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ScienceDirect
Biochemical and Biophysical Research Communications 367 (2008) 590–596
BBRC
www.elsevier.com/locate/ybbrc

Prostaglandin $F_{2\alpha}$ regulates cytokine responses of mast cells through the receptors for prostaglandin E

Izumi Kaneko [a], Takanori Hishinuma [b], Kaori Suzuki [b], Yuji Owada [c], Noriko Kitanaka [c], Hisatake Kondo [c], Junichi Goto [d], Hiroshi Furukawa [a], Masao Ono [a,*]

[a] Department of Pathology, Tohoku University Graduate School of Medicine, 2-1 Seiryo, Aoba-ku, Sendai, Miyagi 980-8575, Japan
[b] Division of Pharmacotherapy, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan
[c] Department of Histology, Tohoku University Graduate School of Medicine, Sendai, Japan
[d] Division of Clinical Pharmacology, Tohoku University Hospital, Sendai, Japan

Received 28 December 2007
Available online 9 January 2008

Abstract

There is an increasing body of evidence that prostanoids modulate mast cell functions and contribute to the development of allergic inflammation. The present study aimed to identify an undetermined function of prostaglandin (PG) $F_{2\alpha}$ in mast cell activation and the signaling mechanism involved in it. Simultaneous quantification of prostanoids by liquid chromatography/tandem mass spectrometry revealed the constitutive release of $PGF_{2\alpha}$, thromboxane B_2, and 6-keto-$PGF_{1\alpha}$ from bone marrow-derived mast cells (BMMCs). Upon activation of BMMCs by lipopolysaccharide, the cytokine production in BMMCs was enhanced when the culture was supplemented with $PGF_{2\alpha}$. However, F prostanoid receptor—a selective receptor for $PGF_{2\alpha}$—was not detected in BMMCs. Further investigations performed using prostanoid receptor antagonists revealed an alternative mechanism wherein the receptors for PGE species—E prostanoid receptors—mediated the $PGF_{2\alpha}$ signal in BMMCs. The present study provides an insight into a novel function of $PGF_{2\alpha}$, i.e., an autocrine accelerator for mast cell activation.

Keywords: Mast cell; Lipid mediator; $PGF_{2\alpha}$; Lipopolysaccharide; E prostanoid receptor; EP; Autocrine

Mast cells play a role in the immune and inflammatory responses by sensing a variety of pathogenic patterns through cell-surface receptors. The recruitment of a high-affinity Fc receptor for IgE (FcεRI) by IgE and antigen (hereafter denoted as IgE/Ag) initiates the release of a variety of inflammatory mediators, including histamines, lipid metabolites, and cytokines, occasionally entailing the onset of immediate hypersensitivity and allergic inflammation. On the other hand, mast cells also recognize pathogens in an innate immune mode through other receptors, thus contributing to host defense against bacterial and viral infections. This recognition is mainly achieved by a member of the toll-like receptor (TLR) family, and it subsequently triggers the release of proinflammatory cytokines, including interferons, interleukins, and tumor necrosis factor (TNF)-α, which act in the elimination of pathogens by activating and mobilizing inflammatory cells toward infected sites. Recent studies have shown that a bacterial component, namely, lipopolysaccharide (LPS), enhances FcεRI-mediated mast cell activation [1,2]. Indeed, the co-occurrence of infectious events and the exacerbation of allergic manifestation has been demonstrated in allergic human populations [3–5]. Current findings implicate the contribution of TLR-mediated mast cell activation to the hypersensitivity in allergic diseases in humans.

Membrane lipid metabolites such as prostaglandins (PGs) and leukotrienes (LTs) have been characterized as early mediators that influence the onset of allergic inflammation in mouse models [6–8]. A recent study showed that when the culture of bone marrow-derived mast cells

* Corresponding author. Fax: +81 22 717 8503.
E-mail address: onomasao@mail.tains.tohoku.ac.jp (M. Ono).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.01.002

(BMMCs) was supplemented with PGE_2 interleukin (IL)-6 production was remarkably enhanced following IgE/Ag simulation [9]. Another study using antagonists specific to an E prostanoid receptor (EP) has shown that EP3 mediates a signal that enhances mast cell activation when supplemented with PGE_2, while EP2 mediates a suppressive signal under the same condition [10]. A study performed using BMMCs derived from EP-deficient strains of mice has proven that EP3 is essential for IL-6 production and degranulation of BMMCs [11]. These findings indicate the contribution of EP2 and EP3 in the regulation of mast cell activation. However, there is little evidence for their role in the production of PGE_1 or PGE_2 in mast cell activation. Paracrine PGE species are considered to be involved in the EP-mediated effects on *in vivo* mast cell activation.

In the present study, we investigated the mechanism of prostanoid-mediated mast cell activation following LPS stimulation using BMMCs. Highly sensitive and simultaneous quantification of prostanoids performed using liquid chromatography/tandem mass spectrometry (LC/MS-MS) revealed constitutive release of $PGF_{2\alpha}$, thromboxane (TX) B_2, and 6-keto-$PGF_{1\alpha}$ into the cultured medium. Functional analyses performed using $PGF_{2\alpha}$ and EP antagonists revealed an enhancing effect of $PGF_{2\alpha}$ on mast cell activation through its heterologous binding to EPs. The present findings provide a novel insight into the role of $PGF_{2\alpha}$ in the regulation of allergenic mast cell response.

