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Modulation of the Surface Expression of CD158 Killer Cell Ig-like Receptor by Interleukin-2 and Transforming Growth Factor-β

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Killer cell Ig-like receptor (KIR) binds to HLA class I molecules on the surface of target cells, and it confers inhibitory signals to NK cells. Although NK cytotoxicity can be affected by the change of the surface expression of KIR on NK cells, the effect of cytokines on the regulation of KIR expression has not been thoroughly investigated. Here in our study, we investigated the effect of several cytokines, including IL-2, TGF-β, IFN-γ, IL-12 and IL-18, on the surface expression of CD158 KIR, which binds to HLA-C, by the use of FACS analysis. In the isolated NK cells, IL-2 obviously increased the surface expression of CD158 KIR after 72 hr *in vitro* culture, and this was evidenced by the increased percentage of $CD158^+$ NK cells and the increased mean fluorescence intensity of CD158 in $CD158^+$ NK cells. In contrast, TGF-β decreased the surface expression of CD158 KIR after 72 hr culture. However, IFN-γ, IL-12 and IL-18 did not change the expression of CD158 KIR. The modulated expression of KIR by IL-2 and TGF-β can be associated with the changed NK-cytotoxic target-discriminating ability of NK cells upon their exposure to IL-2 and TGF-β.

Key Words: NK cells, killer cell Ig-like receptor, IL-2, TGF-β

INTRODUCTION

Natural killer (NK) cell cytotoxicity is determined by the balance between activating and inhibitory signals.[1,2] The inhibitory signal for human NK cells is conferred by killer cell Ig-like receptor (KIR) and CD94/NKG2A, and their ligands are HLA class I molecules.[1,2] KIR belongs to the immunoglobulin superfamily and it is expressed on NK cells and subsets of T cells. Upon its ligation by HLA class I molecules, KIR can deliver inhibitory signals via the immune-receptor tyrosine-based inhibitory motif.[1-3] Therefore, NK cells can recognize the cells that do not express HLA class I molecules as cytotoxic target cells, and KIR plays a role in the cytotoxic target discrimination of NK cells.

CD158, one of the members of KIR, recognizes HLA-C as its ligand.[1,2] On the basis of reactivity to different HLA-C alleles, CD158 is divided into two subgroups, CD158a and CD158b. CD158a recognizes HLA-Cw2, -Cw4, -Cw5 and -Cw6, and it can be detected by EB6 monoclonal antibody (MAb).[4-6] CD158b recognizes HLA-Cw1, -Cw3, -Cw7 and -Cw8, and it can be detected by GL183 MAb.[4-6]

NK cytotoxicity can be affected by the change of the surface expression of the inhibitory receptors such as KIR and CD94/NKG2A on NK cells. For CD94/NKG2A, which has more broad reactivity to HLA class I molecules than KIR,[7,8] it has already been reported that IL-10 or IL-15 can modulate the expression of CD94/NKG2A.[9,10] For KIR, it has also been reported that IL-2 increases the expression of CD158 KIR in the NK cells of normal humans or the patients with rheumatoid arthritis.[11,12] However, the effect of major cytokines on the regulation of KIR expression has not

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been thoroughly studied. The modulated expression of KIR or CD94/NKG2A may subsequently induce an altered threshold in NK cytotoxicity by the altered balance between activating and inhibitory signal to NK cells.

In the present study, we investigated the effect of several cytokines, including IL-2, TGF-β, IFN-γ, IL-12 and IL-18, on the surface expression of CD158 KIR by using FACS analysis after *in vitro* culture of human NK cells. We found that IL-2 increased the surface expression of CD158 KIR, and TGF-β decreased the surface expression of CD158 KIR after 72 hr in vitro culture.

MATERIALS AND METHODS

Isolation of NK cells

Peripheral blood was obtained from normal healthy adult donors. For the isolation of NK cells, RosetteSep™ Human NK Cell Enrichment Antibody Cocktail (StemCell Technologies, Vancouver, Canada) was used according to the manufacturer's instruction. Antibody Cocktail, which contained antibody complexes bispecific for erythrocyte glycophorin A and leukocytes CD3, CD4, CD19, CD36 and CD66b, was added into whole blood and then incubated for 20 min at room temperature. Antibody Cocktail-treated blood was diluted with an equal volume of PBS containing fetal bovine serum (FBS), and the erythrocyte-leukocyte rosettes were removed by Ficoll- Hypaque (Pharmacia, Piscataway, NJ, USA) density gradient centrifugation to obtain the NK cells. In all our experiments, more than 80% of the isolated cells were $CD3^-CD16^+$ or $CD3^-CD56^+$ cells.

