Journal of Immunological Methods 336 (2008) 229–234

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

High-affinity IgE receptor-β chain expression in human mast cells

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ARTICLE INFO

Article history:
Received 26 January 2008
Accepted 7 May 2008
Available online 11 June 2008

Keywords:
Atopic diseases
IgE receptor
Immunoblotting
Immunocytochemistry
Mast cells

ABSTRACT

The high-affinity IgE receptor (FcεRI)-β gene is one of the atopy-associated genes, but its biological significance is largely unknown. In this study, we generated the anti-FcεRI-β chain antibody to clarify β-chain protein expression in human mast cells. The FcεRI-β antibody showed specific binding to a 27 kDa protein with Western blotting and membrane bound immunostaining using cultured mast cells. Monomeric IgE sensitization increased β-chain expression as well as mature α-chain expression in mast cells. Upregulation of β-chain expression with monomeric IgE treatment suggests possible roles of FcεRI-β protein as an atopy-related molecule.

1. Introduction

The high-affinity IgE receptor (FcεRI)-β has been recognized as an atopy-related gene (Cookson et al., 1989 and Shirakawa et al., 1994). Some lines of study using a mouse reconstitution system suggested that the FcεRI-β protein may act as an amplifier for mast mast-cell activation (Lin et al., 1996). Mouse models are often useful tools for biomedical research, but in the case of FcεRI-β, there are significant discrepancies between the mouse and human system. For example, FcεRI-β protein expression is essential for cell-surface expression of FcεRI in mice (Turner and Kinet, 1999), however, human dendritic cells showed a cell-surface FcεRI receptor without β-chain mRNA expression (Bieber et al., 1996). Therefore, the existence of FcεRI without β-chain expression (FcεRI-$\alpha\gamma_2$ subtype) is probable in humans, but not in mice (Hayashi et al., 1999). However, we could not distinguish FcεRI-$\alpha\beta\gamma$ from FcεRI-$\alpha\gamma_2$ in situ due to the lack of reliable anti-human FcεRI-β antibodies for histochemical use. We chose to raise polyclonal rabbit antibodies to a specific peptide of human FcεRI-β in order to help the identification of FcεRI-β protein. One such antibody preparation successfully bound to the expected 27 kDa band on immunoblotting using human mast-cell/basophil lysate.

Another problem related to the functional study of human FcεRI-β protein is a lack of good human cell lines which express FcεRI. Human peripheral-blood-derived basophils and mast cells had been so far recognized as the source for FcεRI, however, the amounts of the cells were practically not sufficient for protein analysis. Recently, Kirshenbaum et al. (2003) had established a cell line (LAD2) from a human mastocytoma patient, which retains the character of native human mast cells and expresses functional FcεRI. Using the LAD2 cell line, we further proceeded to verify the specificity of the antibody with immunoblotting and immunoprecipitation studies. The new antibody reacted with FcεRI-β protein and

Abbreviations: FcεRI, high-affinity IgE receptor; b-mast cells, bone-marrow-derived mast cells.

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doi:10.1016/j.jim.2008.05.006

was useful for immunoblotting and immunocytochemical staining.

2. Materials and methods

2.1. Antibodies

A rabbit anti-serum against unique C-terminal sequences of FcεRI-β (CYSELEDPGEMSPPIDL) was generated by Affinity Research Products Ltd. (Exeter, UK). The anti-serum was purified on a protein-A column (Amersham Plc., Little Chalfont, UK). Other antibodies used in this study included Alexa 488-goat anti-rabbit-F(ab′)$_2$ and Alexa 594-goat anti-mouse IgG-F(ab′)$_2$ (Invitrogen, Carlsbad, CA, USA), chimeric anti-NIP IgE antibody (Serotec, Oxford, UK), rabbit anti-FcεRI-α and rabbit anti-FcεRI-γ polyclonal antibodies (Upstate Biotechnology, Lake Placid, USA), and mouse anti-FcεRI-α monoclonal antibody (clone: CRA1; Kyokuto Pharmaceuticals, Tokyo, Japan).

2.2. Reagents

Reagents used in this study included Kaleidoscope Prestained Protein Standards (Bio-Rad Japan, Tokyo, Japan), Tris–glycine gels (Invitrogen), sodium dodecyl sulfate (SDS), DL-dithiothreitol (DTT), and phosphatidylserine, alcian blue dye (Sigma Japan, Tokyo, Japan), 3-Cyclohexylamino-1-propanesulfonic acid (CAPS; Dojindo, Kumamoto, Japan), complete mini protease cocktail tablet (Roche Ltd., Penzberg, Germany), and HCL Plus Western blotting detection reagents (Amersham). All other reagents used in this study were of analytical grade.

