulsed for 15 min and then analyzed immediately (chase 0 min) or after 45 or 90 min chase. Cells lysates were immunoprecipitated with the anti- α mAb 15-1 and separated by SDS-PAGE. (A) FcεRI expression of the $\alpha\beta\gamma_2$ clone and the $\alpha\gamma_2$ clone used in (B) was measured by flow cytometry after staining with the anti- α mAb 15-1 followed by FITC-GAM. Left panel, $\alpha\gamma_2$ clone; right panel, $\alpha\beta\gamma_2$ clone. Untransfected cells (thin line) were analyzed in parallel. (B) SDS-PAGE analysis showing the immature α bands after 0, 45, and 90 min chase. (C) Plot of the immature α band intensity in arbitrary units as a function of time for the $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ clones shown in (B).

clone even though the $\alpha\beta\gamma_2$ clone expresses more surface α chain than the $\alpha\gamma_2$ clone. In that experiment, the accumulated nascent α chain seen after 30 min is almost cut by half after 60 min chase in the $\alpha\beta\gamma_2$ clone, but it continues to increase in the $\alpha\gamma_2$ clone. Together, these data indicate that the presence of the β chain does not result in an increased α chain synthesis but induces an acceleration of the α chain processing. Thus, in $\alpha\gamma_2$ -expressing cells, there is a relative retention of the newly synthesized α chain while there is a relative disappearance of the nascent α chain in $\alpha\beta\gamma_2$ -expressing cells.

FcεRI α and β Associate Early in the Endoplasmic Reticulum

It is clear from the analyses presented above that the presence of FcεRI β accelerates the processing of nascent α chains and enhances the cell surface expression of α , suggesting that β may affect (1) the tendency of α

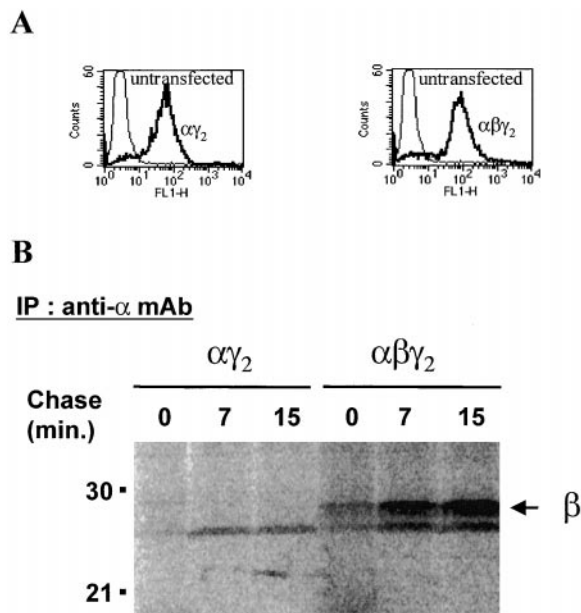


Figure 4. Fc ϵ RI α and β Associate Very Early after Biosynthesis in the ER

(A) Surface Fc ϵ RI expression of the $\alpha\beta\gamma_2$ clone and the $\alpha\gamma_2$ clone used in (B) was measured by flow cytometry after staining with the anti- α mAb 1511 followed by FITC-GAM. Left panel, U937 clone transfected with α and γ cDNAs; right panel, U937 clone transfected with α , β , and γ cDNAs. Untransfected cells (thin line) were analyzed in parallel.

(B) The $\alpha\gamma_2$ and $\alpha\beta\gamma_2$ U937 transfectants shown in (A) were pulsed with a metabolic labeling cocktail (70% [^{35} S]methionine, 15% [^{35} S]cysteine) for 5 min followed by a chase for the indicated times. Lysates were immunoprecipitated with the anti- α mAb 15-1 and resolved by SDS-PAGE.

chains to progress through the endoplasmic reticulum (ER)/Golgi and attain the cell surface and/or (2) the stability of assembled Fc ϵ RI during synthesis or at the cell surface. We hypothesize that for either case to be an important mechanism, α and β association must occur early in the process of biosynthesis. The issue of when the α/β association is first established has not previously been addressed.

