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Effect of IL-12 family cytokines on NK92 cells

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The local immune response by NK cells is an important factor in the progression of periodontal disease. Human NK cells are a homogeneous population that can be divided into five subpopulations based on the surface markers CD16 and CD56. We have confirmed that although NK92 cells do not express CD16, CD56 is highly expressed by FACS analysis. This indicates that NK92 cells are classified as subpopulations of $CD56^{bright}$ $CD16^{-}$. We performed FACS analysis after intracellular staining of intracellular granules and cytokines. We could not observe any effect of IL-12 family cytokines (IL-23, IL-27 and IL-35), other than IL-12 in perforin 1 and IFN-γ production. These results suggest that IL-23, IL-27 and IL-35 may not exert the same effect as IL-12 when stimulated alone in perforin 1 and IFN-γ production, respectively. A small amount of granzyme B production was detected by stimulation of IL-12, IL-27 or IL-35. However, granzyme B production by IL-27 or IL-35 stimulation was smaller than granzyme B production by IL-12 stimulation. These results suggested the possibility that IL-27 or IL-35 has an effect on granzyme B production of NK92 cells. (J Osaka Dent Univ 2017 ; 51 : 115-123)

Key words : Interleukin-12 family cytokines ; $CD56^{bright}$ $CD16^{-}$ natural killer (NK) cells ; Intracellular staining

INTRODUCTION

Periodontitis is a common chronic inflammatory disease affecting periodontal tissues such as gingiva, cementum, the periodontal ligament and alveolar bone. Chronic periodontitis is its most prevalent form. Although infections having a group of gram-negative bacteria (e.g., *Porphyromonas gingivalis*) are essential for inducing periodontitis, the progression of the disease is determined by the host innate and adaptive immune responses.[1-3] Many immunocompetent cells such as lymphocytes and macrophages are involved in the host immune response.[4] Local immune responses by natural killer (NK) cells and other immunocompetent cells are considered a factor in the progression of periodontal disease.

Human NK cells are a population of granular lymphocytes composed of 5-15% lymphocytes in the peripheral blood.[5, 6] NK cells are crucial components of the innate immune system and have the ability to both respond quickly and produce a wide variety of cytokines and chemokines.[7, 8] The effecter functions of NK cells do not require any prior sensitization distinct from T cells and B cells. NK cell function is regulated by both killer cell activating receptors (KAR) and killer cell inhibitory receptors (KIR).[1, 9] The final outcome of NK cell activity results from a balance between KAR and KIR. When the function of NK cells is balanced towards activation, it leads to increased cytotoxicity, increased production of cytokines and chemokines, or both.[1, 10] NK cells are not a homogeneous cell population and can be subdivided into different populations based on the relative expression of the surface markers CD16 and CD56. The two major subsets are $CD56^{bright}$ $CD16^{dim/-}$ and $CD56^{dim}$ $CD16^{+}$, which can be further subdivided into five subpopulations. In human peripheral blood, five NK cell subpopulations can be defined on the basis of the relative expression of the markers CD16 and CD56[5, 11, 12] : (1) $CD56^{bright}$ $CD16^{-}$ (50-70% of $CD56^{bright}$), (2) $CD56^{bright}$ $CD16^{+}$ (30-50% of $CD56^{bright}$), (3) $CD56^{dim}$ $CD16^{-}$, (4) $CD56^{dim}$

$CD16^+$, and (5) $CD56^-$ $CD16^+$. In healthy individuals, populations (3) and (5) are numerically in the minority.[13] $CD56^{dim}$ $CD16^+$ NK cells represent at least 90% of all peripheral blood NK cells, while $CD56^{bright}$ $CD16^{dim/-}$ NK cells account for up to 10% of the remainder.[5, 11, 13] The cytotoxic activity of $CD56^{dim}$ $CD16^+$ NK cells is significantly higher than that of $CD56^{bright}$ $CD16^{dim/-}$ NK cells and they contain much more perforin, granzymes and cytolytic granules.[13, 14] $CD56^{dim}$ subsets have 10-fold higher perforin expression than $CD56^{bright}$ subsets.[13] In contrast, although the $CD56^{bright}$ $CD16^{dim/-}$ NK cells have the capacity to produce abundant cytokines, they have low cytotoxicity.[13] $CD56^{bright}$ subsets are thought to have a capacity for high-level production of cytokines compared with $CD56^{dim}$ subsets.[5, 15] The major cytokines released by $CD56^{bright}$ $CD16^{dim/-}$ NK cells are interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-10 and IL-13.[6, 13] NK cells are one of the most important sources of IFN-γ.

