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Mechanism of interferon-gamma production by monocytes stimulated with myeloperoxidase and neutrophil extracellular traps

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ABSTRACT

Neutrophil extracellular traps (NETs) have an important role in antimicrobial innate immunity and release substances that may modulate the immune response. We investigated the effects of soluble factors from NETs and neutrophil granule proteins on human monocyte function by using the Transwell system to prevent cell–cell contact.

NET formation was induced by exposing human neutrophils to phorbol myristate acetate (PMA). When monocytes were incubated with PMA alone, expression of interleukin (IL)-4, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha mRNA was upregulated, but IL-10, IL-12, and interferon (IFN)-gamma mRNA were not detected. Incubation of monocytes with NETs enhanced the expression of IL-10 and IFN-gamma mRNA, but not IL-12 mRNA. Myeloperoxidase stimulated IFN-gamma production by monocytes in a dose-dependent manner. Both a nuclear factor-kappaB inhibitor (PDTC) and an intracellular calcium antagonist (TMB-8) prevented upregulation of IFN-gamma production. Neither a combined p38alpha and p38beta inhibitor (SB203580) nor an extracellular signal-regulated kinase inhibitor (PD98059) suppressed IFN-gamma production. Interestingly, a combined p38gamma and p38delta inhibitor (BIRB796) significantly decreased IFN-gamma production. These findings suggest that myeloperoxidase induces IFN-gamma production by monocytes via p38gamma/delta mitogen-activated protein kinase.

1. Introduction

Neutrophils have long been thought of as cells involved in innate immunity protecting against microbial invasion. Serine proteases such as cathepsin G, human leukocyte elastase, and proteinase 3 are major proteins found in neutrophil granules. These proteases are commonly thought to be involved in the intralysosomal degradation of engulfed cell debris or microorganisms. Proteolysis has also been suggested to be a fundamental mechanism regulating the activities of various components of the cytokine network [1]. Neutrophil serine proteases act as alternative processing enzymes for the activation of pro-inflammatory cytokines such as interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha [2], IL-18 [3], and matrix metalloproteinase-9 [4]. Thus, these proteases are involved in the regulation of cytokine bioactivity and availability. Neutrophil proteases also modulate other mechanisms regulating inflammation such as inactivation of the anti-inflammatory mediators progranulin [5] and IL-6 [6]. Thus, these serine proteases have a marked influence on innate immune responses [7]. The neutrophil granule enzyme myeloperoxidase plays an important role in antimicrobial responses and is also required for formation of neutrophil extracellular traps [8].

The major immunoregulatory cytokines include interferon (IFN)-gamma, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, and IL-18. These cytokines can dramatically alter both the strength of the immune response and its character. IFN-gamma is a major activator of macrophages [9] and enhances their ability to kill microorganisms [10]. IFN-gamma also upregulates HLA class II antigen expression [11] and promotes T helper type 1 (Th1) differentiation [12], while downregulating the proliferation of Th2 cells. Moreover, IFN-gamma regulates the production of immunoglobulin E and some immunoglobulin G subclasses in humans [13]. In the innate immune response, IFN-gamma is predominantly produced by natural killer cells and natural killer T cells, while it is produced by Th1 CD4 and CD8 cytotoxic T cells in the antigen-specific immune response. Thus, IFN-gamma has a crucial role in immunity against intracellular pathogens and also in control of tumors [14].

Invading pathogens are attacked by both the innate and adaptive arms of the immune system. Chemokines play a critical role in leukocyte trafficking in inflammatory conditions and are key players in the links between innate and adaptive immunity [15]. The chemokine system

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HNE, human neutrophil elastase; IL, interleukin; IFN, interferon; MAPK, mitogen-activated protein kinase; NETs, neutrophil extracellular traps; PMA, phorbol myristate acetate; PAR-2, protease-activated receptor-2; PBMCs, peripheral blood mononuclear cells; RT-PCR, reverse transcription polymerase chain reaction; Th1, T helper type 1; TNF, tumor necrosis factor.

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Table 1
Reagents used in this study.

Reagent	Action
U73122	Phospholipase C inhibitor
TMB-8	Intracellular calcium antagonist
PDTC	NF-kB inhibitor
PD98059	Extracellular signal-regulated kinase inhibitor
SB203580	p38 Mitogen-activated protein kinase inhibitor (p38alpha and p38beta inhibitor)
BIRB796	p38 Mitogen-activated protein kinase inhibitor (p38gamma and p38delta inhibitor)

has been recognized an essential regulator of dendritic cell and lymphocyte trafficking, which are involved in transforming innate immune responses into adaptive responses [16]. Because IFN-gamma induces chemokine production, it enhances adaptive immunity [17].

2. Material and methods

2.1. Ethics statement

All human materials such as peripheral blood used in this study were obtained from nonsmoking healthy volunteers who gave informed consent. The study protocol was approved by the Institutional Review Board of Kumamoto Health Science University.

2.2. Reagents

Human neutrophil elastase (HNE) with an activity of 200 U/L was purchased from SERVA Electrophoresis (Heidelberg, Germany). Human neutrophils were activated by exposure to phorbol 12-myristate 13-acetate (Merck Millipore, Bedford, MA) to induce the formation of neutrophil extracellular traps (NETs). Neutrophil granular proteins such as HNE, cathepsin G (Cosmo Bio Co., Tokyo, Japan), proteinase 3 (Cosmo Bio Co.), and myeloperoxidase (Athens Research and Technology, Athens, GA) were utilized with the micropatterned Transwell system (Corning Incorporated Corning, NY) to investigate cytokine production by monocytes.

