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CD27/CD70 interaction directly induces natural killer cell killing activity

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SUMMARY

The CD27 molecule is expressed on a portion of natural killer (NK) cells as well as T and B cells. To provide the functional capacity of CD27 molecule on NK cells, we here highly purified $CD3^-$ $CD56^+$ NK cells by flow cytometry (purity >98%), and investigated their NK cell activity via CD27/CD70 interaction using a CD70-transfectant by a 4 h ^{51}Cr-release assay. The enhancement of NK activity by purified NK cells in the presence of interleukin-2 (IL-2) or interleukin-12 (IL-12) against CD70/Nalm-6 was not recognized as compared to against mock/Nalm-6. However, after a coculture with the fixed CD70/300–19, the purified NK cells increased the NK cell activity against K562, the value being 10 to 20% higher than coculture with the mock/300–19 in the presence of IL-2 or IL-12. The enhancement of NK activity was blocked by the addition of anti-CD70 monoclonal antibody (mAb). In addition, conjugation of NK cells to the target was increased by coculture with the CD70/300–19 without increased expression of adhesion molecules. In the parallel experiment, there was no increase in the killing capacity of NK cells. These results strongly show that CD27/CD70 interaction directly enhances NK activity in the presence of IL-2 or IL-12 by increasing the effector and target conjugate formation, indicating that CD27/CD70 interaction plays an important role in the cytotoxic function of NK cells.

INTRODUCTION

The CD27 molecule is a 120 000-MW type I glycoprotein[1] and belongs to a newly defined receptor family that includes nerve growth factor receptor (NGFR), tumour necrosis factor receptor (TNFR), CD40 and CD95 (Fas).[2] CD27 is expressed on the majority of peripheral blood T cells,[3] a subpopulation of B cells[4] and some resting natural killer (NK) cells.[5] The cDNA for the CD27 ligand has been found to encode a type II transmembrane protein with homology to tumour necrosis factor (TNF).[6] It is known that the transfectant of CD27 ligand can induce T-cell proliferation in the presence of a suboptimal dose of phytohaemagglutinin (PHA) and enhance the generation of cytotoxic T cells.[6] In addition, it has been shown that CD27 ligand is identical to lymphocyte activation antigen, CD70,[7] which is preferentially expressed on activated $CD45RO^+$ T cells.[8]

Human NK cells are capable of mediating a variety of immunological activity such as destruction of tumour cells and virally transformed cells.[9] NK cytotoxicity can be enhanced by several cytokines such as interleukin-2 (IL-2),[10] IL-4,[11] IL-12,[12] and interferon (IFN),[13] as well as by some surface molecules expressed on NK cells including CD2,[14] CD11a–c/CD18,[15] CD44.[16] NK functional capacity can also be regulated by FcγR III (CD16).[17] It has been shown that IL-2 activated NK cell cytotoxicity of semipurified NK cells can be decreased by the addition of one of CD27 monoclonal antibodies (mAbs).[5]

To provide evidence that CD27 molecule is important for NK cell cytotoxicity, we highly purified NK cells by flow cytometry and analysed the effects of CD27/CD70 interaction on NK cell cytotoxic function using CD70 transfectants.

MATERIALS AND METHODS

Abs and reagents

Anti-CD27 (anti-1A4; IgG1) was described elsewhere.[1] Anti-CD70 (HNE51; IgG1) was purchased from AMAC (Westbrook, ME). Phycoerythrin (PE)-conjugated anti-CD54 (Leu54, immunoglobulin G (IgG)1), fluoroscein isothiocyanate (FITC)-conjugated anti-CD3 (Leu4; IgG2), and anti-CD16 (Leu11c; IgG1) were purchased from Becton Dickinson (San Jose, CA), anti-CD2 (T11), anti-CD56 (N901, IgG1) from Coulter Immunology (Hialeah, FL), CD11a (LFA-1), CD11b from Dako (Denmark), G418 from Life Technologies (Grand Island, NY) and IL-2 from Takeda Seiyaku Co. (Osaka, Japan). IL-12 was kindly provided by Dr M. Kobayashi (Genetics Institute Inc., Cambridge, MA).

Cell preparation

Human peripheral blood mononuclear cells (PBMNC) were isolated from healthy donors by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. $CD3^-$ $CD56^+$

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cells were isolated by sorting with a fluorescence-activated cell sorter (FACStar Plus) (Becton Dickinson) under sterile conditions. $CD3^-$ $CD56^+$ cells thus obtained were >98% $CD56^+$ and >92% $CD16^+$.

