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Molecular Immunology 42 (2005) 581–587
Molecular Immunology
www.elsevier.com/locate/molimm

Distinct regulation of C3a-induced MCP-1/CCL2 and RANTES/CCL5 production in human mast cells by extracellular signal regulated kinase and PI3 kinase

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Received 13 July 2004
Available online 6 November 2004

Abstract

Complement component C3a causes a robust degranulation in human mast cells. Whether C3a also stimulates chemokine production in human mast cells and what signaling pathway it activates is not known. In the present study, we demonstrate that $CD34^+$cell-derived primary mast cells and a human mast cell line LAD 2 express surface C3a receptors at similar levels. Furthermore, C3a caused ∼50% internalization of cell surface C3a receptors in both cell types. We therefore used LAD 2 cells as a model to study C3a-induced biological responses and signaling in human mast cells. We found that C3a stimulated substantial degranulation and induced chemokine monocyte chemoattractant protein 1 (MCP-1/CCL2) and regulated upon activation, normal T-cell expressed and secreted (RANTES/CCL5) production in LAD 2 mast cells. C3a caused a rapid and sustained extracellular-signal-regulated kinase (ERK) phosphorylation and Akt phosphorylation in LAD 2 mast cells. Furthermore, U0126 and LY294002, which respectively inhibit MEK-induced ERK phosphorylation and PI3 kinase-mediated Akt phosphorylation had distinct effects on C3a-induced responses. Thus, U0126, which blocked C3a-induced RANTES/CCL5 production by $50.6 \pm 2.3\%$, inhibited MCP-1/CCL2 generation by $85.2 \pm 0.6\%$. In contrast, LY294002 had no effect on C3a-induced RANTES/CCL5 production but blocked MCP-1/CCL2 generation by $83.7 \pm 1.5\%$. These data demonstrate that C3a activates divergent signaling pathways to induce chemokine production in human mast cells.

Keywords: Mast cells; Complement; Signal transduction; Chemokine

1. Introduction

Mast cells play a central role in the pathogenesis of asthma (Rivera, 2002; Turner and Kinet, 1999). A great deal of research has focused on mast cell activation via the activation of cell surface high affinity IgE (FcεRI) receptors. Cross-linking of FcεRI on mast cells by allergen results in degranulation, leukotriene generation and cytokine synthesis. These mediators increase vascular permeability, recruit inflammatory cells to the airway and promote smooth muscle contraction (Panettieri, 2003).

The complement system forms an important part of innate immunity against bacteria and other pathogens. As a system of 'pattern recognition molecules', foreign surface antigens and immune complexes initiate a proteolytic pathway leading to the formation of a lytic membrane attack complex. The anaphylatoxins C3a and C5a are generated as byproducts of complement activation. C3a and C5a interact with their cognate G protein coupled receptors (GPCR) to activate basophils, eosinophils and monocytes (Bischoff et al., 1990; Daffern et al., 1995; MacGlashan and Warner, 1991). Studies with animal models clearly demonstrated an important role for C3a in the pathogenesis of allergic asthma (Bautsch et al.,

Abbreviations: HMC-1, human mast cell line-1; C3aR, C3a receptor; C5aR, C5a receptor; MCP-1/CCL2, monocyte chemoattractant protein-1; RANTES/CCL5, regulated upon activation normal T-cells expressed and secreted

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doi:10.1016/j.molimm.2004.09.009

2000; Drouin et al., 2002; Humbles et al., 2000). However, the cell type that mediates the effects of C3a in asthma has not been determined. RBL-2H3 cells and murine bone-marrow-derived mast cells, which are commonly used to study FcεRI signaling pathways in mast cells, do not express C3a receptors (C3aR) (Erdei et al., 2004; Erdei and Pecht, 1996). Although an immature human mast cell line HMC-1 natively express C3aR (Ahamed et al., 2004; Hartmann et al., 1997; Nilsson et al., 1996), the inability of C3a to cause mediator release in this cell line limits its usefulness.

Woolhiser et al. (2004) recently showed that C3a stimulates degranulation in primary $CD34^+$ cell-derived human mast cells. However, responsiveness of these mast cells to C3a is variable and appears to depend on the $CD34^+$ cell donor, thus making it difficult to use these cells for signaling studies. Recently, two human mast cell lines LAD 1 and 2 have been developed and characterized, which closely resemble primary cultures of $CD34^+$ cell-derived human mast cells (Drexler and MacLeod, 2003; Kirshenbaum et al., 2003). The purpose of the present study was to utilize LAD 2 mast cells to delineate the C3a-induced responses and signaling in human mast cells. Here, we demonstrate the novel finding that C3a induces chemokine MCP-1/CCL2 and RANTES/CCL5 production in human mast cells. This study also reveals unexpected findings regarding the signaling pathways via which C3a induces chemokine production in human mast cells.