The IL-12 family cytokines are the only family of heterodimeric cytokines, which consist of an α-chain (p19, p28 or p35) and a β-chain (p40 or Epstein-Barr virus-induced protein 3 (Ebi3)).[16-18] There are four members of the IL-12 family cytokines, IL-12, IL-23, IL-27 and IL-35. IL-12 is the first member of the family, which includes the p35 and p 40 subunits.[19] IL-12 family cytokines have unique structural properties in that they share cytokine subunits and cellular receptors with each other. One of the crucial biological activities of IL-12 is to induce IFN-γ production in T cells, macrophages and dendritic cells. IL-12 has also been reported to play a critical role in promotion of Th1 differentiation from CD4 T cells. It has been reported that IL-12 plays a crucial role in inducing IFN-γ production in T cells, macrophages and dendritic cells, as well as in the promotion of Th1 differentiation. In NK cells, IL-12 stimulates the production of IFN-γ and TNF-α, and also enhances cytotoxicity. IL-23 is composed of two subunits p19 and p40. IL-23 is a crucial cytokine for promoting and maintaining IL-17 -producing cells, particularly Th17 cells.[20, 21] However, the effect of IL-23 on NK cells has yet to be clearly elucidated. IL-27 is a heterodimeric cytokine[16, 22] composed of the EBI3 and p28 subunits. IL-27 is an immunoregulatory cytokine that can exert pro- and anti-inflammatory effects during immune responses.[23-25] It has been reported that IL-27 has potent antitumor activities on NK cells.[26-28] However, the effect of IL-27 on NK cells is not well understood. IL-35 was identified as an anti-inflammatory and immunosuppressive cytokine in 2007.[29] In addition to T cells, endothelial cells and vascular smooth muscle cells also produce various inflammation-regulating cytokines.[30] Because IL-35 was discovered relatively recently, knowledge of this molecule is limited, and it has mainly been provided by mouse research. Therefore, there has been little study on the effect of IL-35 on human NK cells. For IL-12 family cytokines other than IL-12, the effect on NK cells has not been elucidated. In this study, we investigated the effect of IL-12 family cytokines on NK92 cells.

MATERIALS AND METHODS

Reagents and Antibodies

We used recombinant human IL-2 purchased from Primmune (Kobe, Japan), recombinant human IL-12, IL-23 and IL-27 purchased from BioLegend (San Diego, CA, USA), and recombinant human IL-35 purchased from PeproTech (Rocky Hill, NJ, USA). PE-conjugated anti-CD16 and PE-conjugated anti-CD56 purchased from BD Biosciences (Mountain View, CA, USA) was used for cell surface staining. PE-conjugated Mouse IgG1, κ Isotype Control purchased from BD Biosciences were used for isotype control of cell surface staining. FITC-conjugated anti-Granzyme B, PE-conjugated anti-Perforin 1 and APC-conjugated anti-IFN-γ were purchased from BioLegend for intracellular staining. FITC Mouse IgG1, κ Isotype Ctrl (ICFC) Antibody, and PE Mouse IgG1, κ Isotype Ctrl (ICFC) Antibody were purchased from BioLegend for isotype control of intracellular staining. APC-conjugated anti-mouse IgG_1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) for isotype control of intracellular staining.

Cells and cell culture

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 (Wako Pure Chemical Industries), 0.2 mM inositol (Merck KGaA), 0.02 mM folic acid (Merck KGaA), 1.5 g/L sodium bicarbonate (Wako Pure Chemical Industries), 0.1 mM 2-mercaptoethanol (Wako Pure Chemical Industries), 100 U/mL recombinant IL-2, 12.5% horse serum (Thermo Fisher Scientific, Waltham, MA, USA) and 12.5% fetal bovine serum (Thermo Fisher Scientific). NK92 cells were incubated at 37°C under 5% CO_2.