Immunofluorescence

Single or two-colour flow cytometric analysis was performed on a FACScan cytometer (Becton Dickinson). FITC-conjugated goat anti-mouse immunoglobulin (Dako, Glostrup, Denmark) was used as a second antibody. Isotype-matched mouse IgG (Coulter Immunology) controls were used throughout the study, and always at levels less than 8% positive in cells.

Preparation of CD70-transfectant

Preparation of a cDNA of CD70 was described previously.[8] Briefly, total RNA was purified by Daudi cells. After amplification by reverse transcriptase–polymerase chain reaction (RT-PCR), the cDNA was cloned into SRα vector. The constructed DNA was transfected into the human pre-B cell line, 300–19 and human pre-B cell line, Nalm-6 by electroporation using a Gene Pulser (Bio-Rad, Hercules, CA). The transfectant was selected by RPMI-1640 (ICN Biomedicals, Inc. Costa Mesa, CA) medium supplemented with 10% fetal calf serum (FCS) (Upstate Biotechnology Inc., Lake Placid, NY) in the presence of G418 (1 mg/ml).

Cell activation and cytotoxic assay

Purified NK cells were incubated with 1% formaldehyde fixed mock/300–19 or CD70/300–19 in the presence of IL-2 (5 U/ml) or IL-12 (10 U/ml) for 20 hr. NK activity was measured against K562 or Nalm-6 cells in a 4-hr ^{51}Cr-release assay as previously described.[18] Briefly, effect cells were mixed with ^{51}Cr-labelled K562 cells or Nalm-6 in a total volume of 200 μl in 96-well U-bottom microtitre plates (Nunc, Roskilde, Denmark) at various effector : target (E : T) ratios ranging from 18 : 1 to 2 : 1. After 4 hr incubation at 37° in a 5% CO_2 atmosphere, 100 μl of supernatant was harvested and counted in a gamma counter (Beckman, Palo Alto, CA). Cytotoxicity of NK cells, expressed as percentage cytotoxicity, was calculated by the following formula:

$$\%\text{Cytotoxicity} = [(\text{experimental release} - \text{spontaneous release}) / (\text{total release} - \text{spontaneous release})] \times 100.$$

Single-cell cytotoxicity assay

This assay was performed as described in our earlier study.[18] Briefly, purified NK cells (2×10^5) were cultured with medium alone, IL-2 or IL-2 plus fixed CD70/300–19 (20% of effector cells) for 1 hr or 18 hr incubation, and mixed with K562 cells in a total volume of 200 μl RPMI-1640 with 10% fetal bovine serum at an E : T ratio of 10 : 1 in 5-ml plastic tubes. After centrifugation at 800 r.p.m. for 5 min, the mixture was incubated at 37° for 30 min. Cells were then gently resuspended with a Pasteur pipette and transferred to a haemocytometer. The fraction of target cells forming conjugates with at least one effect cell was determined after enumeration of at least 200 target cells. Target cells were distinguished from effect cells and fixed CD70/300–19 by their differences in cell size. The percentage of dead target cells in conjugate was determined by counting the number of trypan blue-positive cells in 200 conjugates after 20 hr culture.

RESULTS

High purification of NK cells

To delineate the effect of CD27/CD70 interaction on NK cytotoxicity, at first, we purified NK cells by sorting with a FACStar Plus flow cytometry. As shown in Fig. 1(a), we gated $CD3^-$ $CD56^+$ cells from peripheral blood mononuclear cells and sorted them. More than 98% of the sorted cells were $CD3^-$ $CD56^+$ NK cells (Fig. 1b), and 92% of them $CD16^+$ (Fig. 1c). May–Giemsa staining showed that 98% of the purified cells had granules in the cytoplasm, indicating that most of the sorted cells were granular lymphocytes (data not shown). These data indicate that we could purify $CD3^-$ $CD56^+$ $CD16^+$ or $CD3^-$ $CD56^+$ $CD16^-$ NK cells with contamination of less than 2% of monocytes or T cells.

