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The Ly-49 Family: Regulation of Cytotoxicity and Cytokine Production in Murine CD3+ Cells

John R. Ortaldo, Robin Winkler-Pickett, Anna T. Mason, and Llewellyn H. Mason

The Ly-49 gene families are class I-recognizing receptors on murine NK cells. Most Ly-49 receptors inhibit NK cell lysis upon recognizing their target class I ligands. In this report we have examined the ability of Ly-49A and Ly-49G2 to regulate T cell functions on CD3+ cells, primarily the subset that also expresses NK-1.1 and/or DX5. The majority (>50%) of T cells that express Ly-49 molecules also coexpress NK-1.1 and/or DX5, although some NK-1.1+ and/or DX5+/CD3+ cells express Ly-49 molecules. Lysis of target cells by IL-2-cultured T cells expressing Ly-49A and G2 was enhanced by Abs specific for Ly-49A and G2 as well as by Abs to class I (H-2Dd α1/α2). Murine T cells also were cultured in the presence of targets that express (H-2Dd) which is inhibiting for the Ly-49A and G2 receptors. These cells were examined for a coincident increase in cytokine production (INF-γ, TNF-α, and granulocyte-macrophage CSF). Abs to Ly-49A and G2 or their respective class I ligands blocked the negative signals mediated via the Ly-49 receptors and increased IFN-γ and granulocyte-macrophage CSF production after interaction of these T cells with H-2Dd-expressing tumor targets. Furthermore, an EL-4 T cell line expressing both Ly-49A and G2, when treated with mAb YE148 and 4D11, demonstrated reduced cytokine production and calcium mobilization. These results demonstrate for the first time that Ly-49 class I binding receptors, previously thought to be restricted to mouse NK cells, can mediate important physiological functions of T cell subsets. The Journal of Immunology, 1998, 160: 1158–1165.
Materials and Methods

Mice

All mice were obtained from the Animal Production Area, Frederick Cancer Research and Development Center (Frederick, MD) or The Jackson Laboratory (Bar Harbor, ME). Mice were between 8 and 20 wk of age when euthanized. Animal care was provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86–23, 1985).

Flow cytometric analysis

Cells were stained as previously described (15) and analyzed on a FACSort flow cytometer (Becton Dickinson, Mountain View, CA). Cell sorting was performed on either an EPICS 750 (Coulter Electronics, Hialeah, FL) or a FACStar (Becton Dickinson). Cells were directly stained using phycoerythrin (PE)-conjugated Abs and indirectly stained using a primary Ab followed by an isotype-specific FITC- or PE-conjugated secondary Ab or a biotinylated Ab followed by streptavidin PerCP (San Jose, CA) or streptavidin-Red 670 (Life Technologies, Gaithersburg, MD).

Complete medium

RPMI 1640 (BioWhittaker, Walkersville, MD) was supplemented with 10% FBS (Atlanta Biologics, Norcross, GA), l-glutamine, penicillin/streptomycin, sodium pyruvate, nonessential amino acids, HEPES, and 2-ME. RPMI 1640 (BioWhittaker, Walkersville, MD) was supplemented with 1000 Cetus units (CU)/ml.

Abs used

IgG1 (H-2D b , mouse IgG2a), and 11.4.1 (H2K k mouse IgG2a). T cell reactivity of splenic or liver NK and T cells were isolated from nylon wool-nonadherent lymphocytes. The cells were stained with combinations of CD3 and Ly-5, and were sorted for subpopulations expressing the desired phenotype. Cells then were expanded for 7 to 10 days in complete medium supplemented with 1000 Cetus units (CU)/ml.

NK and T cell isolation

Murine splenic or liver NK and T cells were isolated from nylon wool-nonadherent lymphocytes. The cells were stained with combinations of CD3 and Ly-5, and were sorted for subpopulations expressing the desired phenotype. Cells then were expanded for 7 to 10 days in complete medium supplemented with 1000 Cetus units (CU)/ml.