Cell surface immunofluorescence staining

Expression of surface antigens was measured by Fluorescence-Activated Cell Sorting (FACS) analysis. NK92 cells were incubated at 4°C for 1 h in the dark with PE-conjugated anti-CD16, PE-conjugated anti-CD56 and PE-conjugated Mouse IgG1, κ Isotype Control antibodies. After incubation, cells were washed extensively to remove excess amounts of the antibodies. The cells were washed and analyzed using a BD FACSVerse (BD Biosciences).

Intracellular staining

We performed intracellular staining for assessment of granzyme B, perforin 1 and IFN-γ production. NK 92 cells were transferred to 24-well plates at a density of 1×10^5 cells per well. NK92 cells were stimulated with various cytokines (IL-2, IL-12, IL-23, IL-27 and IL-35) for 19 h at 37°C under 5% CO_2. Monensin (BioLegend) (1 μL/mL) was added to the NK92 cells after 3 h of culture to inhibit cytokine release from the Golgi/endoplasmic reticulum complex. After incubation, NK92 cells were washed with FACS buffer (PBS (-) (Sigma-Aldrich, St. Louis, MO, USA) containing 1% FBS and 0.1% sodium azide (Wako Pure Chemical Industries)). NK92 cells were then fixed with 0.5 mL/tube Fixation Buffer (BioLegend) in the dark for 20 min at room temperature. For use in permeabilization, the cells were diluted with Intracellular Staining Permeabilization Wash Buffer (10 X) (BioLegend) to 1 X in deionized water. The fixed cells were resuspended in diluted Intracellular Staining Permeabilization Wash Buffer and centrifuged at 350 g relative centrifugal force (RCF) for 5 min, and the process was repeated twice. NK92 cells were then intracellularly stained using various antibodies (FITC-conjugated anti-Granzyme B, PE-conjugated anti-Perforin 1 and APC-conjugated anti-IFN-γ) at 4°C for 30 min in the dark. Isotype control of intracellular staining was done using FITC Mouse IgG1κ Isotype Ctrl (ICFC) Antibody, PE Mouse IgG1κ Isotype Ctrl (ICFC) Antibody and APC-conjugated anti-mouse IgG1 at 4°C for 30 min in the dark. NK92 cells were washed with FACS buffer and resuspended in FACS buffer for flow cytometry. NK92 cells were analyzed using a BD FACSVerse.

RESULTS

Expression of CD16 and CD56 on NK92 cells

Expression of cell-surface antigens was measured by FACS analysis. The NK92 cells were then stained with PE-conjugated antibody (anti-CD16, anti-CD56 and anti-Mouse IgG1κ), and the BD FACSVerse was used to assess the expression on the cell surface of CD markers. The level of CD 16 expressed on NK92 cells is shown by a solid line histogram, while a negative control antibody (anti mouse IgG) is shown by a dotted line histogram (Fig. 1, left). We confirmed that CD16 is not ex-

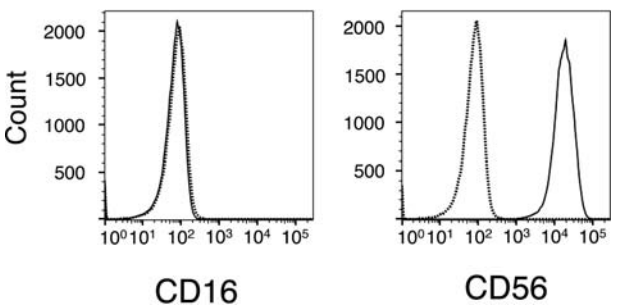

Fig. 1 Expression of CD16 and CD56 on the surface of NK92 cells. NK92 cells were stained with mAb specific for PE conjugated anti-CD16 (left : solid line histogram) and anti-CD56 (right : solid line histogram). Expression with PE conjugated anti-Mouse IgG1κ as control is shown as a dotted line histogram for ease of comparison.

pressed on the surface of NK 92 cells. Levels of CD56 cell-surface expression on NK92 cells are shown in Fig. 1 (right : solid line histogram). Staining with a negative control antibody (anti-Mouse IgG) is shown as a dotted line histogram for ease of comparison. We confirmed that CD56 is highly expressed on the surface of NK92 cells.

