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The MHC Class I Molecule H-2D^p Inhibits Murine NK Cells via the Inhibitory Receptor Ly49A[1]

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MHC class I molecules strongly influence the phenotype and function of mouse NK cells. NK cell-mediated lysis is prevented through the interaction of Ly49 receptors on the effector cell with appropriate MHC class I ligands on the target cell. In addition, host MHC class I molecules have been shown to modulate the in vivo expression of Ly49 receptors. We have previously reported that H-2D^d and H-2D^p MHC class I molecules are able to protect (at the target cell level) from NK cell-mediated lysis and alter the NK cell specificity (at the host level) in a similar manner, although the mechanism behind this was not clear. In this study, we demonstrate that the expression of both H-2D^d and H-2D^p class I molecules in target cells leads to inhibition of B6 (H-2^b)-derived Ly49A$^+$ NK cells. This inhibition could in both cases be reversed by anti-Ly49A Abs. Cellular conjugate assays showed that Ly49A-expressing cells indeed bind to cells expressing H-2D^p. The expression of Ly49A and Ly49G2 receptors on NK cells was down-regulated in H-2D^p-transgenic (B6DP) mice compared with nontransgenic B6 mice. However, B6DP mice expressed significantly higher levels of Ly49A compared with H-2D^d-transgenic (D8) mice. We propose that both H-2D^d and H-2D^p MHC class I molecules can act as ligands for Ly49A. ***The Journal of Immunology,*** **1999, 162: 7010–7014.**

Natural killer cells recognize and kill various tumors, virus-infected cells, and allogeneic cells (1). NK cell function is influenced by the MHC class I expression of the target cell. However, MHC Ags do not need to be expressed on target cells to make the latter sensitive to NK cell-mediated lysis (2). On the contrary, reduced expression of MHC class I makes the target cell more susceptible to NK cell killing (3, 4). As predicted by the "missing-self" hypothesis (5), NK cells express inhibitory receptors specific for MHC class I molecules (6, 7). In the mouse, most of these receptors belong to the Ly49 family. Nine members of this family have been identified so far, of which at least one, Ly49D, is activating rather than inhibitory (8, 9). The Ly49 family maps to the NK gene complex on mouse chromosome 6 (10). The Ly49 receptors are homodimers of C-type glycoproteins (11, 12), and they are expressed on partly overlapping subsets of NK cells (Ref. 13; reviewed in Ref. 14). Each NK cell can thus express more than one type of Ly49 receptor; so far, the available Ab reagents and triple staining techniques have revealed "single receptor"- as well as "double receptor"- and "triple receptor"-expressing subsets. The specificity of each NK cell is ultimately determined by its composition of activating and inhibitory receptors. The process that regulates or selects this composition of receptors to ensure effector function and self tolerance is unknown.

The Ly49A receptor is expressed on approximately 20% of the NK1.1$^+$ cells in B6 mice (6). It recognizes H-2D^d and a class I molecule of the H-2^k haplotype, presumably H-2D^k, as inhibitory ligands (6). We have previously shown that introduction of an H-2D^d transgene on a B6 background, as in D8 mice, confers the ability of NK cells to kill and reject cells that lack H-2D^d but are otherwise syngeneic (15). At least with respect to in vitro killing of Con A blast targets, this H-2D^d-mediated control of NK cell function was exerted mainly, if not entirely, through the Ly49A$^+$ subset, which acquired the ability to kill H-2D^d-negative targets (16). The size of this subset was only marginally affected by introduction of the transgenic ligand (17), although the Ly49A receptor level on each NK cell was reduced 2- to 5-fold (16, 17). At the target cell level, transgenic expression of the H-2D^d resulted in protection from killing by NK cells of H-2D^d-expressing mice, as evaluated by the use of Con A blast targets and bone marrow grafts from the transgenic mice (18).