We performed a pulse-chase experiment to analyze the dynamics of the $\alpha\beta$ association. One $\alpha\gamma_2$ and one $\alpha\beta\gamma_2$ clone with similar levels of surface expression (Figure 4A) were pulsed for 5 min with a [^{35} S]methionine/cysteine labeling cocktail and chased for 0, 7, or 15 min with a cold amino acid mixture. The lysates were then immunoprecipitated with an anti- α monoclonal antibody, 15-1, and immunocomplexes were resolved by SDS-PAGE (Figure 4B). In the $\alpha\beta\gamma_2$ clone, β can be coprecipitated with α after 5 min labeling and 0 min chase. Thus, α and β can be coimmunoprecipitated after no more than 5 min of protein synthesis has taken place. These results indicate that the α/β interaction occurs very early in biosynthesis while both nascent receptor chains are still in the ER.

Increased Stability in Detergent of $\alpha\beta\gamma_2$ Complexes Compared to $\alpha\gamma_2$ Receptors

We next wished to ask what advantage was conferred by the presence of β that resulted in improved surface

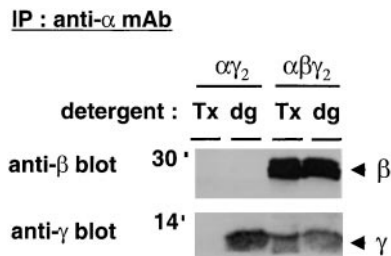


Figure 5. Fc ϵ RI $\alpha\beta\gamma_2$ Are More Stable in the Presence of Detergents Than $\alpha\gamma_2$ Complexes

The same $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ clones as in Figure 4 were lysed in a buffer containing either 0.5% Triton X-100 (Tx) or 1% digitonin (Dg). Lysates were precipitated with anti- α mAb, subjected to SDS-PAGE, and immunoblotted with anti- β (upper panel) and anti- γ (lower panel) Abs.

attainment by new Fc ϵ RI protein complexes. We tested the idea that β chains may enhance the stability of receptor complexes, an effect that would have implications for Fc ϵ RI complexes both during synthesis and when finally assembled at the cell surface.

In the cell, entropy determines that nascent and assembled protein complexes will exist in a state of equilibrium where the tendency to dissociate is balanced by intermolecular forces of attraction and stabilization. We attempted to mimic the action of the forces that tend to dissociate protein complexes *in vivo* using the *in vitro* application of detergents of various stringencies. We compared the stability of $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ Fc ϵ RI complexes *in vitro* in the presence of digitonin and Triton X-100, the latter being a harsher dissociating agent than the former. The data in Figure 5 show the results of this comparison. Postnuclear lysates were prepared from either $\alpha\gamma_2$ - or $\alpha\beta\gamma_2$ -expressing clonal cell lines, using either Triton X-100 or digitonin as the disrupting detergent. Immunocomplexes were purified using an anti- α monoclonal antibody and the presence of copurified β or γ chains was assessed by specific Western analysis. It is clear that the presence of β allows copurification of $\beta\gamma$ complexes in both detergent conditions, while in the absence of β the association between α and γ is completely disrupted by Triton X-100. Thus, where an exogenous dissociating agent is applied, the presence of β helps maintain the Fc ϵ RI complex. We would interpret this to mean that, *in vivo*, the β -containing Fc ϵ RI would be more stable than $\alpha\gamma$ receptors, both during biosynthesis and once expressed at the cell surface.

