Differential involvement of CD40, CD80, and major histocompatibility complex class I molecules in cytotoxicity induction and interferon-γ production by human natural killer effectors

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Abstract: Natural killer (NK) cells are physiologically involved in the immune response against viruses, intracellular bacteria, and parasites as well as against malignant diseases. In addition to the cytotoxic activity, NK lymphocytes mediate a variety of homeostatic effects by producing cytokines. This study focused on the differential role of CD40 and CD80 costimulatory molecules and major histocompatibility complex class I (MHC-I) antigens in the regulation of cytotoxicity and of interferon (IFN)-γ secretion of resting and interleukin (IL)-2-activated human NK cells. CD40 and CD80 molecules were observed to play a specific role in the induction of cytotoxic function but not in IFN-γ production of IL-2-activated NK effectors. In addition, a critical role of CD94-dependent MHC-I recognition for the regulation of IFN-γ production and target lysis was demonstrated. These data provide a possible mechanism underlying functional interactions between NK lymphocytes and CD40/CD80-expressing cell targets, as represented by dendritic cells. *J. Leukoc. Biol.* 72: 305–311; 2002.

Key Words: *NK · dendritic cells · PBMC · FITC*

INTRODUCTION

Natural killer (NK) cells are characterized by a potent cytotoxic activity against tumors [1], virus-infected cells [2], and intracellular parasites [3]. Their interferon-γ (IFN-γ) production is one of the main mechanisms used to control invading pathogens [4]. IFN-γ is secreted by NK lymphocytes in response to interleukin-12 (IL-12), produced by macrophage and dendritic cells (DC) during infections [5]. This cytokine is able to induce the macrophage antimicrobial functions [6] and to promote the T helper 1 differentiation [7].

The outcome of the contact between NK and target cells is orchestrated by several receptor-ligand interactions [8, 9]. According to the "missing self" hypothesis [10], NK cell recognition of major histocompatibility complex class I (MHC-I) molecules is able to turn off their lytic machinery. Human and murine NK cells express several MHC-I recognizing inhibitory receptors. These molecules are classified in two families: the killer immunoglobulin (Ig)-like receptors (KIR) belonging to the Ig gene superfamily expressed in humans; and the lectin-like receptors, Ly49 in mice and CD94/NKG2A in human and in mice [8, 9, 11, 12]. All the inhibitory receptors have been shown to bear an immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic tail, recruiting the protein tyrosine phosphatase SHP-1, which is able to mediate the inhibition of NK cell cytotoxicity [13].

Several activating MHC-I -binding receptors have been identified [8, 9, 14–16]. CD94 can be associated with NKG2C, a molecule lacking the ITIM sequence and having hydrophobic amino acids in the intramembrane domain. The CD94/NKG2C heterodimer is able to activate NK cytotoxicity upon association with the signal-transducing molecule KARAP/DAP12 [15]. Similarly, other ITIM-lacking variants of KIR and Ly49 receptors may activate human and murine NK cytotoxicity. Moreover, the involvement of NK non-MHC-I receptors, such as CD16 [6, 17], CD69 [18], 2B4 [19, 20], and NKG2D-DAP10 [21], in NK cell cytotoxicity activation has been demonstrated. Recently, four NK cell-specific receptors, NKp30, NKp44, NKp46 [22, 23], and NKp80 [24], have been described to initiate resting and IL-2-activated, NK-mediated cell target lysis.

Recent reports, including ours, have described the functional recognition of costimulatory molecules by NK cells in mouse and human models [25–34]. Upon IL-2 activation, the human NK cells express the ligand of CD40, CD154 [35], and they selectively kill CD40-expressing targets [25, 36]. Mouse

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Received November 15, 2001; revised February 21, 2002; accepted March 6, 2002.

tumor cell lines transfected with murine CD40, CD80, or CD86 [26, 27] genes were recognized by NK cells obtained from tylorone-boosted animals or after in vitro IL-2 stimulation of NK cells. Indeed, the expression of CD80 and CD86 molecules on target cells enhances human [30, 31] and mouse [26, 27] NK-dependent cytotoxicity. Still, the involvement of the CD80/CD86-specific ligands already described for T cell costimulation in human and mouse models appears poorly characterized. CD28 antigen expression on mouse [28] and on human [29] NK cells has been detected, suggesting that the CD28-CD80/CD86 interaction could be involved in NK activation. Moreover, data obtained in mouse [27] as well as in human models [31] suggested the NK-mediated, CD80/CD86-dependent cytotoxicity in the virtual absence of CD28/CD152 counter-receptors.

