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Differential usage of COX-1 and COX-2 in prostaglandin production by mast cells and basophils

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ABSTRACT

Basophils have been erroneously considered as minor relatives of mast cells, due to some phenotypic similarity between them. While recent studies have revealed non-redundant roles for basophils in various immune responses, basophil-derived effector molecules, including lipid mediators, remain poorly characterized, compared to mast cell-derived ones. Here we analyzed and compared eicosanoids produced by mouse basophils and mast cells when stimulated with IgE plus allergens. The production of 5-LOX metabolites such as LTB4 and 5-HETE was detected as early as 0.5 h post-stimulation in both cell types, even though their amounts were much smaller in basophils than in mast cells. In contrast, basophils and mast cells showed distinct time course in the production of COX metabolites, including PGD2, PGE2 and 11-HETE. Their production by mast cells was detected at both 0.5 and 6 h post-stimulation while that by basophils was detectable only at 6 h. Of note, mast cells showed 8–9 times higher levels of COX-1 than did basophils at the resting status. In contrast to unaltered COX-1 expression with or without stimulation, COX-2 expression was up-regulated in both cell types upon activation. Importantly, when activated, basophils expressed 4–5 times higher levels of COX-2 than did mast cells. In accordance with these findings, the late-phase production of the COX metabolites by basophils was completely ablated by COX-2 inhibitor whereas the early-phase production by mast cells was blocked by COX-1 but not COX-2 inhibitor. Thus, the production of COX metabolites is differentially regulated by COX-1 and COX-2 in basophils and mast cells.

1. Introduction

Basophils have been sometimes mixed up with mast cells in spite of distinct cell lineages. Basophils are the rarest granulocytes, representing less than 1% of blood-circulating leukocytes, and share some phenotypic features with tissue-resident mast cells. Both types of cells possess basophilic granules in the cytoplasm, express high-affinity IgE receptor FcεRI on the cell surface, and release proinflammatory mediators such as histamine and proteases upon activation [1]. Therefore, basophils have often considered erroneously as minor and non-redundant relatives or even blood-circulating precursors of tissue-resident mast cells. Although the research on basophils had long been hampered by their paucity and the absence of physiologically-relevant cell lines, recent studies utilizing novel analytical tools, including basophil-deficient engineered mice, revealed crucial and non-redundant roles for basophils in various immune responses such as allergy and protective immunity against parasitic infections [2–4]. Nevertheless, basophil-derived effector molecules involved in immune responses, including lipid mediators, remains ill-defined, compared to those derived from mast cells.

Eicosanoids, lipid mediators generated by the metabolism of arachidonic acid, play important roles in a series of biological responses including allergic inflammation and immunomodulation [5–8]. The first step of eicosanoid biosynthesis is the release of arachidonic acid from membrane phospholipids through the action of phospholipases. The next step bifurcates into two major pathways among others, mediated by either lipoxygenase (LOX) or cyclooxygenase (COX). 5-LOX converts membrane-derived arachidonic acid to leukotriene A4

Abbreviations: BMBAs, bone marrow derived basophils; BMMCs, bone marrow derived mast cells; COX, cyclooxygenase; LOX, lipoxygenase; TNP, 2,4,6-trinitrophenyl; OVA, Ovalbumin; HETE, hydroxyeicosatetraenoic acid; PGD2, prostaglandin D2; PGE2, prostaglandin E2; LTA4, leukotriene A4; LTB4, leukotriene B4; LTC4, leukotriene C4; LTD4, leukotriene D4

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(LTA4) and 5-HETE. LTA4 is then catalyzed by LTA4 hydrolase to form LTB4 while it is conjugated with glutathione by LTC4 synthase to form LTC4 that is further converted into LTD4 and LTE4 by extracellular metabolism [8,9]. In the other pathway mediated by COX, two isoforms of the COX enzyme, COX-1 and COX-2, have been identified [10–12]. While COX-1 is constitutively expressed in most cells, COX-2 expression can be up-regulated in response to a variety of stimuli. Both isoforms display the identical biochemical function in spite of relatively low homology (60–65%) at the amino acid level, and convert arachidonic acid to prostaglandin H2 (PGH2) [13]. PGH2 is subsequently catalyzed to PGD2, PGE2, PGI2, PGF2α and thromboxanes by prostaglandin synthase specific to each of them [8,10].

Eicosanoids released from mast cells have been extensively studied by using bone marrow-derived mast cells (BMMCs). When stimulated with IgE and corresponding antigens, BMMCs quickly produce and release LTB4, LTC4, 5-HETE and PGD2 [14,15]. In contrast, basophils were thought for many years to release a much narrower range of eicosanoids, such as LTC4 alone [16–18]. Although recent study expanded the range of eicosanoids released by basophils [19], systematic and comparable studies on the spectrum and quantity of eicosanoids released by activated basophils and mast cells remain to be done. In the present study, we analyzed and compared the time course, repertoire and quantity of eicosanoids produced by mouse basophils and mast cells when stimulated with IgE plus allergens, by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We also determined and compared the catalytic enzymes involved in eicosanoid production by basophils and mast cells, and demonstrated for the first time that COX-1 and COX-2 were differentially used in the production of prostaglandins and 11-HETE by basophils and mast cells.

2. Material and methods

2.1. Mice

C57BL/6 J mice were purchased from CLEA Japan. All animal studies were approved by Institutional Animal Care and Use Committee of Tokyo Medical and Dental University and the Ethics Committee for Animal Experiments of Ono Pharmaceutical Co., Ltd.