Targets

Con A blasts were made using B6 spleen cells that express class I (H-2D d ) or BALB/c spleen cells (H-2D b ), treated for 48 h with 10 μg Con A/2 × 10 6 cells. Tumor targets were maintained in culture as previously described (15). P815 is a rat mastocytoma. WEHI 164 is a methylcholanthrene-induced fibrosarcoma. L5 MF22 is a B cell line that naturally expresses an H-2D b class I molecule. This line has been transfected with a neomycin resistance gene alone (L5Neo3) or with the class I (H-2 b ; L5Cd104)-expressing gene (provided by Dr. I. Nakamura, Buffalo, NY).

Cytotoxicity assays

Tumor targets were labeled with 115I and used in 6-h cytotoxicity assays as previously described (20). Assays involving mAbs included the specific Abs at a concentration of 20 μg/ml for the duration of the cytotoxicity assay. Data are either presented as lytic units per 10 6 cells or as percent specific lysis.

Abs used

The following mAbs to Ly-49 receptors were used: YE1/48 (Ly-49A; A1; YE148), Ly-49C/I (5E6), Ly-49G2 (4D11), and Ly-49D (4E5). Since Ly-49A and Ly-49G2 together are expressed on ~75% of B6 NK cells, we examined T cells for coexpression of CD3, DX5 (a marker that coexpresses CD3, DX5, and GM-CSF), and GM-CSF ELISA kits (R&D Systems, Minneapolis, MN). All samples were measured in duplicate against the standard curve of the assay and are reported as picograms per milliliter. In all assays, the SD of the cytokine measurement was ~0.25 pg/ml.

Calcium mobilization

Analysis of changes in the intracellular Ca 2+ concentration ([Ca 2+ ] i ) of T cells was conducted using a FACSFlow cytometer (Becton Dickinson) and the calcium-sensitive fluorochrome fluo-3 (Molecular Probes, Eugene, OR) (9). [Ca 2+ ] i was monitored with fluo-3-loaded cells suspended at 37°C in Dulbecco’s PBS with Ca 2+ and Mg 2+ supplemented with 5 mM glucose. Increases in [Ca 2+ ] i were detected as increases in fluo-3 fluorescence vs time. Data were analyzed using MultiTime Analysis Software (Phoenix Flow Systems, San Diego, CA).

Results

B6 NK cells express all four known Ly-49 receptors

These can be detected and their function evaluated by the following Abs: Ly-49A (A1; YE148), Ly-49C/I (5E6), Ly-49G2 (4D11), and Ly-49D (4E5). Since Ly-49A and Ly-49G2 together are expressed on ~75% of B6 NK cells, we examined T cells for coexpression of CD3, DX5 (a marker that coexpresses most NK1.1-positive cells) and Ly-49. Figure 1 demonstrates the expression of CD3, DX5, and Ly-49 receptors on freshly isolated spleen cells. Most DX5-positive lymphocytes were CD3 + ; however, 1 to 2% of lymphocytes were CD3 + , DX5 + T cells. A similar percentage of CD3 + , NK1.1 + T cells was observed in B6 splenic lymphocytes (see Fig. 2A). Generally, <3% of T cells express the DX5 marker in normal spleen. As can be seen in Figure 1, the T cell population also contains small subsets of cells that express Ly-49 receptors in B6 mice, with the exception of Ly-49D.

