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Interleukin-12 improves cytotoxicity of natural killer cells via upregulated expression of NKG2D

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KEYWORDS
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Summary Natural killer (NK) cells are crucial components of the innate immune system, providing the first line of defense against infectious pathogens and tumors. Interleukin (IL)-12 is an interleukin produced primarily by antigen-presenting cells that play an essential role in the interaction between the innate and adaptive arms of immunity acting upon T and NK cells to generate cytotoxic lymphocytes. In the present study, we explored the effect of IL-12 upregulation on the NK receptor NKG2D and on the promotion of NK cell function. IL-12 enhanced the cytotoxicity of NK cells to different solid and hematological tumor cell lines and promoted interferon-γ secretion by NK cells. The IL-12-induced cytolytic effect was dependent on the interaction of NKG2D with its ligand, MICA, because blockade of either protein attenuated the effect of IL-12 on NK cytolysis. Reverse transcriptase-polymerase chain reaction and fluorescence-activated cell sorting analyses indicated that IL-12 treatment increased NKG2D transcripts and surface expression in NK cells. Also, IL-12 augmented the expression of cytotoxic effector molecules, TRAIL and perforin, and the phosphorylation of STAT1, STAT4, and ERK1/2, which may also contribute to lysis by NK cells. These results are encouraging for the potential use of IL-12 as part of immunotherapy.

Introduction

Natural killer (NK) cells are central to both the innate and the acquired immune responses [1–3], generating and secreting cytolytic granules, cytokines, and chemokines and expressing several activating and inhibitory receptors on their surfaces. The effector functions of NK cells are regulated by cell signaling responses that follow interactions with target cell ligands [4–6]. The killer cell Ig-like receptors (KIRs) specifically recognize groups of major histocompatibility complex (MHC) class I alleles. Inhibitory KIRs have long cytoplasmic tails, which contain immunoreceptor tyrosine-based inhibition motifs, whereas short-tailed KIRs lack immunoreceptor tyrosine-based motifs and send activating signals to NK cells through interactions with the adaptor molecule, DAP12. The paired activating and inhibitory

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ABBREVIATIONS

CM	culture medium
FasL	Fas ligand
FCS	fetal calf serum
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
KIR	killer cell Ig-like receptors
MFI	mean fluorescence intensity
MHC	major histocompatibility complex
MIC	MHC class I-related chain
NK	natural killer
PBS	phosphate-buffered saline
RT-PCR	reverse transcriptase-polymerase chain reaction
STAT	signal transducer and activator of transcription
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
ULBP	UL16-binding proteins

receptors recognize the same sets of self-MHC class I molecules, differing only in their binding affinities. Importantly, MHC-specific inhibitory NK receptors appear to control NK-mediated cytotoxicity by acting at a very early stage of the signaling cascade of the activating receptors. That is, the effect of the MHC class I-specific inhibitory receptors dominates over NK-cell-activating receptors. However, the activating receptors also play a major role and are believed to be necessary for the initial activation of NK cells [4–6]. In particular, the activating receptor NKG2D is extremely important in the elimination of tumor cells. In contrast with other activating receptors, whose cytotoxicity is inhibited by binding to MHC class I molecules, NKG2D-mediated cytolysis is relatively resistant to MHC-mediated inhibition. Indeed, many tumors coexpressing both NKG2D ligands and classic MHC class I molecules are killed by NK cells via the NKG2D pathway [7,8].

Cytokines also play important roles in the regulation of NK cell activity, with exposure to specific cytokines either enhancing [interleukin (IL)-2, IL-15, IL-12, IL-18, IL-21, and interferon (IFN)-α] or downregulating (transforming growth factor-β and exogenous IFN-γ) NKG2D response [9,10]. In some cases, this modulation is brought about by alteration in the levels of NKG2D. In addition, cytokine-activated NK cells activate antigen-presenting cells, particularly dendritic cells, and induce their maturation and cytokine production [11]. IL-12 is a cytokine that is produced primarily by antigen-presenting cells that plays an essential role in the interaction between the innate and adaptive arms of immunity, acting upon T cells and NK cells to mediate the generation of cytotoxic lymphocytes. IL-12 also induces the polarization of $CD4^+$ T cells to the T helper (Th)-1 phenotype that mediates cell-mediated immunity [12,13]. IL-12 may promote cytotoxicity and IFN-γ production, especially when synergized with IL-18 or IL-15. However, the exact regulatory mechanism of IL-12 on NK cell function remains unclear.

