Alteration of the Natural Killer Repertoire in H-2 Transgenic Mice: Specificity of Rapid Lymphoma Cell Clearance Determined by the H-2 Phenotype of the Target

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Summary

The mechanism behind natural tumor resistance conveyed by a H-2D^d transgene to C57Bl/6 (B6) mice was investigated. Transgenic D8 mice were more efficient than control mice in natural killer (NK) cell mediated rapid elimination of intravenously inoculated radiolabeled lymphoma cells of B6 origin, such as RBL-5. There was no difference between D8 and B6 mice when elimination of YAC-1 targets was monitored. The effect of the transgene on the NK repertoire was related to the H-2 phenotype of the target: the differential elimination of RBL-5 lymphoma cells in D8 and B6 mice was not seen when a H-2 deficient variant of this line was used (efficiently eliminated in both genotypes), nor was it seen with a H-2D^d transfectant (surviving in both genotypes). The data show that a MHC class I transgene can directly control natural killing in vivo by altering the repertoire rather than the general levels of NK activity. Since the NK mediated elimination seen after introduction of a novel gene in the host was neutralized by introducing the same gene (H-2D^d), but not an unrelated class I gene (H-2D^p), in the tumor, the data support the concept of NK surveillance against missing self. This combined transgenic/transfectant system may serve as a tool for a molecular dissection of the interactions between NK cells and their targets in vivo.

Several steps in immune responses are controlled by genes of the MHC. MHC class I and II genes educate, tolerize or activate the T cell subsets (1–5). There is also evidence for a MHC influence on NK cell function. F_1-hybrid resistance mediated by natural killer cells against tumor and bone marrow grafts is one example (6–9). This phenomenon is poorly understood in comparison with the detailed knowledge of how MHC genes control the T cell system (1–5). It is not clear whether the genetic control of natural immunity involves the classical MHC genes, but natural resistance against lymphoma grafts has been mapped close to MHC class I genes in linkage studies (8, 9). We have reported that a H-2D^d transgene introduced in C57Bl/6 (B6)[1] mice convey natural resistance to lymphoma grafts of B6 origin (10). This resistance was dependent on the presence of NK cells, since it was abrogated by treatment of transgenic mice with mAbs against NK1.1 or heteroantiserum against asialo-GM1 (10). The purpose of the present study was to investigate two aspects of this rejection. First, does the H-2D^d transgene directly influence rapid NK mediated elimination, or is the transgene effect indirect, i.e., does it recruit NK cells as effectors in a later stage of a rejection response. Second, if the transgene affects natural killing directly, does it control the general levels of NK activity or the specificity pattern in their "repertoire", i.e., killing of some but not all targets? In particular, is the H-2 phenotype of the target important?

We have studied these questions by following the early events after inoculation of radiolabeled lymphoma cells in transgenic and control mice. The clearance of 5′-[^{125}I]Iodo-2′-deoxyuridine (^{125}I-IUdR) labeled cells has previously been used to measure NK activity in vivo (11–14). One advantage of this rapid assay is that it allows in vivo tests of natural resistance without the interference of the slower T cell mediated reactions against allogeneic or strongly immunogeneic tumors. Our results show that introduction of the H-2D^d gene on B6 background affects early NK mediated elimination against some, but not all targets. Furthermore, the H-2 phenotype of the target appears to be crucial for the transgene effect, as shown most clearly in experiments with H-2 mutant and transfected subclones of the RBL-5 lymphoma.

Materials and Methods

Mice. Mice were bred and maintained at the Department of Tumor Biology, Karolinska Institutet. B6 mice were also purchased from ALAB (Sollentuna, Sweden). The generation of the transgenic

[1] *Abbreviations used in this paper:* B6, C57Bl/6; ^{125}I-IUdR, 5′-[^{125}I]Iodo-2′-deoxyuridine.

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D8 strain has been described earlier (15). Briefly, a 8.0-kb EcoR1 fragment from the plasmid pD^{d}1 (16) containing the H-2D^d gene was microinjected into fertilized B6 embryos and reimplanted into pseudopregnant B6 females. The expression and tissue distribution of the H-2D^d gene paralleled that of the endogenous K^b and D^b genes (17, 18). The D8 strain was a kind gift from Dr. Gilbert Jay.

Tumors. H-2^b tumors: RMA is a subline of the T cell lymphoma RBL-5. RMA-S is a H-2 deficient variant of RMA, generated by repeated cycles of negative selection using mouse allo anti-H-2^b antisera and complement (19, 20). RMA-S has a defect in the association between the class I heavy chains and β_2-microglobulin (β_2 m), resulting in a decreased expression of H-2 on the cell surface (21). RBL-5pD^{d}1 and RBL-5D^p are RBL-5 sublines transfected with the plasmids pD^{d}1 (16) and pRM15 (22), respectively. EL-4 is a benzpyrene induced, and ALC a RAD-LV induced T cell lymphoma. *H-2^a tumors*: YAC-1 is a T cell lymphoma induced by Moloney Leukemia Virus in the A/Sn strain. A.H-2$^-$ is a β_2m deficient variant of YAC-1 with no detectable cell surface expression of class I antigens (21, 23). *H-2^d tumors*: L1210 is a T cell lymphoma and P815 is a mastocytoma, both induced by benzpyrene in the DBA/2 strain. All tumors were maintained as ascites lines in the syngeneic strain and explanted to in vitro culture 1–4 wk before experiments were carried out. Table 1 lists the different cell lines used in this study.