Since these rare NK-like T cells represent a minor population of spleen cells, we used a procedure previously reported (21) from our laboratory to enrich for NK cells from the liver in an attempt to isolate larger numbers of CD3 + , NK1.1 + T cells. Mice were given IL-2 (twice daily) for 3 days, which resulted in the migration of NK1.1 + cells into the liver. Figure 2 demonstrates the advantage of this approach. As can be seen in Figure 2a, the percentage of splenic NK1.1 + cells, both CD3 + and CD3 + , remained similar, while in the liver, the percentage of NK1.1 + cells dramatically increased after IL-2 treatment. CD3 + , NK1.1 + NK cells can reach 50% of the total leukocyte isolate, while CD3 + , NK1.1 + T cells range from 9 to 20% of liver lymphocytes. In addition, as shown in Figure 2B, not only was the percentage of NK1.1 + increased in liver lymphocytes, but the liver was also a site that sustained a dramatic increase in NK1.1 cell numbers. The number of NK1.1 + T cells was <1 × 10 6 cells/liver and increased to >1 × 10 6 after IL-2 treatment. The increase in percentage coupled with the increase in lymphocyte yield resulted in this method being an excellent procedure to recover high percentages and yields of both CD3 + and CD3 + , NK1.1 + lymphocytes for functional studies. Although the spleen contained a similar number of total NK1.1 + cells, their frequency was considerably lower (see Fig. 2A). Therefore, we performed studies to determine whether these liver-derived cells could be a good source of Ly-49-expressing T cells. As shown in Figure 3, the percentage of T cells expressing Ly-49 was very low (often <1%) in spleen, but their percentage was significantly increased (to ~3–5%) in IL-2-treated mice. The patterns of expression of Ly-49 molecules in normal and IL-2-treated liver were similar; T cells expressed Ly-49A, C/I, and G2 but failed to...
express the activating Ly-49D. These results demonstrate that increased numbers of Ly-49+ T cells can be obtained from the livers of IL-2-treated mice, offering a distinct advantage over normal splenocytes.

Since Ly-49 receptors were found on T cells in B6 mice, we next examined numerous other mouse strains for their expression of DX5 and CD3 vs the various Ly-49 molecules. Unlike NK1.1, DX5 is an NK marker that appears to be genetically unrestricted (22). Table I provides a summary of our studies investigating the expression of the Ly-49 molecules on CD3+ spleen cells in various mouse strains. Similar to that in B6 mice, the percentage of DX5+, CD3+ lymphocytes in these various strains was quite low compared with that of the typical DX5+, CD3+ NK cells. In addition, the lack of Ly-49D expression on T cells was consistent within all observed strains. The regulation of expression of Ly-49 molecules is not understood. Once expressed, Ly-49 receptors appear not to be regulated on lymphocytes. The signal(s) that induces expression also is not understood. Therefore, we sought to examine and compare T cells with NK cells for the relative expression on DX5+ lymphocytes. Figure 4 compares the percentages of DX5+ T and NK cells expressing the various Ly-49 receptors. Of interest was that both T and NK cells from the various haplotypes appeared to have a similar level of expression. Strains whose NK cells were high or low for expression of C/I (high: B6, DBA/2; low: AKR or 129/J) and G2 (high: B6, C3H/HeJ; low: AKR or 129/J) also demonstrated concordant expression of T cell-associated Ly-49s. The exception was as previously noted, that Ly-49D was not expressed on T cells. These results are consistent with the hypothesis that these class I binding receptors are similarly regulated in both DX5+ subsets.

Since spleen and liver T cells both express Ly-49 receptors, these cells were examined for their TCR expression and their CD4/CD8 phenotypes. As shown in Table II, both TCRαβ+ and TCRγδ+ cells express Ly-49 receptors. In addition, IL-2-treated mice exhibited small percentages (0.7%) of TCRγδ, Ly-49D+ cells. When the CD4 and CD8 coexpression with Ly-49 was examined, most Ly-49 molecules were expressed preferentially on the CD8 T cell subset (2–4 times higher), although some Ly-49 family members were found on the CD4 subset.

