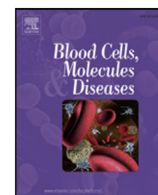
Blood Cells, Molecules and Diseases 55 (2015) 21–26

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

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



# Mechanism of interleukin-13 production by granulocyte-macrophage colony-stimulating factor-dependent macrophages via protease-activated receptor-2

Rui Yamaguchi [a,b], Takatoshi Yamamoto [a], Arisa Sakamoto [a], Yasuji Ishimaru [a], Shinji Narahara [a], Hiroyuki Sugiuchi [a], Eiji Hirose [a], Yasuo Yamaguchi [a,*]

[a] Graduate School of Medical Science, Kumamoto Health Science University, Kumamoto, Japan
[b] Graduate School of Medical Science, Kumamoto University Medical SchooL, Kumamoto, Japan

ARTICLE INFO

*Article history:*
Submitted 19 March 2015
Accepted 27 March 2015
Available online 30 March 2015

*Keywords:*
Extracellular signal-regulated kinase
Granulocyte-macrophage colony-stimulating factor
Interleukin-13
Neutrophil elastase
Protease-activated receptor-2

ABSTRACT

*Background:* Granulocyte-macrophage colony-stimulating factor (GM-CSF) promotes classically activated M1 macrophages. GM-CSF upregulates protease-activated receptor-2 (PAR-2) protein expression and activation of PAR-2 by human neutrophil elastase (HNE) regulates cytokine production.
*Aim:* This study investigated the mechanism of PAR-2-mediated interleukin (IL)-13 production by GM-CSF-dependent macrophages stimulated with HNE.
*Methods:* Adherent macrophages were obtained from primary cultures of human mononuclear cells. After stimulation with HNE to activate the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway, IL-13 mRNA and protein levels were assessed by the reverse transcriptase-polymerase chain reaction and enzyme-linked immunosorbent assay, respectively.
*Results:* PAR-2 protein was detected in GM-CSF-dependent macrophages by Western blotting. Unexpectedly, PD98059 (an ERK1 inhibitor) increased IL-13 production, even at higher concentrations. Interestingly, U0126 (an ERK1/2 inhibitor) reduced IL-13 production in a concentration-dependent manner. Neither SB203580 (a p38alpha/p38beta inhibitor) nor BIRB796 (a p38gamma/p38delta inhibitor) affected IL-13 production, while TMB-8 (a calcium chelator) diminished IL-13 production.
*Discussion:* Stimulation with HNE promoted the production of IL-13 (a Th2 cytokine) by GM-CSF-dependent M1 macrophages. PAR-2-mediated IL-13 production may be dependent on the $Ca^{2+}$/ERK2 signaling pathway.
© 2015 Elsevier Inc. All rights reserved.

## 1. Introduction

We recently reported that stimulation of granulocyte-macrophage colony-stimulating factor (GM-CSF)-dependent macrophages with human neutrophil elastase (HNE) promotes interleukin (IL)-13 production mediated via protease-activated receptor-2 (PAR-2) [1]. IL-13 is a type 2 T helper (Th2) cytokine and is mainly produced by T cells. However, the signaling pathways leading to induction of IL-13 are not well-characterized, even in GM-CSF dependent macrophages. Proliferation and differentiation of T lymphocytes are controlled through signaling pathways that are initiated by the T-cell antigen receptor (TCR). Major pathways induced by TCR stimulation involve mitogen-activated protein kinases (MAPKs), which are a highly conserved family of serine/threonine protein kinases that influence various fundamental cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival. The MAPK pathways are mediated by extracellular signal-regulated kinase (ERK), c-Jun-NH2-terminal kinase (JNK), and p38 protein kinases [2]. TCR stimulation induces distinct patterns of ERK and p38 phosphorylation in human T cells [3]. The activation of ERK is coupled to production of a number of cytokines by T cells and has differing effects on particular cytokines [4]. ERK also plays a critical role in IL-4 expression during TCR-induced differentiation of naive $CD4^{+}$ T cells into T helper (Th) cells [5]. A mitogen-activated protein/extracellular signal-regulated kinase (U0126) has been shown to inhibit IL-13 synthesis by human lymphocytes [6]. The ERK signaling pathway has also been suggested to have a role in increasing the expression of IL-13 [7].