2.3. Cell culture

Human basophils and eosinophils were purified from venous blood with a basophil isolation kit or with anti-CD16 beads, respectively, with Midi MACS (Miltenyi Biotec, Gladbach, Germany). Bone-marrow-derived CD34+cells were purchased from Cambrex North Brunswick, Inc. (North Brunswick, NJ) and bone-marrow-derived mast cells (b-mast) were generated as previously described (Saito et al., 2006). Human blood samples were collected from volunteers with written informed consents, and all procedures were approved by the ethical committees of Kyoto Prefectural University of Medicine and in accordance with the Declaration of Helsinki. Purity of the basophils and eosinophils was checked with alcian blue staining and Hansel staining (Eosino-Stain; Tori-Pharmaceuticals, Tokyo, Japan), respectively, and each showed a >98% purity. Human mast mast-cell line LAD2 was kindly provided by Dr. Arnold Kirshenbaum (NIAID, NIH) and maintained as previously described (Kirshenbaum et al., 2003).

2.4. SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting

LAD2 cells, eosinophils, and basophils were collected, washed twice with phosphate-buffered saline (PBS), and the number of cells was counted. Cells in the amount of 2×10^4 were then solubilized in SDS-sample buffer (62.5 mM Tris–HCl, pH6.8, 2%SDS, 20% Glycerol, and 0.04% bromophenol blue). Next, 50 mM DTT was added to the samples and incubated 15 min at 65 °C. Of each sample, 15 ml was loaded to 12% Tris–glycine gel with Kaleidoscope prestained protein standards. SDS-PAGE and Western blotting were then performed. The electrophoresed protein was transferred to polyvinylidene fluoride (PVDF) membrane (Pall Japan, Tokyo, Japan) using CAPS transfer buffer (10 mM CAPS, pH8.6, and 10% methanol). The membrane was then incubated with primary antibodies (1:1000 dilution in 1% non-fat skim milk) overnight at 4 °C with constant agitation. After washing with PBS containing 0.05% Tween 20 (PBST), the membrane was incubated with a 1:10,000 dilution of HRP conjugated anti-rabbit IgG. (Amersham) for 1 h and then visualized with ECL Plus Western blotting reagents.

2.5. Immunoprecipitation and subsequent immunoblotting

LAD2 cells in the amount of 2×10^7 were collected and washed twice with PBS, and then solubilized with 2 ml of lysis buffer (2 mM L-α-phosphatidylcholine, 10 mM CHAPS, 150 mM NaCl, and 50 mM Tris Tris–HCl pH 8.0 with a complete mini protease inhibitor tablet) and centrifuged at 15,000 rpm for 30 min at 4 °C. The supernatant of the LAD2-cell lysate was pre-cleared with protein-G sepharose for 30 min. Then the supernatant was separated in half, and 1 μg of mouse anti-FcεRI-α monoclonal antibody or the same amount of isotype-matched control (mouse IgG2a) was added to each supernatant. After a 3-hour incubation at room temperature (RT), protein-G sepharose was added and further incubated for 1 h. The protein-G beads were washed 5 times with lysis buffer, then immunoprecipitated protein was solubilized with 50 μl of SDS-sample buffer. The immunoprecipitated protein samples were immunoblotted with anti-FcεRI-β and -γ antibodies as described above.

2.6. Flowcytometry and confocal microscopy analysis

LAD2, b-mast cells, and eosinophils were washed with PBS twice and fixed with 4% paraformaldehyde (PFA) in PBS for 15 min on ice. After PBS washing, the cells were permeabilized with 0.1% saponin in blocking buffer (10% normal goat serum, 1% bovine serum albumin in PBS) for 30 min on ice. Non-specific Fc receptor binding was blocked using an Fc receptor blocking reagent (Miltenyi Biotec). The cells were then washed again with PBS and incubated with anti-FcεRI-β IgG antibody (at the concentration of 20 μg/ml in blocking buffer) for 15 min at RT. As a negative control, the same amount of rabbit IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used. After PBS washes, the samples were incubated with 1 μg/ml of Alexa 488 conjugated anti-rabbit antibody for 30 min. The stained LAD2 cells were also analyzed with a FACS caliber (Becton Deckinson Japan, Tokyo, Japan). For double-staining with FcεRI-α, the fixed LAD2 and b-mast cells were treated with Fc receptor blocking reagents, then incubated with mouse anti-FcεRI-α monoclonal antibody and post-fixed with 4% PFA-PBS. The cells were then permeabilized with saponin and incubated with the anti-FcεRI-β antibody. After PBS washes, the cells were reacted with Alexa 488 conjugated anti-rabbit IgG and Alexa 594 conjugated anti-mouse IgG simultaneously. The stained cells were then visualized with a confocal microscope (Fluoview 300; Olympus, Tokyo, Japan).