In Vivo Rapid Elimination Assay. Tumor cells (10^6/ml) were incubated over night in the presence of 0.5 μCi/ml of 5′-[^{125}I]Iodo-2′-deoxyuridine (^{125}I-IUdR; Amersham, Sweden AB, Solna, Sweden). Before inoculation, the radiolabeled cells were washed 3–4 times with large volumes of PBS and adjusted to 5×10^6/ml in PBS. The activity of the inoculation volume (10^6 cells in 200 μl) was determined and was usually in the range of $2–4 \times 10^5$ cpm. At different timepoints after inoculation, the mice were killed and the lungs, livers, and spleens removed. The remaining radioactivity in each organ was measured in a gamma counter and expressed as percentage of the total activity inoculated.

Pretreatment of Mice with Anti-NK1.1 Antibody. One day before inoculation of tumor cells, the mice were given one single injection of 200 μl ascites prepared anti-NK1.1 mAb (24). This treatment has previously been shown to abrogate natural killer cell activity in vivo (25).

Transfection. RBL-5 cells were electroporated together with the plasmid pD^{d}1, encoding the D^d gene (16), or pRM15, coding for the D^p gene (22), in a BIO-RAD genepulser (BIO-RAD, Richmond, California). Electroporation was carried out in 0.4 ml PBS at 250 Volts and 960 microFahrads. After 10 min at room temperature the cells were put in RPMI with 10% FCS (normal culture conditions). When the number of surviving cells were $5–10 \times 10^6$, the dead and live cells were separated using Ficoll/hypaque (Pharmacia, Uppsala, Sweden) centrifugation, and subsequently sorted on a Fluorescence Activated Cell Sorter (FACS® 4; Becton Dickinson and Co., San Jose, California). In the case of D^p, cotransfection with the neomycin resistance gene was performed, and before sorting, a G418 resistant population was selected to enrich for clones expressing the class I gene. Cells were labeled with H-2D^d specific (34-4-8S, reference 26) or H-2D^p specific (7-16.10, reference 27) mAb and positive cells were sorted on the FACS. After 4–5 such rounds of selection, stable transfectants were obtained that expressed H-2D^d or H-2D^p in addition to the endogenous H-2^b molecules. The resulting transfectants, RBL-5pD^{d}1 and RBL-5D^p were sensitive to H-2D^d and H-2D^p specific CTL respectively, and they were both sensitive to H-2^b specific allo CTL (not shown).

Indirect Immunofluorescence. 10^6 tumor cells were incubated with 100 μl of hybridoma supernatant 30–60 min on ice, washed with PBS, and incubated with 100 μl FITC conjugated rabbit anti-mouse Ig (Dakopatts, Hägersten, Sweden). After washing, the cells were analyzed on the FACS.

Statistical Analysis. Statistical calculations were performed using a two-tailed, non-paired t test. In cases where the variances between the groups were not comparable, the Cochran t test was used.

Results

Survival of H-2^b Lymphomas after Intravenous Inoculation to B6 and H-2D^d Transgenic Mice. Radiolabeled lymphoma

Table 1. *Cell Lines Used in this Study*

				MHC class I haplotype				
				H-2^a	H-2^b	H-2^d	Transfected gene	
Tumor cell	Parental tumor	Strain	Inducing agent	(K^{k}D^d)	(K^{b}D^b)	(K^{d}D^d)	(D^d)	(D^p)
RBL-5	–	C57Bl/6	Raucher virus	–	+	–	–	–
RMA	RBL-5	C57Bl/6	Raucher virus	–	+	–	–	–
RMA-S	RBL-5	C57Bl/6	Raucher virus	–	(+)*	–	–	–
RBL-5pD^{d}1	RBL-5	C57Bl/6	Raucher virus	–	+	–	+	–
RBL-5D^p	RBL-5	C57Bl/6	Raucher virus	–	+	–	–	+
EL-4	–	C57Bl/6	Benzpyrene	–	+	–	–	–
ALC	–	C57Bl/6	RAD-LV	–	+	–	–	–
YAC-1	–	A/Sn	Moloney virus	(+)	–	–	–	–
A.H-2$^-$	YAC-1	A/Sn	Moloney virus	–	–	–	–	–
P815	–	DBA/2	Methylcholantrene	–	–	+	–	–
L1210	–	DBA/2	Methylcholantrene	–	–	+	–	–

* Weak but detectable expression.