Fc ϵ RI β Enhances the Trafficking and Maturation of α Chains

We have shown that Fc ϵ RI β associates very early in the ER with α chains, enhances the stability of receptor complexes, accelerates the processing of nascent α chain, and enhances the surface expression of receptor complexes. If indeed the acceleration of processing of α in the ER due to β and the outcome at the cell surface are connected, then an effect of β on maturation and trafficking of α chains should be present. In that case, one should expect that $\alpha\beta\gamma_2$ cells and $\alpha\gamma_2$ cells with similar levels of Fc ϵ RI on the cell surface have very different steady state levels of immature α chain, and

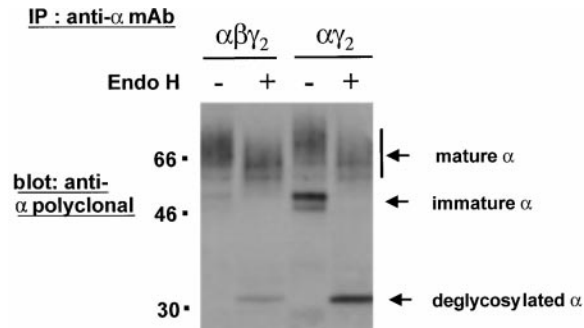


Figure 6. $\alpha\gamma_2$ Clones Contain More Immature α Than $\alpha\beta\gamma_2$ Clones for the Same Level of Surface FcεRI

The same clones as in Figure 4 were analyzed. Cells were lysed and immunoprecipitated with the anti- α mAb 15-1. Immune complexes were treated (plus) or not (minus) with endoglycosidase H (endo H), subjected to SDS-PAGE and transferred to PVDF membrane. Mature and immature forms of α were revealed by immunoblotting with anti- α polyclonal Ab 997.

these steady state levels should be lower in $\alpha\beta\gamma_2$ cells than in $\alpha\gamma_2$ cells.

We have exploited α chain glycosylation to follow processing of the receptor complex in the presence and the absence of the β chain. The α chain is highly and heterogeneously N-glycosylated, with about 50% of the molecular weight of cell surface α due to sugars. The newly synthesized α chain is core-glycosylated (mannose rich) in the ER and is thus sensitive to the action of endoglycosidase H (endo H). It then is transferred to the Golgi, where terminal glycosylation occurs, which replaces mannose rich sugars with complex sugars that are endo H resistant. Thus, these various forms of α can be distinguished by a combination of endo H treatment and SDS-PAGE.

$\alpha\gamma_2$ and $\alpha\beta\gamma_2$ clones with similar levels of FcεRI surface expression (same clones as in Figure 4A) were lysed and the α chains precipitated with anti- α mAb (15-1), which can precipitate both mature and immature forms of α (Letourneur et al., 1995a). The samples were then treated, or not, with endo H and resolved by SDS-PAGE. Western analysis was performed using a polyclonal anti- α antibody (997) (Figure 6). Two forms of α are present in both $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ clones. A form around 46 kDa gives rise, after endo H treatment, to a band at around 30 kDa, which is the expected molecular weight for the core protein. This form represents the immature ER α . A smear around 66 kDa is mainly endo H insensitive and represents mature α . These data show that in two clones expressing similar levels of surface FcεRI, the amount of immature α chain is much greater at steady state in the $\alpha\gamma_2$ clone than in the $\alpha\beta\gamma_2$ clone.

To investigate whether the same observation could be made in clones with very different levels of surface expressed FcεRI, we performed a similar analysis with three $\alpha\beta\gamma_2$ and three $\alpha\gamma_2$ clones, one each with low, medium, or high FcεRI expression levels. For each clone the intensity of the mature α band was plotted as a function of the intensity of the immature α band (Figure 7A). This plot shows that $\alpha\gamma_2$ clones require much more immature α chain in the ER to express the same level of mature α chain than $\alpha\beta\gamma_2$ clones. In order to perform

