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G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: Roles of Mas-related genes MrgX1 and MrgX2

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ABSTRACT

Although human mast cells express G protein coupled receptors for the anaphylatoxin C3a, previous studies indicated that C3a causes mast cell degranulation, at least in part, via a C3a receptor-independent mechanism similar to that proposed for polycationic molecules such as compound 48/80. The purpose of the present study was to delineate the receptor specificity of C3a-induced degranulation in human mast cells. We found that C3a, a C3a receptor "superagonist" (E7) and compound 48/80 induced Ca^{2+} mobilization and degranulation in a differentiated human mast cell line, LAD2. However, C3a and E7 caused Ca^{2+} mobilization in an immature mast cell line, HMC-1 but compound 48/80 did not. We have previously shown that LAD2 cells express MrgX1 and MrgX2 but HMC-1 cells do not. To delineate the receptor specificity for C3a and compound 48/80 further, we generated stable transfectants expressing MrgX1 and MrgX2 in a rodent mast cell line, RBL-2H3 cells. We found that compound 48/80 caused degranulation in RBL-2H3 cells expressing MrgX1 and MrgX2 but C3a did not. By contrast, E7 activated RBL-2H3 cells expressing MrgX2 but not MrgX1. These findings demonstrate that in contrast to previous reports, C3a and compound 48/80 do not use a shared mechanism for mast cell degranulation. It shows that while compound 48/80 utilizes MrgX1 and MrgX2 for mast cell degranulation C3a does not. It further reveals the novel finding that the previously characterized synthetic peptide, C3a receptor "superagonist" E7 activates human mast cells via two mechanisms; one involving the C3a receptor and the other MrgX2.

1. Introduction

The anaphylatoxin C3a is one of the most potent mast cell chemoattractants known (Hartmann et al., 1997; Nilsson et al., 1996). It also induces degranulation in purified human skin mast cells, peripheral blood $CD34^+$ cell-derived mast cells and a newly developed human mast cell line, LAD2 cells (Fukuoka et al., 2008; Lappalainen et al., 2007; Oskeritzian et al., 2005; Venkatesha et al., 2005; Woolhiser et al., 2004). By contrast, C3a does not induce degranulation in murine peritoneal mast cells, bone marrow-derived mast cells or rat basophilic leukemia, RBL-2H3 cells (Erdei et al., 2004; Soruri et al., 2008). C3a, however, causes substantial degranulation in rat peritoneal mast cells via a pathway that appears to be independent of cell surface C3a receptors (Fukuoka and Hugli, 1990; Mousli et al., 1992). These findings raise the interesting possibility that C3a-induced degranulation in human mast cells may involve C3a receptor-dependent and independent pathways (el-Lati et al., 1994).

Studies with synthetic C3a peptides indicated that a 20 amino acid carboxyl terminal fragment of C3a (C3aP: 58–77; Asn-Tyr-Ile-Thr-Glu-Leu-Arg-Arg-Gln-His-Ala-Arg-Ala-Ser-His-Leu-Gly-Leu-Ala-Arg) expresses biological potency equal to natural C3a (Lu et al., 1984). However, incorporation of two tryptophanyl residues at the N-terminus of a 15-residue C3a analogue (E7; Trp-Trp-Gly-Lys-Lys-Tyr-Arg-Ala-Ser-Lys-Leu-Gly-Leu-Ala-Arg), results in ~1500% increase in guinea pig platelet aggregation activity when compared to the C3aP (Ember et al., 1991). The effect of E7 was shown to be specific for the C3a receptor, as it cross-desensitized the ability of C3a but not C5a to induce guinea pig ileum contraction. Furthermore, compared to C3aP, much lower concentrations of E7 were required to induce vascular permeability in guinea pig skin, a response which presumably depends on mast cells (Ember et al., 1991).

Recently, a large family of G protein coupled receptors (Mas-related genes; Mrgs, also known as sensory neuron-specific receptors, SNSR) has been identified in rodents (Dong et al., 2001; Lembo et al., 2002). These receptors are selectively expressed in small-diameter sensory neurons of dorsal root ganglia and are thought to be involved in the sensation and modulation of pain. Interestingly, a subgroup of these receptors (MrgX1–MrgX4), are expressed in human but not murine neurons (Burstein et al., 2006; Dong et al., 2001). Furthermore, MrgX1 and MrgX2 are expressed in human cord blood-derived mast cells and compound 48/80 activates transfected cells expressing MrgX2 but not

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MrgX1 (Tatemoto et al., 2006). Previous studies indicated that C3a could activate both human skin mast cells and rat peritoneal mast cells via a pathway similar to that mediated via compound 48/80 (el-Lati et al., 1994; Mousli et al., 1992; 1994). This raises the intriguing possibility that C3a-induced mast cell degranulation could involve both C3a receptor and MrgX2.

The purpose of this study was to determine the receptor specificity of C3a-induced degranulation in human mast cells. Here, we demonstrate that although compound 48/80 induces mast cell degranulation via MrgX1 and MrgX2, C3a does not utilize these receptors for degranulation. We further show that C3a receptor "superagonist" E7 acts as a dual agonist for C3a receptor and MrgX2.

