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NK cell-mediated cytotoxicity is regulated by both triggering and inhibitory signals. The interaction between MHC class I molecules expressed on target cells and specific MHC class I-binding receptors expressed by NK cells generally leads to inhibition of lysis. We have shown recently that CD80 (B7-1) in mice and CD40 in humans trigger NK cell-mediated cytotoxicity in vitro. In the present study, we show that murine CD40 and CD86 (B7-2) trigger murine NK cell-mediated cytotoxicity in vitro when expressed on tumor cells. Preincubation of the transfected cell lines with anti-CD40 F(ab')2 fragments or cytolytic T lymphocyte-associated Ag-4-Ig (CTLA-4-Ig) before the cytotoxic assay abolished the triggering effect. Furthermore, radiolabeled CD40- and B7-2-expressing cells were rapidly eliminated in vivo in an NK cell-dependent manner. NK cells from CD40 ligand (CD40L)−/− or CD28−/− mice were triggered by tumor cells transfected with CD40 and B7-2, respectively, and these transfectants were rapidly eliminated in vivo when inoculated into CD40L−/− and CD28−/− mice. This suggests that the CD40 and B7-2 molecules can interact with receptors on NK cells other than CD40L and CD28, respectively, and that these may account for some of the reactivities observed in the present study. Collectively, these data demonstrate that 1) costimulatory molecules, other than B7-1, can modulate NK cell responses in vitro, 2) they can also affect NK cell-dependent responses in vivo, and 3) parts of these reactions are independent of CD28 and CD40L. The Journal of Immunology, 1999, 162: 5910–5916.

The spontaneous cytolytic activity observed in spleen cells of different mouse strains tested against the YAC-1 tumor cell line was the first indication of the presence of NK cells in mice (1, 2). Subsequently, NK cells were also implicated in the innate immunity against viruses, intracellular bacteria, and parasites (3, 4). Unlike T and B cells, NK cells do not rearrange genes to acquire a receptor repertoire (5). According to the “missing self” hypothesis (6), NK cells recognize and kill cells with aberrant or absent expression of MHC class I. In recent years, the mechanisms involved in inhibition by MHC class I molecules expressed on target cells have been studied extensively. It is now accepted that progression of the NK-target cell interaction to lysis can be inhibited by MHC class I molecules expressed on target cells, which results in a negative signal transmitted via specific MHC class I-binding receptors (7). In parallel, several molecules have been demonstrated to be involved in triggering of NK cytotoxicity (8–10). Receptors that bind to MHC class I molecules and activate rather than inhibit NK cells have also recently been described, although their significance is presently less clear (11, 12). The presence of activating and inhibitory molecules has led to the current notion that NK function at the molecular level is regulated by a balance between positive and negative signals.

The so-called costimulatory molecules have been characterized extensively in the interaction between T cells and APC. The interaction between B7 molecules and their ligands CD28 and CTLA-41 is critical in the control of T cell responses (13), and the expression of CD40 on dendritic cells (DC) is important for T cell priming and T cell-mediated effector function (14–16). The interaction between CD40 expressed on B cells and CD40L expressed on activated T cells is also crucial in enabling B cells to generate germinal centers, produce Abs, and switch Ig isotype (17, 18).

Some previous studies have suggested that costimulatory molecules may interact with NK cells. YT, a human NK-like leukemia cell line that expresses CD28, is able to lyse some human and murine cell lines expressing B7-1 (19). Murine NK cells have been demonstrated to proliferate and produce cytokines in a CD28-dependent manner (20), and the rejection of B7-1-transfected tumors has been suggested to involve NK cells (21). Recent data from our and other laboratories have demonstrated that B7-1 in mice (22, 23) and CD40 in humans (24) can trigger NK cell-mediated cytotoxicity in vitro. Additional data in the murine system have also indicated that B7-2 may trigger NK cell-mediated cytotoxicity in vitro (23).

In the present study, we have addressed the capacity of CD40 to trigger NK cell-mediated cytotoxicity in a mouse model. We have also included B7-2 transfectants in some experiments to extend our results with CD40 transfectants to a different costimulatory molecule within the same experimental system. We show that transfected tumor cell lines expressing CD40 or B7-2 trigger NK cell-mediated cytotoxicity in vitro, and that the triggering effect is observed even in the absence of CD40L and CD28, respectively, expressed on the effector cells. In addition, we show that radiolabeled CD40- or B7-2-transfected cell lines are rapidly eliminated in vivo in an NK cell-dependent manner. The latter response occurs even in CD40L−/− and CD28−/− mice, respectively.

