The β Subunit of the FceRI Is Associated with the FcγRIII on Mast Cells

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Summary

FceRI is a tetrameric receptor, composed of a ligand recognition subunit, α, a β chain, and dimeric γ chains. Previous studies have indicated that the dimeric γ chain is associated with FcγRIIIA (CD16) on natural killer cells and macrophages as well as the clonotypic T cell receptor. Here we show that in mast cells, in addition to the dimeric γ chains, the β subunit is associated not only with FceRI, but also with FcγRIIIA. Functional reconstitution studies with a mastocytoma cell line indicate that FcγRIII composed of α, β, and γ subunits has the capacity for signal transduction. These studies suggest that through the association of alternative ligand recognition subunits (α, α', α''), a common signal transduction complex (βγ) mediates similar biochemical and effector functions in response to immunoglobulin G (IgG) and IgE.

Materials and Methods

Cell Culture and DNA Transfection. MC9 cells were cultured in DME containing mouse spleen-conditioned medium and 10% FCS. COS-7 cells were cultured in DME containing 10% FCS. Rat FcεRI β subunit cDNA was isolated (11) by PCR from the rat basophilic leukemia (RBL) mRNA, and cloned into the pCEXV-3 expression vector. cDNAs (human and mouse FcγRIIIA-α, human FcεRI-α, human TCR/CD3 γ, and mouse FcεRI-γ) for COS-7 cell transfections were also cloned into pCEXV-3 (1). DNA was transfected into COS-7 cells using DEAE-dextran (135 µg/ml) in the presence of 100 µM chloroquine followed by 20% glycerol shock. For P815 cell transfections, the plasmid containing human FcγRIIIA-α and γ cDNAs and neomycin-resistant gene was constructed (pCNeo-αγ), and FcεRI-β cDNA was cloned into pBabe Puro expression vector (12). P815 cells were transfected with pCNeo-αγ alone or a combination of pCNeo-αγ and β cDNA by the calcium-phosphate method, and selected in the presence of G418 alone or G418 and puromycin. Clones were screened by flow cytometry using 3G8, and expression of the β subunit was checked by Western blotting analysis.

Antibodies. The peptide synthesized from the cytoplasmic domain of mouse FcγRIIIA-α was used to raise the polyclonal antibody. Antibodies against human FcγRIIIA-α, γ, and β chains were already described (13). Antibody against β chain was kindly provided by J.-P. Kinet (National Institutes of Health) (14).

Immunoprecipitation Analysis. MC9 cells and transfected COS-7 cells were iodinated with 125I by using 1,3,4,6-tetrachloro-3α, 6α-diphenylglycouril (Iodo-Gen; Pierce Chemical Co., Rockford, IL). Cells were solubilized in lysis buffer containing 1% digitonin and 0.12% Triton X-100. Cell lysates were sequential incubated (2 h, 4°C for each incubation) with antibodies and with protein A-Sepharose or protein A-Sepharose coated with rabbit anti-mouse IgG antibody. For immunoblotting, samples were separated on SDS-polyacrylamide gels and transferred to an Immobilon-P sheet (Millipore Continental Water Systems, Bedford, MA). Membranes were incubated with 125I-labeled anti-β antibody or with mAb IgG2b anti-β phosphotyrosine antibody; Upstate Biotechnology Inc., Lake Placid, NY) followed by alkaline phosphatase-conjugated goat anti-mouse IgG.
Assay for Cytoplasmic-free Calcium ([Ca\(^{2+}\)]\(^{i}\) Increase, Inositol Phosphate Generation, and Tyrosine Phosphorylation. For [Ca\(^{2+}\)]\(^{i}\) measurements, the cells were incubated at 37°C for 45 min in 20 mM Hepes buffer (pH 7.5) containing 5 mM glucose, 0.025% BSA, 1 mM CaCl\(_2\), and 3 μM fura-2/acetoxymethylester. The loaded cells (10⁶ cells/ml) were stimulated with 3G8 (0.7 μg/ml) and F(ab')\(_2\) of goat anti-mouse IgG (3 μg/ml). Fluorescence changes were monitored with an Alphascan spectrofluorimeter (Photon Technology International Inc.) at an excitation wavelength of 340 nm and an emission wavelength of 505 nm. [Ca\(^{2+}\)]\(^{i}\) was calibrated and computed as described (15). For inositol phosphate measurements, transfectants were labeled with [3H]myo-inositol (10 μCi/ml) for 6 h in inositol-free RPMI 1640 with 10% dialyzed FCS. The cells (5 × 10⁶ cells/ml) were then stimulated with 3G8 (10 μg/ml) in the presence of LiCl. At various time points, the cells were lysed and soluble inositol phosphates were extracted with TCA, and applied to AG1-X8 ion exchange columns (Bio-Rad Laboratories, Richmond, CA) (16). For tyrosine phosphorylation analysis, cells (2 × 10⁶ cells/ml) were stimulated (3 min for each incubation) with 3G8 (5 μg/ml) or F(ab')\(_2\) of goat anti-mouse IgG (10 μg/ml) in addition to 3G8.