Functional involvement of CD40 and CD80 molecule recognition for NK-dependent lysis of allogeneic and autologous DC has been described [28, 36, 37]. In this model, the balance between activating pathways, such as the costimulatory molecules, and MHC-I-dependent inhibitory signals has to be considered as critical for the regulation of the NK-dependent cytotoxicity.

Our study addresses the potential, different role of CD40 and CD80 costimulatory molecules and MHC-I antigens in the regulation of the cytotoxicity and IFN-γ secretion of resting and IL-2-activated human NK cells. The reciprocal interference between the CD40/CD80 molecule and CD94-dependent MHC-I recognition in the regulation of NK functions was analyzed in vitro by using the T2 and T2/TAP1+2 lymphoblastoid tumor cell targets.

MATERIALS AND METHODS

Cell lines

T-B lymphoblastoid hybrid TAP1+2-defective mutant T2 cell was generated as described [38]. The stable MHC-I-reconstituted T2/TAP1+2 cell line was produced by transfection of human TAP1+2 construct into T2 cells as described [39]. All cell lines were cultured in RPMI-1640 medium (Gibco-BRL, Life Technologies Ltd., Paisley, UK) supplemented with 5% heat-inactivated fetal calf serum (FCS; Gibco-BRL) and 2 mM glutamine (Gibco-BRL) at 37°C in 5% CO_2/95% air.

Monoclonal antibodies (mAb), immunofluorescence, and flow cytometry

The anti-CD40 IgM (14G7) and the anti-CD94 (XA185) mAb were a kind gift of Dr. R. van Lear (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands) and of Dr. L. Moretta (Istituto Nazionale Tumori, Genova, Italy), respectively. The control 345.134 mAb, recognizing a glycoprotein widely expressed on human leukocytes, was a kind gift of Dr. S. Ferrone (Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY). The anti-MHC-I mAb (W6.32) was purchased from Sigma-Aldrich (St. Louis, MO). Purified anti-CD80 IgM (BB1/B7-1), fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and Cy-chrome-labeled mAb against IFN-γ (4S.B3), CD28 (CD28.2 and L293), CD154 (TRAP-1), CD152 (BN13), CD40 (5C3), CD3 (SK7), CD56 (MY31), and isotype-matched controls were purchased from Becton Dickinson (Mountain View, CA). Immunofluorescence, flow cytometry, and data analysis were performed by using a FACSCalibur flow cytometer and the CellQuest analysis software (Becton Dickinson).

Polyclonal NK populations

Polyclonal NK cells were obtained from peripheral blood mononuclear cells (PBMC), isolated by centrifugation on Lymphoprep (Nycomed Pharma, Norway) gradients from normal donor buffy coats obtained from the Blood Bank of the Medical School of the Federico II University of Naples. After separation, the PBMC were washed and incubated in complete medium in a horizontally placed plastic flask for 2 h at 37°C to remove adherent cells. The recovered cells were used without pretreatment or incubated for 48 h with 500 IU/ml human recombinant IL-2 (Sigma-Aldrich), as indicated in Results.

Cytotoxicity assay

Cytotoxicity assay was performed with resting or 48-h IL-2-activated polyclonal NK effectors depleted of CD3-positive cells by using anti-CD3-coated magnetic beads (Dynal, Oslo, Norway) and a samarium cobalt magnet. The depletion procedure was repeated twice. Less than 2% of the remaining cells were $CD3^+$, as assessed by fluorescein-activated cell sorter (FACS) analysis (data not shown). Lysis was measured in a conventional 4 h ^{51}Cr-release assay [36]. Target cells were labeled with $Na_2{}^{51}CrO_4$ (100 μCi/2×10^6 cells), and the percent of specific lysis was calculated as [(experimental release−spontaneous release)/(maximum release−spontaneous release)] × 100. The spontaneous release never exceeded 20%. Blocking CD40 and CD80 molecule recognition was performed by pretreatment of targets with the anti-CD40 (14G7) mAb (1:1000 ascites dilution) and the anti-CD80 (BB1/B7-1) mAb (1 μg/ml), alone or in combination or with the control 354.134 mAb, by using the same experimental conditions.