2. Materials and methods

2.1. Materials

All cell culture reagents were purchased from Invitrogen (Gaithersburg, MD). Monoclonal anti-DNP specific IgE and anti-human IgE were purchased from Sigma Life Sciences, Inc (St. Louis, MO). Human IgE was purchased from EMD Biosciences (San Diego, CA). Amaxa cell transfection kits and reagents were purchased from Lonza (Gaithersburg, MD). Plasmids encoding hemagglutinin (HA)-tagged human MrgX1, and MrgX2 in pReceiver–M06 vector were obtained from Genecopeia (Rockville, MD). All recombinant human cytokines were purchased from Peprotech (Rocky Hill, NJ). Cortistatin-14 (CST) and Bovine Adrenal Medulla Docosapeptide (BAM-22P) were obtained from American Peptide (Vista, CA). Human C3a was from Complement Technology (Tyler, TX). Compound 48/80 was from MP Biomedicals (Solon, OH) and Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA).

2.2. Synthesis and purification of C3a receptor peptides

Linear peptides were synthesized by using standard solid-phase methodology and were assembled with a Fmoc protection on an automatic peptide synthesizer (430A, Applied Biosystems, Carlsbad, CA) on a preloaded Fmoc-L-Arginine(Pmc)-HMP resin (Applied Biosystems) (Qu et al., 2011). Peptide deprotection and cleavage was performed using TFA/phenol/water/thioanisole/1,2ethanedithiol, (82.5/5/5/5/2.5) for 3 h at room temperature. All synthetic peptides were purified on a C-18 reverse-phase HPLC column to a ~95% purity and the identity of the peptides was monitored by laser desorbtion mass spectrometry (MALDI-TOF).

2.3. Differentiation of human mast cells from $CD34^+$ progenitors and culture of mast cell lines

Human $CD34^+$ progenitors were cultured in StemPro-34 medium supplemented with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), SCF (100 ng/ml), IL-6 (100 ng/ml) and IL-3 (30 ng/ml) (first week only). Hemi-depletions were performed weekly with media containing SCF (100 ng/ml) and IL-6 (100 ng/ml). Cells were used for experiments after 7–10 weeks in culture (Radinger et al., 2011; Venkatesha et al., 2005). LAD2 cells were maintained in StemPro-34 medium containing nutrient supplements (Invitrogen) supplemented with L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml) and 100 ng/ml SCF (Kirshenbaum et al., 2003; Radinger et al., 2011). Cell culture medium was hemi-depleted weekly with fresh culture medium (Kirshenbaum et al., 2003). Human mast cell line, HMC-1 cells were cultured in Iscove's modified Eagle's medium supplemented with 10% FCS, L-glutamine (2 mM), penicillin (100 IU/ml) and streptomycin (100 μg/ml). RBL-2H3 cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, L-glutamine (2 mM), penicillin (100 IU/ml) and streptomycin (100 μg/ml) (Ali et al., 1994).

2.4. Stable transfection of RBL-2H3 cells

RBL-2H3 cells were detached with versene, washed twice with DMEM and 1×10^6 cells were transfected with plasmids encoding HA-tagged MrgX1 or MrgX2, using the Amaxa nucleofector device and Amaxa kit V according to the manufacturer's protocol. Following nucleofection, cells were cultured in the presence of G418 (1 mg/ml) and cells expressing equivalent receptors were sorted using an anti-HA specific antibody/FITC-conjugated anti-mouse-IgG and used for studies on Ca^{2+} mobilization and degranulation (Subramanian et al., 2011).

2.5. Calcium mobilization

Ca^{2+} mobilization was determined as described previously (Ali et al., 1993; Ali et al., 2000). Briefly, cells (human mast cells; 0.2×10^6 and RBL-2H3 cells; 1.0×10^6) 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 in a Hitachi F-2500 spectrophotometer with an excitation wavelength of 355 nm and an emission wavelength of 410 nm (Ali et al., 2000).

2.6. Degranulation assay

LAD2 mast cells, $CD34^+$ cell-derived mast cells (5×10^3) and RBL-2H3 cells (5×10^4) were seeded into 96-well plates overnight in the presence of human IgE (1 μg/ml) or mouse IgE (1 μg/ml), respectively. The following day, cells were washed and incubated in a total volume of 50 μl of buffer containing 0.1% BSA and exposed to anti-human IgE (human mast cells), DNP-BSA (RBL-2H3) or different concentrations of peptides. 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).

3. Results

3.1. Activation of human mast cells by C3a, a C3a receptor superagonist and compound 48/80

Previous studies showed that recombinant C3a induces degranulation in human skin mast cells, $CD34^+$-derived primary mast cells and a relatively new mast cell line, LAD2 cells (Fukuoka et al., 2008; Lappalainen

Table 1
Amino acid sequences of the peptides used.

Peptides	Amino acid sequence
C3a peptide (C3aP;C3a 58–77)	Asn-Tyr-Ile-Thr-Glu-Leu-Arg-Arg-Gln-His-Ala-Arg-Ala-Ser-His-Leu-Gly-Leu-Ala-Arg
C3a receptor superagonist (E7)	Trp-Trp-Gly-Lys-Lys-Tyr-Arg-Ala-Ser-Lys-Leu-Gly-Leu-Ala-Arg
E7 scrambled peptide (E7S)	Leu-Arg-Ala-Gly-Ser-Arg-Tyr-Lys-Lys-Trp-Ala-Leu-Lys-Trp-Gly
Cortistatin	Pro-[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys]-Lys
BAM-22P	Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-Gly-Arg-Pro-Glu-Trp-Trp-Met-Asp-Tyr-Gln-Lys-Arg-Tyr-Gly