Matrix metalloproteinase 1 produced by CXCL12 stimulation on NK92 cells

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Natural killer (NK) cells play a key role in inflammation and tumor regression through their ability to migrate into tissues. The two major subsets in NK cells are CD56bright CD16dim and CD56dim CD16+. The cytotoxic activity of CD56dim NK cells is significantly greater than that of CD56bright cells. Regarding cytokine production, the situation is inverted. CD56bright NK cells are the most efficient cytokine producers. The role of CD56bright CD16+ cells is not clear. CXCL12 is a chemokine that promotes lymphocyte invasion and migration into tissues; however, the mechanism for this process remains incompletely understood. This study demonstrated that the NK92 cell population, which is the human NK cell line, was CD56bright CD16+. We examined the production of pro-matrix metalloproteinase (MMP) 1 induced by CXCL12 stimulation on NK92 cells. Pro-MMP 1 production was significantly enhanced by CXCL12 stimulation. In addition, the production of pro-MMP 1 was markedly inhibited by SB 203580 (p38 inhibitor). These results suggest that p38, which is the family of mitogen activated protein kinases (MAPKs), was involved in the production of pro-MMP 1 from CXCL12-stimulated CD56bright CD16+ NK92 cells. (J Osaka Dent Univ 2009; 43: 163–167)

Key words: Natural killer (NK) cell; CXCL12; Matrix metalloproteinase (MMP) 1

INTRODUCTION

Natural killer (NK) cells, such as T and B cells, are one of the major lymphocyte subsets that have been identified in all vertebrate species examined.1-4 NK cells participate in both innate and adaptive immunity through the prompt secretion of cytokines and the ability to lyse virally infected cells or tumor cells. The ability of NK cells to migrate appears to be tightly regulated by various molecules, such as integrins, chemokines, and proteinases.5 In human peripheral blood, five NK cell subpopulations can be defined on the basis of the relative expression of the markers CD16 and CD565-8: (1) CD56bright CD16+ (50–70% of CD56bright), (2) CD56bright CD16- (30–50% of CD56bright), (3) CD56dim CD16+, (4) CD56dim CD16-, and (5) CD56- CD16+. In healthy individuals, populations (3) and (5) are numerically in the minority.5 The role of CD56bright CD16+ cells is not clear.

Chemokines produced by inflamed areas enhance cellular adhesion, migration and inflammation. Chemokines are proteins with molecular weights of approximately 7,000 to 10,000 with endogenous leukocyte chemotaxis and activation activities; based on motifs comprising two of the four N-side conserved cysteine residues, they are divided into four families (CXC-, CC-, C- and CX-).10-12 Chemokines regulate lymphocyte trafficking in the body and also selectively induce the migration of lymphocytes into inflammatory sites. CXCL12 is the only known ligand for the chemokine receptor CXCR4.15-19 CXCL12 stimulates migration of specific types of lymphocytes including CD34+ hematopoietic progenitor cells; T, B, and NK cells; and monocytes,16-19 but not neutrophils.16-18, 20, 21

The matrix metalloproteinases (MMPs) are zinc
dependent endopeptidases that play a primary role in the degradation of extracellular matrix (ECM) proteins. So far, there are several MMPs categorized as collagenases: MMP1, MMP8, MMP13, MMP14. These enzymes hydrolyze native collagens to generate one-fourth and three-fourth fragments, which can then act as gelatinases and stromelysins. Because gelatinases and stromelysins fail to degrade native collagens, it is plausible that the catalytic activity of collagenases plays a key role not only in disease status (i.e., inflammation and tumor invasion), but also in the normal development of organogenesis.

The family of mitogen activated protein kinases (MAPKs) is composed of three major groups: the extracellular regulated kinases (ERKs), the C-Jun N-terminal kinases (JNKs) and the p38 MAPKs. p38 is a serine kinase that plays a central role in numerous proinflammatory responses. In this way p38 kinase regulates the production of key inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and cyclooxygenase (COX)-2. p38 kinase regulates multiple pathways in inflammation. The p38 kinase is widely expressed in many cell types, including immune, inflammatory and endothelial cells.