Detection of intracellular IFN-γ

Fresh or IL-2-activated PBMC effectors (2×10^5) and stimulator cells (T2, T2/TAP1+2 cell lines, or human allogeneic DC) were mixed at a ratio of 1:1 and incubated in a sterile 96-well plate (Falcon, Seattle, WA) for a 6-h period at 37°C in RPMI-1640 medium and 10% FCS. To avoid any interference mediated by residual IL-2, the effectors were washed twice with complete medium before the coculture. In the blocking experiments, the PBMC effectors were pretreated with anti-CD94 (XA185) or with the control (354.134) mAb. Alternatively, CD40 and CD80 blocking was performed, pretreating the targets with anti-CD40 14G7 (1:1000 ascites dilution) or with anti-CD80 BB1/B7.1 mAb (1 μg/ml) for 30 min at 4°C before the coculture. To promote intracellular cytokine retention, brefeldin A (BFA; 5 μg/ml; Sigma-Aldrich) was added during the incubation. Intracellular IFN-γ production was detected by using a triple-staining technique and flow cytometry analysis. Briefly, after the incubation, the culture was harvested, and the cells were fixed and permeabilized by using a cytokine-staining kit following the manufacturer's instructions (Caltag Laboratories, Burlingame, CA).

Statistical analysis

Mann-Whitney U test (StatView SE+ Graphics, Abacus Concepts Inc., Berkeley, CA) was performed for the statistical analysis. The differences have been considered meaningful for $P < 0.05$.

RESULTS

CD40 and CD80 costimulatory molecules and MHC-I antigen recognition are involved in the regulation of NK-dependent cytotoxicity against lymphoblastoid tumor targets

To assess whether CD40 and CD80 costimulatory molecules might play a role in anti-tumor NK activity, fresh and IL-2-activated polyclonal NK cells were tested for their ability to lyse the T2 lymphoblastoid cell line. The TAP1+2 gene defect in these cells leads to reduced MHC-I surface expression. To inhibit the recognition of the costimulatory molecules by the NK effector, the target was pretreated with mAb directed against CD40 or CD80 molecules. Pretreatment of T2 cell target with anti-CD40 or with anti-CD80 antibodies was unable to affect the killing activity of fresh polyclonal NK (**Fig. 1A**). In contrast, a strong reduction ($P<0.05$) of the cytotoxicity was

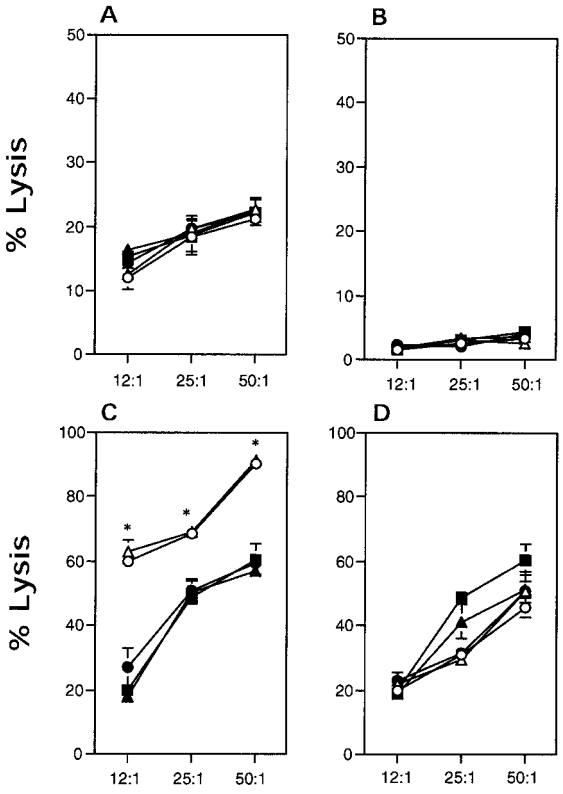

Fig. 1. MHC-I and CD40/CD80 costimulatory molecule recognition significantly affects NK lytic efficiency against the T2 target. Fresh (A and B) and IL-2-activated (C and D) polyclonal NK cells were used as effectors in cytotoxicity assay against T2 (A and C) and T2/TAP1+2 (B and D) cell targets. The targets were pretreated with anti-CD40 mAb (●), with anti-CD80 mAb (■), with a mixture of both mAb (▲), with control 345.134 mAb (△), and with medium alone (○). The percent of lysis and E:T ratio was demonstrated on the abscissas and ordinate axes, respectively. The data presented the mean ± SEM of seven different experiments. *, Results with statistical significance ($P<0.05$).

observed by using IL-2-activated NK cells as effectors (Fig. 1C). No additive or synergistic effect was elicited when a combination of the anti-CD40 and anti-CD80 mAb was used.