Effect of IL12 family cytokines on intracellular granule production of NK92 cells

NK cells participate in the innate immune system through the prompt secretion of cytokines and their ability to lyse virally infected cells without presensitization. It is known that NK cells destroy target cells utilizing lytic mediators perforin and granzyme existing as cytoplasmic granules. We investigated the effect of IL-12 cytokine family stimulation on granzyme B and perforin 1 production of NK92 cells. Intracellular staining was performed for observation of intracellular granules and measured by FACS analysis. Cytokines such as IL-2 and IL-12 are critical regulators of NK cell activation. One of their effects is to increase lytic activity. We used IL-2 and IL-12 as a positive control. Levels of perforin 1 in unstimulated NK92 cells are shown by a dotted line histogram, and a negative control antibody (anti mouse IgG1κ isotype control (ICFC)) is shown by a solid line histogram (Fig. 2, upper left). We found a significant production of perforin 1 even in unstimulated NK 92 cells compared with the negative controls. Levels of perforin 1 produced by IL-2 stimulated NK92 cells (solid line histogram) were slightly increased compared to the levels with unstimulated NK92 cells (dotted line histogram) as a control (Fig. 2, upper middle). Levels of perforin 1 produced by IL-12 stimulated NK92 cells (solid line histogram) were slightly increased compared to the levels with unstimulated NK92 cells (dotted line histogram), and the increased levels were almost the same as with IL-2 stimulation (Fig. 2, upper right). Levels of perforin 1 produced by IL-23 stimulated NK 92 cells (solid line histogram) were about the same as with unstimulated NK92 cells (dotted line histogram), which was used as a control (Fig. 2, lower left).

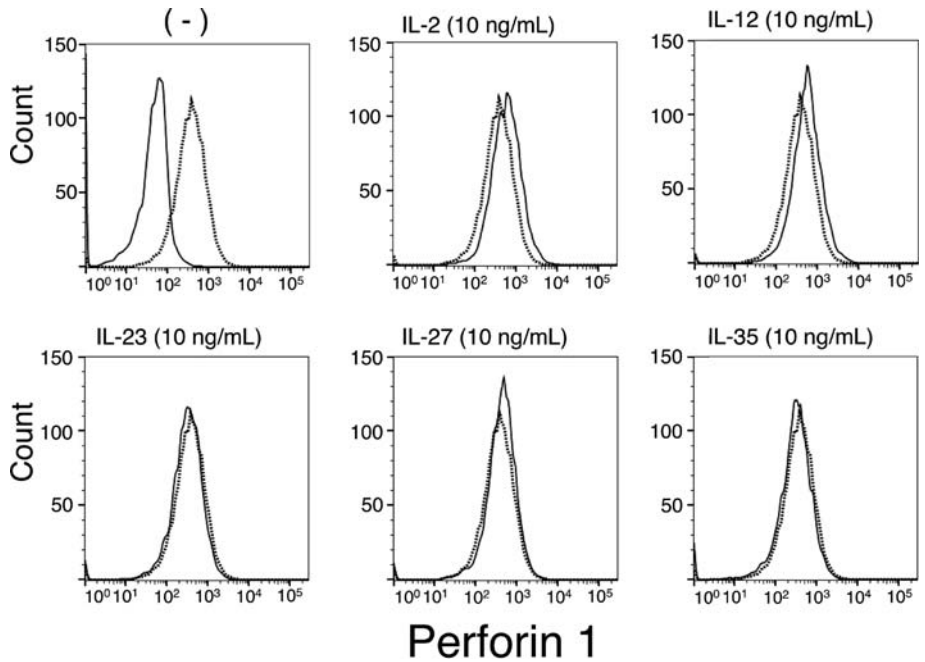

Fig. 2 Effect of IL12 family cytokines on perforin 1 production in NK92 cells. NK92 cells were stimulated with various cytokines (IL-2, IL-12, IL-23, IL-27 and IL-35) for 19 hours at 37°C under 5% CO_2. NK92 cells were fixed, stained with PE-conjugated anti-Perforin 1, and analyzed by flow cytometry. The expression for the stimulus shown at the top of the figure is represented by a solid line histogram. The expression for unstimulated NK92 cells used as a control is shown as a dotted line in all figures.