SB203580 (Wako, Kanagawa, Japan), PD98059 (Wako), BIRB796 (Axon Medchem, Groningen, Netherlands), PDTC (BioVision, Mountain View, CA), TMB-8 (Sigma-Aldrich, Oakville, Ontario, Canada), and U73122 (Merck Millipore) were employed to investigate the intracellular signal transduction pathways involved in IFN-gamma production. All reagent solutions were negative for endotoxin according to the Endospecy test [18]. The actions of these reagents are summarized in Table 1.

2.3. Isolation of adherent monocytes from peripheral blood mononuclear cells

Lymphocyte medium for thawing (BBLYMPH1) was obtained from Zen-Bio, Inc. (Research Triangle Park, NC). Lymphoprep was obtained from Nycomed (Oslo, Norway). Peripheral blood mononuclear cells (PBMCs) were isolated as described previously [19]. Briefly, heparinized blood samples were obtained from nonsmoking healthy volunteers and were diluted 1:1 with pyrogen-free saline. PBMCs were isolated immediately after collection using Lymphoprep gradients, after which the cells were suspended in BBLYMPH1 and incubated for 3 h. For isolation of monocytes by adherence, cells were distributed into 12-well plates (Corning Inc. Costar, NY, USA) at 1×10^6 cells per well and allowed to adhere for 2 h at 37 °C in a 5% CO_2 incubator, followed by washing 3 times with warm phosphate-buffered saline (PBS) to remove nonadherent cells. Then monocytes were cultured in complete medium consisting of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS) and 10×10^3 μg/L gentamicin at 37 °C in humidified air with 5% CO_2. Adherent monocytes were recovered with a cell scraper and their purity was evaluated by staining with CD14-phycoerythrin (PE) mouse anti-human monoclonal antibody (Life Technologies, Staley Road, Grand Island, NY) and flow cytometric (FACS) analysis. Recovery of monocytes was also evaluated by trypan blue staining and counting under a Zeiss microscope (Jena, Germany). Only $CD14^+$ monocytes with >85% purity were used for the present experiments. Monocytes were resuspended in RPMI-1640 medium (Sigma-Aldrich, Oakville, Ontario, Canada) supplemented with 25 mM HEPES (Sigma-Aldrich), 100 mM/L L-glutamine (Sigma-Aldrich), 100×10^3 U/L penicillin, and 100×10^3 μg/L streptomycin (Sigma-Aldrich), and then were stimulated with HNE for 6 h.

2.4. Isolation of human neutrophils

Human neutrophils were isolated from the peripheral blood of healthy nonsmoking volunteers as previously described [20]. Briefly, a suspension containing neutrophils was prepared by dextran sedimentation, Ficoll-Paque centrifugation, and hypotonic lysis of residual

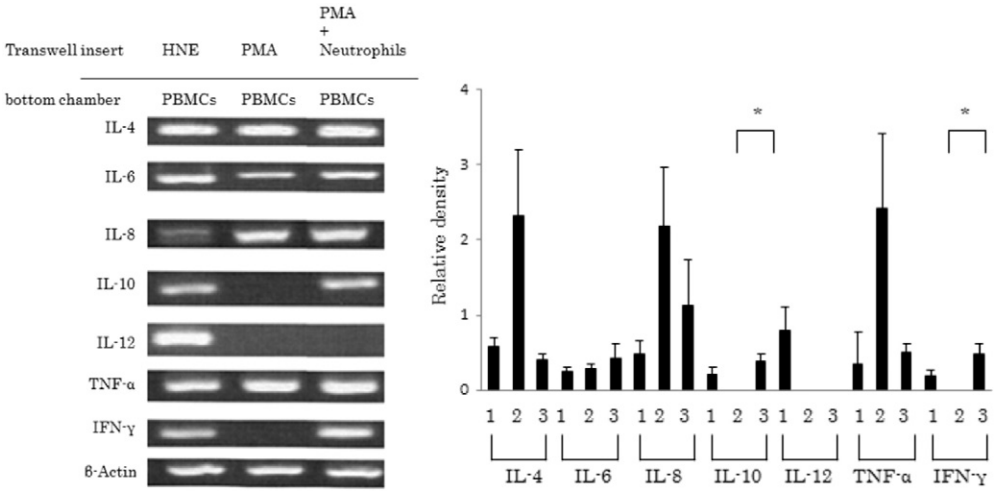

Fig. 1. Cytokine mRNA expression by monocytes exposed to NETs. RT-PCR showed that PMA alone did not upregulate expression of IL-10, IL-12, and IFN-gamma by monocytes, while NETs from PMA-stimulated human neutrophils upregulated IL-10 and IFN-gamma mRNA expression. HNE also upregulated IL-10 and IFN-gamma mRNA expression. The relative density of the bands normalized to β-actin is shown on the right. PMA: phorbol 12-myristate 13-acetate, HNE: neutrophil elastase, and RT-PCR: reverse transcription polymerase chain reaction. Data were obtained by using samples from three volunteers in each group and are represented as the mean ± SE. *P < .01; N.S., not significant. 1. Neutrophil elastase (85×10^3 μM/mL). 2. Phorbol myristate acetate (200×10^3 mM/L). 3. Phorbol myristate acetate (200×10^3 mM/L) + Human neutrophils (2×10^5 cells).