The CD40 and CD80 ability to trigger the NK cytotoxicity was also evaluated in the presence of the T2/TAP1+2 transfected cell line. In this system, the transfection of TAP1+2 genes restored the MHC-I surface expression, significantly reducing the NK-mediated killing (Fig. 1B and D). The residual IL-2-activated NK susceptibility was unaffected after anti-CD40 and anti-CD80 mAb treatment of T2/TAP1+2 target (Fig. 1D).

To assess whether differences in the expression of CD40 and CD80 costimulatory molecules on T2 and T2/TAP1+2 targets might account for the previous observation, the surface levels of CD40 and CD80 antigens were analyzed in both cell lines. As shown in **Figure 2**, the staining profiles revealed similar levels of both antigens on T2 (C and E) and T2/TAP1+2 (D and F) lymphoblastoid cell lines, and the expected difference in MHC-I expression was confirmed (A and B).

As IL-2 stimulation of effectors was required to elicit CD40/CD80-dependent cytotoxicity, we analyzed the expression of CD28 and CD152 receptors on NK cells after the cytokine treatment. Notably, the staining on the 48-h IL-2-activated $CD3^-$ $CD56^+$ NK cells showed only a slight expression of CD28 (**Fig. 3D**) by using the anti-CD28 L293 clone mAb [29]. In contrast, no staining was observed on fresh NK cells (Fig. 3C) and by using the anti-CD28 CD28.2 clone mAb (data not shown). In addition, no CD152 staining was observed on fresh (Fig. 3E) and IL-2-activated polyclonal NK cells (Fig. 3F). As a control, the expression of the CD154 [25] on human fresh and IL-2-activated NK effectors (Fig. 3, A and B) was assessed.

MHC-I antigens and CD40/CD80 costimulatory molecules are involved differentially in triggering NK-dependent IFN-γ secretion

To assess whether the functional recognition of CD40 and CD80 molecules and of MHC-I antigens on target cells could modulate the NK-dependent IFN-γ secretion, intracellular production of such cytokine was detected by using a triple-staining technique and flow cytometry analysis.

The ability of NK effectors to produce IFN-γ was first investigated in the presence of lymphoblastoid tumor targets. T2 and T2/TAP1+2 cell lines were used as stimulators and were cocultured for 6 h with fresh or IL-2-activated polyclonal NK cells. To maintain the complexity of the physiological microenvironment, the CD3-undepleted NK lymphocytes were

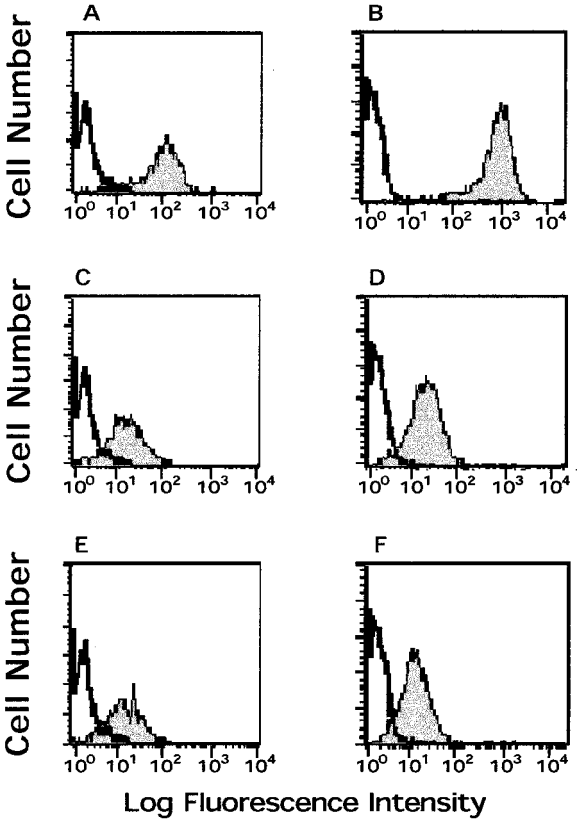

Fig. 2. T2 and T2/TAP1+2 lymphoblastoid cell lines show similar surface expression of CD40 and CD80 molecules. Staining profiles obtained by using anti-MHC-I (A and B), anti-CD40 (C and D), and anti-CD80 mAb (E and F) on T2 (A, C, and E) and on T2/TAP1+2 cell lines (B, D, and F). Open and shaded curves represent staining profiles obtained with isotype controls or specific mAb, respectively.