2. Materials and methods

2.1. Chemicals and reagents

Purified C3a was obtained from Advance Research Technologies (San Diego, CA). C5a and LY294002 were purchased from Sigma (St. Louis, MO) and U0126 from Calbiochem (La Jolla, CA). Indo-1 AM and pluronic F-127 were from Molecular Probes (Eugene, OR). Polyclonal C3aR antibody and normal rabbit IgG were obtained from Torrey Pines Biolabs Inc. (Houston, TX). Human recombinant stem cell factor (SCF) and interleukin-6 (IL-6) were purchased from Peprotech (Rocky Hill, NJ). Interleukin-3 (IL-3) was purchased from Biosource (Camarillo, CA). DNP-BSA was purchased from Biosearch Technologies (Novato, CA). Anti-DNP-IgE was generous gift from Dr. Juan Rivera (NIH). Goat-anti-rabbit IgG conjugated to FITC and all tissue culture reagents were purchased from Invitrogen (Gaithersburg, MD). Reagents for ELISA kits were purchased from R & D Systems (Minneapolis, MN). Phosphoplus p44/42 MAP kinase (ERK) (Thr202/Tyr204) and Phosphoplus Akt (Ser 473) kits were from Cell Signaling (Beverly, MA).

2.2. Cell culture

Human $CD34^+$ cell-derived mast cells were cultured in serum-free media StemPro-34 (Invitrogen) supplemented with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), recombinant human SCF (100 ng/ml), recombinant human IL-6 (100 ng/ml) and rhIL-3 (30 ng/ml) (first week only) (Kirshenbaum et al., 1999). Hemidepletions were performed weekly with media containing rhSCF (100 ng/ml) and rhIL-6 (100 ng/ml). Human mast cell line, LAD 2 cells were maintained in StemPro-34 medium supplemented with 100 ng/ml stem cell factor (SCF) (Kirshenbaum et al., 2003). Human mast cell line, HMC-1 cells were cultured in IMDM supplemented with 10% FCS, glutamine (2 mM), penicillin (100 IU/ml) and streptomycin (100 μg/ml).

2.3. Flow cytometry

The expression of C3aR on the cell surface was evaluated by flow cytometry as described (Ahamed et al., 2001). Briefly, 0.2×10^6 cells were washed with HEPES buffered saline containing 0.1% BSA. The cells were then incubated with rabbit IgG or rabbit anti-human C3aR antibody (1:100 dilutions) for 1 h at 4 °C. After washing, cells were incubated with FITC-labeled goat anti-rabbit IgG secondary antibody. Cells were fixed in 1% paraformaldehyde and analyzed on a FACStar^{PLUS} flow cytometer (BD Biosciences, Mountain View, CA).

2.4. Calcium measurements

Ca^{2+} mobilization was determined as described previously (Ali et al., 1994). Briefly, cells (1×10^6) were loaded with 1 μM indo-1 AM in the presence of 1 μM pluronic F-127 for 30 min at room temperature. Cells were washed and resuspended in 1.5 ml of HEPES-buffered saline. Ca^{2+} mobilization was measured after ligand stimulation in a Hitachi F-2500 spectrophotometer with an excitation wavelength of 355 nm and an emission wavelength of 410 nm and the data were expressed as a ratio of 355/410.

2.5. Assay of degranulation

LAD 2 mast cells (5×10^3) were seeded into 96-well plates in a total volume of 50 μl of buffer containing 0.1% BSA and exposed to different concentrations of C3a or C5a for 30 min. For total β-hexosaminidase release, control cells were lysed in 50 μl of 0.1% Triton X-100. Aliquots (15 μl) of supernatants or cell lysates were incubated with 15 μl of 1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine for 1.5 h at 37 °C. Reaction was stopped by adding 250 μl of a 0.1 M Na_2CO_3/0.1 M $NaHCO_3$ buffer and absorbance was measured at 405 nm (Ali et al., 1994).

2.6. Assay of MCP-1/CCL2 and RANTES/CCL5 production

LAD 2 mast cells (0.2×10^6 per well in 400 μl basal medium) were stimulated with C3a (100 nM) or C5a