In vitro NK cells culture with cytokines

The isolated NK cells were cultured in RPMI 1640 containing 10% FBS without any exogenous cytokine, or they were stimulated with one of the following cytokines: 100 U/mL of recombinant IL-2 (Genzyme, Cambridge, MA, USA), 20 ng/mL of TGF-β (Genzyme), 200 U/mL of IFN-γ (Genzyme), 10 ng/mL of IL-12 (Leinco Technologis, Inc., St. Louis, Mo, USA) and 50 ng/mL of rIL-18 (Endogen, Woburn, MA, USA). After 24, 48 and 72 hr, the cultured NK cells were harvested for FACS analysis.

Immunofluorescence staining of CD158 KIR and FACS analysis

To examine the cell surface expression of CD158 KIR, immunofluorescence staining and FACS analysis were performed. The cultured NK cells were washed with PBS, then resuspended in RPMI 1640 containing 1% FBS and incubated with EB6 MAb (Beckman Coulter, Miami, FL, USA), GL183 MAb (Beckman Coulter) or A803g MAb (ATgen Co., Seoul, Korea). A803g is a mouse MAb that was produced against recombinant p58 KIR protein and it can detect specific types of CD158a or CD158b such as KIR2DL1, KIR2DL3, and KIR2DS4.[13] The cells were washed with PBS containing 1% FBS, and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig (Becton Dickinson, Lincoln Park, NJ, USA) was added and the mixture was allowed to incubate. The cells were washed, and propidium iodide was added for the exclusion of dead cells. FACS analysis was performed using a FACSCalibur (Becton Dickinson) and the data was analyzed with WinMDI software.

RESULTS

There was no difference for the surface expression of CD158 KIR between cytokine-untreated NK cells and the NK cells treated with any of the cytokines after 24 hr *in vitro* culture (data not shown). After 48 hr and 72 hr culture with IL-2, the surface expression of CD158b was examined with GL183 MAb, and this was compared to the untreated NK cells. IL-2 scarcely increased the CD158b expression after 48 hr, but IL-2 obviously increased the surface expression of CD158b after 72 hr culture (Fig. 1A). IL-2 increased not only the percentage of $CD158^+$ NK cells in the total NK cell population, but it also increased the mean fluorescence intensity (MFI) of CD158 in the $CD158^+$ NK cells. In one representative case in Fig. 1A, the percentage and the MFI of CD158+ NK cells were increased from 27.1% to 27.8% and from 35.2 to 38.6 at 48 hr of IL-2 stimulation, respectively.

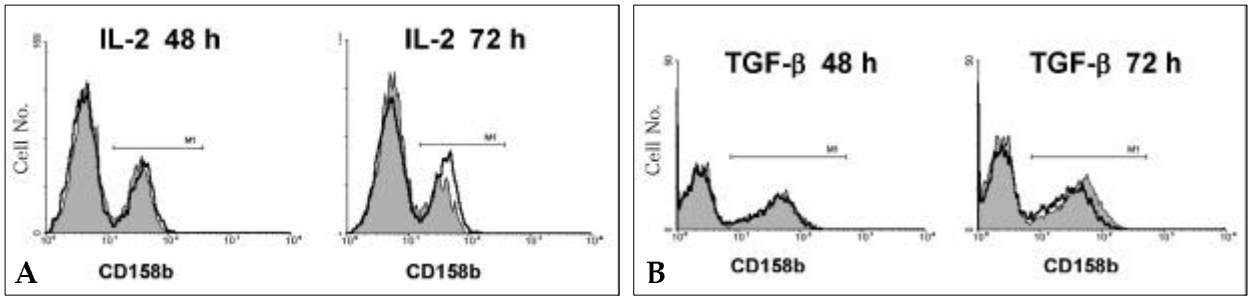

Fig. 1. The effect of IL-2 and TGF-β on the surface expression of CD158b KIR. The isolated NK cells were cultured for 48 hr and 72 hr with 100 U/mL of IL-2 (A) or with 20 ng/mL of TGF-β (B). The cultured NK cells were stained with GL183 MAb for the detection of CD158b KIR, and flow cytometric analysis was performed. The first peak of each figure represents $CD158b^-$ NK cells, and the second peak represents $CD158b^+$ NK cells. The gray filled area represents the cultured NK cells without IL-2 or TGF-β; the black line represents cultured NK cells with IL-2 or TGF-β. The changes of $CD158^+$ NK cells and the MFI were described in the text. The figure shows a representative experiment of three performed experiments.