Levels of perforin 1 produced by IL-27 stimulated NK 92 cells (solid line histogram) were little changed compared to the levels with unstimulated NK92 cells (dotted line histogram) which were used as a control (Fig. 2, lower middle). Levels of perforin 1 produced by IL-35 stimulated NK 92 cells (solid line histogram) were at the same level as with unstimulated NK92 cells (dotted line histogram) which were used as a control (Fig. 2, lower right). Under our present experimental conditions, the effects of the IL-12 family cytokines (IL-23, IL-27 and IL-35) other than IL-12 in perforin 1 production, could not be confirmed.

Levels of granzyme B in unstimulated NK92 cells are shown by a dotted line histogram, and a negative control antibody (anti mouse IgG1κ isotype control (ICFC)) is shown by a solid line histogram (Fig. 3, upper left). We found that a significant amount of granzyme B is produced even in unstimulated NK 92 cells compared to negative controls. Levels of granzyme B produced by IL-2 stimulated NK92 cells (solid line histogram) were slightly increased compared with unstimulated NK92 cells (dotted line histogram) as a control (Fig. 3, upper middle). The levels of granzyme B produced by IL-12 stimulated NK92 cells (solid line histogram) increased slightly compared to unstimulated NK92 cells (dotted line histogram) and the increased level was ever so slightly higher than that of stimulated IL-2 (Fig. 3, upper right). Levels of granzyme B produced by IL-23 stimulated NK 92 cells (solid line histogram) were at the same level as unstimulated NK92 cells (dotted line histogram), which were used as a control (Fig. 3, lower left). Levels of granzyme B produced by IL-27 stimulated NK 92 cells (solid line histogram) showed only a small increase compared with unstimulated NK92 cells (dotted line histogram), cells which were used as a control (Fig. 3, lower middle). Levels of granzyme B produced by IL-35 stimulated NK 92 cells (solid line histogram) showed only a small increase compared with unstimulated NK92 cells (dotted line histo-