We recently reported that GM-CSF upregulates PAR-2 protein production by macrophages [1]. PARs are G protein-coupled receptors

*Abbreviations:* ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; HNE, human neutrophil elastase; IL, interleukin; MAPK, mitogen-activated protein kinase; PAR-2, protease-activated receptor-2; PBMCs, peripheral blood mononuclear cells; RT-PCR, reverse transcription polymerase chain reaction; Th2, type 2 T helper cell.

* Corresponding author at: Graduate School of Medical Science, Kumamoto Health Science University, Kitaku Izumi-machi 325, Kumamoto 861-5598, Japan.
*E-mail address:* yamaguti@kumamoto-hsu.ac.jp (Y. Yamaguchi).

http://dx.doi.org/10.1016/j.bcmd.2015.03.006
1079-9796/© 2015 Elsevier Inc. All rights reserved.

**Table 1**
Reagents used.

| Reagent | Action |
|---|---|
| TMB-8 | Intracellular calcium antagonist |
| PD98059 | Extracellular signal-regulated kinase 1 inhibitor |
| U0126 | Extracellular signal-regulated kinase 1/2 inhibitor |
| SB203580 | p38 mitogen-activated protein kinase inhibitor (p38alpha and p38beta inhibitor) |
| BIRB796 | 38gamma and p38delta inhibitor |

that are activated by proteolytic cleavage of the amino-terminus and these receptors act as sensors for extracellular proteases. The activation of PAR-2 stimulates MAPK [8]. The MAPK/ERK pathway (also known as the Ras/Raf/MEK/ERK pathway) is a chain of proteins that transfer signals from a receptor on the cell surface to DNA in the cell nucleus, with the MAPKs p44ERK1 and p42ERK2 having a crucial role in intracellular signaling. PAR-2 activation enhances ERK1/2 phosphorylation in a time-dependent manner [9]. ERK1 and ERK2 are regulated similarly and contribute to intracellular signaling by phosphorylating a largely shared set of substrates in both the cytosol and the nucleus [9]. ERK2 positively regulates normal and Ras-dependent cell proliferation, whereas ERK1 affects the overall level of cell signaling by antagonizing ERK2 activity. Thus, ERK1 and ERK2 have differing effects on Ras-dependent cell signaling [10]. The mechanism of IL-13 production via the MAPK/ERK signaling pathway was investigated after stimulation of GM-CSF-dependent macrophages with HNE.

## 2. Material and methods

### 2.1. Ethics statement

Human peripheral blood samples were obtained from healthy volunteers and this study was approved by the Institutional Review Board of Kumamoto Health Science University. Written informed consent was obtained from all of the volunteers.

### 2.2. Chemicals and reagents

Human neutrophil elastase (HNE) with an activity of 200 U/L was purchased from Serva Electrophoresis (Heidelberg, Germany). SB203580 (Wako, Kanagawa, Japan), PD98059 (Wako), U0126 (Promega, Madison, WI), BIRB796 (Axon Medchem, Groningen, Netherlands), and TMB-8 (Sigma-Aldrich, Ontario, Canada) were employed to investigate the intracellular signaling pathways involved in IL-13 production. All reagent solutions were negative for endotoxin according to the Endospecy test [11]. The actions of these reagents are summarized in Table 1.

### 2.3. Isolation of adherent monocytes from peripheral blood mononuclear cells

Lymphocyte thawing medium (BBLYMPH1) was obtained from Zen-Bio, Inc. (Research Triangle Park, NC). Peripheral blood mononuclear cells (PBMCs) were isolated as described previously [12]. Briefly, heparinized blood samples were obtained from nonsmoking healthy volunteers and were immediately diluted 1:1 with pyrogen-free saline, followed by the isolation of PBMCs using Lymphoprep gradients (Axis-Shield PoC As, Norway). Then the cells were suspended in BBLYMPH1 and incubated for 3 h. For the isolation of monocytes by adherence to plastic, cells were distributed into 12-well plates (Corning Inc. Costar, NY, USA) at $1 \times 10^6$ cells per well and left to adhere in a 5% $CO_2$ incubator for 2 h at 37 °C, followed by washing 3 times with warm phosphate-buffered saline (PBS) to remove nonadherent cells. The monocytes thus obtained were cultured in complete medium consisting of RPMI 1640 (Sigma-Aldrich, Oakville, Ontario, Canada) supplemented with 10% heat-inactivated fetal calf serum (FCS) and $10 \times 10^3$ μg/L gentamicin at 37 °C in 5% $CO_2$ humidified air.