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2 Abbreviations used in this paper: CTLA-4, cytolytic T lymphocyte-associated antigen-4; CD40L, CD40 ligand; DC, dendritic cells.
Collectively, these data suggest that costimulatory molecules may trigger NK cell-mediated cytotoxicity in vitro and in vivo.

Materials and Methods

Mice

C57BL/6 (B6) and BALB/c mice (4–8 wk old) were from the Microbiology and Tumor Biology Center, Karolinska Institutet (Stockholm, Sweden). CD40L−/− and CD28−/− mice, generated by homologous gene recombination, have been described previously (18, 25). All mice were maintained at the Microbiology and Tumor Biology Center, Karolinska Institutet. Animal care was in accordance with institutional guidelines.

Cell lines, transfections, and flow cytometry

RMA is a variant of the Rauscher-virus-induced T cell lymphoma RBL-5 of B6 origin. RMA-S is an MHC class I-deficient (TAP-2-deficient) variant of RMA. B16.F1 is a subclone of the B16 spontaneous melanoma of B6 background. YAC-1 is a highly NK cell-sensitive, Moloney virus-induced T cell lymphoma of A/Sn background. All cell lines were grown in complete RPMI 1640 medium containing 10 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/m of streptomycin, and 2 × 10−5 M mercaptoethanol, at 37°C in a humidified 5% CO2 atmosphere in air. Vectors containing mouse CD40 or B7-2 cDNAs were transfected by electroporation, as described (21). Transfected cells were selected in complete medium containing 2 mg/ml of G418 (Life Technologies) for CD40 transfectants, or 20 μg/ml of ganciclovir, and 2 μg/ml of Xantine (Sigma) for B7-2-transfected cell lines. Surviving cells were selected by cell sorting in a FACS Vantage (Becton Dickinson). CD40 and CD70 expression on cell lines generated and used in the present study is shown in Table I. Phenotype of the tumor cell transfectants used in this study

Generation of Con A-activated T cell blasts

For the generation of Con A-activated T cell blasts, 107 erythrocyte-depleted splenocytes were cultured in complete RPMI medium supplemented with 3 μg/ml of Con A (Sigma) for 48 h, and then used as targets in a standard 4-h 51Cr release assay.

In vivo studies

Cell lines transfected with CD40 or B7-2 were labeled with 3HCr for 1 h at 37°C, washed three times in PBS, and resuspended in PBS. A total of 106 cells per mouse was injected i.v. Twenty-four hours after inoculation, mice were sacrificed, and the remaining radioactivity in the lungs was measured in a gamma counter. The results are expressed as percentage of the total activity inoculated. To deplete NK cells in vivo, 200 μg of the anti-NK1.1 PK136 mAb (American Type Culture Collection (ATCC), Manassas, VA) was injected i.p. 48 h before inoculation of the transflectants (26). Control mice received 200 μg of nonimmune mouse IgG (Sigma). CD28−/− mice were pretreated with the hamster anti-mouse CTLA-4 UC10-4F10-11 mAb (PharMingen), 200 μg/mouse, 48 and 24 h before inoculation of the radiolabeled cells.

Purification of splenic DC

Splenic DC were enriched by plastic adherence, as described (27). Briefly, spleens from B6 mice were cut into small fragments and digested with collagenase type IV (Sigma). Cells were cultured in plastic dishes for 90 min, after which nonadherent cells were washed away. The adherent cells were cultured in complete RPMI medium. During this time, the DC detached from the plates. After overnight culture, the cells were stained with FITC-labeled anti-B220 mAb RA3-6B2 (PharMingen), or with rat anti-MHC class II mAb M5/114 (ATCC) plus hamster anti-CD11c mAb N418 (ATCC), followed by FITC-labeled streptavidin (Dakopatts, Glostrup, Denmark). Analysis of the expression of the transfected molecules and MHC class I was performed in a FACSScan (Becton Dickinson).

