Clinical Immunology (2013) 149, 379–387

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

CD27 engagement by a soluble CD70 protein enhances non-cytolytic antiviral activity of CD56^{bright} natural killer cells by IFN-γ secretion☆

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Received 26 April 2013; accepted with revision 16 September 2013
Available online 26 September 2013

KEYWORDS
Natural killer cells;
CD27;
CD70;
IFN-γ;
Hepatitis C virus

Abstract We investigated regulation of human NK cell function by CD27 engagement using a recombinant soluble CD70 protein. CD27 was preferentially expressed on CD56^{bright} NK cells, and soluble CD70 protein bound to CD27^{+}CD56^{bright} NK cells. While soluble CD70 protein enhanced IFN-γ secretion by CD56^{bright} NK cells in the presence of IL-12, it augmented neither cytolytic activity nor proliferation of NK cells. Thus, we next asked if soluble CD70 protein could be used to induce non-cytolytic antiviral activity of NK cells using an *in vitro* hepatitis C virus (HCV) infection system. Soluble CD70 protein stimulated NK cells to suppress HCV replication by enhancing NK cell IFN-γ secretion without killing infected cells. Taken together, we demonstrate that CD27 engagement by a soluble CD70 protein enhances non-cytolytic antiviral activity of CD56^{bright} NK cells by IFN-γ secretion. Thus, this soluble CD70 protein may be useful for the treatment of viral infections such as HCV infection.

☆ This work was supported by a National Research Foundation grant funded by the Korea government (MEST) (2010–0030075) and a grant from the Ministry of Health & Welfare (BCM0081211) in Korea. This work was also partly supported by the KAIST Future Systems Healthcare Project from the Ministry of Education, Science, and Technology.
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http://dx.doi.org/10.1016/j.clim.2013.09.007

1. Introduction

Natural killer (NK) cells are innate immune cells capable of killing tumor cells and virus-infected cells [1]. Their cytolytic activity is regulated by signals from both activating and inhibitory NK cell receptors [2]. The activating receptors include NKG2D and natural cytotoxicity receptors (NCRs) [2,3] while killer cell immunoglobulin-like receptors (KIRs) are major NK cell inhibitory receptors that bind to MHC class I molecules expressed on target cells [2,4]. As tumor cells and virus-infected cells tend to downregulate MHC class I expression and increase expression of ligands for NKG2D or NCRs, they become targets for NK cell-mediated cytolysis [5]. In addition, activated NK cells secrete cytokines such as IFN-γ and TNF-α that exert effector and immunomodulatory functions [2,6].

Two functionally distinct subsets of human NK cells have been identified according to their levels of CD56 and CD16 expressions [7]. Most human NK cells express high levels of CD16 (FcγRIII) and low levels of CD56, and are referred to as $CD56^{dim}$ NK cells. $CD56^{dim}$ NK cells express high amounts of cytotoxic molecules such as perforin and granzymes, and a major effector function of these cells is the cytolysis of target cells [8]. $CD56^{dim}$ NK cells also exert antibody-dependent cell-mediated cytotoxicity (ADCC) *via* CD16 engagement [7,8]. In contrast, $CD56^{bright}$ NK cells are a minor population of human NK cells with the absence or low levels of CD16 [9]. A major function of $CD56^{bright}$ NK cells is the secretion of cytokines such as IFN-γ and TNF-α. IFN-γ secreted by activated NK cells contributes to the antiviral and immunomodulatory functions of NK cells [6,7,9].

CD27 is a type I transmembrane glycoprotein expressed on lymphocytes including T cells, B cells and NK cells. In T cells, CD27 is a costimulatory receptor that when engaged by its ligand, CD70, enhances signals through the T cell receptor (TCR) [10–12]. Although $CD56^{bright}$ NK cells are known to express CD27 [13–16], a role for CD27 in regulation of NK cell activity has not been fully elucidated. In previous studies, blocking the CD27–CD70 interaction with either anti-CD27 or anti-CD70 antibodies attenuated cytolytic activity of cytokine-activated human NK cells [10,17,18]. In contrast, CD27 crosslinking by a specific anti-CD27 antibody did not enhance cytolytic activity of mouse NK cells [19]. Instead, CD27 crosslinking on mouse NK cells induced IFN-γ secretion and proliferation [19]. In addition, *in vitro* and *in vivo* analyses of NK cells from CD27-deficient mice yielded inconsistent data. In this regard, while *in vivo* analyses demonstrated reduced cytolytic activity and IFN-γ secretion by CD27-deficient NK cells, *in vitro* analyses failed to yield similar results [20].