H-2D^d can thus define "self" as perceived by the NK cell system, with multiple functional effects as a consequence of in vivo expression of this inhibitory ligand of Ly49A. However, the "NK self phenotype" may not be defined in a unique manner by each single H-2 allele. In a recent study, we demonstrated that Con A-activated T cell blasts from mice transgenic for another H-2D allele, H-2D^p (B6DP mice), are also protected from NK cells of H-2D^d-expressing mice, e.g. BALB/C and D8 mice (19). Similarly, B6DP bone marrow grafts were strongly, although not totally, protected from rejection by NK cells of H-2D^d-transgenic D8 mice, while nontransgenic B6 marrow was efficiently rejected. The introduction of the H-2D^p transgene also conferred the capacity of the mice to reject nontransgenic B6 bone marrow. These experiments indicated that H-2D^d and H-2D^p MHC class I molecules control NK specificity in a similar manner. The most likely explanation was that H-2D^d and H-2D^p molecules are recognized by the same NK cell subset. This could occur either by coexpression of two different inhibitory receptors on the NK cell subset, or by a common motif on H-2D^d and H-2D^p recognized by the same inhibitory receptor. There is evidence from earlier studies of "F1 hybrid resistance" to bone marrow grafts that different H-2 haplotypes may contain gene products that "cross-react" with respect to NK cell recognition (20, 21).

The aim of this study was to analyze whether H-2D^d and H-2D^p inhibit the same NK cell subset and, if so, whether this involved the same inhibitory receptor on this subset. We demonstrate that

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tumor cells transfected with either H-2D^d or H-2D^p are protected from lysis by the Ly49A$^+$ NK subset in B6 mice. Ab-blocking experiments indicated both that MHC class I molecules are recognized by the Ly49A receptor and that conjugation studies support that Ly49A and H-2D^p interact physically. In addition, analysis of the expression of inhibitory receptors (Ly49A, C/I, and G2) in B6DP mice showed that the NK cells expressed reduced levels of Ly49A and Ly49G2. However, the level of Ly49A was significantly higher in B6DP mice than in D8 mice, which may suggest that Ly49A binds H-2D^p with a lower affinity than H-2D^d, yet still sufficiently well to enable Ly49A receptor-mediated inhibition.

Materials and Methods

Mice

C57BL/6 (B6)[3] (H-2^b), H-2D^d-transgenic B6 (D8), and H-2D^p-transgenic B6 (B6DP) mice were bred and maintained at the Microbiology and Tumor Biology Center, Karolinska Institute.

Cell lines

RBL-5 and RMA lymphoma cell lines are of B6 origin. The H-2D^d and H-2D^p transfectants of RBL-5 were generated in our laboratory.

Abs and reagents

PE-conjugated anti-NK1.1 (PK136) mAb were purchased from PharMingen (San Diego, CA). Protein G (Pharmacia, Uppsala, Sweden)-purified anti-Ly49A (A1) mAb were either conjugated with biotin (Pierce, Rockford, IL) according to standard protocols or purchased from PharMingen. FITC-conjugated anti-Ly49C/I (SW5E6) mAb were from PharMingen, and anti-Ly49G2 mAb were purified from the 4D11 hybridoma obtained from American Type Culture Collection (ATCC, Manassas, VA) and conjugated to FITC (Sigma, St. Louis, MO) according to a standard protocol. FITC-conjugated streptavidin was purchased from Dakopatts (Glostrup, Denmark), and Red-670-conjugated streptavidin from Life Technologies (Täby, Sweden). The H-2L^d-specific Ab (30-5-7) was used as isotype-matched control Ab for the anti-Ly49A mAb. PE-conjugated goat anti-mouse IgG (H+L) was purchased from Caltag (San Diego, CA). F(ab′)$_2$ fragments of A1 mAbs were generated by pepsin digestion. Undigested mAbs and F(ab′)$_2$ fragments were separated on protein G columns. The purity of the preparation was analyzed with SDS-PAGE under reducing and nonreducing conditions.

Cytotoxicity assay

Nylon wool nonadherent spleen cells (2.5–3 $\times$ 10^6/ml) were cultured in complete media supplemented with 1000 U/ml rIL-2 as previously described (16). On day 3, the adherent cells were stained with biotinylated A1 mAb followed by Red 670-streptavidin and PE-NK1.1. Ly49A-positive and -negative cells were sorted on a FACS Vantage (Becton Dickinson, Mountain View, CA) and recultured for 1–2 days the dissociate the Abs used for sorting. Tumor cell targets were labeled with ^{51}Cr as previously described (18). In the blocking experiments, the effector cells were preincubated with 150 μg/ml of anti-Ly49A F(ab′)$_2$ mAb. After incubating effector and target cells in 96-well plates for 4 h at 37°C, 100 μl of supernatant were harvested, and the radioactivity was measured in a gamma counter.