The ability of Ly-49A and Ly-49G2 receptors to regulate NK lysis has been previously shown; therefore, we performed experiments to determine whether these molecules regulated T cell-mediated cytolysis. In these experiments, IL-2-treated B6 lymphocytes isolated from the liver were sorted and expanded in 1000 CU/ml of IL-2 and evaluated for their function on days 9 to 12. Table III presents two typical experiments using CD3+. Ly-49G2+ cells as effectors for the lysis of P815 (H-2Dd) targets.

FIGURE 1. Expression of DX5 and Ly-49 receptors in T cells. Freshly isolated B6 spleens were examined for expression of CD3, DX5, and Ly-49s in normal spleen. The expression of Ly-49 receptors (using Abs (A, YE148; C, 5E6; G2, 4D11; D, 4E5)) is shown vs that of CD3 for T cells. The coexpression of CD3 and DX5 also is shown in the lower right panel. The percentage of positive gated lymphocytes in each quadrant is shown. This experiment is representative of >10 experiments performed.

FIGURE 2. Distribution of NK1.1+ CD3+ T cells in spleen and liver. A. Freshly isolated B6 spleen and liver cells from normal (NT) or IL-2-treated animals were examined for CD3 (anti-CD3-biotin/avidin Perp) and NK1.1 (PK136-PE) expression. The percentage of positive gated lymphocytes in each quadrant is shown. B, The absolute number of CD3+ NK1.1+ or CD3− NK1.1+ cells in spleen and liver per animal was calculated for control and IL-2-treated animals. This experiment is representative of >10 experiments performed.
often used to study the role of Ly-49 molecules in NK-mediated lysis). The effector function of the CD3$^+$, Ly-49G2$^+$ cells against P815 was tested either alone or in the presence of mAb specific for Ly-49G2 or H-2Dd. Both T and NK effector cells potently killed the prototypic NK target, YAC-1 (data not shown). As can be seen in Table III, the basal level of NK (CD3$^+$, Ly-49G2$^+$) and T (CD3$^+$, Ly-49G2$^+$) cell-mediated lysis of P815 was increased by the inclusion of Abs that reversed the inhibitory signals mediated through Ly-49A and Ly-49G2 (Expt. I and II) or by Abs to the appropriate class I, H-2Dd (Expt. II). Since the two major inhibitory Ly-49s on H-2D b (B6) effectors were Ly-49A and Ly-49G2 (both recognizing H-2D d ), they had a complementary effect, reversing inhibition of lysis. In addition, an increase in lytic capability was seen for both NK and T cells when anti-class I Abs to the α1/α2 domain of H-2Dd were used, but not with other anti-class I Abs (17). Expt. III examines the ability of CD3$^+$, NK1.1$^+$ (often used to study the role of Ly-49 molecules in NK-mediated lysis). The effector function of the CD3$^+$, Ly-49G2$^+$ cells against P815 was tested either alone or in the presence of mAb specific for Ly-49G2 or H-2Dd. Both T and NK effector cells potently killed the prototypic NK target, YAC-1 (data not shown). As can be seen in Table III, the basal level of NK (CD3$^+$, Ly-49G2$^+$) and T (CD3$^+$, Ly-49G2$^+$) cell-mediated lysis of P815 was increased by the inclusion of Abs that reversed the inhibitory signals mediated through Ly-49A and Ly-49G2 (Expt. I and II) or by Abs to the appropriate class I, H-2Dd (Expt. II). Since the two major inhibitory Ly-49s on H-2D b (B6) effectors were Ly-49A and Ly-49G2 (both recognizing H-2D d ), they had a complementary effect, reversing inhibition of lysis. In addition, an increase in lytic capability was seen for both NK and T cells when anti-class I Abs to the α1/α2 domain of H-2Dd were used, but not with other anti-class I Abs (17). Expt. III examines the ability of CD3$^+$, NK1.1$^+$ (often used to study the role of Ly-49 molecules in NK-mediated lysis). The effector function of the CD3$^+$, Ly-49G2$^+$ cells against P815 was tested either alone or in the presence of mAb specific for Ly-49G2 or H-2Dd. Both T and NK effector cells potently killed the prototypic NK target, YAC-1 (data not shown). As can be seen in Table III, the basal level of NK (CD3$^+$, Ly-49G2$^+$) and T (CD3$^+$, Ly-49G2$^+$) cell-mediated lysis of P815 was increased by the inclusion of Abs that reversed the inhibitory signals mediated through Ly-49A and Ly-49G2 (Expt. I and II) or by Abs to the appropriate class I, H-2Dd (Expt. II). Since the two major inhibitory Ly-49s on H-2D b (B6) effectors were Ly-49A and Ly-49G2 (both recognizing H-2D d ), they had a complementary effect, reversing inhibition of lysis. In addition, an increase in lytic capability was seen for both NK and T cells when anti-class I Abs to the α1/α2 domain of H-2Dd were used, but not with other anti-class I Abs (17). Expt. III examines the ability of CD3$^+$, NK1.1$^+$