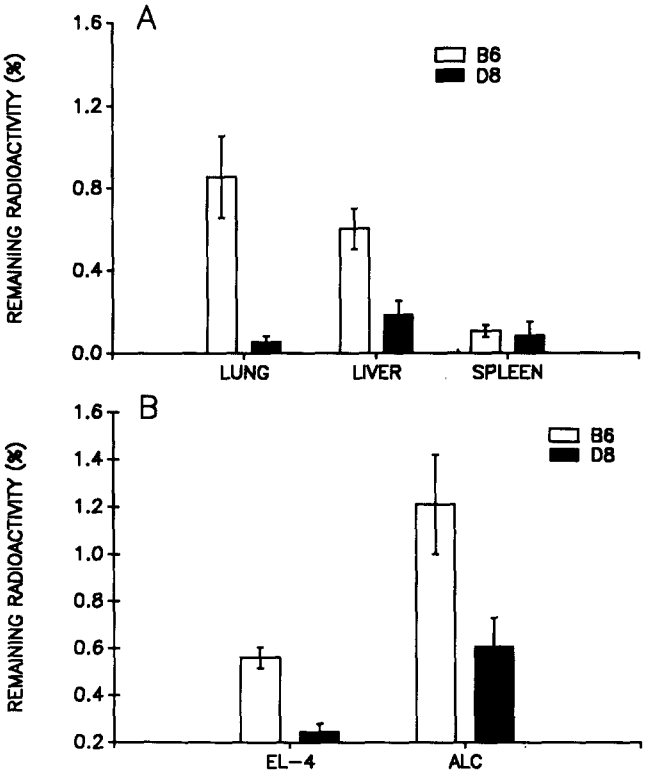

Figure 1. Elimination of RBL-5 (*A*) and of EL-4 and ALC (*B*) in C57Bl/6 (B6) and D8 mice. The remaining radioactivity in the different organs indicates the amount of surviving cells, and is expressed as a percentage of the total amount radioactivity inoculated. In both experiments the mice were killed 12 h after tumor cell inoculation. In (*B*), only lung values are shown. All figures in this paper show arithmetic mean and SE from at least four mice in each group, unless anything else is indicated. The differences between B6 and D8 in (*A*) were statistically significant in the lung ($p < 0.01$) and in the liver ($p < 0.05$) but not in the spleen. In (*B*), the differences between B6 and D8 were significant ($p < 0.001$) for both EL4 and ALC. Data for D8 in (*A*) is mean value from three mice.

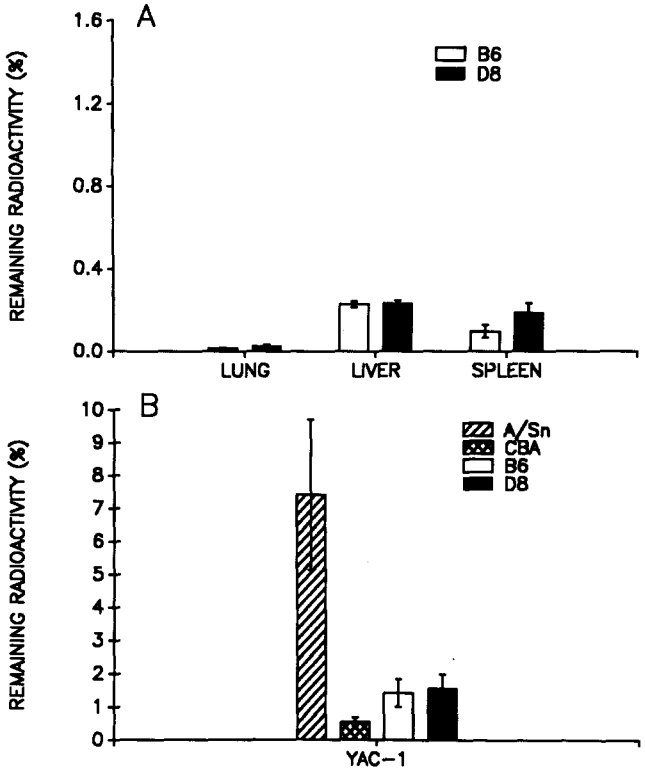

Figure 2. Elimination of YAC-1 in B6 and D8 mice after 12 h (*A*) and in B6, D8, CBA, and A/Sn after 3 h (*B*). Data in (*A*) is obtained from the same experiments as in Fig. 1 *A*. In (*B*), only lung values are shown. The difference between A/Sn and CBA in (*B*) were statistically significant ($p < 0.05$).

cells were inoculated into B6 and D8 mice. Survival of inoculated cells was estimated by measuring remaining radioactivity in different organs at different time points. There was a significant difference in pulmonary clearance of RBL-5 lymphoma cells between control B6 and transgenic D8 mice at 12 h after inoculation. The same pattern was seen in the liver, while there was a marginal difference in the spleen (Fig. 1 *A*). The terms clearance or elimination will be used for this difference in endpoint survival of lymphoma cells, since kinetic studies (further discussed below) showed that there was no difference in homing patterns of lymphoma cells in the different genotypes of mice. To study whether this differential elimination was a general phenomenon or unique to RBL-5, we inoculated two other lymphomas with high H-2^b expression, ALC and EL-4. The same selective elimination in the D8 mice was seen also for these lymphomas (Fig. 1 *B*).