2.2. Generation and activation of BMBAs and BMMCs

Bone marrow-derived basophils (BMBAs) and bone marrow-derived mast cells (BMMCs) were generated as described previously [20,21] with minor modification. In brief, mouse bone marrow cells were cultured with 10 ng/mL murine IL-3 and 2 ng/mL murine SCF for 40 days to obtain BMMCs while they were cultured with 0.1 ng/mL IL-3 for 7 days to obtain BMBAs. In case of BMBAs, the basophil fraction in cultured bone marrow cells was purified with positive sorting of $CD49b^+$ cells by using MACS pro system (Miltenyi Biotec). The purity of basophils ($CD49b^+CD200R3^+cKit^-$) in the BMBA preparation and mast cells ($CD200R3^+cKit^+$) in the BMMC preparation was > 90%. Identity of basophils and mast cells was verified by their selective expression of *Mcpt8* and *Mcpt6* mRNAs encoding serine proteases mMCP-8 and mMCP6, respectively [20,22,23]. BMBAs and BMMCs were sensitized overnight with 1 μg/mL of hapten 2,4,6-trinitrophenyl (TNP)-specific IgE (IGELb4, ATCC-TIB141), and then incubated with 10 ng/mL TNP-conjugated ovalbumin (TNP_{12}-OVA, Biosearch Tech.) or control ovalbumin (OVA, Invivogen) for indicated time periods. In some experiments, before stimulation, cells were pretreated for 10 min with of 3 μmol/L of inhibitors, including BW-A4C (5-LOX inhibitor, Sigma), SC-560 (COX-1 inhibitor, Cayman Chemicals), and celecoxib (COX-2 inhibitor, Sigma).

2.3. Preparation and activation of primary basophils

$CD49b^+$ cells were enriched from mouse bone marrow cells by using MACS pro system, sensitized for 3 h with 1 μg/mL of anti-TNP-IgE, and then incubated with 10 ng/mL of TNP-OVA or control OVA for 30 min. After treatment with 0.1%NaN_3, $CD49b^+CD200R3^+c\text{-}Kit^-$ basophils were sorted by FACS AriaIII (BD biosciences), and subjected to quantitative RT-PCR analysis.

2.4. Quantitative RT-PCR

Total RNA was prepared using RNeasy™ Mini Kit (Qiagen), followed by cDNAs synthesis with Superscript VILO™ Master Mix (Thermofisher). Quantitative PCR of the cDNA was performed on Agilent Mx3005P system using a Taqman Fast Universal PCR Master Mix (Applied Biosystems) and Taqman gene expression assay primer and probe mix as follows: *Gapdh* (Mm_99999915_g1), *Mcpt6* (Mm 01301240_g1), *Mcpt8* (Mm_00484935_g1), *Alox5* (Mm_01182747_m1), *Ptgs1* (Mm00477214_m1), *Ptgs2* (Mm_00478374_m1). Gene expression levels were normalized by *Gapdh* expression levels.

2.5. Quantitative analysis of eicosanoids

Eicosanoids were extracted in the presence of deuterated internal standard (LTB4-6,7,14,15-d4 and 15(S)-HETE-5,6,8,9,11,12,14,15-d8, Cayman chemicals) by using $CHCl_3$/methanol/acetic acid (49.5:49.5:1). For LC-MS/MS analysis, a 5500 QTRAP triple quadrupole mass spectrometer (Sciex Co., Ltd.) equipped with an UFLC Poroshell 120 SB-C18 column (2.7 μm particle size, 2.1 × 150 mm; Agilent) was used. Samples were eluted with mobile phase A consists of ultra-pure water with 0.1% acetic acid, and B of acetonitrile/methanol (4:6 v/v) with 0.1% acetic acid. Gradient separations were performed from 27% of B for 5 min, ramped to 70% after 15 min, ramped to 85% after 25 min, and ramped to 100% after 30 min and held for 10 min, with flow rates of 0.2 mL/min. MS/MS analyses were conducted in negative ion mode. Eicosanoids were identified by multiple reaction monitoring (MRM) using transitions of a set of parent ion mass (*m/z*) > daughter ion mass (*m/z*) (Table 1). Concentration of each eicosanoid was carried out in a five-point calibration from the peak area. Eicosanoids for LC-MS/MS standards (LTB4, 5-HETE, PGD2, PGE2, 11-HETE, and 12-HETE) were purchased from Cayman Chemicals.

3. Results and discussion

3.1. Basophils release 5-LOX metabolites, LTB4 and 5-HETE at the early phase after stimulation

We first analyzed and compared the time course of production and repertoire of 5-LOX metabolites released from activated basophils and mast cells. To this end, bone marrow-derived basophils (BMBAs) and bone marrow-derived mast cells (BMMCs) were generated from mouse bone marrow cells, and stimulated with TNP-specific IgE plus the corresponding antigen TNP-OVA or control OVA. Culture supernatants were collected at 0, 0.5 and 6 h post-stimulation, and subjected to LC-MS/MS analysis. The production of LTB4 and 5-HETE was detected in

Table 1
Parameters for identification of eicosanoids.

Targets	IS	RT (min)	Transition (*m/z*) Parent > Daughter	LLOQ (ng/mL)
LTB4	LTB4-d4	19.72	335.0 > 195.0	0.1
5-HETE	15-HETE-d8	24.88	319.0 > 115.0	0.05
PGD2	LTB4-d4	16.70	351.0 > 189.0	0.5
PGE2	LTB4-d4	16.50	351.0 > 271.0	0.05
11-HETE	15-HETE-d8	23.94	319.0 > 167.0	0.05

IS; Internal standard, RT; Column retention time, LLOQ; Lower limit of quantification.