CD3 $\rightarrow$

**FIGURE 3.** Expression of Ly-49 receptors on CD3$^+$ T cells in spleen and liver. Freshly isolated B6 spleen and liver cells from normal (NT) or IL-2-treated animals were examined for CD3 (anti-CD3-biotin/SA-PCP) and Ly-49-FITC (using Abs (A, YE148; C, 5E6; G2, 4D11; D, 4E5). The percentage of positive gated lymphocytes in each quadrant is shown. This experiment is representative of more than five experiments performed.
lymphocytes to lyse a B cell line, L5 MF22 (H-2D\textsuperscript{b}), and an H-2D\textsuperscript{d}-transfected line, L5cD\textsuperscript{d}104 (H-2D\textsuperscript{b/d}). When these NK1.1\textsuperscript{1} T cells were tested against the parental H-2D\textsuperscript{b} cell line, considerable lysis was observed. However, this lysis was diminished in the presence of H-2D\textsuperscript{d} (reduction from 46.6 to 31.4% lysis). The inclusion of individual Abs to either Ly-49A or Ly-49G2 reversed the lysis; however, the addition of both anti-Ly-49A and anti-G2 together or anti-H-2D\textsuperscript{d}\textsuperscript{a1/a2} inhibited the negative signal and reversed the lysis to the control (H-2D\textsuperscript{b}) level. Similar effects on the H-2D\textsuperscript{d}-expressing line L5cD\textsuperscript{d}104 were not seen with mAb to Ly-49C or H-2D\textsuperscript{b}. Therefore, the functional inhibition of NK1.1\textsuperscript{1} T cells also was seen with an H-2D\textsuperscript{d}-transfected B cell line.

FIGURE 4. Expression frequency of Ly-49s on DX5\textsuperscript{+} lymphocytes. Splenic lymphocytes were isolated from normal mice of the indicated strains. The NK (CD3\textsuperscript{+}, DX5\textsuperscript{+}; □) and DX5\textsuperscript{+} (CD3\textsuperscript{+}, DX5\textsuperscript{+}; ■) T cells were examined for expression of Ly-49s. Data represent the percentage of Ly-49s that are expressed on the DX5\textsuperscript{+} subsets for Ly-49A (A), Ly-49C/I (B), Ly-49D (C), and Ly-49G2 (D).

Table II. Expression of Ly-49 receptors on T cell subsets

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<td>Spl IL-2</td>
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Table IV. Regulation of cytotoxicity by Ly-49 receptors