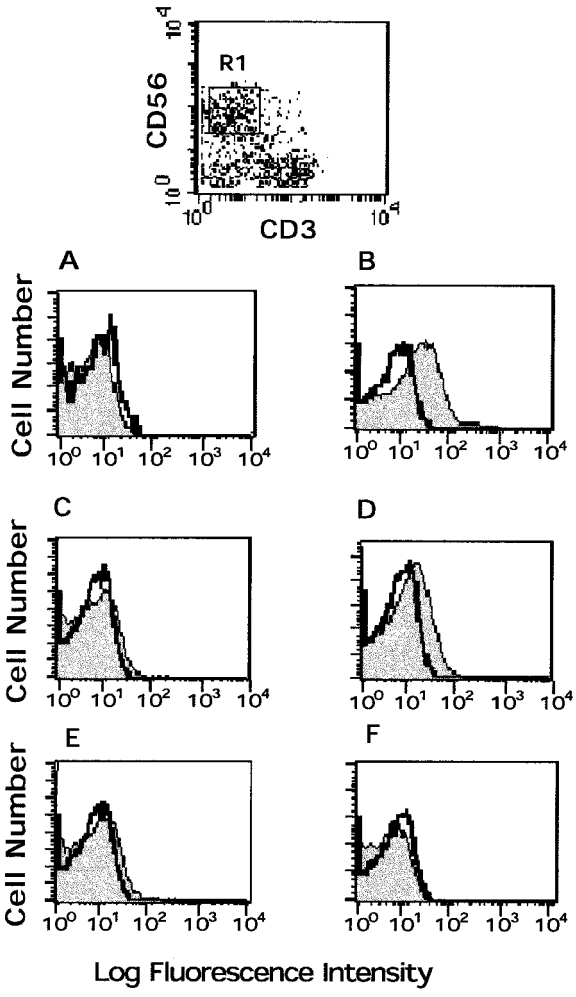

Fig. 3. Analysis of CD154, CD28, and CD152 expression on human NK cells. The figure shows one representative FACS analysis for the staining of CD154 (A and B), CD28 (C and D), and CD152 (E and F) on human fresh (A, C, and E) and 48-h IL-2-activated, polyclonal NK cells (B, D, and F). PBMC were stained with Cy-chrome anti-CD56, FITC anti-CD3, and PE anti-CD154 (A and B), anti-CD28 (C and D), or anti-CD152 (E and F) mAb. A live gate (indicated as R1 region) was set such that only $CD56^+$ lymphocytes were collected for analysis. The staining obtained with the control isotype mAb is shown as an open profile, and the specific staining is indicated by the shaded curves.

analyzed. Notably, a significant staining ($P<0.05$) for intracellular IFN-γ was detected in 48-h IL-2-activated $CD3^-$ $CD56^+$ NK cells (set as R1 region in **Fig. 4**) when incubated with T2 (B) but not with the T2/TAP1+2 cell line (C). Moreover, the same results were obtained in the presence of purified $CD3^-$ $CD56^+$ NK population. No intracellular IFN-γ was detected in the presence of medium (A) or incubating fresh NK cells with both targets (data not shown). In addition, $CD3^+$ $CD56^-$ lymphocytes (R2 region) always showed no intracellular IFN-γ production, providing the specificity for a T2-dependent NK cell triggering. These results were consistent with the already observed high and low NK susceptibility of T2 and T2/TAP1+2 targets, respectively (Fig. 1), suggesting that MHC-I recognition could regulate the IFN-γ secretion and the NK cytotoxic activity.

To investigate whether the IFN-γ production also might be modulated by CD40 and CD80 recognition, we performed experiments in which the 48-h IL-2-activated NK effectors were incubated with T2 target pretreated with anti-CD40, anti-CD80, or with the 345.134 control mAb (Fig. 4B). Interestingly, the analysis performed in the presence of anti-CD40 or anti-CD80 mAb treatments never elicited significant changes in the IFN-γ production by NK cells. No additive or synergistic effect was induced when a combination of the anti-CD40 and anti-CD80 mAb was used. Notably, the same antibodies were able to significantly modulate the NK-dependent cytotoxic activity against T2 target (Fig. 1C). The results were confirmed by analyzing the IFN-γ presence in the culture supernatants after a 24-h incubation period of the 1:1 effector-target mixture in the absence of BFA treatment (data not shown). In addition, the anti-CD154 or anti-CD28 mAb treatment of the 48-h IL-2-activated NK effectors, regardless of the presence of exogenous IL-2 and the absence of the target, was unable to affect IFN-γ production (data not shown).