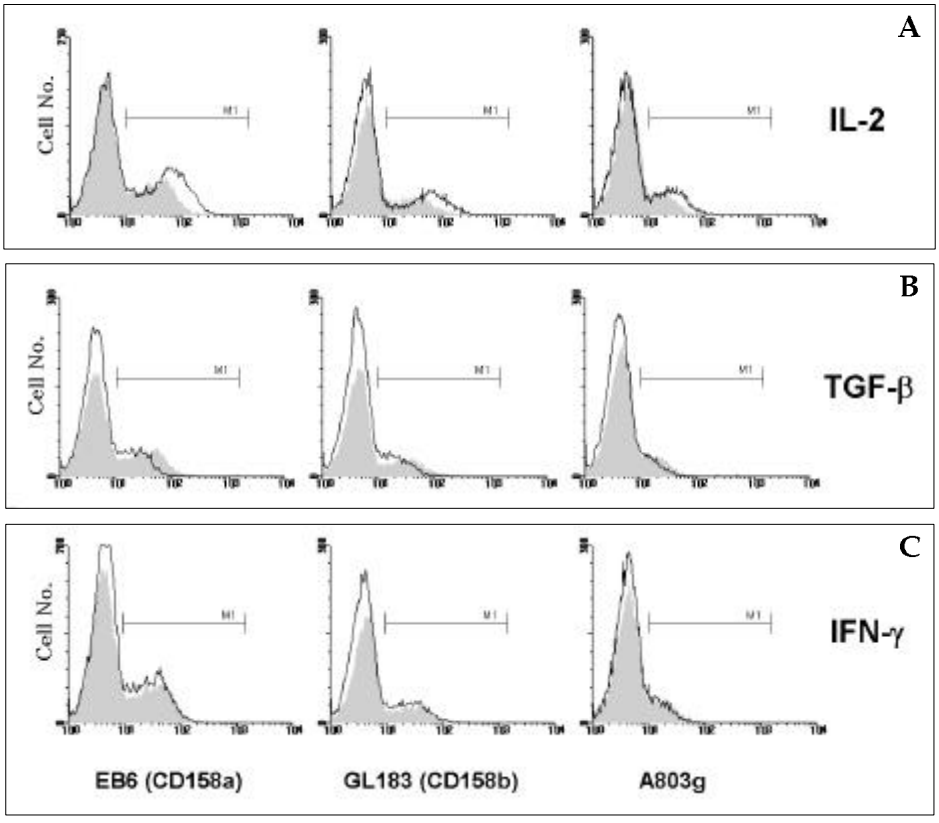

Fig. 2. The effect of IL-2, TGF-β and IFN-γ on the surface expression of CD158 KIR. The isolated NK cells were cultured without exogenous cytokine, or they stimulated with 100 U/mL of IL-2 (A), 20 ng/mL of TGF-β (B), and 200 U/mL of IFN-γ (C) for 72 hr. The cultured NK cells were stained with EB6 MAb, GL183 MAb or A803g MAb, and flow cytometric analysis was performed. The first peak of each figure represents $CD158^-$ NK cells, and the second peak represents $CD158^+$ NK cells. The gray filled area represents cultured NK cells without cytokine; the unfilled area (black line) represents cultured NK cells with cytokine. The figure shows a representative experiment of four experiments for IL-2, and three experiments performed for TGF-β and IFN-γ respectively.

After 72 hr of IL-2 stimulation, the percentage was increased from 25.0% to 34.0% and the MFI was increased from 34.0 to 40.7. The increased expression was observed for all the tested CD158 subgroups, CD158a, CD158b and A803g-reactive CD158 (Fig. 2A), indicating that the IL-2-induced up-regulation of CD158 KIR is not just specific for CD158b, but the IL-2-induced up-regulation also occurred for all subgroups of CD158 KIR.

The change of the CD158 KIR expression was obvious after 72 hr culture for all of the other cytokines, that is, if the cytokines induce any change of the CD158 KIR expression. Therefore, the representative data are presented for the other

