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Selective depletion of basophils ameliorates immunoglobulin E-mediated anaphylaxis

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ABSTRACT

Basophils, which are the rarest granulocytes, play crucial roles in protective immunity against parasites and development of allergic disorders. Although immunoglobulin (Ig)E-dependent responses via receptor for IgE (FcεRI) in basophils have been extensively studied, little is known about cell surface molecules that are selectively expressed on this cell subset to utilize the elimination *in vivo* through treatment with monoclonal antibody (mAb). Since CD200 receptor 3 (CD200R3) was exclusively expressed on basophils and mast cells (MCs) using a microarray screening, we have generated anti-CD200R3 mAb recognizing CD200R3A. In this study we examined the expression pattern of CD200R3A on leukocytes, and the influence of the elimination of basophils by anti-CD200R3A mAb on allergic responses. Flow cytometric analysis showed that CD200R3A was primarily expressed on basophils and MCs, but not on other leukocytes. Administration with anti-CD200R3A mAb led to the prominent specific depletion of tissue-resident and circulating basophils, but not MCs. Furthermore, *in vivo* depletion of basophils ameliorated IgE-mediated systemic and local anaphylaxis. Taken together, these findings suggest that CD200R3A is reliable cell surface marker for basophils *in vivo*, and targeting this unique molecule with mAb for the elimination of basophils may serve as a novel therapeutic strategy in ameliorating the allergic diseases.

1. Introduction-

Basophils are the least granulocytes, typically representing less than 1% of blood leukocytes, that have been implicated in the protective immunity against several pathogens, including parasites, and in the development of allergic disorders [1–3]. While basophils and mast cells (MCs) share some characteristics, including expression of the high-affinity receptor for immunoglobulin (Ig)E (Fcε receptor, FcεR) and release of allergy-inducing mediators such as histamine, basophils are distinct from MCs in several aspects, including development and anatomical localization, and life-span [1–3]. Although these apparent differences between basophils and MCs might suggest that they may have distinct roles *in vivo*, the literatures on the features of basophils have been limited owing to their rarity and similarities with MCs.

CD200 receptors (CD200Rs), members of the Ig superfamily, are composed of five distinct proteins, CD200R1, CD200R2, CD200R3, CD200R4, and CD200R5 in mice [4–6]. Although CD200R1 or CD200R2 has been implicated to function as high- or low-affinity receptor for CD200, the specific interaction between other CD200Rs and CD200 remains to be debated [4–7]. Whereas CD200R1 and CD200R2 is reportedly expressed on lymphoid cells and myeloid cells, including T cells, dendritic cells, MCs and basophils, CD200 have shown to be broadly expressed on a variety of cell types derived from hematopoietic and non-hematopoietic origins [4–7]. Previous studies have demonstrated that CD200R1 acts as an inhibitory receptor for subpopulations of macrophage lineages to control several immune responses [8,9]. Furthermore, the interaction between CD200 and CD200R1 reportedly led to the inhibition of degranulation and cytokine production in MCs and basophils upon FcεR stimulation [10,11]. Relative to CD200R1 and CD200R2, the characterization of

Abbreviations: BMMCs, bone marrow-derived mast cells; CD200R, CD200 receptor; DNP, 2,4-dinitrophenol; DNP-BSA, DNP-conjugated bovine serum albumin; FcεR, Fcε receptor; FcγR, Fcγ receptor; GFP, green fluorescent protein; Ig, Immunoglobulin; IL, Interleukin; IRES, internal ribosome entry site; ITAM, immunoreceptor tyrosine-based activation motif; mAb, monoclonal antibody; RBC, red blood cells; PE, Phycoerythrin; PSA, passive systemic anaphylaxis

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the other members of CD200R has not been well addressed.

Unlike other CD200Rs, CD200R3 appears to be mainly expressed on MCs and basophils with possible eight different splice versions (CD200R3A-H) in terms of variances of extracellular portion and cytoplasmic tails, and three different types of cytoplasmic tails might influence the association with an immunoreceptor tyrosine-based activation motif (ITAM)-bearing signaling adaptor molecules, including the Fcγ receptor (FcγR) chain as part of FcεR and both DAP10 and DAP12 [5,6,12,13]. It has been shown that cross-linking of CD200R3 with mAb (clones Ba91 and Ba103) induced degranulation in MCs and production of the cytokine in basophils *in vitro* [12]. However, the ligand(s) and the precise function(s) of CD200R3 remain still unclear.

Anaphylaxis is a rapid-onset, potentially life-threatening allergic reaction caused by the excessive release of allergic mediators after allergen exposure [14,15]. It has been well known that anaphylaxis is mainly caused by cross-linking of IgE-FcεR complexes on MCs [14,15]. Previous studies have suggested that basophils are one of the major mediators in the IgG-, but not IgE-mediated systemic anaphylaxis, distinctively from MCs, in mice when mAb to CD200R3 (clone Ba103) was used for specific elimination of basophils *in vivo* [16].

In this study, we examined the expression pattern of CD200R3A on leukocytes, and the influence of the depletion of CD200R3A$^+$ leukocytes on IgE-mediated experimental anaphylaxis, known as passive systemic anaphylaxis (PSA), in mice by utilizing anti-CD200R3A mAb (clone 6C4H2) [13]. In addition, we further addressed the role of CD200R3A in FcεRI-mediated activation of MCs.