Results and Discussion

The mouse mast cell line MC9 expresses the α, β, and γ subunits of FcεRI and the α subunit of FcyRIIIA, as assessed by Northern blotting (data not shown). Previous studies have indicated that γ associates with FcyRIIIA (6-8). To determine if the β chain of FcεRI also associates with FcyRIIIA, MC9 cells were surface labeled with [125I] in the presence of 0.01% digitonin, solubilized, and immunoprecipitated using anti-FcεRIIIA-α antibody. Anti-FcεRIIIA-α coimmunoprecipitated the ligand binding subunit α, the γ chain, and in addition, a 30-kD protein. This 30-kD protein migrated with the same apparent molecular mass as the FcεRI β subunit, shown by using anti-β antibody (Fig. 1 A). The association of the 30-kD protein was not the result of cross reactivity of the anti-FcyRIIIA-α antibody with FcεRI-α, as demonstrated by Fig. 1 B. COS cells transfected with FcεRI-α, β, and γ chains were surface labeled and immunoprecipitated with specific antibodies. Anti-β and anti-γ antibodies coimmunoprecipitated these three chains, whereas anti-FcyRIIIA-α antibody did not (Fig. 1 B).

To identify the 30-kD protein as β, we prepared cell lysates from MC9 cells and immunoprecipitated with anti-FcyRIIIA-α antibody. The immunoprecipitated samples were separated on 12.5% SDS-polyacrylamide gels, transferred to Immobilon-P sheet, and incubated with [125I]-labeled anti-β antibody.

Figure 1. Association of 30-kD protein with FcyRIIIA. Immunoprecipitation analysis of MC9 cells (A) and COS cells transfected with FcεRI-α, β, and γ subunits (B). Cell lysates were prepared with digitonin lysis buffer, were incubated with indicated antibodies (C, control antibody), and immune complexes were separated on 10% Tricine-SDS-polyacrylamide gels.
2). These results indicate that in mast cells, FcεRI-β is not only one component of FcεRI but is also complexed with FcγRIIIA-α and γ chains.

To gain further insight into the nature of the association of β with FcγRIIIA, we used a transient cDNA expression system in COS cells. We have previously shown that FcγRIIIA-α interacts directly with γ or TCR/CD3 ζ chain in the endoplasmic reticulum. In the absence of γ or ζ chains, the α subunit is rapidly degraded (13). One function of the γ subunit is in the assembly of the FcγRIIIA complex by protecting the α subunit from degradation. Therefore, we studied the requirement of β chain for surface expression of FcγRIIIA-α by flow cytometry analysis. As has been reported (6-8), cotransfection of the α subunit with γ resulted in a 20-fold stimulation of surface expression of FcγRIIIA-α. The β subunit was unable to substitute for the γ chain to enhance surface expression of FcγRIIIA-α. The efficiency of the cell surface expression of COS cells transfected with α, β, and γ was nearly identical to the cell surface expression observed with α and γ (data not shown). No difference in the kinetics of α degradation was observed in pulse-chase experiments of COS cells transfected with either α, β, γ or α, β, γ. These results are similar to what has been observed for human FcεRI, in which γ is sufficient for cell surface expression of human FcεRI-α (17).