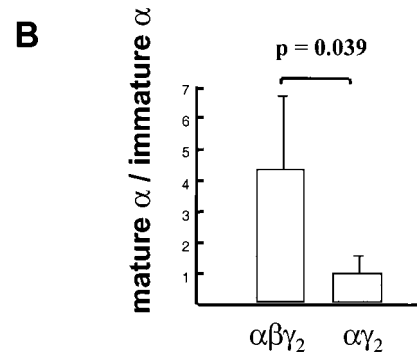
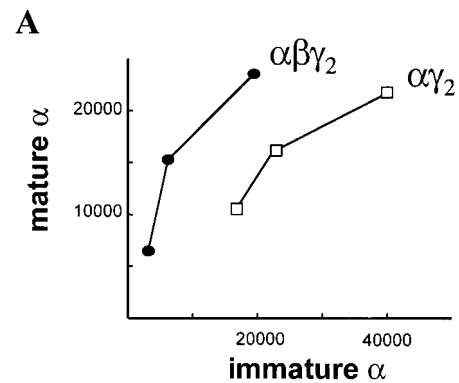


Figure 7. The Presence of the β Chain Favors the α Chain Maturation

(A) Three $\alpha\beta\gamma_2$ and three $\alpha\gamma_2$ clones with low, medium, and high FcεRI expression levels were analyzed by Western blotting after immunoprecipitation with an anti- α Ab. The intensities of the bands corresponding to mature and immature α (as defined in Figure 6) were measured by scanning densitometry. For each clone the intensity of the mature α bands was plotted as a function of the intensity of the immature α bands.

(B) The ratio between mature α and immature α in three ($\alpha\beta\gamma_2$) and two ($\alpha\gamma_2$) uncloned stably transfected populations and three $\alpha\gamma_2$ and three $\alpha\beta\gamma_2$ clones was calculated from experiments similar to the one depicted in (A). Results are expressed as mean $\pm$ SD. The means $\pm$ SD for each group were calculated and compared using an unpaired t test.

statistical calculations, we then analyzed more samples (five of each type) and calculated the ratio between mature α and immature α (Figure 7B). The mature α /immature α ratio is significantly higher in the $\alpha\beta\gamma_2$ cells (4.33 ± 2.38 , mean $\pm$ SD) than in the $\alpha\gamma_2$ cells (1.03 ± 0.6) ($p = 0.039$). Taken together with the pulse-chase experimental results that demonstrate an accelerated processing of α chain in the presence of the β chain, these data demonstrate that α maturation is considerably improved in the presence of the β chain. Remarkably, the increased ratio (4-fold increase) that reflects the improvement of the processing of α correlates closely with the increased surface expression described in Figure 1 (4- to 6-fold increase), suggesting that the majority of the surface expression increase is due to improved processing. Thus, α maturation is considerably improved in the presence of the β chain, linking the early association of α and β and the enhanced stability of β -containing

complexes to the phenotype of β amplification of receptor surface expression.

Discussion

Human Fc ϵ RI is expressed as two isoforms, $\alpha\beta\gamma_2$ and $\alpha\gamma_2$. These two isoforms differ in their capacity to signal, the tetramer $\alpha\beta\gamma_2$ being 3- to 5-fold more active than the trimer $\alpha\gamma_2$. This difference is due to the β chain, which acts as an amplifier of signals generated by the γ chain, causing an enhancement in Syk tyrosine phosphorylation, Syk kinase activity, and intracellular calcium mobilization (Lin et al., 1996). These effects translate directly in vivo to enhanced systemic anaphylactic responses (Dombrowicz et al., 1998). Here we show that in addition to this enhancement of signaling by mature surface-expressed receptors, β is an amplifier of receptor expression.