Results

Table I. Cell surface MHC class I, CD40, and B7-2 expression on cell lines used in the present study

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Background</th>
<th>Kb</th>
<th>Db</th>
<th>CD40</th>
<th>B7-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMA</td>
<td>3</td>
<td>271</td>
<td>237</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>RMA-CD40 clone.16</td>
<td>4</td>
<td>294</td>
<td>197</td>
<td>268</td>
<td>NT</td>
</tr>
<tr>
<td>RMA-B7-2 clone.39</td>
<td>10</td>
<td>288</td>
<td>212</td>
<td>NT</td>
<td>826</td>
</tr>
<tr>
<td>RMA-B7-2 clone.35</td>
<td>15</td>
<td>301</td>
<td>201</td>
<td>NT</td>
<td>44</td>
</tr>
<tr>
<td>B16.F1</td>
<td>3</td>
<td>15</td>
<td>18</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B16.F1-CD40 clone.7</td>
<td>4</td>
<td>16</td>
<td>18</td>
<td>97</td>
<td>NT</td>
</tr>
<tr>
<td>Splenic DC</td>
<td>7</td>
<td>1347</td>
<td>1154</td>
<td>404</td>
<td>1226</td>
</tr>
</tbody>
</table>

* Mean fluorescence intensity.

NH, not tested.

Statistics

Statistical analysis from the in vivo experiments was performed using a paired Student’s t test. A p value of <0.05 was considered statistically significant.

Results

Phenotype of the tumor cell transfectants used in this study

Generation of Con A-activated T cell blasts

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Purification of splenic DC

Splenic DC were enriched by plastic adherence, as described (27). Briefly, spleens from B6 mice were cut into small fragments and digested with collagenase type IV (Sigma) at 37°C for 60 min to release DC. Low density cells were selected by centrifugation on a 35% BSA gradient (Sigma), cultured in plastic dishes for 90 min, after which nonadherent cells were washed away. The adherent cells were cultured in complete RPMI medium. During this time, the DC detached from the plates. After overnight culture, the cells were stained with FITC-labeled anti-B220 mAb RA3-6B2 (PharMingen), or with rat anti-MHC class II mAb M5/114 (ATCC) plus hamster anti-CD11c mAb N418 (ATCC), followed by biotin-labeled goat anti-rat (Caltag, South San Francisco, CA) plus FITC-labeled goat antihamster (PharMingen), and PE-labeled streptavidin. DC were identified as B220+CD11c+MHC class II+ and at least 80% of the cells were DC by these criteria.

Statistical analysis

Statistical analysis from the in vivo experiments was performed using a paired Student’s t test. A p value of <0.05 was considered statistically significant.
CD40 and B7-2 were monitored every second to third week, and no alteration was observed over time.

**IL-2- and IFN-activated NK cell effectors are triggered by CD40 and B7-2**

Two different types of activated NK cells were used to address the question as to whether NK cells could be triggered by the expression of the costimulatory molecules on target cells. In a first set of experiments, splenocytes were activated in vitro with IL-2. In the second set of experiments, splenocytes from mice that had been injected previously with the IFN-inducer Tilorone were used. B6-derived IL-2- or IFN-activated NK cells did not kill RMA, RMA-injected previously with the IFN-inducer Tilorone were used. B6-derived NK cells were tested for their ability to kill RMA and B16.F1 cell lines transfected with CD40 or B7-2. The cell lines tested in the individual experiments were: A and B, RMA (○), RMA-neo (▲), RMA-gpt (▼), RMA-CD40 clone.16 (△), RMA-B7-2 clone.39 (▲); C, RMA-neo (○), RMA-CD40 clone.16 (○), RMA-CD40 clone.10 (□), RMA-CD40 clone.13 (▼); D, RMA-gpt (●), RMA-B7-2 clone.35 (○), RMA-B7-2 clone.39 (□), RMA-B7-2 clone.47 (▼); E, B16.F1 (●), B16.F1-neo (○), B16-F1 clone.7 (▼). The experiments were repeated seven times (IL-2-activated effector cells), three times (IFN-activated effector cells), and two times (CD40 and B7-2 clones, other than RMA-CD40 clone.16 and RMA-B7-2 clone.39, and B16.F1), with similar results. One representative experiment is shown in each panel.

To test critically whether the observed effects were mediated by effector cells other than NK cells, we studied killing of target cells by BALB/c-derived NK cells. BALB/c-derived NK cells killed RMA-CD40 clone.16 more efficiently than RMA-neo (Fig. 2B), and with these effectors, the RMA-CD40 clone.16 was as susceptible as YAC-1 cells. Notably, the difference between RMA-neo and RMA-CD40 clone.16 was, if anything, larger when BALB/c-derived NK cells were used.