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<td>+ rat-lgG</td>
<td>2.2 (1.21)</td>
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<td>+ anti-Dd</td>
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<td>1.4 (1.97)</td>
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<td>+ anti-Db</td>
<td>50.6 (1.4)</td>
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<table>
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<th>Experiment 1</th>
<th>Effector Populations</th>
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<td>Treatment</td>
<td>CD3−49G2</td>
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<td>Lymphocyte</td>
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<td>+ anti-Ly-49A and G2</td>
<td>12.6 (3.9)</td>
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<tr>
<td>+ anti-Ly-49C</td>
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<td>+ anti-CD2</td>
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<td>+ anti-CD3</td>
<td>4.0 (1.19)</td>
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<tr>
<td>+ rat-lgG</td>
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We examined EL-4, a murine T cell line that expresses Ly-49 molecules. We found that this cell line expressed high levels of Ly-49A and low levels of Ly-49G2, with >99% of the cells being Ly-49A⁺ (not shown). Therefore, we used EL-4 as a model to study the effects of the Ly-49 molecules. Preliminary experiments demonstrated that phosphorylation of Ly-49A and Ly-49G2 was induced by pervanadate treatment (not shown), an effect similar to that observed for NK cells and NK cell lines. Since EL-4 can produce IL-2, we also evaluated EL-4 for the ability of Ly-49A and Ly-49G2 receptors to inhibit IL-2 production during the interaction of EL-4 with P815 cells. A representative experiment is shown in Figure 5. EL-4 spontaneously produces IL-2, and this production was not altered by addition of rat IgG2a, anti-Ly-49A, anti-Ly-49G2 (not shown), or anti-Ly-49A plus anti-Ly-49G2 or anti-Ly-49C/I. However, the addition of P815 expressing an inhibitory class I (H-2Db) resulted in diminished spontaneous production of IL-2 by EL-4 cells. The addition of anti-Ly-49A and G2 reversed this inhibition, while irrelevant IgG or anti-Ly-49C/I failed to block the inhibitory signal being delivered from the H-2Db on the P815 target. An anti-CD3 Ab that cross-linked to P815 via its FcR served as a positive control.

Table IV. Regulation of cytokine production by Ly-49 receptors

<table>
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<tr>
<th>Treatment</th>
<th>Effector Populations</th>
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<td>CD3−49G2</td>
</tr>
<tr>
<td>Lymphocyte and P815</td>
<td>0</td>
</tr>
<tr>
<td>+ anti-Ly-49A and G2</td>
<td>127</td>
</tr>
<tr>
<td>+ anti-Ly-49C</td>
<td>34</td>
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<tr>
<td>+ anti-Dd</td>
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<tr>
<td>+ anti-Db, Dk</td>
<td>42</td>
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<tr>
<td>+ anti-K</td>
<td>12</td>
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<tr>
<td>+ anti-CD3</td>
<td>48</td>
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<th>Treatment</th>
<th>Effector Populations</th>
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<td></td>
<td>CD3−49G2</td>
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<tr>
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<td>222</td>
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<tr>
<td>P815 only</td>
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<td>PMA/ionomycin</td>
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We examined EL-4, a murine T cell line that expresses Ly-49 molecules. We found that this cell line expressed high levels of Ly-49A and low levels of Ly-49G2, with >99% of the cells being Ly-49A⁺ (not shown). Therefore, we used EL-4 as a model to study the effects of the Ly-49 molecules. Preliminary experiments demonstrated that phosphorylation of Ly-49A and Ly-49G2 was induced by pervanadate treatment (not shown), an effect similar to that observed for NK cells and NK cell lines. Since EL-4 can produce IL-2, we also evaluated EL-4 for the ability of Ly-49A and Ly-49G2 receptors to inhibit IL-2 production during the interaction of EL-4 with P815 cells. A representative experiment is shown in Figure 5. EL-4 spontaneously produces IL-2, and this production was not altered by addition of rat IgG2a, anti-Ly-49A, anti-Ly-49G2 (not shown), or anti-Ly-49A plus anti-Ly-49G2 or anti-Ly-49C/I. However, the addition of P815 expressing an inhibitory class I (H-2Db) resulted in diminished spontaneous production of IL-2 by EL-4 cells. The addition of anti-Ly-49A and G2 reversed this inhibition, while irrelevant IgG or anti-Ly-49C/I failed to block the inhibitory signal being delivered from the H-2Db on the P815 target. An anti-CD3 Ab that cross-linked to P815 via its FcR served as a positive control.
with the SE. Data are representative of three experiments performed. 