Target Cell Specificity of the Transgene Effect. In contrast to the H-2^b lymphomas, YAC-1 cells were eliminated equally efficiently in B6 and D8 mice at 12 h (Fig. 2 *A*). YAC-1 is known to be efficiently killed by NK cells within hours in vitro as well as in vivo (14). It was possible that the 12-h time point was suboptimal to detect a H-$2D^d$ mediated difference in clearance, but we failed to observe any difference in survival in assays after 3 h (Fig. 2 *B*). The previously known genetic differences in NK activity between A/Sn (low), B6 (intermediate) and CBA (high) (14) were readily detectable at this time point.

The L1210 lymphoma and P815 mastocytoma, both of DBA/2 (H-2^d) origin, gave the same pattern as YAC-1. They were equally well eliminated in D8 and B6 mice, whether tested after 3 (P815, Fig. 3 *A*), 12 (P815, Fig. 3 *B*) or 21 (L1210, Fig. 3 *C*) h. RMA (Fig. 3 *B*) or RBL-5 (Fig. 3 *C*) were included as a control for transgene induced elimination.

Expression of H-$2D^d$ was a common feature for the three tumors for which there was no difference in elimination between control and transgenic mice. To test whether the lack of transgene effect on clearance of YAC-1 cells was dependent on the H-$2D^d$ product (or class I expression in general) at the tumor cell level, we inoculated cells from the YAC-1 variant line A.H-2^-. This line does not express any class I gene products at the cell surface due to a translational block of the β_2m mRNA (21, 22). Nevertheless, this cell was equally well eliminated from D8 and B6 mice (Fig. 4). The

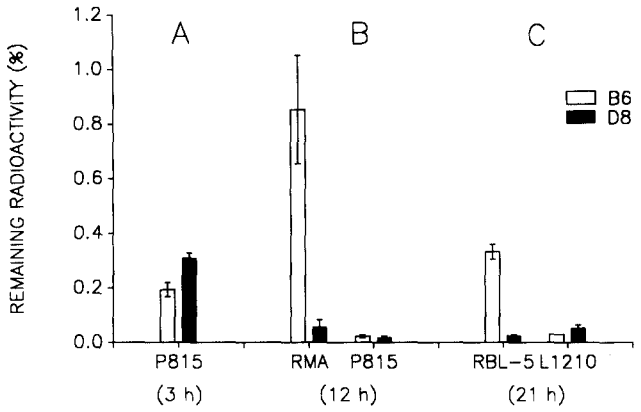

Figure 3. Remaining radioactivity in the lungs of of B6 and D8 mice for P815 (*A*), RMA and P815 (*B*) and RBL-5 and L1210 (*C*). For each experiment, the different timepoints are indicated in parentheses. Data for RMA to D8 in (*B*) was obtained from three mice. The differences between B6 and D8 for RMA ($p < 0.01$ in Fig. 3 *B*) and RBL-5 ($p < 0.001$ in Fig. 3 *C*) were statistically significant.

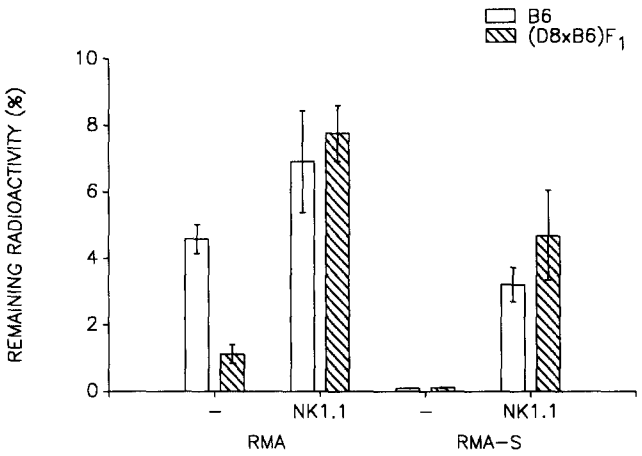

Figure 5. Effect of anti-NK1.1 treatment on 12-h survival of RMA and H-2 deficient variant RMA-S in the lungs of B6 and (D8 × B6)F_1 mice. In this experiment all data for B6 and data for RMA-S in untreated (D8 × B6)F_1 were obtained from groups of three mice. The difference between B6 and (D8 × B6)F_1 for RMA was statistically significant ($p < 0.001$).

differences in general levels of NK activity between A/Sn and CBA (14) were detected for this cell line also (Fig. 4).