We have used a reconstitution system to demonstrate the expression amplifying role of β . In this system transfectants were generated with either the α , β , and γ cDNAs or only the α and γ cDNAs. In the hematopoietic cell line U937, $\alpha\beta\gamma_2$ transfectants express on average 4-fold more receptors than $\alpha\gamma_2$ transfectants. β also acts as a receptor amplifier in a heterologous cell line, the NIH 3T3 fibroblast cell line, where $\alpha\beta\gamma_2$ transfectants express on average almost 6-fold more receptors than $\alpha\gamma_2$ transfectants (Figure 1). That β is directly responsible for this expression amplification is demonstrated by its ability to upregulate Fc ϵ RI surface expression when transfected transiently in stable $\alpha\gamma_2$ -expressing cells (Figure 2). We also present biochemical data on the mechanism by which this amplification function of β is mediated. We show that β acts by accelerating the processing of nascent α chain (Figure 3) and by promoting the maturation of the IgE binding α chain (Figures 6 and 7). This effect is likely mediated by an early association between α and β in the ER (Figure 4) and possibly by an increased stability of the nascent Fc ϵ RI complex, as shown by the higher resistance to detergent dissociation of $\alpha\beta\gamma_2$ complexes when compared to $\alpha\gamma_2$ complexes (Figure 5).

The important receptor expression amplifying function of β has not been studied or described previously. When we investigated the signal amplifying function of β , it was critical that we compare transfected cell lines (and engineered mouse strains) expressing comparable levels of surface Fc ϵ RI. Therefore, for functional analysis we selected $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ clones with matching Fc ϵ RI expression. This strategy allowed us to demonstrate that the signal amplifier function is an intrinsic property of β and not a consequence of higher Fc ϵ RI expression. However, it masked the receptor expression-amplifying function of β , which became apparent only when we analyzed all the $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ selection-resistant clones.

In human Fc ϵ RI, in contrast to rodent Fc ϵ RI, β is dispensable for receptor expression. However, the human receptor expression-amplifying function of human β suggests that the difference between the human and rodent systems may be more of degree than nature, with a requirement for β in the rodent system and an actively positive effect of β in the human system. Therefore, it appears likely that what makes β mandatory

for rodent Fc ϵ RI expression is also responsible for the expression amplifier effect in the human system. The inability of rodent α to be expressed at the cell surface with γ in the absence of β points toward a feature specific to rodent α . Thus, α is either incapable (rodent), or poorly capable (human) of trafficking to the cell surface with the γ chain alone. This dependence on β for α expression at the cell surface could be due to the presence of an ER retention motif in the α chain that would be masked by the β chain, by analogy with a similar motif in the intracellular tail of α that is masked by the γ chain (Letourneur et al., 1995b). The additional retention motif that we propose here would be located in the extracellular part of rodent α , since the extracellular part of human but not rodent α can be expressed by itself as a soluble protein (Blank et al., 1991).

The recently elucidated crystal structure of the extracellular part of the Fc ϵ RI α may provide insight into its site of interaction with β . The two Ig domains of α are positioned at an acute angle to one another, forming a convex surface at the top of the molecule, where some of the amino acids previously implicated in IgE binding are located, and a marked cleft enclosed by the two globular Ig domains that faces the plasma membrane and is not involved in IgE binding. This configuration suggests that residues located in this cleft could be involved in the interaction with β and γ .