Recent studies have demonstrated that mAb 5E6 recognizes H-2Dd/H-2Dk. This recognition of class I ligand by the prototype molecule, Ly-49A, when expressed on NK cells has been shown to bind the class I molecules play in NK cell biology. The importance of generating mAb against the remaining, undefined members of the Ly-49 gene family is paramount to understanding the functional roles these molecules may play in NK cells via Ly-49 molecules may be similar to those observed in NK cells.

**Discussion**

Nine distinct members of the Ly-49 gene family have been identified (Ly-49A through Ly-49I), although specific mAb are only available for Ly-49A (A1, YE1/32, and YE1/48), Ly-49C/I (5E6) (15), Ly-49G2 (4D11), and Ly-49D (4E5). The availability of these mAb has helped to further define the functional roles of these class I-binding NK receptors. The importance of generating mAb against the remaining, undefined members of the Ly-49 gene family is paramount to understanding the functional roles these molecules play in NK cell biology. The prototype molecule, Ly-49A, when expressed on NK cells has been shown to bind the class I molecules H-2D^d/H-2D^k. This recognition of class I ligand by the Ly-49A receptor has been shown to inhibit the lytic properties of Ly-49^A NK cells. A similar effect has been observed for Ly-49G2^"NK cells, but only against selected target cells that appear to express high levels of H-2D^d and/or H-2L^d (20, 23). The lack of an effect with the 5E6 Ab is complex and is somewhat disparate from previous results. Recent studies have demonstrated that mAb 5E6 reacts with both Ly-49C and Ly-49I (24). Although results (13, 25) indicate that Ly-49C could receive a negative signal from H-2^d MHC, in vivo studies indicate that 5E6^+ cells play an important role in the rejection of H-2^d bone marrow (26). In addition, 5E6^+ cells in vitro either receive a weak or not inhibitory signal from H-2^d Con A blasts (27). Collectively, the ability of H-2^d to deliver an inhibitory signal to Ly-49C must be reinterpreted after using Ly-49I-specific and Ly-49C-specific Abs.

Our examination of lymphocytes from normal mice indicated that the Ly-49A, C/I, and G2 receptors may be coexpressed on T cells. When Ly-49^+ T cells are examined, they are primarily NK1.1 and/or DX5 positive. Expression of Ly-49 on T cells has been demonstrated on both TCRa/b and TCRy/6 cells. In addition, when T cells coexpress Ly-49 receptors, they are predominantly found in the CD8^" subset, however, some CD4^+ T cells can also express Ly-49 receptors. Finally, analysis of the expression of Ly-49 receptors in 11 strains of murine T cells strongly indicates that the Ly-49 genes are similarly regulated in both NK and T cells. Strains that exhibit high percentages of Ly-49 receptors on NK cells are expressed in a parallel fashion in DX5^+ T cells. Of considerable interest was the apparent lack of expression of Ly-49D on murine T cells. This potentially activating Ly-49D receptor is abundantly expressed on NK cells from several mouse strains, but is absent on their DX5^- T cells. The functional relevance of this observation is presently unclear. Also, this study has demonstrated for the first time the presence of functional Ly-49 molecules on B6 T cells. Ly-49G2 and Ly-49A, which have strong inhibitory effects on NK cell functions, appear to function similarly in T cells. As a percentage of the total T cell population, Ly-49 expression is quite low (<1%), and the functional role of these unique cells in vivo is not understood. However, the selection and the expansion of T cell populations in which high percentages of the cells are NK1.1^- or DX5^- demonstrate that the spontaneous lytic process of these cells can be regulated by interactions with class I molecules. This effect was shown by addition of Abs specific for Ly-49A and G2, which are known to inhibit H-2Dr target cell lysis by B6 effectors, and also by inclusion of Abs to H-2Dr on the target.