Survival of RBL-5 in Transgenic and Normal B6 Mice; Role of NK1.1 Positive Cells and Lymphoma Class I Expression. Another property that distinguishes the B6 lymphomas from YAC-1, P815 and L1210 is that only the former expresses H-2^b. To test whether the transgene induced difference in clearance was dependent on H-$2K^bD^b$ expression in RBL-5, we inoculated the class I low variant line RMA-S. There was no difference between B6 and (D8 × B6)F_1 mice for this variant, which has a 95% reduction in H-2^b expression compared to RBL-5 (19, 20). It was efficiently cleared from the lungs in both genotypes (Fig. 5).

The difference between (D8 × B6)F_1 and B6 mice in lung clearance of RBL-5 cells was abrogated by pretreatment of hosts with anti-NK1.1 mAb (Fig. 5). This treatment also greatly reduced the difference in clearance between RBL-5 and H-2 deficient RMA-S cells in B6 hosts.

The NK mediated elimination of normal RBL-5 cells in D8 or (D8 × B6)F_1 mice and of syngeneic but H-2 deficient RBL-5 cells (RMA-S) in B6 mice suggested that self match with respect to H-2 phenotype prevented natural killing (Fig. 5, references 19, 20). The effect of the transgene would then be a consequence of a redefinition of "self" in the transgenic mice, creating a situation of incomplete match to the H-2^b lymphomas. This predicted that the difference in elimination of these lymphomas between D8 and B6 mice should disappear if the H-$2D^d$ gene was introduced in the

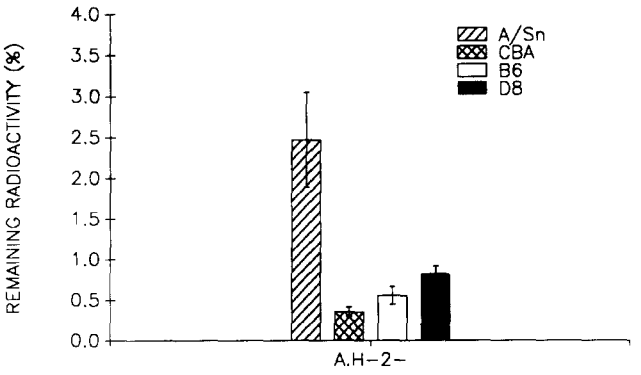

Figure 4. Elimination of H-2 negative YAC-1 variant A.H-2^-. The figure shows remaining radioactivity in the lungs of B6, D8, CBA and A/Sn mice 3 h after inoculation. Mean of two experiments. The difference between A/Sn and CBA was significantly different ($p < 0.01$).

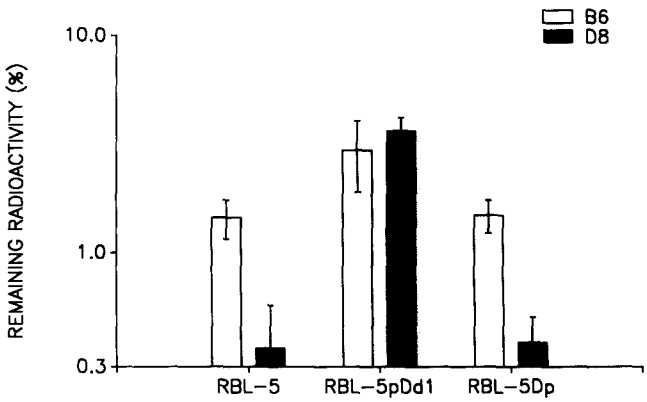

Figure 6. Remaining radioactivity (after 12 h) of normal, H-$2D^d$ transfected RBL-5 or H-$2D^p$ transfected RBL-5 in the lungs of B6 and D8 mice. The figure is a summary of two independent experiments. The number of mice in each host/tumor combination was 8 except for RBL-5 to B6 where 9 mice were used. The difference between B6 and D8 was statistically significant for RBL-5 ($p < 0.05$) and RBL-5D^p ($p < 0.01$), but not for RBL-5pD^{d}1. Just before the experiments, the transfected cells were analyzed for the expression of the transfected genes, as described in materials and methods. Fluorescence values (lin) from a representative analysis were for K^b, D^b, D^d, D^p and no antibody respectively: RBL-5 (353, 453, 24, 12, 13), RBL-5pD^{d}1 (267, 312, 217, n.d., 15) and RBL-5D^p (310, 370, n.d., 147, 11). n.d., = not determined.