Effect of CD70 on NK cell cytotoxicity

It has been shown that the anti-CD27 mAb can decrease the cytotoxic function of activated NK cells. To clarify the mechanism of the decrease in NK cell cytotoxicity by anti-CD27 mAb, whether a negative signal or blockage of CD27 and its ligand, we developed CD70-transfectant and investigated its effect on highly purified NK cells. By the addition of the fixed CD70/300–19 in the presence of IL-2 or IL-12, NK activity was

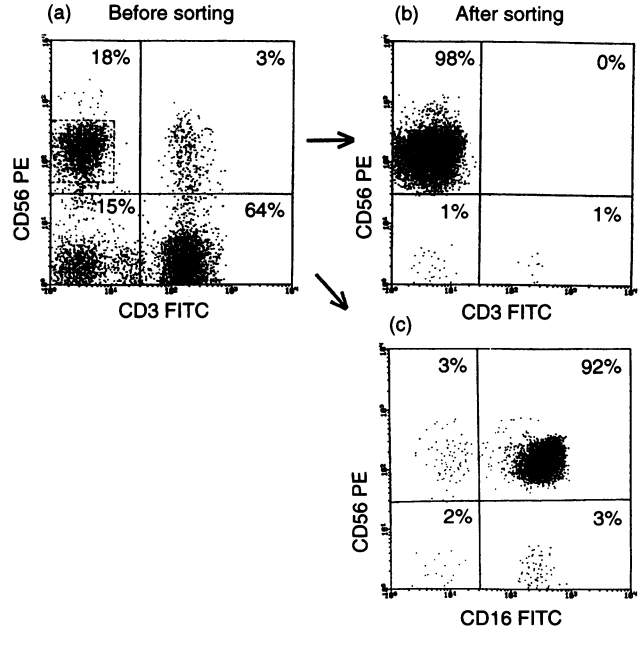

Figure 1. Purification of NK cells. MNCs were stained with a PE-conjugated anti-CD56 mAb and a FITC-conjugated anti-CD3 mAb (a), and $CD3^-$ $CD56^+$ lymphocytes were isolated using a FACStar Plus flow cytometry (b). Purified $CD3^-$ $CD56^+$ lymphocytes were checked by staining with a FITC conjugated anti-CD16 mAb (c). These data indicate that we could purify $CD3^-$ $CD56^+$ $CD16^+$ or $CD3^-$ $CD56^+$ $CD16^-$ NK cells with contamination of less than 2% of monocytes or T cells.

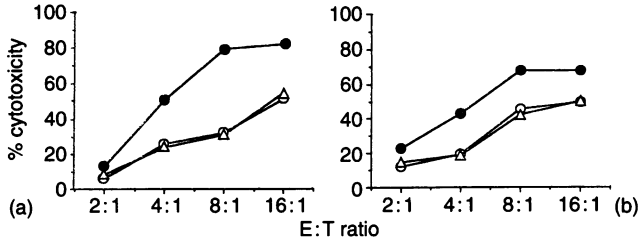

Figure 2. Effects of CD70-transfectant on NK cell cytotoxicity. Highly purified NK cells were incubated with CD70/300–19 (closed circles), Mock/300–19 (open triangles), or medium alone (open circles) in the presence of IL-2 (a) or IL-12 (b) for 20 hr and then tested for cytotoxicity against K562 at various E : T ratios. Results were expressed as the percentage of cytotoxicity. The representative data in the four separate experiments are shown.

increased as compared to that of medium alone, whereas mock/300–19 had no effect on NK cell activity (Fig. 2). No enhancement of NK cell activity was recognized by coculture with CD70/transfectant for 20 hr without IL-2 or IL-12 (data not shown).

We next added anti-CD70 mAb at the initial time into the NK cell culture in the presence of IL-2 or IL-12. As shown in Fig. 3, anti-CD70 mAb completely blocked the enhancement of NK cell cytotoxicity induced by CD70-transfectant, whereas control mAb, anti-CD56, had no effect on NK cell cytotoxicity by coculture with CD70-transfectant. Similar results were obtained when we used anti-CD27 mAb, anti-1A4 (data not shown). These data indicate that NK cell cytotoxicity was increased by CD27/CD70 interaction in the presence of IL-2 or IL-12, and the blockage of anti-CD70 mAb between the interaction of CD27 on NK cells and CD70 inhibited the enhancement of NK cell cytotoxicity induced by CD70 molecule. However, when we cocultured whole mononuclear cells were with fixed CD70/transfectant for 20 hr in the presence of IL-2 or IL-12 and used them as the effector, no apparent enhancement of NK cell activity was investigated (data not shown).