The Fc ϵ RI expression amplifier role of β may explain the fact that mast cells and basophils, which transcribe β , express 10–100 more surface Fc ϵ RI than monocytes and dendritic cells, which lack β . Eosinophils express very low surface levels of Fc ϵ RI, however, the α and γ proteins, but not β , can be detected inside the cells (Seminario et al., 1999). This suggests that in the absence of β , most $\alpha\gamma_2$ complexes do not proceed to the surface. This phenomenon parallels what we have observed with transfected cells, where a larger amount of immature α compared to mature α is present inside the cell in $\alpha\gamma_2$ transfectants than in $\alpha\beta\gamma_2$ transfectants (Figures 6 and 7). These two observations suggest a mechanism for the expression amplification function of β by which β would act as a sort of chaperone for α during maturation. Such a mechanism has been demonstrated for other multimeric membrane proteins such as, for example, the voltage-gated potassium channel Kv1.2. One of the β subunits, Kv β 2, increases efficiency of Kv1.2 surface expression by associating cotranslationally with the Kv1.2 α subunit, promoting N-glycosylation of Kv1.2 α and increasing stability of Kv β 2/Kv1.2 complexes (Shi et al., 1996). Fc ϵ RI shares some characteristics with this system. First, glycosylation of the α chain is required for efficient processing in eukaryotic cells, which indicates that it is necessary for interaction with the eukaryotic cell machinery but is not required for expression of the protein per se (Letourneur et al., 1995a). Second, α and β associate very early after de novo protein synthesis (Figure 4). Third, Fc ϵ RI $\alpha\gamma_2$ complexes dissociate more easily than $\alpha\beta\gamma_2$ complexes in detergent (Figure 5). Fourth, α glycosylation maturation is more efficient in the presence of β (Figures 6 and 7).

We have measured the increase in Fc ϵ RI attainment of the cell surface in the presence and absence of the β chain. Overall, β appears to increase receptor expression at the cell surface 4- to 6-fold. When combined

with the 3- to 5-fold signal amplification that we have calculated β to confer upon FcεRI responses to antigen, the overall effect of having β in an FcεRI complex is likely to be a 12- to 30-fold amplification of downstream events. FcεRI β is thus an extremely important modulator of outcomes in the generation and maintenance of an allergic response.

This property of β raises the important question of why a mechanism for generating such a substantial amplification of antigen-derived signals has evolved. One possibility would be to compensate for the relatively low levels of serum IgE present even in patients with parasitic infections, a situation in which the protective benefit of an IgE response has been suggested (Hagan et al., 1991). The flip side of this effect is the fact that mast cell responses to tiny amounts of antigen/IgE may be amplified to potentially devastating levels of effector response, resulting in allergic and anaphylactic pathology.

In humans, FcεRI-expressing cells are divided into two groups, β⁺ and β⁻. Our data make it clear that this difference will translate to vastly different cellular responses to antigen/IgE. Human cells expressing β-containing FcεRI are those involved in allergic mediator secretion, whereas cells lacking β are primarily thought to use FcεRI for antigen presentation, since antigen presentation by monocytes is much more efficient when antigen contacts these cells via binding of antigen-specific IgE to FcεRI than in the absence of specific IgE (Maurer et al., 1995, 1998). By analogy, eosinophils could also be involved in antigen presentation (Gounni et al., 1994; Kita et al., 1999). Lack of the β chain on monocytes and dendritic cells, and possibly eosinophils, may reflect the following: first, that lower FcεRI surface levels are more appropriate for antigen presentation than for FcεRI-mediated effector cell activation, thus, there is no need for the β chain to promote the energetically expensive trafficking of high levels of FcεRI to the surface. Second, additional signal amplification, as provided by the β chain, may be unnecessary on cells where no or little effector response is to be induced by antigen. The lack of β chain may even serve to actively avoid any induction of inappropriate signaling pathways using monocyte or dendritic cell signaling proteins that are present in order to transduce signals from other receptors such as the FcγR family. Some minimal signaling is observed after ligation of monocyte αγ₂ receptor (Maurer et al., 1994), and the biological significance and outcomes of these signals in the context of an IgE-driven immune response remain to be investigated.

Since β has been proposed as a candidate gene for atopy, the new expression-amplifying function described here, in addition to the known signaling-amplifier function of β, reinforces the importance of β as a key modulator in allergy, and any process able to increase β expression will translate into drastic enhancement of FcεRI-mediated response.