Conjugation assay

Con A blasts (prepared as described in Ref. 18) and Ly49A$^+$ RMA cells, were washed in RPMI 1640 5% FCS, labeled at 37°C for 20–30 min with 5 μM Cell Tracker Green CMFDA or Orange CMTMR diluted in RPMI 1640 5% FCS (Molecular Probes, Eugene, OR), washed 2 times, and resuspended in RPMI 1640 with 5% FCS. The cells were incubated at 37°C for 30 min to allow excess of fluorochrome to diffuse off the cells and then washed 1 time in RPMI 1640 with 5% FCS. The RMA cells were subsequently incubated with anti-Ly49A Ab, isotype-matched control Ab, or medium only at 4°C for 30 min. Cells of each type (2.5 $\times$ 10^5) (i.e., Con A blasts and RMA cells) were then mixed in triplicate in a total volume of 150 μl RPMI 1640 with 5% FCS in 96-well plates, centrifuged at 50 $\times$ g for 1 min, and incubated 15 min at 4°C. The mixed cells were carefully resuspended with a multichannel pipette in 350 μl PBS with 1% FCS, transferred to precipitin tubes, and analyzed by flow cytometry for conjugates. Percentages of conjugates were calculated according to the formula: 100 $\times$ {[number of FL1$^+$/FL2$^+$ cells]/[number of (FL1$^+$/FL2$^+$) + (FL1$^-$/FL2$^+$) + (FL1$^+$/FL2$^-$) + (FL1$^-$/FL2$^-$) cells]}.

[3] Abbreviations used in this paper: B6, C57BL6; D8, H-2D^d-transgenic B6; B6DP, H-2D^p-transgenic B6.

Table I. *Lysis of RBL-5, RBL-5D^d, and RBL-5D^p tumor cell targets by Ly49A$^+$/NK1.1$^+$ and Ly49A$^-$/NK1.1$^+$ cells from B6 mice (% specific lysis)*[a]

Target Cell	Effector Cells: Ly49A$^+$	Ly49A$^-$	Ly49A$^+$ vs Ly49A$^-$[b]
RBL-5	24.1 ± 10.9	25.5 ± 11.3	p = 0.8
RBL-5D^d	**7.2 ± 4.8**	28.3 ± 8.8	p<0.0001
RBL-5D^p	**10.3 ± 7.7**	19.9 ± 8.6	p<0.0001

[a] Mean lysis values (± SD) from 10 independent experiments at E:T ratio 10:1. Values significantly ($p < 0.05$) different from RBL-5 tumor cell target are in bold. Statistically significant differences were assessed using paired Student's *t* test.

[b] Comparison of the lysis by Ly49A$^+$ and Ly49A$^-$ NK cells of each target. Statistically significant differences were assessed using paired Student's *t* test.

Flow cytometry analysis

Erythrocyte-depleted spleen cells were passed through nylon wool (Polyscience, Eppelheim, Germany) columns. The columns were incubated for 1 h at 37°C, and the nonadherent cells were collected and stained with either biotinylated anti-Ly49A (A1) mAb, FITC-conjugated anti-Ly49G2 (4D11) mAb, or FITC-conjugated anti-Ly49C/I (SW5E6) mAb. Streptavidin RED-670 was used as a second step reagent for the anti-Ly49A mAb. All samples were stained with PE-conjugated NK1.1 mAb and subsequently analyzed on a FACScan flow cytometer (Becton Dickinson).

Statistics

Statistical analysis of the data was performed using a paired Student *t* test. A value of $p < 0.05$ was considered statistically significant.

Results

Protection of H-2D^p- and H-2D^d-transfected tumor cells against killing by Ly49A$^+$ NK cells from B6 mice