As reported elsewhere,[19] CD80-transfected P815 can be killed by T cells in the presence of anti-CD3 mAb after only 4 hr incubation. Furthermore, we used CD70/Nalm-6 as the target of IL-2 or IL-12 activated NK cells. IL-2 or IL-12 activated NK cells did not kill the CD70-transfected Nalm-6 more than mock/Nalm-6, indicating that CD27/CD70 interaction is not sufficient to increase NK activity at only 4 hr reaction (data not shown).

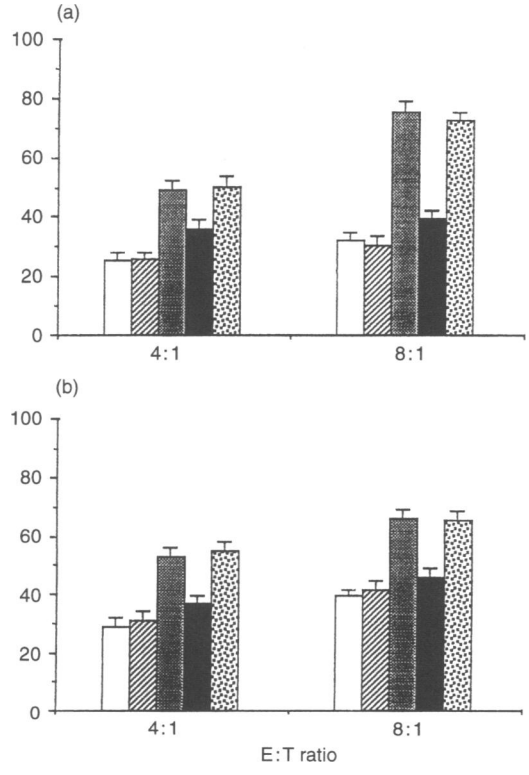

Figure 3. Inhibition of NK activity by anti-CD70mAb. Purified NK cells were incubated with medium alone (open bar), mock/300–19 (slant bar) CD70/300–19 (shaded bar), CD70/300–19 plus anti-CD70mAb (solid bar) or CD70/300–19 plus anti-CD56mAb (spot bar) in the presence of IL-2 (a) or IL-12 (b). These cells were used as effectors against K562, then tested by a standard ^{51}Cr-release assay at E:T of 4:1 and 8:1. The representative data in the three separate experiments are shown.

Effect of CD70-transfectant on target binding and killing capacity of NK cells

We cocultured the purified NK cells with fixed CD70-transfectant in the presence of IL-2, then investigated their conjugate formation with K562 and the percentage of dead target cells in conjugate. CD70-transfectant did not increase the conjugate formation between NK and K562 cells in only 1 h culture (Fig. 4). In contrast, CD70-transfectant induced the conjugate formation between the purified NK cells and K562 by 20-hr incubation. Since mock-transfectant had no effect on the conjugate formation (data not shown), CD27/CD70

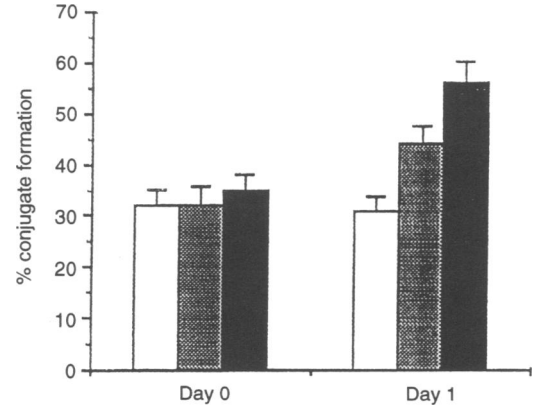

Figure 4. Effects of CD70-transfectant on conjugate formation. Freshly purified NK cells were cultured with medium alone (open bar), IL-2 (shaded bar) or IL-2 + CD70/300–19 (solid bar) for 30 min or 20 hr. Then the cells were mixed with K562 cells at an E:T ratio of 10:1, and conjugate formation assays were carried out as described in the Materials and Methods. The representative data in the two separate experiments are shown. CD70-transfectant did not increase the conjugate formation between NK and K562 cells in only 1 hr culture. In contrast, CD70-transfectant induced the conjugate formation between the purified NK cells and K562 by 20 hr incubation.

interaction can increase the conjugate formation between NK and target cells.