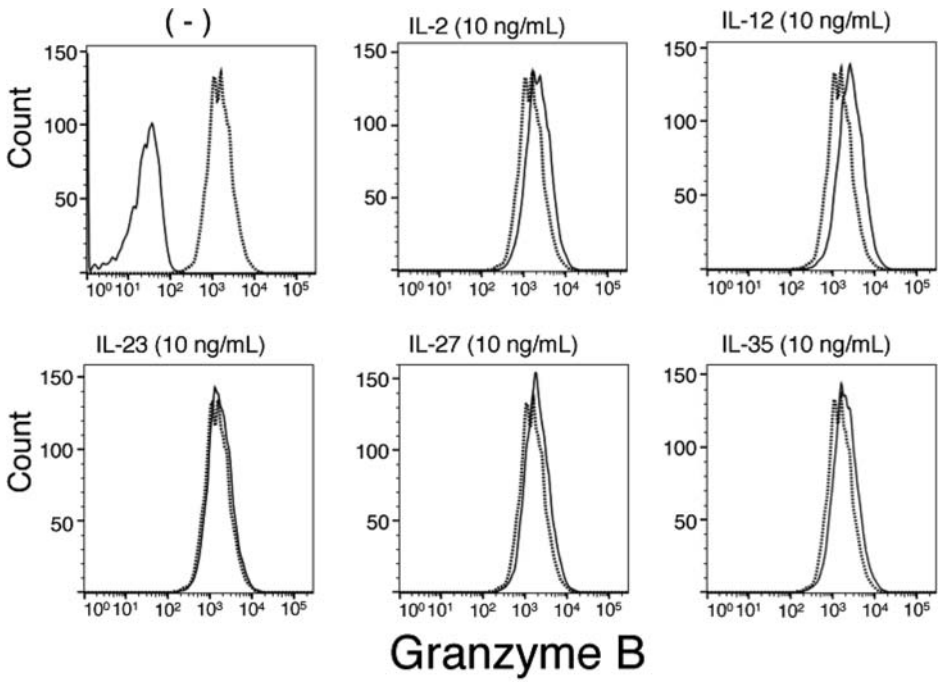

Fig. 3 Effect of IL12 family cytokines on granzyme B production in NK92 cells. NK92 cells were stimulated with various cytokines (IL-2, IL-12, IL-23, IL-27 and IL-35) for 19 hours at 37°C under 5% CO_2. NK92 cells were fixed, stained with FITC-conjugated anti-granzyme B and analyzed by flow cytometry. The expression for the stimulus shown at the top of the figure is represented by a solid line histogram. The expression for unstimulated NK92 cells used as a control is shown as a dotted line in all figures.

gram), which were used as a control (Fig. 3, lower right). The increased levels of granzyme B produced by IL-35 stimulated NK 92 cells were almost the same as the situation with IL-27 stimulated NK 92 cells. A small amount of granzyme B production was confirmed by stimulation of IL-12, IL-27 and IL-35 under our experimental conditions. The increase in the level of granzyme B produced by IL-12 stimulated NK 92 cells was greater than that with IL -27 or IL-35 stimulated NK 92 cells.

Effect of IL12 family cytokines on IFN-γ production of NK92 cells

It is known that NK cells are one of the most important producers of IFN-γ. We investigated the effect of IL-12 cytokine family stimulation on IFN-γ production of NK92 cells. Intracellular staining was performed for observation of IFN-γ production and measured by FACS analysis. Cytokines such as IL-2 and IL-12 are important regulators of NK cell activation and have an effect on the production of IFN-γ, in addition to the production of intracellular granules. As with experiments with intracellular granule production, we used IL-2 and IL-12 as a positive control. Levels of IFN-γ in unstimulated NK92 cells are shown by a dotted line histogram, and a negative control antibody (anti mouse IgG1) is shown by a solid line histogram (Fig. 4, upper left). We found that small amounts of IFN-γ are produced even in unstimulated NK 92 cells compared to negative controls. Levels of IFN-γ produced by IL-2 stimulated NK92 cells (solid line histogram) were slightly increased compared with unstimulated NK92 cells (dotted line histogram) which were used as a control (Fig. 4, upper middle). Levels of IFN-γ produced by IL-12 stimulated NK92 cells (solid line histogram) were increased compared to that by unstimulated NK92 cells (dotted line histogram), which were used as a control (Fig. 4, upper right). Increased levels of IFN-γ produced by IL-12 stimulation were larger than that by IL-2 stimulation. Levels of IFN-γ produced by IL-23 stimulated NK 92