In light of the observation that Ly49A$^+$ NK cells are inhibited by H-2D^d-expressing targets (6) and that H-2D^d and H-2D^p acted similarly with respect to protective capacity (19), we tested whether target cells expressing H-2D^p also could inhibit Ly49A$^+$ NK cells. Ly49A$^+$ and Ly49A$^-$ NK cells from B6 mice were sorted by flow cytometry and used as effector cells against RBL-5 (H-2^b) or RBL-5 tumor cells transfected with either H-2D^p or H-2D^d class I molecules. The Ly49A$^+$ NK cells consistently showed a reduced lysis of RBL-5D^p as well as RBL-5D^d tumor cells, compared with the lysis of untransfected RBL-5 tumor cells (Table I, Fig. 1). In occasional experiments (e.g. Fig. 1), we observed somewhat less efficient protection of Ly49A$^+$ NK cell lysis by the RBL-5D^p tumor cell targets as compared with RBL-5D^d. However, there was no significant difference between the two different transfectants when the data from all experiments were analyzed (Table I, data not shown). The Ly49A$^-$ NK cells killed all the tumor targets to a similar extent, although they appeared to kill RBL-5D^p targets somewhat less well than RBL-5 and RBL-5D^d targets. There was a significant difference for the RBL-5D^d as well as the RBL-5D^p targets but not for untransfected RBL-5, when comparing the lysis mediated by Ly49A$^+$ and Ly49A$^-$ NK cells (Table I). We conclude that Ly49A$^+$ NK cells are inhibited by both H-2D^p- and H-2D^d-transfected tumor cells.

The role of the Ly49A receptor: evidence from functional and physical binding assays

By blocking the Ly49A receptor on the effector cells with anti-Ly49A F(ab′)$_2$ Ab fragments, we were able to induce efficient lysis

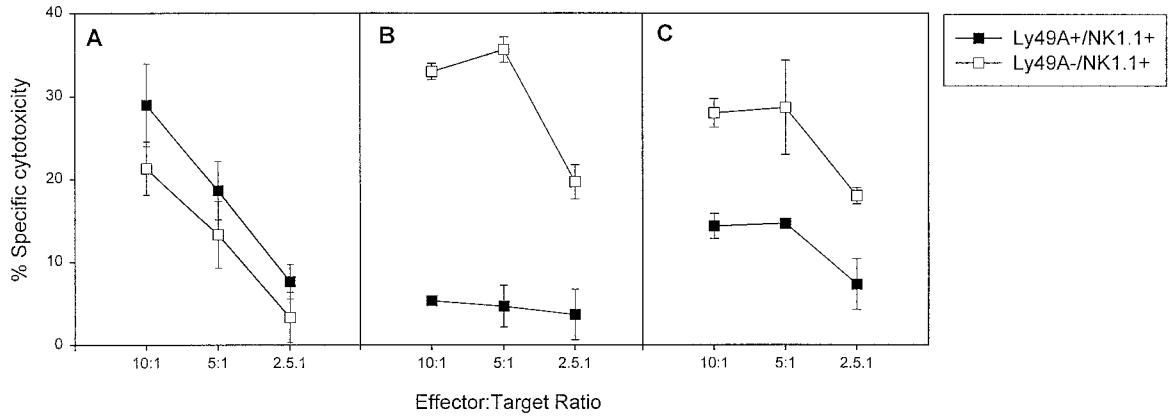

FIGURE 1. Ly49A$^+$ NK cells are inhibited by H-2D^p-transfected RBL-5 (H-2^b) tumor cells. Ly49A$^+$/NK1.1$^+$ (filled squares) and Ly49A$^-$/NK1.1$^+$ cells (open squares) were sorted from IL-2-activated splenocytes from B6 mice and tested against ^{51}Cr-labeled (*A*) RBL-5, (*B*) RBL-5D^d, and (*C*) RBL-5D^p tumor cells. Data are presented as the percentage of specific cytotoxicity ± SD of triplicates. The data are a representative result from 1 of 10 experiments.

of RBL-5D^p and RBL-5D^d tumor cells (Fig. 2). This demonstrates that the inhibition by both MHC class I molecules was indeed mediated through the Ly49A receptor. To investigate whether Ly49A receptor physically interacts with H-2D^p, we used a cellular binding assay. Fluorochrome-labeled Ly49A-expressing RMA cells and Con A blasts from different mouse strains were mixed, and the numbers of conjugates were analyzed by flow cytometry. To assess the role of the Ly49A receptor, the test was performed in the presence or absence of anti-Ly49A Abs. Ly49A-expressing RMA cells formed conjugates with both D8 and B6DP Con A blasts (Fig. 3 and Table II). In both cases, the conjugate formation was blocked by the addition of anti-Ly49A Abs, while addition of isotype-matched control Abs did not alter the number of conjugates. Some conjugate formation (~7%) was also observed between B6 Con A blasts and Ly49A-expressing RMA cells. However, blocking with anti-Ly49A Abs did not decrease the number of conjugates, suggesting that this binding was not mediated through the Ly49A receptor (Fig. 3). It should be noted that the Ly49A-expressing RMA cells repeatedly formed less conjugates with B6DP Con A blasts than with D8 Con A blasts (Table II). These experiments support that H-2D^p and H-2D^d both physically and functionally interact with the receptor Ly49A, suggesting that these MHC molecules share a common inhibitory motif.