The coculture with T2 or T2/TAP1+2 transfectants revealed significant differences in the IFN-γ production by NK effectors. Therefore, a critical involvement of MHC-I-dependent pathways in the cytotoxicity and the IFN-γ production of NK cells might be hypothesized.

To characterize the ability of MHC-I recognition in modulating the NK-dependent IFN-γ production, we tested the effect of anti-CD94 (XA185) mAb pretreatment of NK effectors. This approach focused on CD94-associated receptor involvement. As shown in **Figure 5**, the pretreatment with the anti-CD94 mAb was able to induce a clear IFN-γ production in the $CD3^-$ $CD56^+$ NK subset (R1; $P<0.05$), and no increase in the cytokine intracellular levels was detected in the $CD3^+$ $CD56^-$ counterpart (R2). No effect was observed in the presence of the 345.134 control mAb.

These observations point consistently to an involvement of CD94-mediated MHC-I recognition in the modulation of IFN-γ production in human NK cells, and only a marginal role for CD40 and CD80 recognition was demonstrated.

DISCUSSION

A complex balance between activating and inhibitory signals regulates the NK-effector functions [8–10, 12–14, 22, 35]. The outcome of such interactions is not limited to modulating cytotoxicity, but also represents a key event in the regulation of cytokine secretion and proliferation of NK cells [32–34]. In this context, recognition of MHC-I molecules is able to turn off the NK lytic machinery [8–10]. Recent data have been suggesting the involvement of costimulatory pathway recognition in enhancing the NK cell-mediated anti-tumor [25–31] and anti-microbial immunity [32].

CD40 and CD80 costimulatory molecules exhibit a tissue-specific expression. They are expressed mainly on DC, monocytes, B cells, and hematopoietic-derived tumors [26, 27, 35, 40]. In addition, CD40 antigen expression on normal [41] or transformed, nonhaematopoietic cells was also described [42].

This study focused on the potentially different role of CD40 and CD80 costimulatory molecules and MHC-I antigen recognition in the regulation of the cytotoxicity and IFN-γ secretion by resting and IL-2-activated human NK cells.