In the parallel experiment, we assayed the effect of CD27/CD70 interaction on the killing capacity of NK cells by estimating the percentage of dead conjugates. There was no increase in the percentage of dead target cells in conjugate when assayed after 20 hr incubation: the value (mean) was 24·8% with CD70/300–19 and 24·2% with mock/300–19 in two separate experiments.

Effect of CD70-transfectant on the expression of adhesion molecules on NK cells

By coculture with NK cells and fixed CD70-transfectant in the presence of IL-2 for 20 hr, the surface expressions of CD2, CD54, CD11a or CD11b on NK cells were not increased as compared to that cultured with mock-transfectant (data not shown). These data indicate the possibility that CD27/CD70 interaction has an important role in enhancement of contact between NK cells and the target.

DISCUSSION

The present study provides evidence that the cytotoxic function of highly purified $CD3^-$ $CD56^+$ NK cells is enhanced by the addition of the fixed CD70-transfectant in the presence of IL-2 or IL-12, indicating that CD27/CD70 interaction plays an important role in the cytotoxic function of NK cells.

It is important to have highly purified NK cells to remove the effect of T cells on the analysis of NK cell functions. However, the purity is only 70 to 85% by means of negative selection using antibodies and immunobeads (data not shown). NK cells are composed of a variety of populations such as $CD3^-$ $CD16^{bright}$ $CD56^{dim}$, $CD3^-$ $CD16^{dim}$ $CD56^{bright}$, and $CD3^-$ $CD16^-$ $CD56^{bright}$.[20] NK activity is mainly effected by $CD16^{bright}$ $CD56^{dim}$ NK cells because this population occupies over 80% of NK cells and has high NK activity. In our positive selecting system of NK cells by flow cytometry, $CD3^-$ $CD56^+$ cells contain two populations, $CD16^-$ $CD56^{bright}$ and $CD16^{bright}$ $CD56^{dim}$ NK cells (purity >98%).

Anti-CD27 mAb, anti-1A4, inhibits NK activity of semipurified, long time-cultured NK cells in the presence of IL-2.[5] Since NK cells express CD70 strongly after activation (data not shown), the inhibition may be due to the blockage of CD27/CD70 interaction between NK cells themselves or NK cells and contaminated T cells. As demonstrated previously, NK cells expressed CD27 and its expression increased after activation.[5] Although resting NK cells expressed CD27 mildly in our experiment, NK activity was increased by coculture purified NK cells and CD70-transfectant in the presence of IL-2 or IL-12 within 24 hr (Fig. 2). The cytotoxic functions of resting NK cells and activated NK cells against CD70-transfected target cells were not enhanced in the 4-hr ^{51}Cr release assay, indicating that CD27/CD70 interaction may gradually induce cytotoxic functions of NK cells. When we used mononuclear cells (MNCs) as effectors, the enhancement of NK activity was not recognized by the addition of CD70-transfectant. Recently, we found that CD27-transfectant decreased pokeweed mitogen (PWM)-driven B cell IgG synthesis by the blockage between $CD27^+$ B cells and CD70 molecule.[21]. On the basis of these findings, T cells, which highly express CD27 molecule, may disturb the interaction between CD27 on NK cells and CD70 transfectant. Additionally, since the CD70 transfectant, 300–19, used here expressed no adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1) and leucocyte function-associated antigen 1 (LFA-1) except CD70 as demonstrated previously,[22] the effect of CD70-transfectant is mainly because of the CD70 molecule.

The CD27/CD70 interaction-induced augmentation of NK cell activity depends largely on the increase in the target binding, but not killing, capacity. Unexpectedly, the augmented target binding of NK cells by CD27/CD70 interaction was not accompanied by an increase in their surface expression of adhesion molecules such as CD2, CD54, CD11a or CD11b on NK cells. Thus, CD27/CD70 interaction can augment contact of NK cells to the target cells, in which such popular adhesion molecules may not be involved. CD27/CD70 interaction may promote the maturation of NK cells in the target recognition capacity.

Our study has verified that CD27/CD70 interaction plays an important role in the cytotoxic function of NK cells through its augmentative effect on the conjugate formation between NK cells and K562 target cells in the presence of IL-2 and IL-12.

Further investigation is needed to know if the increase in NK activity by CD27/CD70 interaction is related to perforin and the Fas/Fas ligand system.

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