H-2D^p expressed as a transgene modulates the Ly49A and Ly49G2 expression levels on NK cells

Having demonstrated that Ly49A can recognize also H-2D^p MHC class I molecules and mediate inhibition of NK cells, we analyzed whether H-2D^p expressed as a transgene on a B6 background could influence the NK cell receptor repertoire in a similar manner as the H-2D^d gene (16). We examined the expression of Ly49A (with A1 mAb), Ly49C/I (SW5E6 mAb), and Ly49G2 (4D11 mAb) on NK1.1$^+$ cells from B6, D8, and B6DP mice by flow cytometry. As shown previously (16, 17), the expression of Ly49A was reduced on NK1.1$^+$ cells from D8 mice, as compared with nontransgenic B6 NK cells (Table III). NK cells from B6DP mice also expressed significantly reduced levels of Ly49A; interestingly, the Ly49A levels were "intermediate" compared with the "high" and "low" Ly49A-expressing NK cells from B6 and D8 mice, respectively (Table III). The Ly49A expression on B6DP was approximately 70% compared with B6 NK cells (= 100%). NK cells from B6DP mice, as well as from D8 mice, were also found to express significantly reduced levels of Ly49G2 compared with NK cells from B6 mice. There was no significant difference in the level of Ly49C/I expression between the mice. As to the number of Ly49A$^+$ NK cells, these were similar in B6DP and B6

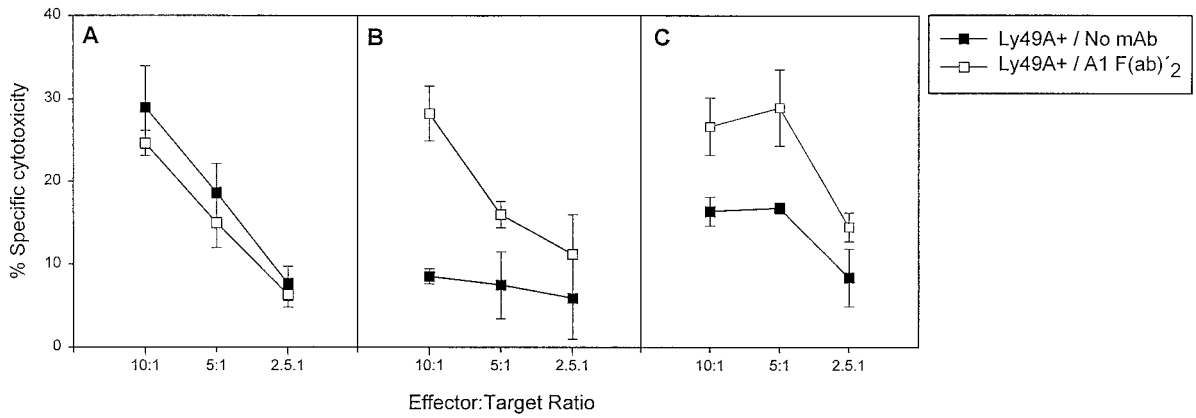

FIGURE 2. Ly49A$^+$ NK cells preincubated with anti-Ly49A F(ab′)$_2$ mAbs kill RBL-5D^p and RBL-5D^d tumor cells. Ly49A$^+$/NK1.1$^+$ were sorted from IL-2-activated splenocytes from B6 mice and tested against (*A*) RBL-5, (*B*) RBL-5D^d, and (*C*) RBL-5D^p tumor cells in the absence (filled squares) or presence (open squares) of anti-Ly49A F(ab′)$_2$ mAb. Data are presented as the percentage of specific cytotoxicity ± SD of triplicates. The data are a representative result from one of three experiments.