**In vitro cytotoxicity is due to NK cells and not to T cells**

The IL-2-activated spleen cell culture contains a mixture of different cell subpopulations, and it cannot formally be excluded that the observed effects were mediated by effector cells other than NK cells. To confirm that the killing of the transfectants was mediated by NK cells, NK1.1+ / TCR- cells were sorted from IL-2-activated B6 splenocytes, and assayed against RMA-CD40 clone.16 and RMA-B7-2 clone.39. As shown in Fig. 3, only NK cells (NK1.1+ / TCR-), but not T cells (TCR+ / NK1.1+), were able to kill the RMA-CD40 clone.16 and the RMA-B7-2 clone.39. The results were not restricted to specific subpopulations of NK1.1+ cells according to their expression of specific Ly49 receptors (data not shown). Yet, it is still an open question as to whether the current triggering effect observed is an effect attributed to a particular subpopulation of NK cells, or the whole population per se.

**Triggering of cytotoxicity is related to the expression of the transfected molecule on the cell surface**

To test critically whether the observed effects were due specifically to the expression of the transfected molecules on the cell surface, blocking experiments were performed with rat anti-mouse CD40-F(ab′)2 fragments or with CTLA-4-Ig fusion protein before the cytotoxic assay. As shown in Fig. 4A, the killing of the RMA-CD40 clone.16 by IL-2-activated NK cells was reduced to wild-type levels when the target cells had been incubated with rat anti-mouse CD40-F(ab′)2 fragments, while addition of CTLA-4-Ig fusion protein had no effect. Conversely, the NK-mediated killing of RMA-B7-2 clone.39 was reduced to wild-type levels when target cells were preincubated with CTLA-4-Ig, but not when incubated with F(ab′)2 anti-CD40 (Fig. 4B). These data demonstrate that the increased sensitivity of the transfected cell lines to lysis by NK cell effectors is dependent on the presence of CD40 and B7-2 at the cell surface.
IL-2-activated NK cells are triggered by RMA-CD40 clone.16 and RMA-B7-2 clone.39 even in the absence of CD40L and CD28.

Is triggering of cytotoxicity by CD40 and B7-2 dependent on CD40L and CD28 expressed by the NK effectors? To address this question, we performed experiments in which IL-2-activated NK cells were prepared from CD40L−/− and CD28−/− mice. As shown in Fig. 5, A and B, IL-2-activated NK cells from both B6 and CD40L−/− mice killed efficiently RMA-CD40 clone.16. In a similar manner, IL-2-activated NK cells from both B6 and CD28−/− mice killed RMA-B7-2 clone.39 (Fig. 5, C and D).

These observations were not entirely unexpected since the expression of CD40L (this study) and CD28 (22) was not detected by NK cells, but not T cells, are involved in the killing of the CD40- and B7-2-transfected cell lines. NK1.1+/TCR− and NK1.1+/TCR+ populations were sorted from bulk IL-2-activated spleen cell cultures, and tested for their ability to kill RMA-CD40 clone.16 (A and B) and RMA-B7-2 clone.39 (C and D). The cell lines tested were YAC-1 (○), RMA-neo (○), RMA-CD40 clone.16 (▼), RMA-gpt (■), and RMA-B7-2 clone.39 (□). The experiment was repeated three times with similar results. One representative experiment is shown.
flow cytometry on resting or on in vitro activated NK1.1⁺ B6-derived cells. CTLA-4 is stored by T cells in the endoplasmic reticulum, and is rapidly transported to the cell surface upon activation (28). To exclude that a similar mechanism could account for the CD40L expression shortly after NK cells contact with target cells in the cytotoxicity assay, we plated IL-2-activated NK cells with RMA-CD40 clone.16 at 50:1 and 10:1 E:T ratios, and get cells in the cytotoxicity assay, we plated IL-2-activated NK cell lines are efficiently eliminated in vivo in an NK cell-dependent manner

Previous studies have shown that MHC class I-deficient RMA (H-2b) cell lines are efficiently eliminated in vivo in an NK cell-dependent manner when injected into B6 mice, whereas MHC class I-positive RMA cells are not (29; this study). As shown in Fig. 6, B6 mice efficiently eliminated RMA cells transfected with CD40 and B7-2, whereas control cells were spared. NK cell depletion resulted in an increase in the remaining radioactivity in the lungs, indicating that the elimination of the transfected cell lines is diminished in the absence of NK cells (Fig. 6).