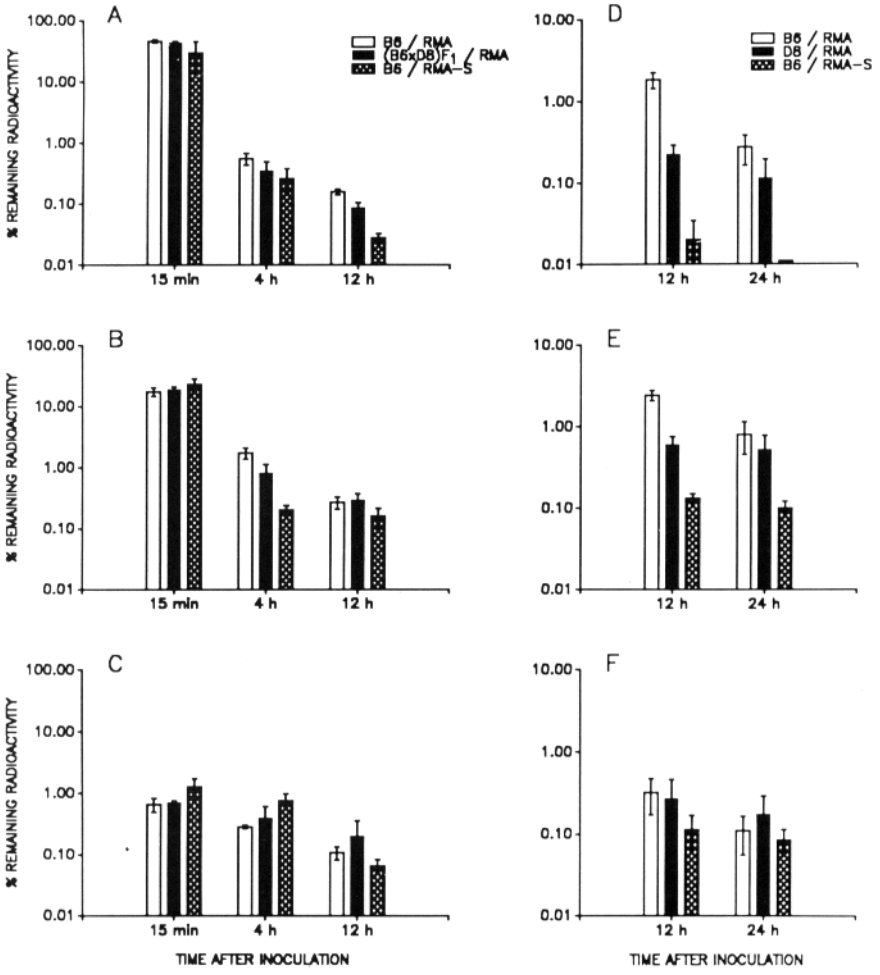

Figure 7. Time kinetics of elimination in B6, D8 and (D8 × B6)F_1 mice. The figures show remaining radioactivity in the lungs (*A* and *D*), liver (*B* and *E*) and spleen (*C* and *F*) at three different timepoints after inoculation. The host/tumor combinations were RMA to B6 (*open bars*), RMA to D8 or (D8 × B6)F_1 (*filled bars*) and H-2 deficient variant RMA-S to B6 (*cross hatched bars*). *A–C* and *D–F* represent two different experiments. The lung differences at 12 h between normal and transgenic B6 mice in the ability to eliminate RMA, were in both experiments statistically significant ($p <$ 0.001 in *A*, and $p < 0.001$ in *D*). Similarly, the differences between RMA and RMA-S in lung survival in B6 mice were in both cases significant ($p <0.01$ in *A*, and $p < 0.001$ in *D*).

lymphoma; it should now survive in both hosts. To test this, the RBL-5 lymphoma was transfected with the H-2D^d gene. When the resulting transfectant (RBL-5pD^d1) was used, the selective elimination in the D8 mice was abrogated (Fig. 6). As a control, RBL-5 was transfected with the H-2D^p gene and used in the same experiments. For this transfectant the pattern of elimination was similar to that of untransfected RBL-5.

H-2 Transgene in the Host Versus H-2 Deficiency in the Target: Kinetics and Organ Distribution of Clearance in Two Different Situations. The survival of RBL-5 in B6 mice at different time points was used as a control for elimination caused by the H-2D^d transgene in the host (comparison with RBL-5 into (D8 × B6)F_1 or D8 elimination caused by the H-2 deficiency of the target (comparison with RMA-S into B6). Neither comparison revealed any significant differences at 15 min or 4 h after inoculation (Fig. 7 *A–C*). However, at 12 h, a significant difference between B6 and (B6 × D8)F_1 (Fig. 7 *A*, Exp. 1), or between B6 and D8 (Fig. 7 *D*, Exp. 2) in the ability to eliminate RMA was seen in the lungs. In the same comparison, there was also a significantly better elimination of RMA-S than of RMA in normal B6 mice (Fig. 7, *A* and *D*). In all cases this difference was more pronounced than the difference between RMA in B6 and transgenic mice. The differences were smaller in the liver and could not be seen in the spleen (Fig. 7 *B*, *E*, *C*, and *F*).