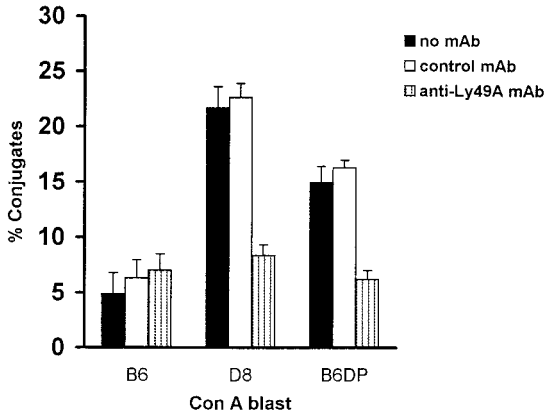

FIGURE 3. Ly49A$^+$ RMA cells form conjugates with Con A blasts expressing H-2D^p or H-2D^d MHC class I molecules. Conjugates between Ly49A-expressing RMA cells and Con A blasts from B6, D8, and B6DP were analyzed using a flow cytometry-based technique (see *Material and Methods*). Data are presented as the percentage of conjugates formed between RMA and Con A blasts in the absence or presence of anti-Ly49A mAb or isotype-matched control Ab (± SD of triplicates). The data are a representative result from one of two experiments.

mice ($p > 0.05$), while D8 mice had a somewhat reduced number of Ly49A$^+$ NK cells, as previously shown by Salcedo et al. (17).

Discussion

Our results from this study provide an explanation for the previously observed similarity between H-2D^d and H-2D^p with respect to influence over NK cell specificity: both class I molecules interact with the Ly49A receptor. Ly49A$^+$ NK cells from B6 mice showed a reduced ability to kill RBL-5 tumor cells transfected with either H-2D^p or H-2D^d, as compared with their killing of untransfected RBL-5 cells. Blocking of Ly49A with anti-Ly49A F(ab′)$_2$ Abs restored efficient lysis of RBL-5D^p and RBL-5D^d cells, while killing of RBL-5 cells was unaffected by Ly49A receptor blocking. Ly49A-expressing cells formed a significantly higher number of conjugates with Con A blasts from H-2D^p- and H-2D^d-transgenic mice than with Con A blasts from nontransgenic B6 mice. Again, these interactions involving H-2D^d and H-2D^p class I molecules could be blocked by anti-Ly49A mAb.

Interestingly, Ly49A-expressing RMA tumor cells consistently formed a lower number of conjugates with B6DP Con A blasts than with D8 Con A blasts ($p < 0.05$, Table II). This observation suggests that Ly49A has a slightly lower affinity for H-2D^p than for H-2D^d. However, we cannot rule out the possibility that the B6DP Con A blasts merely express a lower number of Ly49A receptor ligands compared with D8 Con A blasts. To test this, a binding assay with known concentrations of purified molecules must be used. Notably, by using purified MHC class I molecules, Kane and colleagues have demonstrated that Ly49A binds H-2D^k with lower affinity than H-2D^d (22, 23). However, Ly49A$^+$ NK cells from B6 mice are still inhibited by H-2^k-expressing cell lines (probably through H-2D^k) (6). The receptor-calibration model (16, 24) proposes that the effector or target cell needs to express sufficient levels of receptor and ligand, respectively, to generate an inhibitory signal. According to this model it can be hypothesized that NK cells need to express Ly49A at higher levels to be inhibited by H-2D^p- (or H-2D^k-) expressing targets than to be inhibited by H-2D^d targets, in a situation where the targets express the same number of MHC class I molecules.

It has been demonstrated that the cell surface expression of Ly49A is markedly decreased on fresh NK cells derived from mice transgenic for H-2D^d compared with their nontransgenic littermates (16, 17, 25, 26). Here we demonstrate that Ly49A is also down-regulated in mice transgenic for the H-2D^p class I molecule. However, the Ly49A expression in B6DP mice was not down-regulated to the same extent as in D8 mice. The difference in Ly49A expression between D8 and B6DP mice was significant ($p < 0.05$, Table III). As discussed above, different affinity between Ly49A/H-2D^d and Ly49A/H-2D^p or low expression of H-2D^p on the cells that participate in the calibration of Ly49A receptor may explain the observed Ly49A expression pattern in B6DP and D8 mice. In contrast to Ly49A, B6DP and D8 mice were found to have similar levels of Ly49G2 expression ($p > 0.05$, Table III). These were reduced compared with those of nontransgenic B6 mice, suggesting that H-2D^p may interact also with Ly49G2. Additional work is needed to determine whether H-2D^p is able to bind and inhibit Ly49G2$^+$ cells. It is noteworthy that B6DP mice had similar numbers of Ly49A$^+$/NK1.1$^+$ cells as B6

Table II. *Conjugate formation between Ly49A$^+$ RMA cells and Con A blasts from B6, D8, and B6DP mice*[a]

