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Functionality of the IgA Fc receptor (FcαR, CD89) is down-regulated by extensive engagement of FcεRI

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Abstract Besides mast cells and basophils, the high-affinity IgE Fc receptor (FcεRI) is exclusively expressed on certain FcαR (IgA Fc receptor)-expressing immune cells such as neutrophils in allergic patients. Transfected rat basophilic leukemia cell line (RBL-2H3) co-expressing FcεRI and FcαR was analyzed for effects of simultaneous receptor engagement by their specific antibodies on degranulation and signaling. Whereas supraoptimal FcεRI engagement decreased degranulation, which is known as a bell-shaped dose–response curve, such inhibitory effect was not observed with FcαR engagement. However, simultaneous engagement of FcεRI and FcαR showed that supraoptimal FcεRI engagement down-regulates FcαR-mediated degranulation. This inhibition was associated with extensive phosphorylation of inositol polyphosphate 5′-phosphatase SHIP1 and FcεRIβ, and reversed by adding actin-depolymerizing drug, latrunculin B. The results suggest an endogenous mechanism by which FcαR functionality is down-regulated in an 'allergic environment' where FcεRI is co-expressed and extensively cross-linked on FcαR-expressing effector cells.
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Introduction

The high-affinity Fc receptor (FcR) for IgE (FcεRI) on mast cells and basophils is composed of an α-subunit (FcεRIα), a β-subunit (FcεRIβ), and two disulfide-linked γ-subunits (FcRγ) [1,2]. Whereas the FcεRIα subunit binds to the Fc portion of IgE, the FcεRIβ and FcRγ subunits contain immunoreceptor tyrosine-based activation motif (ITAM) in their cytoplasmic tails, and mediate the signal transduction of FcεRI. Aggregation of FcεRI by binding of multivalent allergen to FcεRI-bound IgE leads to rapid release of proinflammatory mediators. Although this type of cell activation is implicated well as a key mechanism leading to allergic inflammation, the dose-dependent FcεRI-mediated activation by increasing concentrations of multivalent antigen (Ag) or IgE displays a bell-shaped dose–response curve, where degranulation is suppressed at high Ag or IgE concentrations. This inhibitory effect was thought to be due to the formation of nonstimulatory monomeric Ag-IgE-FcεRI complexes resulting from competition of an excess of polyvalent Ag for IgE. However, it has recently been shown that as Ag concentration increases, some intracellular signals do not decrease but in turn do increase [3,4]. These include FcεRIβ and SH2-domain-containing inositol

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phosphate 5′-phosphatase-1 (SHIP1), whose tyrosine phosphorylation is strongly induced by extensive FcεRI engagement. Moreover, bone marrow-derived mouse mast cells (BMMCs) from SHIP1 knockout mice have recently demonstrated to fail to display a bell-shaped dose–response curve for FcεRI-mediated degranulation [3], suggesting that an endogenous inhibitory mechanism involving SHIP1 determines the upper limit of FcεRI aggregation that triggers degranulation.

FcεRI was initially thought to be limited to mast cells and basophils in rodents; however, in human, there are many reports describing FcεRI expression extending to other cell types such as epidermal Langerhans cells [5–7], monocytes [8], eosinophils [9], circulating dendritic cells [10], platelets [11], and neutrophils [12]. Furthermore, increased FcεRI expression on these cells has been shown to be associated with allergic diseases [6,8,10,12,13]. Among them, neutrophils [14,15], monocytes [14,15], and eosinophils [16] also express FcR for IgA (FcαR, CD89) on their cell surface, and are activated by IgA-immune complexes, leading to a wide range of immunological responses [17–19]. These include phagocytosis, antibody (Ab)-dependent cell-mediated cytotoxicity, superoxide production, release of inflammatory mediators and cytokines, and internalization and presentation of IgA-complexed Ags, which play important roles in host defense against invading microorganisms. Like FcεRI, FcαR associates with the FcRγ chain and uses it for its signal transduction [20–22], although FcRγ-less FcαR has been shown to perform some functions such as endocytosis [23]. Cells can simultaneously receive multiple signals through different membrane receptors, whose integration determines cell responses. In order to gain molecular basis of functional interaction between FcεRI and FcαR, we established a cell line co-expressing both receptors by gene transduction of FcαR cDNA into FcεRI-expressing rat basophilic leukemia cell line RBL-2H3. The receptors expressed on these cells were separately or simultaneously cross-linked by Abs against FcεRI and FcαR. The data demonstrate that extensive engagement of FcεRI suppresses not only cellular responses initiated by its own but also functionality of co-expressed heterologous FcαR.