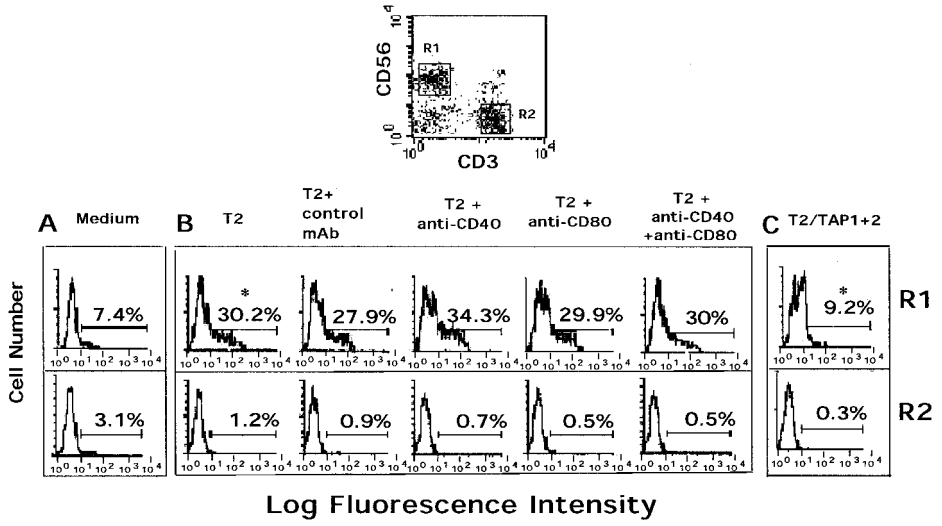

Fig. 4. The CD40- and CD80-dependent pathways are unable to affect IFN-γ secretion by NK effectors. The figure shows the FACS analysis for the intracellular IFN-γ staining in 48-h IL-2-activated PBMC incubated for a 6-h period with medium alone (A), with T2 (B), or T2/TAP1+2 (C) cell lines. PBMC were stained with Cy-chrome anti-CD56, PE anti-CD3, and FITC anti-IFN-γ mAb. The target cells were gated out based on their high forward scatter (data not shown). The live gates were set such that only single $CD56^+$ or $CD3^+$ cells (indicated as R1 and R2 regions, respectively) were collected for the subsequent analysis. The percent of IFN-γ^+ cells is indicated. (B) Intracellular staining for IFN-γ in R1 and R2 cells incubated with the T2 cell line alone or pretreated with control mAb, with anti-CD40 mAb, or both, as indicated. Data are representative of one out of seven independent experiments. *, In R1, the differences, obtained with T2 versus medium or with T2/TAP1+2 versus T2 incubations, were statistically significant ($P<0.05$) in the seven experiments.

Our results strongly suggest the critical role of MHC-I molecules in the regulation of NK cytotoxicity and IFN-γ production in a human model. In addition, the involvement of CD40 and CD80 costimulatory molecules in triggering NK-dependent cytotoxicity but not IFN-γ production was revealed.

The MHC-I-defective T2 target was sensitive to NK lysis and able to promote the IFN-γ production by IL-2-activated NK effectors. The restored expression of the MHC-I molecule on T2/TAP1+2 targets inhibited the killing activity and IFN-γ production by the same effectors. Furthermore, blocking of the CD94-associated inhibitory receptor allowed a significant production of IFN-γ by NK cells stimulated with T2/TAP1+2 transfectants. Notably, the levels of IFN-γ were significantly lower as compared with the ones obtained in the presence of the MHC-I-defective homologous counterpart T2 targets. In addition, no intracellular IFN-γ was detected incubating fresh NK cells with both targets (data not shown).

These data might indicate a function for the CD94-dependent MHC-I recognition in modulating the IFN-γ production in human NK. Moreover, such observation highlights a difference with the mouse model, in which the CD94 was found not responsible for the inhibition of production [43]. However, because the cytokine level was only partially increased in our experiments, the CD94 could not be the unique molecule involved in IFN-γ secretion. In this regard, the recently described role for the KIR3DL1 receptor [44] strongly supports this hypothesis. Therefore, the need for additional studies focusing on the involvement of other NK receptors recognizing

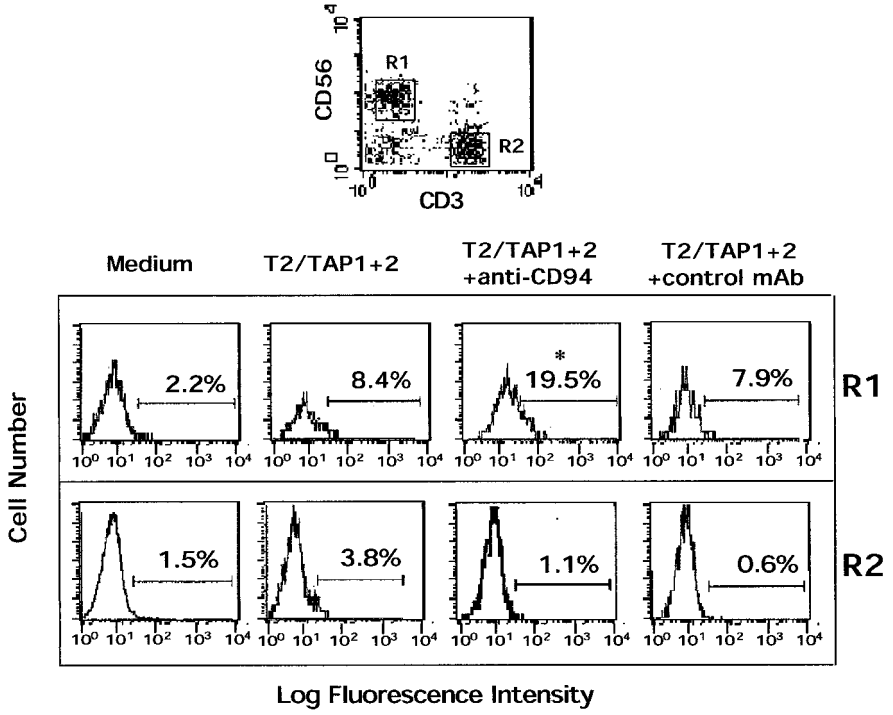

Fig. 5. CD94 recognition modulates IFN-γ production of human NK lymphocytes. The figure shows the intracellular IFN-γ staining in 48-h IL-2-activated PBMC pretreated with anti-CD94 or with control 345.134 mAb, as indicated, and incubated with T2/TAP1+2 transfectant. PBMC were stained with Cy-chrome anti-CD56, PE anti-CD3, and FITC anti-IFN-γ mAb. The live gates were set such that only single $CD56^+$ or $CD3^+$ cells (indicated as R1 and R2 regions, respectively) were collected for the subsequent analysis. The percent of IFN-γ^+ cells is indicated. The data represent the results obtained in one out of five independent experiments. *, Result was statistically significant ($P<0.05$) in the five experiments.

