Immunology Letters 141 (2011) 45–54

Contents lists available at ScienceDirect

Immunology Letters

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

Distinct PGE_2-responder and non-responder phenotypes in human mast cell populations: "All or nothing" enhancement of antigen-dependent mediator release

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ARTICLE INFO

Article history:
Received 31 January 2011
Received in revised form 7 July 2011
Accepted 10 July 2011
Available online 20 July 2011

Keywords:
Mast cells
PGE_2
Degranulation
Cytokine release
Signaling

ABSTRACT

Reports indicate that prostaglandin (PG)E_2 markedly enhances antigen-mediated degranulation in mouse bone marrow-derived mast cells (BMMCs) but not in human mast cells (HuMCs). We have examined the underlying mechanism(s) for this disparity in HuMCs derived from the peripheral blood of multiple donors in addition to mouse BMMCs. HuMCs from half of these donors failed to respond to PGE_2 and the PGE_2 EP3 receptor agonist, sulprostone. However, HuMCs from the remaining donors and the LAD2 human MC line responded to PGE_2 and sulprostone with marked enhancement of antigen-mediated degranulation and IL-8 production in a similar manner to that observed in mouse BMMCs. The EP2 agonist, butaprost, failed to modulate antigen-mediated responses in any type of MCs. These distinct phenotypes could not be explained by differences in EP2 or EP3 expression nor by differences in the ability of PGE_2 to elevate levels of cAMP, a signal recognized to down-regulate mast cell activation. Moreover, both responder and non-responder HuMC populations exhibited similar activation of phosphatidylinositol 3-kinase, and MAP kinases. However, translocation of PLCγ_1 to the cell membrane and the associated calcium signal were enhanced only in the responder HuMC population indicating that the link between EP3 and PLCγ is impaired in the non-responder HuMCs.

Conclusions: These data provide a cautionary note for the translating of observations in the mouse to human mast cell-dependent disorders, but may also provide a basis for examining the effects of co-activating receptors in patients susceptible to allergic conditions.

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1. Introduction

Inflammatory mediators released from activated mast cells play a major role in the initiation of allergic reactions associated with anaphylaxis, atopy, rhinitis and allergic ocular disorders [1]. The manifestations of these reactions are the direct consequence of activation of a complex signaling cascade within mast cells upon antigen/IgE-dependent aggregation of cell surface high affinity receptors for IgE (FcεRI) [2]. Mast cells also express a variety of other classes of receptors that have the capacity to potentiate FcεRI-mediated mast cell responses [3–5]. Such receptors include the growth factor receptor KIT [6,7], Toll like receptors 2, and 4 [8], the IL-33 receptor [9], and specific G protein-coupled receptors (GPCRs) including the EP3 prostaglandin (PG)E_2 receptor, the A2b and A3 adenosine receptors and the C3a complement receptor [10–12].

The ability of these co-activating receptors to enhance antigen-mediated mast cell activation has led to the suggestion that, under specific circumstances, these receptors have the capacity to contribute to allergic responses *in vivo* [13,14]. However, the extent to which they actually influence mast cell activation in a physiological setting has yet to be determined. Nevertheless, studies conducted in both mouse bone marrow-derived mast cells (BMMCs) and human mast cells derived from peripheral blood progenitor cells (HuMCs) have revealed that the KIT ligand, stem cell factor (SCF), markedly enhances antigen-mediated mast cell degranulation and cytokine production [6,7]. The synergistic interactions between KIT and the FcεRI are regulated by an amplification pathway involving the phosphoinositide 3 kinase (PI3K)/Btk axis leading to enhanced phospholipase (PL)Cγ activation and an elevated calcium signal [15,16]. At least in the case of degranulation, these events may be coordinated by the transmembrane adap-

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0165-2478/$ – see front matter Published by Elsevier B.V.
doi:10.1016/j.imlet.2011.07.002

tor molecule, linker for activation of T cells 2 (LAT2; also known as LAB or NTAL) [7,17]. As with SCF, both IL-33 [9] and specific TLR [8] agonists markedly enhance FcεRI-mediated cytokine generation in mouse BMMCs and/or the mouse MC9 mast cell line. However, in contrast to the SCF-mediated response, the consensus of reports [8,18], including our own unpublished observations, suggest that neither agonist enhances mast cell degranulation or the release of arachidonic acid which is necessary for production of eicosanoids. This may be explained by the fact that both of these agents signal through the adaptor molecule MyD88 [19] which appears to lack the ability to generate the required calcium signal [8].

Of the various G protein coupled receptor (GPCR) agonists known to potentiate the actions of antigen, we found that PGE_2 was the most effective and that it acted primarily through the EP3 subset of PGE_2 receptors [20]. Although the synergistic actions of PGE_2 and SCF were remarkably similar, in that both degranulation and cytokine production were enhanced [6,15,20], the underlying signaling events regulating these responses were different. The synergistic actions of PGE_2 on mediator release were not dependent on phosphatidylinositol 3-kinase (PI3K), as was the case of SCF [6], but rather on a cross-synergy in the membrane translocation and activation of PLCβ and PLCγ [20]. PGE_2 acts through PLCβ via the EP3 receptor via $G\alpha_i$ in contrast to the activation of PLCγ by antigen via FcεRI [20].