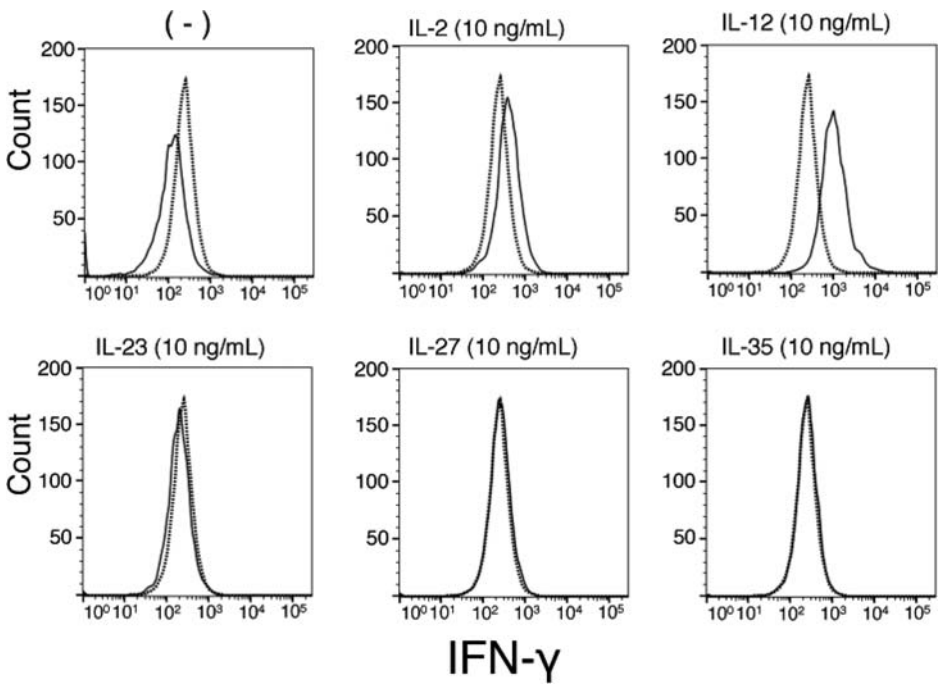

Fig. 4 Effect of IL12 family cytokines on IFN-γ production in NK92 cells. NK92 cells were stimulated with various cytokines (IL-2, IL-12, IL-23, IL-27 and IL-35) for 19 hours at 37℃ under 5% CO_2. NK92 cells were fixed, stained with APC-conjugated anti-IFN-γ and analyzed by flow cytometry. The expression for the stimulus shown at the top of the figure is represented by a solid line histogram. The expression for unstimulated NK92 cells used as a control is shown as a dotted line in all figures.

cells (solid line histogram) showed little change compared with unstimulated NK92 cells (dotted line histogram), which were used as a control (Fig. 4, lower left). Levels of IFN-γ produced by IL-27 stimulated NK 92 cells (solid line histogram) were at the same level as by unstimulated NK92 cells (dotted line histogram), which were used as a control (Fig. 4, lower middle). Levels of IFN-γ produced by IL-35 stimulated NK 92 cells (solid line histogram) were at the same level as by unstimulated NK92 cells (dotted line histogram), which were used as a control (Fig. 4, lower right). Under our present experimental conditions, the effect of IL-12 family cytokines (IL-23, IL-27 and IL-35) other than IL-12 on IFN-γ production could not be confirmed.

DISCUSSION

NK cells are well known to have prompt secretion of cytokines and the ability to lyse virally infected cells or tumor cells without prior sensitization. Rather than being a homogeneous population, human NK cells can be divided into various subsets according to their surface markers.[6] Based on the cell surface density of CD16 and CD56, NK cells can be subdivided into five subpopulations :[5, 11, 12] (1) $CD56^{bright}$ $CD16^{-}$, (2) $CD56^{bright}$ $CD16^{+}$, (3) $CD56^{dim}$ $CD16^{-}$, (4) $CD56^{dim}$ $CD16^{+}$, and (5) $CD56^{-}$ $CD16^{+}$. In the experiments reported here, we used an NK cell line, NK92, to show that CD16 is not expressed on the surface of NK92 cells and that CD56 is highly expressed on the surface of NK92 cells. This result indicates that NK92 cells are classified as CD 56^{bright} $CD16^{-}$ among the five subpopulations.

