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Granulocyte–macrophage colony-stimulating factor primes interleukin-13 production by macrophages via protease-activated receptor-2

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ABSTRACT

Chronic inflammation is often linked to the presence of type 2-polarized macrophages, which are induced by the T helper type 2 cytokines interleukin-4 and interleukin-13 (IL-13). IL-13 is a key mediator of tissue fibrosis caused by T helper type 2-based inflammation. Human neutrophil elastase (HNE) plays a pivotal role in the pathogenesis of pulmonary fibrosis.

This study investigated the priming effect of granulocyte–macrophage colony-stimulating factor (GM-CSF) on IL-13 expression by macrophages stimulated with HNE.

Adherent macrophages were obtained from primary cultures of human mononuclear cells. Expression of IL-13 mRNA and protein by GM-CSF-dependent macrophages was investigated after stimulation with HNE, using the polymerase chain reaction and enzyme-linked immunosorbent assay.

GM-CSF had a priming effect on IL-13 mRNA and protein expression by macrophages stimulated with HNE, while this effect was not observed for various other cytokines. GM-CSF-dependent macrophages showed a significant increase in the expression of protease activated receptor-2 (PAR-2) mRNA and protein. The response of IL-13 mRNA to HNE was significantly decreased by pretreatment with alpha1-antitrypsin, a PAR-2 antibody (SAM11), or a PAR-2 antagonist (ENMD-1068).

These findings suggest that stimulation with HNE can induce IL-13 production by macrophages, especially GM-CSF-dependent macrophages. Accordingly, neutrophil elastase may have a key role in fibrosis associated with chronic inflammation.

1. Introduction

Interleukin (IL)-13 is a typical cytokine that drives and modulates the immune response together with IL-4. IL-13 is a key mediator of tissue fibrosis caused by Th2-based inflammation since it induces tissue fibrosis by selectively stimulating the production and activation of transforming growth factor-beta1 [1]. Macrophages are cells with an important role at the interface between innate immunity and adaptive immunity, which are generally classified into classically activated (M1) and alternatively activated (M2) macrophages. Classically activated (M1) macrophages undergo activation by exposure to pro-Th1 cytokines, whereas alternatively activated (M2) macrophages are generated in a Th2-dominant environment.

Macrophage colony-stimulating factor (M-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) were originally identified as hemopoietic growth factors [2]. These mediators are involved in regulating the size and function of various macrophage populations and have been shown to contribute to macrophage heterogeneity. Stimulation of human monocytes with GM-CSF leads to the formation of M1 cells, while M2 cells develop after culture with M-CSF [3,4]. In addition, GM-CSF stimulates the growth of myelomonocytic precursor cells from the bone marrow in humans, as well as the growth of mature granulocytes and macrophage effector cells. Furthermore, GM-CSF primes cells for the production of reactive oxygen species by promoting accumulation of phosphatidylinositol 3,4,5-trisphosphate and activation of Rac in response to chemoattractants such as *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine [5]. Thus, GM-CSF has a strong priming effect on cytokine-producing cells and it has been reported to prime human monocytes in a dose- and time-dependent manner to enhance

Abbreviations: GM-CSF, granulocyte–macrophage colony-stimulating factor; HNE, human neutrophil elastase; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; PAR-2, protease-activated receptor-2; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; Th2, T helper cell type 2; TNF-alpha, tumor necrosis factor-alpha

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lipopolysaccharide-stimulated synthesis of tumor necrosis factor-alpha (TNF-alpha) [6].

Neutrophils form the first line of the innate immune defenses against infection. Activated neutrophils release the contents of intracellular granules after migrating to sites of inflammation and various granule proteins (including cathepsin G, human leukocyte elastase, and proteinase 3) play a central role in the early inflammatory response [7]. While neutrophils have long been known to participate in acute inflammation, evidence for a role of these cells in chronic inflammation and autoimmune diseases is now emerging. For example, it was reported that neutrophils release high levels of neutrophil elastase extracellularly during chronic inflammation [8] and it has been suggested that neutrophil elastase may be involved in regulating chronic inflammation in various diseases [9].

IL-13 is a key mediator of tissue fibrosis, which is a characteristic feature of a wide spectrum of diseases. It has been suggested that IL-13-dependent fibrosis is mediated via a mechanism involving TGF-beta1 and matrix metalloproteinase-9 [10], although Pungpapong et al. [11] reported that IL-13 activates tissue fibrosis via a TGF-beta-independent mechanism. It was also reported that mice lacking neutrophil elastase and mice treated with a neutrophil elastase inhibitor developed less pulmonary fibrosis [12,13]. These reports suggest a fibrogenic effect of neutrophil elastase in inflamed lungs. Based on the above findings, the present study was undertaken to investigate whether GM-CSF acts as a priming signal for the production of IL-13 by stimulation with human neutrophil elastase.