To analyze the involvement of CD40L and CD28 in the in vivo elimination of the transfecteds, we performed rapid elimination tests in which radiolabeled transfecteds were inoculated into CD40L⁻/⁻ and CD28⁻/⁻ mice, respectively. The elimination of RMA-CD40 clone.16 and RMA-B7-2 clone.39 by CD40L⁻/⁻ and CD28⁻/⁻ mice, respectively, did not differ significantly from that observed when the cells were inoculated into B6 mice (Fig. 7). These data show that the in vivo elimination of the transfecteds can occur in the absence of CD40L (for RMA-CD40 clone.16) or CD28 (for RMA-B7-2 clone.39) on the host effector cells.

Discussion

The main finding in the present study is that CD40 expressed on tumor cell targets triggers cytotoxicity in vitro by both IL-2- and IFN-activated murine NK cells. This was directly related to the presence of the transfected molecules on the cell surface. The triggering by CD40 expressed on tumor targets occurs even in the absence of CD40L on NK effectors. Furthermore, transfected cells were rapidly eliminated in vivo after inoculation into syngeneic B6 mice. Data obtained with B7-2-transfected tumor cell lines were similar to those observed with CD40, indicating that the observed effects were not restricted to the costimulatory molecule CD40.

In recent years, it has been understood that many NK cell-mediated effector functions are controlled by triggering as well as inhibitory signals (7–10). The inhibitory receptors and signals are fairly well characterized, and there is now a more intensive interest in the characterization of molecules involved in the triggering of NK cells. It has been demonstrated, both in the murine and in the human system, that the expression of costimulatory molecules by different tumor cell lines can result in an increased susceptibility to lysis by NK cells. The transfection of murine B7-1 in different tumor cell lines triggers NK cell-mediated cytolysis in vitro (22, 23). Additional data have indicated that the same effect occurs with the murine B7-2 molecule (23). Moreover, human NK cell lines are triggered to kill in vitro the mastocytoma cell line P815 transfected with human CD40 (24).

The present study shows that the transfection of murine CD40 and B7-2 into the MHC class I⁺ thymoma cell line results in an increased sensitivity to NK cells. That the transfected cell lines are killed but the mock transfecteds are spared indicates that the inhibitory signal delivered through MHC class I is overcome by the positive signal delivered by CD40 or B7-2.

In humans, a relatively high proportion of melanomas has been shown to express CD40 (30). The killing of the murine melanoma cell line B16.F1 transfected with CD40 extends the finding to a second tumor cell line, other than RMA, and opens the possibility that this cell type can be targeted by NK cells in vivo when expression of costimulatory molecules occurs.

B7 molecules expressed on APC interact with CD28 and CTLA-4 expressed on T cells (13). In a similar manner, CD40 expressed by B cells and DC interacts with CD40L expressed on activated T cells (14–18). Expression of CD28/CTLA-4 or CD40L on NK cells has been shown to be dependent on the maturation state, the method of activation, and the origin of the NK cells. Although CD28 is expressed on human fetal NK cells (31), it has been claimed to be lost after maturation and is absent on peripheral blood NK cells in adults. In mice, data regarding the expression of CD28 are controversial. CD28 expression has been reported on bone marrow-derived NK cells upon activation with IL-2 (32), but
has not been detected on 4-day IL-2-activated splenic NK cells (22). In humans, CD40L has been reported to be expressed upon short-term culture of NK cells (24). In our system, CD40L expression was not detected on resting or on in vitro IL-2-activated NK cells, and no evidence for expression of CD40L on murine NK cells is available to our knowledge. In this context, the in vitro killing of RMA-CD40 and RMA-B7-2 cells by CD40L−/− and CD28−/−–derived NK cells, as well as the in vivo elimination of RMA-CD40 clone.16 and RMA-B7-2 clone.39 by CD40L−/− and CD28−/− mice, are not entirely surprising. The NK cell–mediated killing of tumors expressing costimulatory molecules in the absence of the “expected” ligand on effector cells has already been described. Chambers and coworkers (22) found that IL-2–activated NK cells derived from CD28−/− mice were able to kill tumor cells expressing B7-1 molecule. Moreover, NK cells derived from patients with a mutation on CD40L are able to kill in vitro the mastocytoma cell line P815 transfected with the human CD40 molecule (E. Carbone, unpublished data). Taken together, these and previous results do not exclude a role for CD28 and CD40L on NK cells under conditions in which these molecules are expressed, but they also indicate that receptors other than CD28 and CD40L, with a B7-1/B7-2- and CD40-binding capacity, respectively, may be expressed on NK cells.