classical and nonclassical MHC-I molecules appears to be critical.

We suggest the involvement of CD40/CD80 recognition in triggering cytotoxicity but not of IFN-γ production by human IL-2-activated NK lymphocytes. Indeed, masking of CD40 and CD80 molecules on the T2-susceptible target was able to down-modulate NK-mediated cytotoxicity significantly, but not to modify the coinduced IFN-γ production in NK effectors. Similar results were obtained by using human monocyte-derived, allogeneic DC targets (data not shown).

The ability of CD40 and CD80 recognition to trigger IL-2-stimulated but not freshly isolated NK effectors could indicate the functional interactions between costimulatory molecules and their putative receptors expressed only on activated NK cells. In this context, the expression of the CD40/CD80-specific ligands involved in T cell costimulation [25, 32–35, 41] was also analyzed in our model. Our results confirmed the dependence from 48-h IL-2 stimulation of CD154 expression on NK, according to previous observations [25]. The NK receptor patterns able to recognize the CD80-dependent costimulation appear less understood [26–34]. In this regard, despite the strong activating effect of the CD80 recognition on NK cytotoxicity, we detected only a very slight expression of the CD28 antigen on IL-2-activated NK effectors. This observation might suggest the involvement of still undefined CD80-binding structures likely distinct from CD28/CD152 molecules on human NK cells.

Taken together, our observations suggest a differential regulation in cytokine production and cytotoxic activity by the CD40/CD80 costimulatory and MHC-I molecules during NK activation. Indeed, cytotoxicity and IFN-γ secretion are inhibited by MHC-I molecules, confirming their role as a dominant signal in the tuning of NK functions. In contrast, the CD40/CD80 recognition pathway enhanced the NK killing activity but was unable to affect the IFN-γ secretion. In this context, the possible, selective involvement of specific pathways underlying such functional dichotomy, as suggested for Vav $1^{-/-}$ mice [45], needs further investigation.

Does this differential regulation of NK functions reflect an immunomodulatory role for NK lymphocytes?

There is an emerging consensus that NK cells might provide a regulatory feedback during the immune response. More specifically, the interaction between NK and DC has been indicated as an important step in the regulation of the subsequent adaptive immune response [37]. The outcome of this interaction might depend on the balance between activating pathways, such as the costimulatory molecules and MHC-I antigens expressed on DC. In this context, the CD80-dependent, NK-mediated killing of autologous DC has been described recently [27, 28]. In addition, our previous findings suggested a role for CD40 molecules in the NK-dependent killing of immature IL-10-treated DC [36], and IL-10 exposure was shown to lead to a reduction of IFN-γ production by NK cells in the absence of significant effect on their cytotoxic activity [46]. Therefore, a distinct, functional control for cytokine secretion and cytotoxic activity in NK lymphocytes could be hypothesized.

These data suggest a possible role for the NK effectors in clearing the CD40/CD80-expressing targets, i.e., the DC exposed to high levels of IL-10 in the local tissue [47, 48]. In this context, the triggering of NK lytic machinery by costimulatory pathways, without significant effect on IFN-γ secretion, might be of some relevance.

Moreover, the ability of CD40/CD80 and of MHC-I molecules to promote/inhibit specific NK-dependent functions might identify a relevant mechanism underlying the physiologic cross-talk between innate and adaptive immune responses in humans.

ACKNOWLEDGMENTS

This work was supported partly by grants from the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica Ita (MURST; Cluster 03, Piano Operativo del Piano di Ingegneria Molecolare), the Scuola Superiore di Immunologia "Ruggero Ceppellini" (Naples), and the Associazione Italiana Celiachia (AIC). G. T. was a fellow of MURST. We thank Drs. Valeria Martignetti, Giuseppe Matarese, and Veronica Sanna for helpful discussion and critical reading of the manuscript.

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