Much of the experimental evidence for the enhancement of mast cell activation by co-activating receptors has been obtained from studies of mouse BMMCs [3] although the synergy between KIT and FcεRI has been replicated in HuMCs [6,7]. This is in contrast to the reports that PGE_2 enhances antigen-mediated activation of BMMCs [21,22] but inhibits antigen-induced degranulation in HuMCs [23,24]. In preliminary studies we too were unable to detect any potentiation of degranulation by PGE_2 in HuMCs derived from $CD34^+$-peripheral blood progenitor cells. Thus, at least in terms of the PGE_2 response, the mouse model may not reflect the situation in humans. We thus investigated this apparent dichotomy in HuMCs derived from multiple donors and in BMMCs.

As reported here, in contrast to previous observations, we find that as in BMMCs, PGE_2 can synergistically enhance mast cell degranulation and cytokine production in HuMCs. However, this enhancement was donor dependent as mast cells from half the human donors failed to respond to PGE_2, hereafter respectively referred to as the responder and non-responder HuMC populations. The differences between PGE_2-responders and non-responders were unrelated to EP receptor expression, but rather reflected differences in downstream signaling. These data suggest caution must be applied when observations in the mouse are translated to human systems and reminds us of the heterogeneity of human immunologic responses. Regardless, as will be discussed, the data may also provide insight as to how co-activating receptors may impact mast cell driven disease in the human.

2. Materials and methods

2.1. Human and mouse mast cell cultures

$CD34^+$ peripheral blood progenitor cells for this study were selected at random from stored samples obtained from normal donors. These samples were obtained following informed consent under a protocol approved by the NIAID IRB. Primary human mast cells (HuMCs) were obtained from these progenitor cells by culturing in StemPro-34 culture media containing recombinant human IL-3 (30 ng/ml) (first week only), IL-6 (100 ng/ml), and SCF (100 ng/ml) (Peprotech) as described [25,26]. Experiments were conducted on these cells 7–10 weeks after the initiation of culture. The LAD2 human mast cells were cultured in StemPro-34 culture media containing SCF (100 ng/ml) (Peprotech) as described [27].

BMMCs were obtained by flushing bone marrow cells from the femurs of C57BL/6 J mice (The Jackson Laboratory), then culturing the cells for 4–6 weeks in RPMI 1640 supplemented with 10% FBS, glutamine (4 mM), sodium pyruvate (1 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), non-essential amino acids (Sigma), HEPES (25 mM), β-mercaptoethanol (50 μM) and mouse recombinant IL-3 (30 ng/ml) (Peprotech). Cultures were maintained at 37 °C in a humidified incubator of 95% air, 5% CO_2. Mouse studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee at NIH.

2.2. Cell activation

For the initial activation experiments, HuMCs were grown from 8 individual donors (H1–H8). Subsequent signaling studies were conducted on a subset of cells obtained from selected donors displaying specific phenotypes, depending on availability. HuMCs or BMMCs were sensitized overnight with an optimal concentration (100 ng/ml) of biotinylated human IgE or mouse SPE-7 (IgE anti-DNP) (Sigma) respectively, in cytokine- and SCF-free medium. The following day, the cells were washed with cytokine-free medium or HEPES buffer (10 mM HEPES [pH 7.4], 137 mM NaCl, 2.7 mM KCl, 0.4 mM $Na_2HPO_4{\cdot}7H_2O$, 5.6 mM glucose, 1.8 mM $CaCl_2{\cdot}2H_2O$, 1.3 mM $MgSO_4{\cdot}7H_2O$) containing 0.04% BSA (Sigma) to remove excess IgE and then resuspended in medium or HEPES buffer at the required cell density for a specific assay. BMMCs or HuMCs were stimulated with DNP-HSA (10 ng/ml) or with streptavidin (SA, 10 ng/ml) at 37 °C respectively.

2.3. Degranulation and cytokine production

For degranulation, mast cells were sensitized overnight as described above and then rinsed with HEPES buffer containing 0.04% BSA. HuMCs and LAD2 cells (1×10^4 cells/well) and BMMCs ($3–5 \times 10^4$ cells/well) were aliquoted into individual wells of a 96 well plate and triggered in the same buffer with antigen/SA (0–100 ng/ml) and/or indicated GPCR agonists (Sigma) for 30 min. Degranulation was monitored by the release of β-hexosaminidase into the supernatants [7,20] and calculated as a percentage of the total content (cells and media) after cell activation.

For cytokine release studies, cells were sensitized as above, washed with cytokine-free medium. HuMCs (1×10^6 cells/1 ml/well) were triggered in this media for 6–8 h with SA (10 ng/ml), PGE_2 (100 nM), and/or SCF (100 ng/ml). Cytokines were measured in the cell culture supernatant by human IL-8 Quantikine ELISA kits (R&D Systems).

2.4. Fractionation of cells and immunoblotting

Membrane fractions were prepared as described [20]. Briefly, HuMCs (1×10^6 cells/sample) were sensitized as described above and washed with HEPES (0.04% BSA) buffer, then stimulated with SA (10 ng/ml) and/or PGE_2 (100 nM) at 37 °C for 2 min. After lysis of the cells and sonication, the resultant preparations were centrifuged at 20,000 × g for 30 min. Proteins in the pellet fractions were then solubilized with 1% Triton X-100, 1% NP40, and 0.1% SDS in lysis buffer followed by centrifugation at 15,000 × g for 15 min to collect the solubilized membrane fraction.

For immunoblot analyses, HuMCs (0.5×10^6 cells/sample) were sensitized with IgE and washed with HEPES (0.04% BSA) buffer, then stimulated with SA (10 ng/ml) and/or PGE_2 (100 nM) at 37 °C for 2 and 10 min. HuMC lysates were prepared as described [20,28]. Total cell lysates or membrane fractions were subjected