Discussion

Our results indicate a direct influence of a MHC transgene on rapid elimination of tumor grafts mediated by NK cells. This extends previous studies with MHC congenic mice, where elimination within 4–36 h after inoculation of radiolabeled cells has been shown to be T cell independent (11–13). Another main conclusion is that the transgene effect varied depending on the target cell, i.e., it affected the repertoire rather than general levels of killing. The effect of the transgene could be seen against several lymphomas of H-2^b background, but could not be detected against three allogeneic lymphomas (P815, L1210, and YAC-1), nor against a completely H-2 negative lymphoma variant (A.H-2^-) and a H-2 deficient mutant of the H-2^b lymphoma RBL-5 (RMA-S). We interpret this as a consequence of efficient natural killing of these cells in control as well as in transgenic mice, rather than absence of NK effects in both genotypes. The recovered levels of radioactivity were low and YAC-1, L1210, and RMA-S

are known to be efficiently cleared by NK cells in B6 and B10 mice (11, 13, 14, 28). Efficient NK mediated elimination of RMA-S in both (D8 × B6)F_1 and B6 mice was also demonstrated directly by the use of host treatment with anti-NK1.1 mAb (Fig. 5).

Why was the elimination efficient against some cells in normal B6 mice, while other targets were killed only in the B6 mice carrying the H-2D^d gene? In previous studies, the match or mismatch of H-2 between host and target has been emphasized as an important factor contributing to rapid elimination (11, 13, 29). There were two possible explanations of the data in that respect: (*a*) Expression of a particular class I molecules on the target (e.g., H-2D^d, common for P815, L1210, and YAC-1) triggered the NK elimination in B6 as well as D8 mice, resulting in equally efficient elimination in both strains. (*b*) Complete H-2 class I match between target and host (e.g., RBL-5 to B6) prevented elimination. Our data support the second alternative. The differences in elimination between D8 and B6 mice seen for tumor cells of the H-2^b genotype could be abrogated by interfering with this match, either by reducing endogenous H-2^b expression in the lymphoma cells, or by introducing the "transgene" (H-2D^d) in the lymphoma cells by transfection. In the former case efficient elimination occurred in both hosts, while in the latter lymphoma cells survived in both hosts. Thus, the H-2 match in the syngeneic (RBL-5 to B6) or "transsyngeneic" (RBL-5pD^{d}1 to D8) situation prevented elimination.

A recent survey of multiple host/donor combinations supports a role for self match in prevention of rapid elimination of grafted lymphocytes in the rat (30). However, also active recognition of allogeneic MHC by NK cells has been postulated in the rat (30, 31). It is unlikely that the allogeneic lymphomas in our study were actively recognized and eliminated in B6 mice because they expressed foreign MHC. The H-2 negative YAC-1 variant A.H-2$^-$ does not translate its β_2m transcripts, and is therefore unable to express any class I molecules on the cell surface (21). Yet, it was at least as efficiently eliminated as YAC-1 in D8 and B6 mice. This showed that expression of H-2D^d (or any class I molecules) on the cell surface was not required for elimination. Furthermore, introduction of foreign MHC genes (H-2D^d or H-2D^p) did not result in increased elimination in B6 (Fig. 6). Thus, we find no evidence that allogeneic H-2 in the target cell can trigger a response. However, it must be noted that in bone marrow graft experiments, H-2D^d introduced as a transgene to B6 mice resulted in a NK mediated rejection by non transgenic B6 mice (32). The reasons for this differential effect of H-2D^d on lymphoma and bone marrow cells are not known. One possibility is that the transgene suppresses expression of endogenous H-2K^b,D^b molecules in bone marrow but not in lymphoma cells, a possibility that is now being tested. Another possibility is that different subsets of NK1.1$^+$ cells are active in marrow and lymphoma rejection. Recently, a CD3$^+$, NK1.1$^+$ cell has been shown to mediate marrow graft rejection (33). Our results do not distinguish between CD3$^-$, NK1.1$^+$, and CD3$^+$, NK1.1$^+$ as responsible effector cells. However, the latter are thymus dependent (33, 34), while the rapid elimination of H-2 mismatched lymphoma cells has been demonstrated also in nude mice (11).

The allogeneic tumors used in this study share one allele with the D8 mice, H-2D^d, but none with B6. However, they did not survive better in H-2D^d positive D8 than in B6 mice. We propose that the partial match with respect to H-2D^d is insufficient to compensate for the lack of match with respect to H-2K^b and D^b. A prediction from this is that H-2^d cells, such as L1210, transfected with the relevant H-2^b sequences should become resistant to elimination in both D8 and B6 mice.

A.H-2$^-$ showed differential sensitivity to elimination by CBA and A/Sn mice (Fig. 4), two strains with a known difference in levels of NK activity against YAC-1 (14). Since A.H-2$^-$ lacks MHC expression at the cell surface, it can be regarded as an "indicator" of NK activity, irrespective of match or mismatch between host and target MHC genotypes. Since there was no difference between D8 and B6 for A.H-2$^-$, we conclude that the the H-2D^d transgene has not altered the general levels of NK activity in the B6 strain.