In this study, we confirmed that the NK 92 cell population was CD56brightCD16+. We then examined the effects of CXCL12 on NK92 cells. We found that pro-MMP 1 production was significantly enhanced by CXCL12 stimulation and that the production of pro-MMP 1 was markedly inhibited by p38 inhibitor SB 203580.

**MATERIALS AND METHODS**

**Cells**

The NK92 cell line was obtained from American Type Tissue Collection (ATCC, Rockville, MD, USA). NK92 cells were maintained in Alpha Minimum Essential Medium (α-MEM) (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 2 mM L-glutamine, 0.2 mM inositol, 0.02 mM folic acid (Sigma-Aldrich Co., St. Louis, MO, USA), 1.5 g/L sodium bicarbonate, 0.1 mM 2-mercaptoethanol (Wako Pure Chemical Industries), 100 U/mL recombinant IL-2 (Shionogi Co. Ltd., Osaka, Japan), 12.5% horse serum (Invitrogen Co., Carlsbad, CA, USA) and 12.5% fetal bovine serum (Equitech-Bio, Inc., Kerrville, TX, USA).

**Regents and Antibodies**

Anti-CXCR4 (12 G 5) antibody and human recombinant CXCL12 were purchased from R&D Systems (Minneapolis, MN, USA). SB 203580 and anti-MMP 1 polyclonal antibody were purchased from Calbiochem (Darmstadt, Germany). Fluorescein isothiocyanate (FITC)-conjugated anti-CD16 and phycoerythrine (PE)-conjugated anti-CD56 antibodies were obtained from BD Biosciences (San Jose, CA, USA). Mouse IgG1 κ (MOPC) was purchased from Sigma-Aldrich Co. Alexa Fluor 488 goat anti-mouse IgG (H+L) was purchased from Invitrogen Co.

**Fluorescence-Activated Cell Sorting (FACS) Analysis**

Expression of surface antigens was measured by FACS analyses. NK92 cells were incubated at 4°C for 30 minutes with FITC-conjugated anti-CD16 and PE-conjugated anti-CD56 antibodies and washed extensively to remove excess amounts of the antibodies. For detecting CXCR4 on NK92 cells, the cells were incubated with anti-CXCR4 antibody, washed, and incubated with Alexa Fluor 488 goat anti-mouse IgG (H+L). The cells were washed extensively and analyzed using an FACS Caliber (BD Biosciences, Mountain View, CA, USA).

**Western Blot Analysis**

NK92 (1×10⁵) cells were incubated in serum-free α-MEM with CXCL12 for 24 hours. Conditioned media were collected, centrifuged to remove debris, and concentrated in Amicon Centriprep concentrators (Millipore Corporation, Bedford, MA, USA) up to 10-fold, to visualize proteins by Western blotting. Total cell lysates were prepared by dissolving cells in sodium dodecyl sulfate (SDS)-sample buffer and briefly sonicated to shear DNA. In some studies, NK92 cells were preincubated with 10 μM SB 203580 for 30 minutes at 37°C before incubation with CXCL12. Samples were separated on 10%
SDS polyacrylamide gels (SDS-PAGE) under reducing conditions. Proteins were transferred to polyvinylidene difluoride (Immobilon-P) membranes (Sigma-Aldrich Co.). Membranes were incubated for 3 hours with primary antibodies in PBS containing 0.05% Tween 20 and 10% Blocking One (Nakalai Tesque, Kyoto, Japan). Peroxidase-conjugated secondary antibody (GE Healthcare UK Ltd., Buckinghamshire, UK) was used at a 1 : 1,000 dilution, and immunoreactive bands were visualized using Super Signal West Pico chemiluminescent substrate (Thermo Scientific., Rockford, IL, USA). Signals on each membrane were analyzed with VersaDoc 5000 (Bio-Rad, Hercules, CA, USA).