In the present study, we investigated regulation of human NK cell function in response to CD27 engagement. To crosslink CD27, we prepared a recombinant, soluble CD70 protein. CD27 was confirmed to be preferentially expressed by $CD56^{bright}$ NK cells, and the soluble CD70 protein bound to these cells. Strikingly, while soluble CD70 protein treatment enhanced IFN-γ secretion, it did not enhance cytolytic activity of NK cells. We also assessed the ability of soluble CD70 protein treatment to augment non-cytolytic antiviral activity of NK cells using an *in vitro* hepatitis C virus (HCV) infection system. Using this infectious model, we found that soluble CD70 protein treatment stimulated NK cells to suppress HCV replication by enhancing IFN-γ secretion without killing infected host cells.

2. Materials and methods

2.1. Cell culture and antibodies

Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood of healthy normal donors by Ficoll–Hypaque density gradient. NK cells were isolated from PBMCs by MACS Negative Isolation Kit (Miltenyi Biotec, Auburn, CA) and treated with Fc blocking reagent (Miltenyi Biotec) before being used. Huh-7.5 cells were provided by Apath (Brooklyn, NY). The following fluorochrome-conjugated antibodies were used for flow cytometric analyses: anti-CD3-Pacific Blue, anti-CD4-PE-Cy7, anti-CD8-APC-H7, anti-IFN-γ-APC, anti-CD107a-PE, anti-NKG2D-APC or -PE, anti-NKG2A-FITC, anti-CD158a-FITC, anti-CD158b-FITC, anti-NKp30-PE, anti-TRAIL-PE, anti-FasL-PE, anti-granzyme B-FITC, anti-perforin-PE (all from BD Biosciences, San Jose, CA), anti-NKp46-FITC (R&D Systems, Minneapolis, MN), anti-CD27-FITC, anti-CD56-APC (Miltenyi Biotec), and anti-Myc-AlexaFluor-488 (Millipore, Billerica, MA).

2.2. Preparation of recombinant proteins

A soluble form of CD70 consisting of the extracellular domain (amino acids 39–193) of human CD70, the Fc portion of human IgG1, and a Myc tag was generated. The soluble CD70 fusion protein was expressed in HEK293E cells and purified from culture supernatant through a protein A-Sepharose column (Amersham Biosciences, Sunnyvale, CA) according to the manufacturer's instructions [21]. Human IgG1 Fc without the extracellular domain of CD70 was also expressed, purified using the same methods, and used as a negative control. The purified recombinant proteins were dialyzed with PBS and analyzed by SDS-PAGE. Protein preparations were further analyzed with the Limulus Amebocyte Lysate Test Kit (Lonza, Basel, Switzerland) to determine endotoxin levels, which were all less than 0.01 EU/μg. Cytotoxic effect of soluble CD70 protein was assessed by LDH assay, and it was not toxic to Huh-7.5 cells up to 100 μg/mL. A soluble form of CD27 (the extracellular domain, amino acids 21–191) was also prepared in the same manner as described above for the CD70 protein.

2.3. Flow cytometric analysis & intracellular cytokine staining

PBMCs or isolated NK cells were treated with FcR blocking reagent and stained with fluorochrome-conjugated antibodies for 30 min at 4 °C. For intracellular cytokine staining, isolated NK cells (200,000 cells/well) were stimulated with IL-12 (1 ng/mL) and/or soluble CD70 protein in 96-well U-bottom plates. One hour later, anti-CD107a-PE and brefeldin A (BD Biosciences) were added, and the culture was maintained for an additional 12 h. Cells were fixed, permeabilized, and stained with anti-IFN-γ-APC. Flow cytometry was performed using an LSRII (BD Biosciences), and the data were analyzed using FlowJo software (Treestar, San Carlos, CA).