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Treatment of murine mast cells with IgEκ and protein L enhances apoptotic cell death induced by IL-3 withdrawal

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ABSTRACT

Engagement of the high-affinity IgE receptor (FcεRI) can be either protective or non-protective against apoptotic cell death (ACD) in bone marrow-derived murine mast cells (BMMCs) after IL-3 withdrawal, depending on the avidity between IgE and its antigen. We recently reported that protein L (PpL), a bacterial Igκ-binding soluble protein, is able to stimulate intracellular signaling to induce activation of BMMCs by interacting with the IgEκ–FcεRI complex. However, it is unclear if cross-linking of FcεRI with IgEκ and PpL prevents or enhances IL-3-dependent ACD in BMMCs. In the present study, we found that IL-3-dependent ACD of BMMCs is accelerated by loading soluble PpL in the presence of IgEκ-occupied FcεRIα. For this purpose, soluble PpL was incorporated into the BMMCs. Unlike soluble PpL, immobilized PpL failed to enhance ACD, although both forms of PpL induced IL-6 production equally in BMMCs. In addition, we observed that DNS_5-BSA protected anti-DNS IgE-sensitized BMMCs from IL-3 depletion-mediated ACD by inducing the production of autocrine IL-3. In contrast, DNS_5-PpL enhanced IL-3 withdrawal-induced ACD of anti-DNS IgE-sensitized BMMCs and reduced the production of autocrine IL-3. These findings suggest that PpL increases IL-3 withdrawal-induced ACD of IgEκ-sensitized BMMCs by incorporating PpL into the BMMCs and that this internalized PpL may interfere with survival signals via FcεRI.

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1. Introduction

Mast cells (MCs) express the high-affinity IgE receptor (FcεRI) on their cell surface. FcεRI is engaged by IgE and its specific multivalent antigens, triggering FcεRI-dependent intracellular signals that lead to MC activation and associated cellular processes, such as the degranulation response, cytokine production, and cell survival [1]. However, the diverse responses of MCs to FcεRI activation depend on the quality of FcεRI cross-linking. The interaction of a low-affinity antigen with the IgE–FcεRI complex or the engagement of small numbers of FcεRIs promotes cell survival and cytokine production without inducing a strong degranulation response [2,3]. By contrast, a high-affinity antigen or large numbers of aggregated FcεRIs evokes a robust degranulation response without cell survival or cytokine production [4,5].

Finegoldia magna is one of the opportunistic pathogens present in the oral cavity and in the intestinal and urogenital tracts [6,7]. These bacteria express a B cell superantigen, protein L (PpL), with multiple (four or five) Igκ binding regions [8–10]. Interestingly, stimulating FcεRI with a murine IgEκ mAb and PpL elicits cytokine production but fails to induce a robust degranulation response [11]. This finding potentially indicates that the avidity between murine IgEκ and PpL is as low as the avidity between IgE and a low-affinity antigen and that the engagement of FcεRI with IgEκ and PpL leads to cell survival in murine MCs.

To address this possibility, we investigated the effects of cross-linking FcεRI with murine IgEκ and PpL during IL-3 depletion-induced apoptotic cell death (ACD) in bone marrow-derived murine MCs (BMMCs). Here, we report that stimulation of FcεRI with murine IgEκ and PpL enhanced the ACD induced by IL-3 withdrawal. Furthermore, we investigated how PpL increases IL-3-dependent ACD in BMMCs.

Abbreviations: ACD, apoptotic cell death; BMMCs, bone marrow-derived murine mast cells; FcεRI, high-affinity IgE receptor; FITC, fluorescein isothiocyanate; PpL, protein L; RBL, rat basophilic leukemia.

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2. Materials and methods

2.1. Reagents

Two commercially available anti-TNP IgEκ (15.3.2 and C48-2) and anti-DNS IgEκ (27.72) mAbs were purchased from BD Biosciences (CA, USA). The recombinant PpL and anti-DNP IgE (SPE-7) were obtained from Sigma (MO, USA). DNS_5-PpL (5 mol DNS per mol of BSA), DNS_5-BSA (5 mol DNS per mol of BSA), and PpL–fluorescein isothiocyanate (FITC) were prepared in our laboratory, and the recombinant murine (rm) IL-3 was purchased from PeproTech (NJ, USA).

2.2. Preparation of BMMCs

C57BL6/J mice were obtained from Charles River Laboratories in Japan (Kanagawa, Japan). For some experiments, thighbones from FcεRIα$^{-/-}$ and FcεRIα$^{+/+}$ mice were provided by Dr. Toshiaki Kawakami (La Jolla Institute of Allergy and Immunology). After obtaining animal care committee approval, all experiments were performed in accordance with the Nihon University guidelines for the care and use of laboratory animals. BMMCs were prepared from the femurs of C57BL/6J mice as previously described. Rat basophilic leukemia (RBL)-2H3 cells were cultured in DMEM supplemented with 10% (v/v) FBS (Invitrogen/Gibco, CA, USA).