Con A Blast	No mAb[b]	Control mAb	Anti-Ly49A mAb[c]
B6	7.1 ± 3.1	4.5 ± 2.6	7.1 ± 2.7
D8	**18.3 ± 3.8**	17.6 ± 7.0	**9.8 ± 4.8**
B6DP	**14.2 ± 3.9**[d]	12.2 ± 5.7	**7.4 ± 2.5**

[a] Mean percentage of conjugates (± SD) between Ly49A-expressing RMA cells and Con A blasts in absence or presence of anti-Ly49A mAb or isotype-matched control mAb (anti-H-2L^d). The anti-Ly49A-blocking data are derived from nine separate experiments. The control mAb was included in two of these experiments.

[b] Values statistically different ($p < 0.05$) from B6 Con A blasts in the absence of mAb are shown in bold. Statistically significant differences were assessed using paired Student's *t* test.

[c] Values statistically different ($p < 0.05$) from Con A blasts in the absence of mAb are shown in bold. Statistically significant differences were assessed using paired Student's *t* test.

[d] Significantly ($p < 0.05$) fewer number of conjugates formed with B6DP compared with D8 Con A blasts. Statistically significant differences were assessed using paired Student's *t* test.

Table III. *Analysis of the inhibitory receptor repertoire in B6, D8, and B6DP mice*

	Expression Level of Ly49 Receptors on NK1.1$^+$ Cells[a]			Number of Ly49$^+$/NK1.1$^+$ Cells[b]		
Mouse strain	Ly49A	Ly49C/I	Ly49G2	Ly49A	Ly49C/I	Ly49G2
B6	100	100	100	21 ± 2	57 ± 11	49 ± 5
D8	33 ± 5	94 ± 4	73 ± 8	18 ± 3	47 ± 18	37 ± 8
B6DP	67 ± 6[c]	111 ± 11	68 ± 3	25 ± 4	49 ± 11	48 ± 5

[a] MFI (± SD) from three to five independent experiments. Ly49A, stained with A1 mAb; Ly49G2, 4D11 mAb; Ly49C/I, SW5E6 mAb. The fluorescence intensity on B6 was set to 100.

[b] Mean number of NK1.1$^+$ cells ± SD expressing Ly49A, Ly49G2, or Ly49C/I.

[c] Significantly ($p < 0.05$) different from the expression on D8 NK1.1$^+$ cells. Statistically significant differences were assessed using paired Student's *t* test.

mice. In contrast, D8 mice have a somewhat decreased number of Ly49A$^+$/NK1.1$^+$ cells (Table III) (17). Notably, the reduced number of Ly49A$^+$ cells in D8 mice compared with B6 mice could be accounted for by a reduction in the "double positive" Ly49A$^+$/G2$^+$ subset only, whereas the number of Ly49A$^+$/G2$^-$ NK cells was rather elevated in D8 mice (M. Johansson, unpublished observations). These data can be explained within a model where NK cells expressing several different inhibitory receptors for the same self MHC I molecules are selected against (reviewed in Ref. 14).

The binding experiments and Ly49A receptor expression analysis suggest that the interaction between H-2D^d and H-2D^p molecules and the Ly49A receptor may not be completely identical. This is in line with the observation that B6DP bone marrow cells were not completely protected from rejection by D8 mice and that B6DP mice had a lower ability to reject B6 bone marrow cells and RBL-5 tumor cells compared with D8 mice (19). In addition, Glas et al. have demonstrated that RBL-5D^p tumor cells are less efficiently protected from NK cell-mediated rejection than RBL-5D^d tumor cells when grafted into H-2^d SCID mice (27). A structural explanation could be that H-2D^d and H-2D^p share some, but not all, critical motifs for Ly49A binding. The protection has previously been mapped to the $\alpha1/\alpha2$ domains of H-2D^d (6), and, more recently, Sundbäck et al. have shown that the motif(s) determining allelic specificity of the Ly49A receptor is located in the $\alpha2$ domain of H-2D^d (28). This does not exclude that conserved amino acid residues present in the $\alpha1$ domain may also contribute to the interaction with Ly49A. To map the protective motif(s) shared between H-2D^d and H-2D^p class I molecules, it will be necessary to perform extensive site-directed mutagenesis studies on the MHC class I molecules and ultimately structural analysis of Ly49-MHC complexes.

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