We have recently reported that receptors can inhibit cytokine production by NK cells (28). In the present report we have examined several activation pathways in T cells and analyzed the possible inhibitory role of Ly-49 class I-binding receptors. The induction of cytokines as a result of target cell interactions was shown to be regulated by Ly-49 family members for both IFN-y and GM-CSF. While the addition of H-2Dr-expressing targets had no effect on stimulation by anti-CD3 or by pharmacologic triggering with PMA and ionomycin,
no conclusions can be drawn from these limited data. Although these findings were of interest, detailed studies with anti-CD3 were beyond the scope of this study, and the present experiments were not designed to examine the complex TCR signaling by Ly-49s. In the present study anti-CD3 was used only as a positive control for T cells, and the emphasis of the present study is target cell interactions that result in cytokine production.

Cytokine production by NK cells is likely to be an important physiologic event in a number of settings. For example, IFN-γ production by NK cells is critical for antiviral effects against leukokorionemeningitis virus (29). Recent studies have demonstrated important marrow-regulating effects of NK cells in perforin and Fas knockout mice (30). Collectively, these data support the hypothesis that cytokine production by NK cells and perhaps by T cells might be paramount in the regulation of hybrid resistance and marrow transplantation. Our current data with NK and T cells also suggest that Ly-49 receptors can regulate cytokines such as IFN-γ and GM-CSF, consistent with the findings of these previous studies. The abilities of Ly-49+ lymphocytes to recognize various class I molecules on hemopoietic stem cells and subsequently regulate their cytokine production could have a dramatic influence on stem cell repopulation. However, the in vivo function of CD3/1, NK1.1/DX5+ T cells and their potential role in marrow transplantation are unclear. Most studies to date have employed reg agents such as NK1.1 and Abs to Ly-49 family members to modify marrow engraftment in preclinical models. It is clear that these reagents will not discriminate between NK and T cells in vivo. Therefore, a potential role for the CD3/1/DX5+ T cells may be a redundancy with the classical NK cells for marrow engraftment, or these cells may have some unique, as yet undiscovered, function. Further studies to directly examine these alternatives are required.

In humans, class I binding killer cell-inhibitory receptors that regulate human T cell functions have been reported on memory T cells. Therefore, a potential role for the CD3/1, DX5+ T cells and perhaps by T cells might be paramount in the regulation of hybrid resistance and marrow transplantation. Our current data with NK and T cells also suggest that Ly-49 receptors can regulate cytokines such as IFN-γ and GM-CSF, consistent with the findings of these previous studies. The abilities of Ly-49+ lymphocytes to recognize various class I molecules on hemopoietic stem cells and subsequently regulate their cytokine production could have a dramatic influence on stem cell repopulation. However, the in vivo function of CD3/1, NK1.1+/DX5+ T cells and their potential role in marrow transplantation are unclear. Most studies to date have employed reg agents such as NK1.1 and Abs to Ly-49 family members to modify marrow engraftment in preclinical models. It is clear that these reagents will not discriminate between NK and T cells in vivo. Therefore, a potential role for the CD3/1/DX5+ T cells may be a redundancy with the classical NK cells for marrow engraftment, or these cells may have some unique, as yet undiscovered, function. Further studies to directly examine these alternatives are required.

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In summary, the present study describes for the first time that Ly-49 class I-binding molecules that are known to inhibit both spontaneous killing and cytokine production in NK cells also mediate these effects on a subset of murine T cells. Our studies demonstrate that the production of GM-CSF and IFN-γ, which are important hemopoietic regulatory cytokines, is inhibited in this subset of T cells and T cell lines via their interaction with class I target cells. We speculate that inhibitory effects mediated via these interactions may be important for the success or failure of marrow grafts.

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References


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