2. Materials 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 0.2 U/L was purchased from Serva Electrophoresis (Heidelberg, Germany). GM-CSF and M-CSF were obtained from Tocris Bioscience (Bristol, UK). Alpha1-antitrypsin (Lee Biosolutions, MO), an antibody for protease-activated receptor-2 (PAR-2) (SAM11; Santa Cruz Biotechnology, Santa Cruz, CA), and a selective PAR-2 antagonist (ENMD-1068; Abcam, Cambridge, UK) were employed to study the signal transduction pathways involved in IL-13 mRNA expression. Two PAR-2 agonists, AC-264613 [2-oxo-4-phenylpyrrolidine-3-carboxylic acid [1-(3-bromo-phenyl)-(E/Z)-ethylidene]-hydrazide] (Tocris Bioscience) and 2-furoyl-LIGRLO-amide (Tocris Bioscience), were employed to investigate the signal transduction pathways involved in the activation of PAR-2. All reagents were negative for endotoxin by the Endospecy test [14,15].

2.3. Isolation of peripheral blood mononuclear cells (PBMCs)

Heparinized blood samples were obtained from nonsmoking healthy volunteers and were diluted 1:1 with pyrogen-free saline, after which PBMCs were isolated by density gradient centrifugation with Ficoll-Hypaque and washed three times [16]. The viability of the cells thus obtained exceeded 95% according to the trypan blue dye exclusion test. PBMCs were resuspended in RPMI-1640 medium supplemented with 25 mM HEPES, 100 mmol/L L-glutamine, 100×10^3 U/L penicillin, 100×10^3 μg/L streptomycin, and 10% fetal calf serum.

2.4. Isolation of monocytes

For isolation of monocytes as adherent cells, PBMCs (2×10^6 per well) were distributed into 12-well plates (Corning Inc. Costar, NY), and were cultured in a 5% CO_2 incubator for 2 h at 37 °C in 1 mL of RPMI-1640 medium containing 0.3 g/L L-glutamine (Sigma) supplemented with 5% (v/v) heat-inactivated fetal calf serum (FCS; Sigma), 100×10^3 U/L penicillin (Sigma), and 0.1×10^3 mg/L streptomycin (5% complete medium). Then nonadherent cells were removed and the adherent cells were carefully washed twice with prewarmed 5% complete medium [17].

2.5. Induction of macrophages from PBMCs and stimulation with HNE

GM-CSF and M-CSF are known to induce different macrophage populations [3]. Accordingly, monocytes were seeded at 2×10^6 cells/mL into 12-well tissue culture plates containing RPMI-1640 medium with

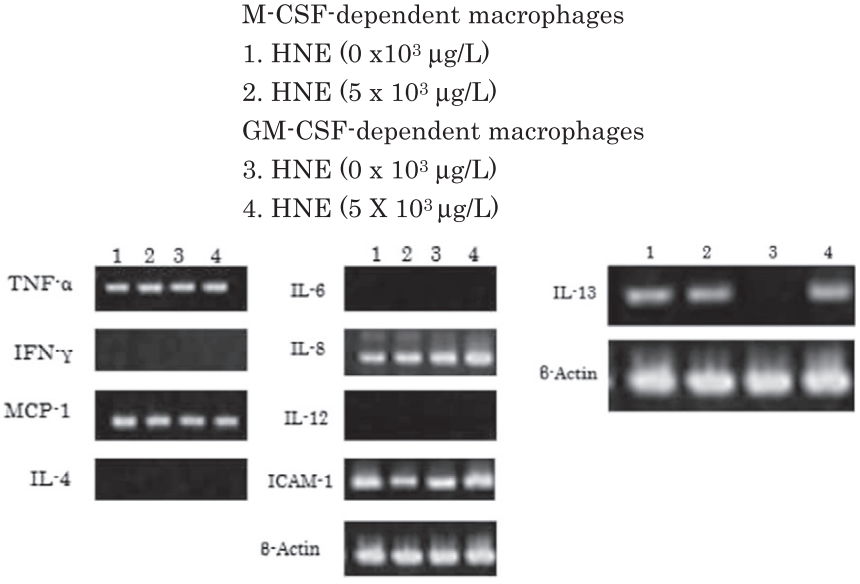

Fig. 1. Representative results of PCR for the detection of TNF-alpha, interferon-gamma, MCP-1, Il-4, IL-6, IL-8, IL-12, IL-13, and ICAM-1 mRNA expression by M-CSF-dependent and GM-CSF-dependent macrophages after incubation with or without HNE. There was no priming effect of GM-CSF on the expression of mRNAs for any of these cytokines and factors. Samples were run in triplicate and three separate experiments were performed.