RESULTS

Expressions of cell surface markers and receptor on NK92 cells
After the NK92 cells were stained with anti-CD16 FITC and anti-CD56 PE antibodies, the FACS Caliber was used to assess the expression of cell surface markers. While expression of CD56 was confirmed, expression of CD16 was not seen on the NK92 cells (Fig. 1 A). These results suggest that the NK92 cell subset is CD56<sup>high</sup> CD16<sup>-</sup>. The NK92 cells were then stained with primary antibody (anti-CXCR4 antibody) and secondary antibody (Alexa Fluor 488 goat anti-mouse IgG (H+L)), and the FACS Caliber was used to assess the expression of CXCL12 receptor. The majority of NK92 cells expressed CXCR4, which is the only known chemokine receptor for the ligand CXCL12 (Fig.1 B).

Production of pro-MMP 1 from CXCL12-stimulated NK92 cells
When NK92 cells were cultured in the absence or presence of CXCL12, active MMP1 in the serum-free conditioned medium was constitutively produced. However, CXCL12 did not increase production of active MMP1. On the other hand, in the presence of CXCL12 the production of pro-MMP 1 was significantly enhanced compared with the situation of unstimulated NK cells. Furthermore, we found that CXCL12 enhanced the production of pro-MMP 1 in a dose dependent manner (Fig. 2).

Inhibition by p38 inhibitor of the production of pro-MMP 1 from CXCL12-stimulated NK92 cells
Because MAPK such as p38, MEK 1/2, and JNK
are involved in MMP production in various cell types, we tested specific inhibitors of these MAP kinases for their ability to inhibit pro-MMP 1 production from CXCL12-stimulated NK92 cells. The production of pro-MMP 1 was significantly inhibited by SB 203580 (p38 inhibitor) (Fig. 3), indicating that p38 plays a key role in pro-MMP 1 production from CXCL12-stimulated NK92 cells. These results suggested that production of pro-MMP 1 from CXCL12-stimulated NK92 cells is regulated by mechanisms involving MAPK, including p38.

DISCUSSION

Human NK cells can be subdivided into different populations based on the relative expression of the surface markers CD16 and CD56. The two major subsets are CD56\textsuperscript{bright} CD16\textsuperscript{dim} and CD56\textsuperscript{dim} CD16\textsuperscript{+}, respectively. In this study, our FACS analysis demonstrated that NK cell line NK92 is a subset of CD56\textsuperscript{bright} CD16\textsuperscript{+}. The CD56\textsuperscript{dim} CD16\textsuperscript{+} NK cells represent at least 90% of all peripheral blood NK cells and are therefore the major circulating subset. A maximum of 10% are CD56\textsuperscript{bright} NK cells. They are abundant cytokine producers but are only weakly cytotoxic before activation. In tonsils and lymph nodes, –75% of NK cells belong to the CD56\textsuperscript{bright} subset. In sites of peripheral inflammation, CD56\textsuperscript{bright} NK cells represent 40–60% of all NK cells and display an activated phenotype (CD69\textsuperscript{+}). In this type of bidirectional interaction, a direct physical contact between NK cells and monocytes is required for a maximal effect.

The migration of lymphocytes through the tissues in response to chemokines is a multistep process that requires cell adhesion coupled with other mechanisms to overcome the physical tissue barrier. MMPs have been demonstrated to play a key role in the degradation of ECM and hence are important in cellular invasion and migration into tissues. The secretion of MMPs, such as MMP1, is important for invasion in lymphocytes. We demonstrate that CXCL12 significantly enhanced the production of pro-MMP 1 from NK92 cells in a dose dependent manner. Our results indicated that CXCL12/CXCR4 ligation specifically induces pro-MMP 1 from NK92 cells. Pro-MMP 1 production from CXCL12-stimulated NK92 cells was significantly inhibited by SB 203580, which is a p38 inhibitor. These results suggested that p38 of MAPK is involved in the production of pro-MMP 1 from CXCL12-stimulated NK92 cells. However, the enhanced production of pro-MMP 1 from NK cells by CXCL12 was not completely inhibited by SB 203580. In this regard, the production of pro-MMP 1 from CXCL12-stimulated NK cells provide another signaling pathway that is not involved in p38 MAPK.

In conclusion, our studies suggested that the CXCL12 stimulation plays an important role for the pro-MMP 1 production of NK92 cells. Additional study is needed on the degradation and invasion of collagen on CXCL12-stimulated NK92 cells.

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