After recovery of monocytes with a cell scraper, purity was evaluated by fluorescent staining with CD14-phycoerythrin (PE) mouse anti-human monoclonal antibody (Life Technologies, Staley Road Grand Island, NY) and FACS analysis. Viability of the recovered monocytes

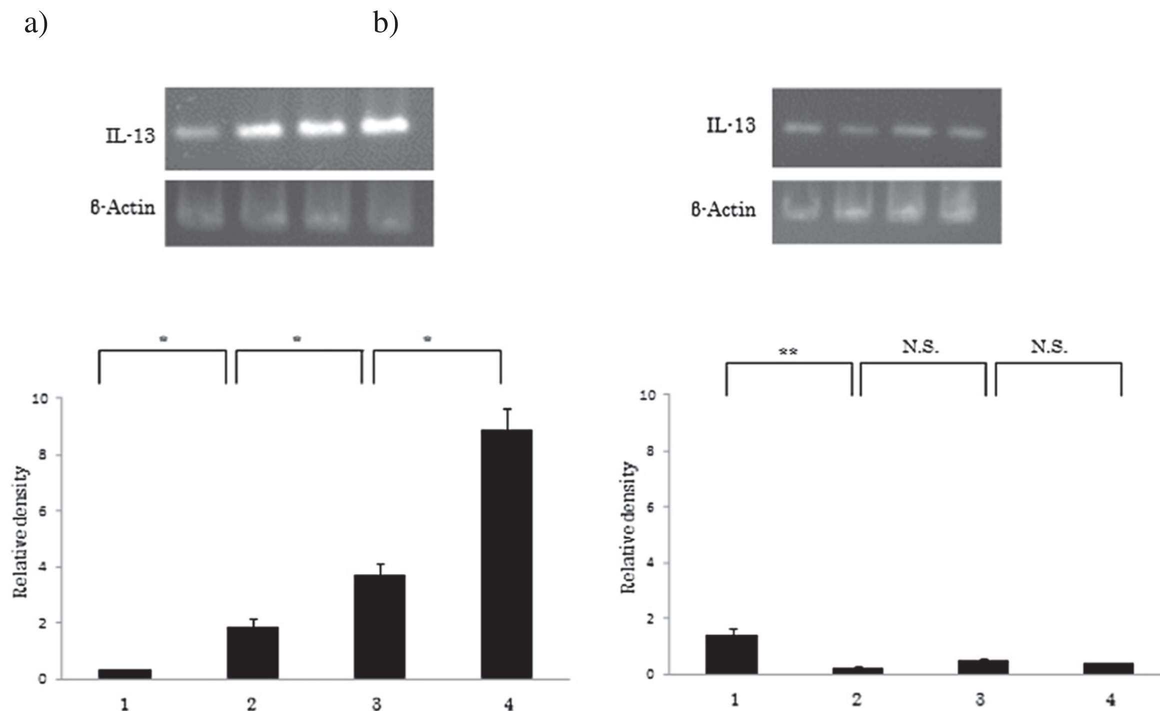



**Fig. 1.** IL-13 mRNA expression by GM-CSF-dependent macrophages and monocytes after stimulation with HNE. GM-CSF-dependent macrophages (a) and monocytes (b) were incubated with HNE (5, 10, 20, or $50 \times 10^3$ μM/L) for 6 h and the IL-13 mRNA level was determined by RT-PCR. The relative density of the bands is normalized to that of β-actin. GM-CSF: granulocyte macrophage colony-stimulating factor, HNE: human neutrophil elastase, RT-PCR: reverse transcriptase-polymerase chain reaction. Data were obtained by using cells from three different donors in each group and represent the mean ± SE. *P < .01; **P < .05; N.S., not significant. 1. HNE ($5 \times 10^3$ μM/L). 2. HNE ($10 \times 10^3$ μM/L). 3. HNE ($20 \times 10^3$ μM/L). 4. HNE ($50 \times 10^3$ μM/L).

Download English Version:

https://daneshyari.com/en/article/2827067

Download Persian Version:

https://daneshyari.com/article/2827067

Daneshyari.com