The γ chain is closely related to the ζ chain of TCR/CD3 complex (17-19) and is found complexed with the FcγRIIIA-α chain in NK cells (20, 21). Association of β with FcγRIIIA-α, γ, or ζ in COS cells was therefore examined by coimmunoprecipitation experiments. The β subunit was complexed with the γ or ζ subunit (Fig. 3 A). COS cells transfected with FcγRIIIA-α, β, and γ chains formed the complex observed in MC9 cells (Fig. 3 B), suggesting that the interaction of these three chains do not require cell type-specific proteins. Similar results were obtained when these subunits were translated in vitro in the presence of microsome membranes (data not shown). We have consistently observed in both MC-9 cells (Fig. 1 A) and COS cell transfectants (Fig. 3) that the stoichiometry of β in complexes with α, γ, or ζ subunits varies depending on the antibody used for coimmunoprecipitation. In Fig. 1 A, the antibodies against α, or γ chain are far more efficient in precipitating α chain than is the anti-β antibody. Similarly, in Fig. 3 A, anti-β antibody precipitates the β-γ complex more efficiently than the β-ζ complex. These differences might be related to the stability of these various complexes in the presence of the antibodies used, partial dissociation of the receptors after solubilization, or may reflect the distribution of these complexes in vivo.

FcγRIIIA on NK cells and macrophages is composed of at least three polypeptide chains: a ligand-recognition subunit (FcγRIIIA-α) and the associated dimeric γ and ζ chains (5-8, 20, 21), whereas on mast cells it is composed of α, β, and γ chain. To characterize potential functional differences between these FcγRIIIA isoforms (α, γ and α, β, γ), these complexes were reconstituted in transfected cells. Fc receptors for IgE and IgG are incapable of evoking proximal or distal signals in heterologous cells, such as fibroblasts. To study the signaling capacity of these isoforms, we used the mouse mastocytoma cell line P815 (22) for transfection of the human FcγRIIIA-α and γ subunits or, alternatively, the α, β, and γ subunits. Stable transfectants were obtained, and the density of cell surface expression for these two isoforms was quantitated by flow cytometry using the mAb 3G8. Comparable levels of FcγRIIIA-α were observed for each isoform.

Immunoprecipitation experiments confirmed the association of α with the γ subunit, or alternatively with the β and γ subunits, respectively (data not shown). The ability of 3G8 to activate an increase in [Ca2+]i and phosphatidyl-inositol 4,5-biphosphate (PIP2) hydrolysis in these transfected cells was evaluated. In both transfectants, a substantial increase in [Ca2+]i was seen in response to 3G8, which was further enhanced by crosslinking using F(ab')2 of goat anti-mouse IgG (Fig. 4 A). Stimulation of FcγRIIIA with 3G8 resulted in the generation of inositol phosphates in both transfectants (Fig. 4 B). Since activation of a tyrosine kinase
pathway by stimulation of TCR/CD3 (23), FcεRI (24), and FcγRIIIA (25). Using Western blot analysis employing a mAb against phosphotyrosine, we revealed a 70-kD protein whose phosphorylation was stimulated by 3G8 in the α,γ transfectant, whereas in the α,β,γ transfectant, tyrosine phosphorylation of this protein required additional crosslinking of the receptor. In contrast, tyrosine phosphorylation of a 90-kD protein in the α,β,γ transfectant was stimulated with 3G8 only (Fig. 4 C). These results indicate that both the α,γ complex and the α,β,γ complex have the capability for transmitting early responses. Antibodies to endogenous murine FcγRs elicit similar signals in P815 cells (22, 29).

The demonstration that FcγRIIIA on mast cells is composed of α, β, and γ subunits raises the possibility that NK cells and macrophages, the other two cell types known to express FcγRIIIA, express a tetrameric form of this receptor. Although by RNA and protein analysis β expression is restricted to mast cells, structurally related molecules have been described on other hematopoietic cell types (26–28) and may be candidates for association with FcγRIIIA. Indeed, the presence of such molecules may account for the ability of P815 cells to signal in the absence of a transfected β chain. The identification of these related molecules and their functional dissection will certainly shed light on the complexities of these receptors. In mast cells, however, Fc-mediated signaling has converged so that structurally distinct ligands result in a common signaling pathway by virtue of the exchange of ligand recognition subunits in a tetrameric receptor complex.

Figure 4. Signal transducing properties of P815 cells expressing α,γ and α,β,γ. [Ca²⁺]i increase (A), inositol phosphate generation (B), and tyrosine phosphorylation (C) after stimulation of α,γ and α,β,γ transfectants. Each lane of tyrosine phosphorylation patterns represents lysates from ~2 × 10⁶ cells.

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