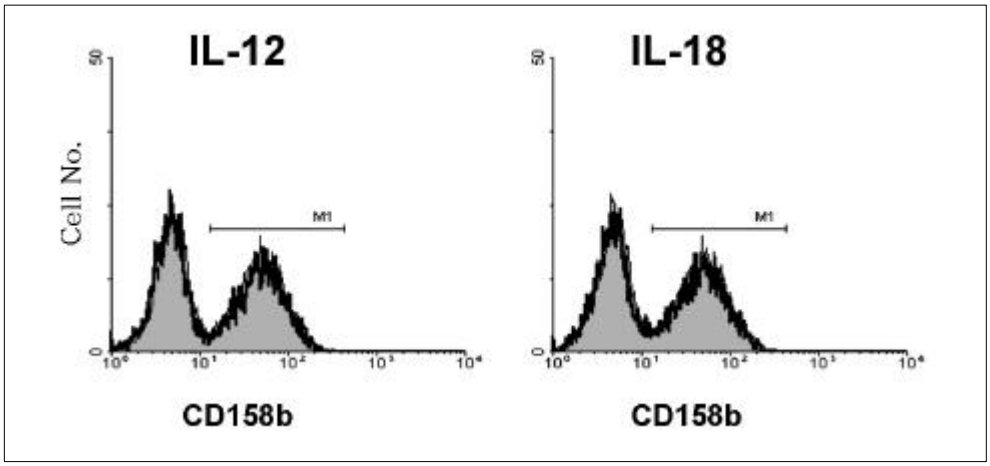

Fig. 3. The effect of IL-12 and IL-18 on the surface expression of CD158b KIR. The isolated NK cells were cultured without exogenous cytokine, or they were stimulated with 10 ng/mL of IL-12 or 50 ng/mL of IL-18 for 72 hr, respectively. The cultured NK cells were stained with GL183 MAb for the detection of CD158b KIR, and flow cytometric analysis was performed. The first peak of each figure represents CD158b$^-$ NK cells, and the second peak represents CD158b$^+$ NK cells. The gray filled area represents cultured NK cells without IL-12 or IL-18; the black line, which was overlapped on gray filled area, represents the cultured NK cells with IL-12 or IL-18. The figure shows a representative experiment of two separately performed experiments.

cytokines after 72 hr culture (Fig. 2 and 3). In contrast to IL-2, TGF-β decreased the percentage of CD158$^+$ NK cells from 43.9% to 42.8% at 48 hr of TGF-β stimulation and from 44.1% to 40.8% at 72 hr of TGF-β stimulation, and it also decreased the MFI of CD158$^+$ NK cells from 40.8 to 38.6 at 48 hr TGF-β stimulation and from 41.4 to 30.1 at 72 hr of TGF-β stimulation (Fig. 1B). The decreased expression of CD158$^+$ NK cells by TGF-β stimulation was observed for all the tested CD158 subgroups (Fig. 2B). However, IFN-γ, IL-12, and IL-18 neither changed the level of CD158 expression or the percentage of CD158$^+$ NK cells, even after 72 hr culture (Fig. 2C and 3).

DISCUSSION

Since the identification of KIR, it has been believed that the expression of KIR is very stable and it is not regulated by cytokine stimulation. In contrast to KIR, it has been already reported that the expression of CD94/NKG2A is modulated by IL-10 or IL-15.[9,10] Recently, it has also been reported that IL-2 increases the expression of CD158 KIR in the NK cells of normal humans or in NK cells of patients with rheumatoid arthritis,[11,12] however, the level of CD158 expression in CD158$^+$ NK cells and the percentage of CD158$^+$ NK cells were not precisely analyzed in these previous reports.

In the present study, we investigated the effect of several cytokines, including IL-2, TGF-β, IFN-γ, IL-12 and IL-18, on the surface expression of CD158 KIR. We chose IL-2, IFN-γ IL-12 and IL-18, as these cytokines are known to exert potent activating effects on the immune response. We also picked TGF-β as an inhibitory immune response cytokine. Among these cytokines, we found that IL-2 increased the surface expression of CD158 KIR. In contrast to IL-2, TGF-β decreased the surface expression of CD158 KIR, and IFN-γ IL-12 and IL-18 did not change the expression of CD158 KIR. The surface expression of KIR was slowly increased with time after 24 - 48 hr of IL-2 treatment. In a recent experiment, the surface expression of NKp30, which is one of natural cytotoxicity receptors,[14] and NKG2D receptor,[15] were at a maximum level after 7 days when NK cells were incubated with IL-2,[16] suggesting that the exposure time of NK cells to cytokine is one of important factors determining NK-mediated cytotoxicity. Hypothetically, the modulated expression of KIR may induce the changed thresh-

old in NK cytotoxicity by the altering the balance between activating and inhibitory signals to the NK cells. It is interesting that IL-2, a well-known NK cell activating cytokine, increased the surface expression of CD158 KIR, implying that NK cells may acquire the improved cytotoxic target-discriminating ability upon exposure to IL-2.[17] A similar hypothesis can be raised for TGF-β, that NK cells have decreased ability for cytotoxic target discrimination upon the exposure to TGF-β. It was reported that TGF-β inhibited the IL-2-mediated surface expression of NKG2D and NKp30 receptors, and consequently inhibit the NK-mediated killing of target cells.[16] In human CD8+ T cells, TGF-β induced the expression of CD94/NKG2A inhibitory receptors.[18] These data suggest that the balance of various cytokines on the expression of activating and inhibitory receptors including KIR influences the NK cell activity against the target cells.

A further study on the molecular mechanisms of the modulated expression of CD158 KIR by IL-2 and TGF-β at the transcriptional level is necessary for understanding the regulation of KIR.

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