2. Materials and methods

2.1. Mice

Female C57BL6/J mice were purchased from CLEA Japan (Tokyo, Japan). Animals were maintained in specific pathogen-free conditions in the animal facility at University of Miyazaki. All experimental procedures were performed with mice between 7 and 12 weeks of age in accordance with institutional guidelines of the Animal Experiment Review Board.

2.2. Cells

To prepare single-cell suspensions from spleen and lung, tissue samples were digested with collagenase type III (Worthington Biochemical, Lakewood, NJ) at 37 °C for 40 min, and were ground between glass slides. Splenocytes were treated with red blood cells (RBC) lysis buffer (Sigma-Aldrich, St. Louis, MO) before suspension. Bone marrow (BM) cells were flushed from the femurs and tibias. To prepare peritoneal exudate cells (PEC), the peritoneal cavity was lavaged with 10 ml of PBS using a 10 ml plastic syringe and 18-gauge needle. Single-cell suspensions were obtained by forcing through a 100-μm cell strainer (BD Biosciences, San Jose, CA). For preparation of transfectants expressing mock-green fluorescent protein (GFP) or CD200Rs-GFP, RBL2H3 cells were retrovirally transfected with pMX-internal ribosome entry site (IRES)-GFP vector (mock-GFP) as a control or pMX-CD200Rs-IRES-GFP vectors as described previously [13]. BM-derived MCs (BMMCs) were generated as previously described [12]. Briefly, BM cells were cultured in RPMI-1640 medium (Wako Pure Chemicals, Osaka, Japan) supplemented with recombinant murine interleukin (IL)-3 (4 ng/ml, Wako Pure Chemicals), 10% heat inactivated FBS, antibiotic-antimycotic (GIBCO BRL, Rockville, MD), 1 mM $_L$-glutamine, and 0.05 mM 2-mercaptoethanol for 4 weeks, and the cell population confirmed by flow cytometry in which CD49b$^+$FcεRIα$^+$ BMMCs represented 90% of the cells.

2.3. Flow cytometry

Cells were stained with fluorescein-conjugated mAbs to mouse CD3ε (145-2C11), CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD11c (HL3), CD49b (HMα2), B220 (RA3-6B2), CD117 (2B8), isotype-matched control mAb (BD Biosciences), FcεRIα (MAR-1) (eBioscience, San Diego, CA). For CD200R3 staining, cells were stained with biotinylated mAb to CD200R3A (6C4H2), which was generated in our laboratory as described previously [13], plus streptavidin-PE (BD Biosciences). For staining with recombinant fusion proteins, cells were stained with Fc fragment of human IgG (huIgFc) or CD200-huIgFc [13] followed by anti-human IgG-PE (eBioscience). Fluorescence staining was analyzed with a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR).

2.4. In vivo depletion of basophils

For the depletion of CD200R3A$^+$ leukocytes *in vivo*, mice were intravenously (i.v.) injected with anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) or isotype-matched control Ig (100 μg/mouse; Sigma-Aldrich) through the tail vein one day before the antigenic sensitization.

2.5. Degranulation assay

Degranulation of MCs was measured by the release of β-hexosaminidase according to the previous report [12,17] with some modifications. Briefly, BMMCs were incubated overnight with 2,4-dinitrophenol (DNP)-specific IgE (clone SPE-7, 1 μg/ml; Sigma-Aldrich) in RPMI-1640 medium. After overnight incubation, the cells were stimulated with or without DNP-conjugated bovine serum albumin (DNP-BSA, 250 ng/ml; LSL, Tokyo, Japan) in the presence or absence of isotype-matched control Ig (10 μg/ml) or anti-CD200R3A mAb (clone 6C4H2; 10 μg/ml), or ionomycin (1 μm; Sigma-Aldrich) at 37 °C for 30 min in Tyrode's buffer. The supernatant was collected to determine the β-hexosaminidase activity and the cells were lysed with 1%-Triton X (Wako Pure Chemicals) in Tyrode's buffer. The supernatant and lysate were incubated with p-nitrophenyl *N*-acetyl β-gulcosaminide (Wako Pure Chemicals) in citrate buffer at 37 °C for an hour. The reaction was stopped by glycine and absorbance was measured with a spectrophotometer at 405 nm. The calculation of the release of β-hexosaminidase was determined by the following equation: (supernatant optical density)/(supernatant optical density plus cell lysate optical density) ×100.

2.6. Induction of passive systemic anaphylaxis

Induction and evaluation of passive systemic anaphylaxis were performed according to the previous report [16] with some modifications. In brief, mice were i.v. sensitized with DNP-specific IgE (50 μg in 300 μl PBS/mouse), and then the sensitized mice were injected i.v. with or without isotype-matched control Ig (100 μg/mouse) or anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) 1 h after sensitization. Twenty-four hours after sensitization, the mice were i.v. challenged with DNP-BSA (50 μg in 300 μl PBS/mouse), and rectal temperature was measured by a digital thermometer (TD-300; Shibaura Electronics, Tokyo, Japan) every 5 min for 60 min.

2.7. Passive cutaneous anaphylaxis

Induction and evaluation of passive cutaneous anaphylaxis were performed as described previously [12] with some modifications. In brief, mice were intradermally (i.d.) sensitized with or without DNP-specific IgE (100 ng in 100 μl PBS/mouse) or control PBS in each ear, and then the sensitized mice were injected i.v. with or without isotype-matched control Ig (100 μg/mouse) or anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) 1 h after sensitization. Twenty-four hours after sensitization, the mice were i.v. challenged with DNP-BSA (125 μg in 200 μl PBS/mouse) containing 0.8% Evan's blue dye (Sigma-Aldrich).