Materials and methods

Preparation and activation of BMMC. BMMCs were obtained by culturing mouse bone marrow cells in the RPMI 1640 supplemented with 10% FCS, 1 mM sodium pyruvate, non-essential amino acids, 50 μM 2-mercaptoethanol (SIGMA, St. Louis, MO), and 5 ng/ml murine IL-3 (R&D Systems, Minneapolis, MN). BMMCs were activated with 1 μg/ml trinitrophenyl (TNP) hapten-specific IgE (TNP-IgE) and 1 ng/ml of TNP-conjugated ovalbumin (TNP-OVA) (fraction VII, SIGMA). LPS (*Escherichia coli* 055:B5, SIGMA) was used for the stimulation at 0.1 μg/ml. A cyclooxygenase (Cox) inhibitor, 5-lipooxygenase (Lox) inhibitor, or EP antagonist was incubated for 30 min prior to the supplementation of PG. $PGF_{2\alpha}$, PGE_2, PGD_2 (Cayman chemical, Ann Arbor, MI), and FP agonist Fluprostenol (BIOMOL, Plymouth Meeting, PA) were dissolved in ethanol and were added to the BMMC culture, then incubated for 20 min prior to the stimulation. EP antagonists—ONO-8711 (EP1 antagonist), ONO-AE3-240 (EP3 antagonist), and ONO-AE3-208 (EP4 antagonist)—were kindly donated from Ono pharmaceutics Co. Ltd., Osaka, Japan. Indomethacin (WAKO Pure Chemical, Osaka, Japan), NS-398, SC-560, and NDGA (Cayman chemical) were dissolved in DMSO or ethanol and were added to the BMMC culture with a constant solvent concentration (0.1% DMSO or ethanol). All experiments were performed under non-toxic conditions for any inhibitor dose, which were assessed by cell viability test (data not shown).

Measurements of cytokines and degranulation of BMMC. The concentration of IL-6 and TNF-α in the cultured medium were measured at 6 h after stimulation by enzyme-linked immunosorbent assay (ELISA) kits (BD PharMingen, San Diego, CA).

Liquid chromatography/tandem mass spectrometry (LC/MS-MS) analysis. Simultaneous quantification of PGs and TXB_2 were performed as previously described [12]. Briefly, the cultured medium collected at 1 h after the stimulations were subjected to analysis by LC/MS-MS. $PGF_{2\alpha\text{-}d4}$, $PGE_{2\text{-}d4}$, $PGD_{2\text{-}d4}$, 6-keto-$PGF_{1\alpha\text{-}d4}$, and $TXB_{2\text{-}d4}$ (each 2 ng) were added into the cultured medium (0.2 ml) as an internal standard. The sample was acidified and passed through an Empore C18 HD disk cartridge (3 M Industry, St. Paul, MN). The bound fraction was collected in hexane-ethylacetate (1:2, v/v, 1 ml) as a PG-enriched fraction. After evaporating solvent, the residue was reconstituted in mobile phase (30 μl), sonicated for 30 s, and filtered. The reconstituted sample was transferred to an autosampler vial; 10 μl was subjected to the LC/MS-MS analysis. For the HPLC part, chromatography was performed on a C18 Capcell Pak MGII (1.5 × 150 mm, 5 μm) (Shiseido, Tokyo, Japan) using isocratic elution with acetonitrile–water–acetic acid (40:60:0.02 v/v) at a flow rate of 100 μl/min at 40 °C. The selected reaction monitoring was performed as previously described conditions [12].

Reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was purified from kidney, brain, and BMMCs by using a TRIzol™ reagent (Invitrogen, Carlsbad, CA). The complementary DNA (cDNA) was prepared from 1 μg of total RNA with random hexamers and reverse transcriptase SuperScript III™ (Invitrogen). Polymerase chain reaction (PCR) was performed with following primers: 5′-GCTCTTGGTGT TTCCTTCTCG-3′ and 5′-TGCTTGCTGGCTCTCCTTCTC-3′ for a mouse F prostanoid receptor (FP) (446 bp), and 5′-CAGGAGATGG CCACTGCCGCA-3′ and 5′-CTCCTTCTGCATCCTGTCAGCA-3′ for mouse β-actin (276 bp). The annealing temperature in the PCR was set at 60 °C for FP and 52 °C for β-actin.

Statistical analysis. Difference between two mean values was evaluated by two-tail *t*-test or, in case of multiple comparison, the *t*-test combined with Bonferroni correction following ANOVA. P value less than 0.05 was regarded as statistically significant.

Results and discussion

Autocrine PGs influence cytokine production on TLR4-mediated BMMC activation

To estimate the role of autocrine PGs and LTs in LPS-dependent BMMC activation, the inhibitory effect of the Cox or 5-Lox inhibitor was measured by examining the effect on cytokine production. The Cox and Lox inhibitors significantly suppressed IL-6 production (Fig. 1A). The Cox-selective inhibitors NS398 and SC560 did not significantly inhibit TNF-α production; however, the other inhibitors significantly suppressed TNF-α production (Fig. 1B). These findings indicate the role of autocrine PGs and LTs in enhancing cytokine production following TLR4-mediated BMMC activation.

BMMCs constitutively produce $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$

To identify the autocrine PGs from BMMCs, we simultaneously measured the cumulative amounts of $PGF_{2\alpha}$, PGE_2, PGD_2, 6-keto-$PGF_{1\alpha}$ as PGI_2 metabolites and PGJ_2 and TXB_2 as TXA2 metabolites in the BMMC culture medium by LC/MS-MS, as described previously [12]. The following three culture conditions were tested: untreated, treated with LPS (100 ng/ml), and treated with IgE/Ag (1 ng/ml TNP-OVA). A significant amount of $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$ was detected under all the conditions (Table 1). $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were undetectable in the culture medium, and an increase of TXB_2 was detected in the presence of BMMC, indicating that $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$ are constitutively released from BMMCs. The stimulation with IgE/Ag but