In general, $CD56^{bright}$ NK cells are considered to be the major source of high levels of immunoregulatory cytokines, whereas $CD56^{dim}$ NK cells are considered to be highly cytotoxic for NK-sensitive target cells.[12, 31] The cytotoxic activity of $CD56^{dim}$ NK cells is significantly higher than that of $CD56^{bright}$ NK cells and they have slightly less cytotoxicity than $CD56^{bright}$ $CD16^{-}$ NK cells.[5, 13, 32] In contrast, the CD 56^{bright} NK cells have the capacity to produce abundant cytokines compared with $CD56^{dim}$ NK cells.[5] $CD56^{bright}$ NK cells are the most efficient cytokine producer and the most prominent cytokines produced are TNF-α and IFN-γ. Furthermore, NK cells have been reported to secrete various factors including immunoregulatory cytokines such as IL-5, IL-8, IL-10, IL-13 and GM-CSF.[31, 33] Because the subset of NK 92 cells is $CD56^{bright}$ $CD16^{-}$, it is thought that although NK92 cells produce abundant immunoregulatory cytokines, they are less effective mediators of cytotoxicity. However, it has been reported that after activation with IL-2 or IL-12, CD 56^{bright} NK cells exhibit similar or enhanced cytotoxicity against NK targets compared to $CD56^{dim}$ NK cells.[15, 32, 34] From this study, where we examined the effect of IL-12 family cytokines on NK92 cells, we believe that IL-12 family cytokines may be effective for both cytokine production and cytotoxic activity as with IL-12 stimulation. Therefore, we examined the cytotoxic activity by investigating the effect of IL -12 family cytokine stimulation on the production of granzyme B and perforin 1 by NK92 cells by analyzing intracellular staining performed for observation of intracellular granules. Regarding the production of perforin 1 in NK92 cells under our present experimental conditions, even in IL-2 or IL-12, which were used as positive controls, we were only able to confirm slight production. Under our present experimental conditions we were not able to confirm the effect of IL-12 family cytokines (IL-23, IL-27 and IL-35) other than IL-12 in perforin 1 production. Under our present experimental conditions, the production of granzyme B by IL-2 or IL-12 stimulation, which was used as a positive control, was at the same low level as in the experiment of perforin 1 production. A small amount of granzyme B production was confirmed by stimulation of IL-12, IL-27 and IL-35 under our present experimental conditions. However, granzyme B production produced by IL-27 or IL-35 stimulation was smaller than that by IL-12 stimulation. This suggests the possibility that IL-27 or IL-35 may have an effect on the granzyme B production of NK92 cells.

It is known that $CD56^{bright}$ NK cells have considerable capacity to produce cytokines. IFN-γ is one of the major cytokines released by $CD56^{bright}$ NK cells. Therefore, we examined the cytokine production capacity by investigating the effect of IL-12 family

cytokine stimulation on the production of IFN-γ by NK92 cells by analyzing intracellular staining performed for observation of intracellular cytokines. With regard to the production of IFN-γ in NK92 cells under our present experimental conditions, we were able to detect small amounts of production when IL-2 or IL-12 was used as a positive control. In addition, the amount of IFN-γ produced was higher with IL-12 stimulation than with IL-2 stimulation. However, under our present experimental conditions we could not confirm the effect of IL-12 family cytokines (IL-23, IL-27 and IL-35) other than IL-12 in IFN-γ production.

It is known that various combinations of cytokines exhibit a synergistic effect on the production of cytokines and the activation of cells. Indeed, the optimal combination of cytokines for the production of each cytokine by $CD56^{bright}$ NK cells appears to be different. For example, the combination of IL-12 and IL-18 is the best to induce a strong production of IFN-γ, which is 20-30 times higher in $CD56^{bright}$ than in $CD56^{dim}$ NK cells.[5, 35] On the other hand, whereas the combination of IL-12 and IL-18 is the optimal stimulus for the production of IFN-γ, the combination of IL-15 and IL-18 results in optimal production of GM-CSF, and the combination of IL-12 and IL-15 induces the highest levels of expression of IL-10 by $CD56^{bright}$ NK cells.[5, 15] In addition, IL-27 alone has no apparent direct stimulatory properties, although combinations with IL-12 and/or IL-2 can induce IFN-γ production by T cells and NK cells.[16, 36] In this way, even cytokines that are either ineffective or minimally effective by direct action can exert a remarkable effect with the synergistic action of being combined with other cytokines. With our experimental conditions, we were able to find very little influence of IL-12 family cytokines other than IL-12 in either cytokine production or cytotoxic activity of NK92 cells. Even when IL-2 or IL-12 stimulation was used as a positive control, it was not possible to observe significant enhancement. It is considered necessary to revise the stimulation conditions in the future. Since stimulation by either IL-27 or IL-35 produced only slight changes in the granzyme B production, there may be a significant change if this stimulation condition can be modified. In addition, a review of stimulation conditions may result in marked changes in perforin 1 production and IFN-γ production that failed to confirm the effect of IL-12 family cytokines. In the future we will consider the synergistic effect with other cytokines such as IL-18, which is known to have a synergistic effect with IL-12.

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