Experimental Procedures

Cell Culture, Antibodies, and Reagents

U937- and NIH 3T3-transfected cell lines were generated and maintained as described (Lin et al., 1996). Transfected KU812 cells were maintained in RPMI 1640 supplemented with 16% fetal bovine serum, 2 mM L-glutamine, and 0.6 mg/ml G418. The rabbit anti-FcεRI

β 961, the monoclonal (mAb) (15-1) and polyclonal (997) anti-human FcεRI α, and the polyclonal anti-γ chain antibodies (Ab) were prepared as described (Letourneur et al., 1991, 1995; Wang et al., 1992; Maurer et al., 1996). Human IgE was from Dr. Z. Eshhar (Schwarzbaum et al., 1989).

Construction of Stable Transfectants

The human α and γ cDNAs were subcloned in the pCDL SRα296 eukaryotic expression vector (Yokota et al., 1986), the human β cDNA in the pBJ1neo eukaryotic expression vector containing a neo resistance cassette (Lin et al., 1990). When the α and γ but not β cDNAs were cotransfected, the empty pBJ1neo was cotransfected. U937 and NIH 3T3 cells were cotransfected as described (Lin et al., 1996). Stable transfectants in the KU812 cells were generated by electroporation (300 volts, 960 μF) with the α and γ constructs and selected with G418.

Transient Transfection

KU812 stable transfectants expressing FcεRI αγ₂ (5 × 10⁶ cells) were cotransfected by electroporation with 10 μg pBJ1neo β construct and 1 μg green fluorescent protein construct (pGreen Lantern, Life Science, Bethesda, MD).

Assessment of FcεRI Expression by Surface Staining and Flow Cytometry

Cells were stained with 1 μg biotinylated IgE, followed by streptavidin-phycoerythrin (SA-PE) (1:200) (PharMingen, San Diego, CA) and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Alternatively, cells were stained with anti-α mAb 15-1 followed by FITC-conjugated goat-anti mouse. Untransfected cells were processed in parallel and used as negative control.

Immunoprecipitation and Western Blotting

Cell lysis and immunoprecipitation were performed as previously described (Lin et al., 1996). Briefly, cells were lysed at a ratio of 3 × 10⁷ cells/ml of lysis buffer (0.5% Triton X-100, 150 mM NaCl, 200 mM boric acid [pH 8.0]), supplemented with the "Complete" protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN). In some experiments, cells were lysed in 1% digitonin instead of 0.5% Triton X-100. Where indicated, immunoprecipitates were treated with endo-β-N-acetylglucosamidase (endo H) (New England Biolabs, Beverly, MA) as previously described (Letourneur et al., 1995a). Samples were resolved on SDS 13% polyacrylamide gel, transferred to PVDF membrane, and blotted with the antibodies indicated. Immunoreactive proteins were visualized using alkaline phosphatase-coupled second-step reagents and enhanced chemi-luminescence (ECF, Amersham). Fluorescence was quantified using a Storm scanner (Molecular Dynamics, Sunnyvale, CA).

Pulse-Chase Analysis

Cells were incubated in cysteine- and methionine-free RPMI medium containing 10% dialyzed FBS (Biofluids, Rockville, MD) for 20 min at 37°C. The cells at 5 × 10⁶/ml were then pulsed with 0.2 mCi/ml TRAN ³⁵S-LABEL (70% [³⁵S]methionine, 15% [³⁵S]cysteine) (ICN Biomedicals, Costa Mesa, CA) for 5–15 min at 37°C. An aliquot of 15 × 10⁶ cells was collected at time 0, and the remaining cells were washed and resuspended in complete medium supplemented with cysteine and methionine for various periods of chase. At each time point, 15 × 10⁶ cells were pelleted by centrifugation, washed twice with ice-cold PBS, and resuspended in 0.5 ml of ice-cold lysis buffer (0.5% Triton X-100, 300 mM NaCl, 50 mM Tris [pH 7.5] in the presence of protease inhibitors).

Statistics

Averages are expressed ± SD. Comparisons between groups were performed with the Student's unpaired t test.

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