Materials and methods

Cell culture and retroviral gene transduction

Rat basophilic leukemia RBL-2H3 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). Gene transfer of human FcαR cDNA [24], which was subcloned into the Moloney murine leukemia virus-based retroviral vector (pMX-puro), was performed as described [25]. The FcαR$^+$ transfectants were grown in DMEM supplemented with 10% FCS and 1 μg/ml puromycin.

Reagents

Monoclonal anti-FcαR (clone A59) and anti-rat FcεRIα Abs were purchased from BD Biosciences (San Jose, CA) and Upstate (Lake Placid, NY), respectively, and used in all applications including cell stimulation, and flow cytometric and immunoprecipitation analyses. Mouse IgG1, and FITC-conjugated rabbit anti-mouse IgG were purchased from BD Biosciences. Anti-FcεRIβ (clone JRK) has been described [26]. Anti-FcRγ was obtained from Upstate. Horseradish peroxidase (HRP)-conjugated anti-mouse IgG, HRP-conjugated anti-rabbit IgG, and HRP-conjugated anti-phospho-tyrosine (PY-20) were purchased from GE Healthcare (Buckinghamshire, UK). Anti-SHIP1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

RT-PCR

Total RNA was isolated by the phenol-guanidium-isothiocyanate method using Isogen (Nippongene, Tokyo, Japan), and subjected to RT-PCR using the one step RNA PCR kit (Takara Shuzo, Tokyo, Japan) according to the manufacturer's protocol. The specific primers used were: for human FcαR, forward 5′-GTTCGTCATTGACCACATGG-3′, and reverse 5′-GAGGCTTCCTTGTTCAGTGC-3′; for rat FcεRIα, forward 5′-GCAATGGATACTGGAGGATC-3′, and reverse 5′-AGATTGGAGCAGCAGCCACT-3′; for rat β-actin, forward 5′-ACCCACACTGTGCCCATCTA-3′, and reverse 5′-CGGAACCGCTCATTGCC-3′. The PCR reaction was performed in a thermal cycler for 20 cycles, each consisting of 30 s at 95 °C, 30 s at 60 °C, and 1.5 min at 72 °C.

Determination of FcR cell surface expression

Cells (5×10^5) were washed with phosphate-buffered saline (PBS) and stained with anti-human FcαR, anti-rat FcεRIα, or isotype-matched mouse IgG1 at 4 °C for 30 min. The cells were washed again with PBS, then stained with FITC-conjugated rabbit anti-mouse IgG, and analyzed by a flow cytometry (BD Biosciences).

Degranulation

Cells (5×10^4/well) were seeded in 96-well plates and cultured overnight. After being washed with Tyrode's buffer containing 10 mM HEPES (pH 7.4), 130 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.6 mM $MgCl_2$, 5.6 mM glucose, and 0.1% BSA, FcRs were cross-linked with anti-FcαR or/and anti-FcεRIα at varying concentrations in 50 μl of Tyrode's buffer at 37 °C for 30 min. For Fig. 4, cells were pretreated with the actin monomer-sequestering agent latrunculin B (LatB, 10 μM; Sigma, St Louis, MO) for 10 min before cross-linking. Degranulation was determined by β-hexosaminidase release as described [25].

Immunoprecipitation and immunoblotting

Cells (1×10^7) were lyzed in lysis buffer containing 20 mM Tris–HCl (pH 7.4), 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 1 mM Na_3VO_4, 2 mM EDTA, 0.2 mM PMSF, 20 μM leupeptin, and 0.15 U/ml aprotinin on ice for 30 min. The lysates were centrifuged at 8000 ×*g* at 4 °C for 10 min. For Fig. 1C, supernatants were incubated with anti-FcαR or anti-FcεRIα and protein G-conjugated magnetic beads (Miltenyi Biotec, Gladbach, German) on ice for 30 min, and then applied to μcolumn in the magnetic field of the μMACS separator (Miltenyi Biotec). The columns were washed with lysis buffer four times, once with low salt wash buffer containing 50 mM

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