Our results show that the triggering of cytotoxicity in vitro is a direct consequence of the presence of CD40 and B7-2 on the cell surface of the tumor cells, since blocking of these molecules results in reduced sensitivity to NK cell–mediated lysis. This and other observations has led to the suggestion of the existence of alternative CD40– and B7-binding molecules expressed on NK cells. In this scenario, the triggering would imply a direct contact between CD40 and B7-2, and these alternative receptors. Such CD40 and B7-2 receptors would then be expressed on NK cells after IL-2 or IFN activation, as effectors obtained in both ways mediate cytotoxicity against CD40 or B7-2 transfecteds. An alternative hypothesis is that the observed effects are mediated by unrelated molecules. In this situation, CD40 and B7-2 would interact with molecules on the surface of the target cell, and these molecules would in turn trigger the NK cells. The contact between such molecules and CD40 or B7-2 would be disrupted when incubating the transfecteds in vitro with anti-CD40 mAb or with CTLA-4, respectively.

Clones expressing high levels of B7-2 are killed, while the one expressing low levels is not, indicating that the costimulatory molecule must be present at a certain level on the cell surface to trigger NK cell–mediated cytotoxicity in vitro. We did not obtain transfecteds expressing different levels of CD40, and therefore we cannot conclude that the observed effect is a general feature of the costimulatory molecules in its interaction with NK cells. One possibility to explain the different susceptibility of B7-2 clones is that the response is governed by a balance between positive and negative signals. With low levels of expression, the positive signal delivered through the costimulatory molecule is not sufficient to overcome the inhibitory one delivered through MHC class I. Alternatively, the outcome of the response is the result of a threshold effect; in this scenario, a certain level of costimulatory molecule would be required to trigger killing, irrespective of MHC class I expression.

The question of whether NK cells can interact directly with costimulatory molecules has been addressed mainly in in vitro experimental systems. These have demonstrated that different tumor cell lines transfected with such molecules trigger NK cell–mediated cytotoxicity. The role of NK cells in in vivo immune responses has to date been studied by depletion of this lymphocyte population with mAbs. These data support the notion that, indeed, NK cells are involved in the in vivo elimination of tumors lacking MHC class I expression (29), and in clearance of viruses and parasites (3, 4). Moreover, recent data have demonstrated their involvement in the course of autoimmune and inflammatory diseases (33, 34). A novel finding in our study is that the expression of the costimulatory molecules on tumor cells also results in their rapid elimination after i.v. inoculation into mice, and that mice depleted of NK cells before inoculation have an impaired capacity to clear the transfecteds. The effect is therefore dependent, at least in part, on NK cells, although we cannot exclude the possibility that other cells of the innate immune system could also be involved. In this sense, granulocytes have shown to be important in the antitumor immune response induced by B7-transfected tumors (21, 35). Furthermore, the fact that RAG−/− mice eliminate B7-1 transfecteds as efficiently as normal B6 mice do (B. J. Chambers, unpublished data) indicates that the rapid elimination is independent of the presence of NK T or T cells.

Another in vivo evidence on the role of costimulatory molecules in triggering NK cells comes from in vivo experiments with tumor cells transfected with B7 molecules. The transfection of B7 molecules into tumors has indeed become a useful tool to induce strong antitumor cytotoxic T cells (36), and in some particular experimental models, the involvement of NK cells in the antitumor response has also been implicated (21, 35).

To which extent the interaction between costimulatory molecules and NK cells is a relevant event in the course of the immune response is presently unknown. It is important to keep in mind that costimulatory molecules are expressed almost exclusively by APC, which have a key role in initiating, developing, and terminating immune responses. Therefore, it is tempting to speculate that the interaction between NK cells and APC could be a mechanism to control the generation of adoptive immune responses. In line with this idea, previous reports have suggested that NK cells might interfere with DC function (37, 38). In addition, NK cells mediate the killing of DC in a partially CD40-dependent manner. However, results proving a role for NK cell–mediated killing of APC in vivo are lacking. Finally, we should also consider the possibility that interaction of NK cells with APC through costimulatory molecules can lead to production of cytokines (19) or augmentation of NK cell proliferation (20).

Collectively, our report extends previous data on the capacity of costimulatory molecules to trigger NK cell–mediated cytotoxicity, and shows for the first time that murine CD40 is able to trigger murine NK cells.

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