2.3. Evaluation of cell death

ACD was evaluated by double staining with FITC-conjugated annexin V and propidium iodide (PI) as previously described [12]. BMMCs (1×10^6) were or were not sensitized with each 0.5 μg/ml IgEκ mAb overnight. The IgEκ-sensitized or unsensitized BMMCs (1×10^5) were stimulated with soluble PpL in the presence or absence of 5 ng/ml rmIL-3 for 30 h. Alternatively, the IgEκ-sensitized BMMCs (1×10^5) were stimulated with immobilized PpL for 30 h. After stimulation, the BMMCs were labeled with annexin V and PI and then analyzed using a FACSCalibur flow cytometer (BD Biosciences, CA, USA). The following cell populations were identified: annexin V$^+$/PI$^-$ (early apoptotic cells), annexin V$^+$/PI$^+$ (late apoptotic cells), and annexin V$^-$/PI$^-$ (living cells).

2.4. Degranulation and cytokine production assays

BMMCs (1×10^6) were sensitized with 0.5 μg/ml IgEκ mAb overnight. In some experiments, unsensitized BMMCs were also analyzed. The IgE-sensitized and unsensitized BMMCs (2×10^5) were washed with PBS and then stimulated or not stimulated with at the indicated concentrations for 0.5 h (for degranulation) or 6 h (for cytokine production). In some experiments, the IgEκ-sensitized BMMCs were stimulated with immobilized PpL for 6 h. In addition to PpL, antigens (DNS_5-BSA and DNS_5-PpL), anti-DNP IgE (SPE7), and A23187 were used as stimuli in this study. Degranulation was detected according to β-hexosaminidase release as described previously [13]. The percentage of β-hexosaminidase released was calculated as follows: (supernatant optical density of cells)/(total cell lysate optical density of cells) × 100. IL-3 and IL-6 production was analyzed using specific ELISA kits (Affymetrix eBioscience, CA, USA).

2.5. Analysis of DNA fragmentation and caspase-3 activation

After stimulation with PpL for 18 h, the BMMCs were harvested and washed twice with PBS. DNA was prepared from the cells using an Apoptosis Ladder Detection Kit (Wako Chemical, Tokyo, Japan). Fragmented DNA was resolved on a 1% agarose gel and visualized with ethidium bromide. Activated caspase-3 was assayed using a Caspase-3 Detection Kit (FITC-DEVD-FMK) (Merck Millipore, MA, USA) according to the manufacturer's protocol. Stained cells were evaluated using a FACSCalibur flow cytometer.

2.6. Confocal microscopy

RBL-2H3 cells (2×10^5) were sensitized with 0.5 μg/ml 15.3.2 or C48-2 in a cover-glass culture overnight. The cells were stimulated with PpL for 0.5 h, washed twice with PBS, fixed with 4% paraformaldehyde for 0.5 h, and permeabilized in PBS containing 0.1% Triton-X100 for 15 min at room temperature. The cells were washed twice with 1 ml of PBS and stained with FITC-conjugated PpL (1:1000) for 1 h in the dark. The cover glasses were washed with PBS and then mounted. Confocal microscopy was performed using the FV1000 system and the FLUOVIEW software (OLYMPUS CORPORATION, Tokyo, Japan).

2.7. FcεRI internalization assay

Receptor internalization was detected by changes in the cell surface FcεRI expression after PpL stimulation. Briefly, RBL-2H3 cells (2×10^5) were sensitized or not sensitized with 0.5 μg/ml 15.3.2 or C48-2 overnight. The sensitized cells were washed with PBS and stimulated or not stimulated with 30 nM PpL for the indicated times. The cells were labeled with 0.1 μg/ml anti-mouse IgE mAb-FITC for 15 min on ice. The labeled cells were analyzed with a FACSCalibur flow cytometer.

2.8. Statistical analysis

The data shown are means ± SE or SD. The statistical analyses were performed using Student's *t*-test. *p*-Values less than 0.05 were considered to indicate statistically significant differences.

3. Results

3.1. Engagement of FcεRI with IgEκ mAb (15.3.2) and soluble PpL augments MC death induced by IL-3 withdrawal

We first confirmed whether the engagement of FcεRI with IgE and a low-affinity antigen is protective against MC death induced by IL-3 depletion. For this purpose, BMMCs were sensitized with anti-DNS IgE (27.74) and then stimulated with 30 nM DNS_5-BSA in the absence of IL-3. Fig. 1A shows that 27.74-sensitized BMMCs were protected from MC death after IL-3 withdrawal in the presence of DNS_5-BSA but not BSA. The 27.74 antibody is an IgEκ mAb that cannot bind PpL [11]. As shown in Fig. 1B, PpL loading did not affect survival during IL-3-dependent cell death in 27.74-sensitized BMMCs. Unlike 27.74, the 15.3.2 antibody is a PpL-binding IgEκ mAb [11]. We observed that PpL loading enhanced IL-3-dependent cell death in BMMCs sensitized with 15.3.2 in a concentration-dependent manner (Fig. 1B). Fig. 1C and D show that unsensitized WT and 15.3.2-sensitized FcεRIα$^{-/-}$ BMMCs were resistant to PpL-induced cell death. In addition, the enhanced cell death in MCs stimulated with 15.3.2 and PpL was not rescued by lactose, which inhibits the interaction between IgE and alternative IgE binding proteins, such as those of the galectin family [14–16] (Fig. 1E). These results clearly indicate that FcεRIα, the IgE binding subunit of FcεRI, is responsible for the PpL-dependent augmentation of cell death induced by IL-3 deprivation.

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