Restoration of MHC class I expression by transfection has been reported to protect from NK lysis in several MHC deficient targets, even if this is not a general rule (reviewed in reference 35). The present study extends these findings by two original observations: (*a*) The transfected gene protected from NK mediated elimination in vivo, and (*b*) The recipient line did not from the start have a primary general H-2 deficiency. Furthermore, we show that the protective effect was specific for a class I allele that corrected the transgene induced mismatch, since the control transfectant RBL-5D^p was not accepted in D8 mice. Presence of the H-2D^d transgene in D8 donor mice also led to protection of their bone marrow from rejection in H-2D^d expressing B10.D2 mice (32), although it was not formally shown that the protective effect of the transgene was exerted in the grafted cell, as in the present study. Conversely, bone marrow (reference 36, Höglund, manuscript submitted for publication) and Con A induced T cell blasts (Höglund, P., C. Öhlén, E. Carbone, L. Franksson, H.-G. Ljunggren, A. Latour, B. Koller, and K. Kärre, manuscript submitted for publication) from mice deficient in β_2m/MHC class I, are recognized and killed by NK cells from β_2m expressing mice. Interestingly, β_2m deficient NK cells failed to kill the β_2m deficient bone marrow and Con A blasts, further supporting the role of the class I environment in calibrating the NK repertoire (Höglund, P., C. Öhlén, E. Carbone, L. Franksson, H.-G. Ljunggren, A. Latour, B. Koller, and K. Kärre, manuscript submitted for publication, C. Öhlén, manuscript in preparation).

"Protective epitopes" have been mapped to the α-1 and α-2 domains in a study of HLA transfected human NK targets (37), but the mechanism behind the effect is not known. It could occur either through a MHC class I mediated/presented negative signal to effector cells - the "effector inhibition" model (35) - or by interference with another NK target antigen - the "target interference" model (35). If the target antigen interfered with in the latter case was polymorphic and MHC

linked, this model can be reconciled with the recessive Hh antigen model (7). A recessive target antigen has recently been proposed for recognition of PHA blasts by alloreactive $CD3^-$, $CD16^+$ human NK cells. Resistance to lysis was dominantly inherited and segregated with HLA haplotype in a family study of lymphocyte target donors (38). If resistance is controlled by an HLA class I (like) gene in this human system, it may represent an analogue to the H-$2D^d$ mediated protection from NK mediated rejection of bone marrow (32) and lymphoma grafts (this study) in allogeneic or transgenic recipients expressing H-$2D^d$.

The rejection of H-2^b lymphomas in the D8 strain was most pronounced in the lungs but was also observed in the liver. The small difference in splenic clearance was surprising, since the spleen is the standard source of NK cells for in vitro assays. For some previously studied tumors, the spleen was the primary organ for in vivo clearance by NK cells (13), while other tumors were cleared more efficiently in the lungs as in this study (14). The reasons for these differences are not known but they may reside in the choice of strains or tumor cells under study. Some tumor cells may express adhesion molecules specific for capillaries of the lung or liver, while others home primarily to the spleen. It is also possible that tumor cells arrested in the lungs can recruit NK cells from the circulation and the spleen, leaving fewer cells for tumor elimination in this organ. It should also be noted that highly active NK cells can be isolated from the liver as well as from the lungs (39).

The elimination of RMA-S in B6 resembled that of RBL-5 in D8 mice both with respect to organ distribution and kinetics (Fig. 7). The main difference was in the strength of killing, which was one order of magnitude higher for elimination of RMA-S in B6 than for RBL-5 in D8 (in both cases using RBL-5 to B6 as control). This fits well with the rejection potential of subcutaneous lymphoma grafts in the corresponding host/tumor combinations (up to 10^5 RBL-5 cells rejected by D8 mice, but up to 10^6 RMA-S cells rejected in B6) (19, 20, 28). The degree of H-2 mismatch between host and target in the two combinations may contribute to this differential strength. For RMA to D8 the only mismatch is the transgene, H-$2D^d$, while RMA-S lacks both K^b and D^b in comparison with D8 and B6. The NK mediated rejection potential against totally MHC class I/β_2m deficient bone marrow grafts is also remarkably strong (36).

We conclude that an H-$2D^d$ transgene can alter the NK repertoire in a specific manner in B6 mice, as most clearly demonstrated by the experiments with the RBL-5 lymphoma and its mutant and transfected sublines. How then can the H-$2D^d$ gene instruct NK cells in mice of B6 background to kill targets they would spare in non transgenic mice? One step towards the answer will be to determine the cell type in which the H-$2D^d$ gene must be expressed. An interesting possibility is that MHC genes determine the repertoire of NK cells in situ, i.e., transgene expression in the $NK1.1^+$ cell itself is necessary and sufficient. This and other alternatives can be tested through expression of transgenes controlled by tissue or cell type specific controlling regions. The neutralizing effect seen after introduction of the H-$2D^d$ gene also to the target can be further explored with other transfectants expressing totally allogeneic, mutant or chimeric H-2 genes. Thus, our combined transgenic/transfectant system, may contribute to a molecular understanding of interactions between NK cells and targets in vivo.

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