The NK cell lines NK92 and NKL established from patients with NK malignancies [14,15] have played an important role in the study of NK cell biology during the past several years [16–18]. Here, we use these human NK cell lines, along with freshly purified human NK cells, to elucidate the effector mechanisms underlying the interaction of IL-12-stimulated NK cells with target tumor cells. We characterized the effects of IL-12 on NK cell cytotoxicity, IFN-γ production, and NKG2D expression and determined that IL-12 can upregulate NKG2D expression, thereby improving NK cellular responsiveness. IL-12 also augmented IFN-γ secretion and the expression of the cytotoxic effector molecules perforin (also known as pore-forming protein) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Finally, we demonstrated that IL-12 promotes the phosphorylation of cell-signaling molecules signal transducer and activator of transcription (STAT)-1, STAT4, and ERK1/2.

Subjects and methods

Cells and cell culture

The human NK cell line NK92, which is derived from a patient with non-Hodgkin's lymphoma [14], was purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in α-minimum essential modified medium with 12.5% fetal calf serum (FCS), 12.5% horse serum, and 100 U/ml IL-2. The human NK cell line NKL, which is derived from aggressive human natural killer cell leukemia [15], was kindly provided by Professor Jin (Department of Immunology, Fourth Military Medical University, Xi'an, People's Republic of China). NKL cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS and 100 U/ml IL-2. The human tumor cell lines analyzed in this study were preserved in our laboratory and were all maintained in RPMI1640 supplemented with 10% heat-inactivated FCS. These included the human cervical carcinoma cell lines HeLa and Caski, the human larynx cancer cell line Hep2, the human melanoma cell line M21, the human hypermetastatic lung cancer PG, the human hepatocellular carcinoma cell line HepG2, the human gastric carcinoma cell line SGC7901, the human breast carcinoma cell line MDA231, the human erythroleukemia K562 cells, and the human Burkitt's lymphoma cell line Raji. Cell culture media, FCS, and horse serum were Gibco (Invitrogen, Carlsbad, CA) cell culture products.

Cytokines and antibodies

Purified rhIL-12 was obtained from R&D Systems (Minneapolis, MN). rhIL-2 (1.5×10^7 U/mg) was purchased from Changchun Changsheng Gene Pharmaceutical Co. (Changchun, China). All cytokines were reconstituted in sterile phosphate-buffered saline (PBS) with 0.1% human serum albumin and were endotoxin free. Neutralizing antibodies against NKG2D (anti-human NKG2D monoclonal antibody, mouse IgG1) and MICA (anti-human MICA-blocking mAb, mouse IgG1) were purchased from R&D Systems. PE-conjugated mouse anti-human NKG2D mAb (mouse IgG1) and the PE-conjugated mouse IgG1 isotype were purchased from BD PharMingen (San Diego, CA). PE-conjugated mouse anti-human MICA mAb (murine IgG_{2B}) and PE-conjugated mouse IgG_{2B} isotype were obtained from R&D Systems. Anti-phospho-STAT1 (Tyr701), anti-phospho-STAT4 (Ser721), and anti-phospho-ERK1/2 (Thr202/Thr204) goat polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and were used in Western blotting at a dilution of 1:1000. Anti-STAT1 (42H3) rabbit mAb, anti-STAT4 (C46B10) rabbit mAb, and anti-ERK1/2 (137F5) rabbit mAb were products from New England Biolab (Cell Signaling Technology, Beverly, MA) and used at a dilution of