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NATURAL RESISTANCE AGAINST LYMPHOMA GRAFTS CONVEYED BY H-2D^d TRANSGENE TO C57BL MICE

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Genetic factors are known to influence host resistance to certain tumors. In the mouse, resistance has been linked to the MHC locus on chromosome 17 (1–4). *H-2* genes or other closely linked genes may be responsible, and natural resistance to transplantable lymphomas represents one case where these two possibilities have not been resolved (5). A distinct set of H-2-linked "hemopoietic histocompatibility" (Hh)[1] antigens has been postulated to explain the competence to reject small lymphoma grafts (5). We have used the transgenic strain D8 (6) to analyze the genetic control of host resistance to tumors. The transgenic mouse D8 was generated by introducing an 8.0-kb genomic fragment containing the *H-2D^d* gene and 2.5-kb 5′ and 2.0-kb 3′ flanking sequences into C57BL/6 (B6) zygotes (6). The transgene product was expressed in different tissues in the same way as the endogenous H-2^b haplotype products, without alterations in the expression of the latter (6, 7). The expressed antigen was also recognized as self, and could function as a restriction element for T cells (7). We here report that the D8 mice have acquired "natural" resistance to C57BL lymphoma grafts in parallel with expression of the transgene product.

Materials and Methods

Mice. All strains and crosses were bred and maintained in the animal facilities of the Johns Hopkins University (Baltimore, MD) or at the Karolinska Institute (Stockholm, Sweden). The D8 transgenic strain was described previously (6). The C57BL/6 (B6) mice from which the D8 line was derived, as well as the original breeders of B10 congenic strains, were from the Jackson Laboratory, Bar Harbor, ME. B6 mice were also purchased from ALAB, Sollentuna, Sweden.

Tumors. RBL-5 MA is a subline of the Raucher virus–induced T cell lymphoma RBL-5 of B6 origin (8). P52-127.166 and EL-4 (both T cell lymphomas of B6 origin) were induced by RAD-LV and benzpyrene, respectively. All tumors were maintained as ascites lines in the syngeneic strain.

Tumor Growth Experiments. Graded doses of RBL-5 MA ascites cells were inoculated sub-

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[1] *Abbreviation used in this paper:* Hh, hemopoietic histocompatibility.

cutaneously into the right flank of age-matched mice. The growth of the solid tumors was followed by weekly palpations. Tumors grew equally well in B6 mice of the National Institutes of Health (Bethesda, MD) colony, the progenitor strain of the transgenic mice, and B6 mice from the Stockholm colony, our usual host for the serial transplantation of the RBL-5 lymphoma.

In Vivo NK Cell Depletion. 1 d before inoculation of tumor cells, each mouse either received an intravenous dose of 30 μl anti-asialo GM_1 antiserum, or an intraperitoneal injection of 200 μl anti-NK 1.1 mAb (ascites preparation) (8). In the case of anti-asialo GM_1 antiserum, the mice received additional inoculations on day 3 and 7 after tumor cell inoculation.

In Vitro H-2 Typing. (D8 × B6)F_1 × B6 backcross mice were typed for expression of the D^d antigen by a one step complement-dependent cytotoxicity test. The 34-5-8S (anti D^d) mAb, (obtained from the American Type Culture Collection, Rockville, MD) was used on inguinal lymphnode cells removed during general anesthesia. All mice that showed >50% specific killing were typed as D^{d+}. The majority of mice typed as positive showed >80% dead cells, while the majority of mice typed as negative showed <20% dead cells. Of 126 mice typed so far, 63 have been found D^{d+} and 63 D^{d-}.

Results and Discussion

Tumor growth was followed after subcutaneous inoculation of graded doses of RBL-5 lymphoma cells (H-2^b) in mice of the C57BL/6 (B6) strain and of the *H-2D^d* transgenic strain D8. Transgenic D8 mice were more resistant to inocula of 10^3 and 10^4 cells than the B6 mice (Table I). F_1 hybrids between D8 and B6 were all D^{d+} and rejected the RBL-5 lymphoma in a similar fashion as D8.

Tumor resistance segregated with D^d in (D8 × B6)F_1 × B6 backcross mice. An inoculum of 10^4 cells grew out as tumors in 3 of 18 D^{d+} and in 17 of 18 D^{d-} backcross mice (Fig. 1). The 1:1 distribution confirmed that the transgene segregated as a single dominant gene (6). The integration site of the transgene is unknown, but linkage analysis studies have excluded a location site within or near the MHC (9).

It has been previously found that the RBL-5 tumor is sensitive to F_1 hybrid resistance (10), defined as the rejection of transplanted hematopoietic tissue or tumors of parental origin by F_1 hybrids of two inbred strains (1, 5). RBL-5 cells were rejected by all F_1 hybrids and all backcross mice that carried the *D^d* gene (10, Table I). As shown by the dose titration experiments, (D8 × B6)F_1 hybrids and B6 to congenic *H-2D^d* strain F_1 hybrids rejected RBL-5 cells equally well (Table I).

Resistance of the D^{d+} backcross mice could be completely abrogated by either 400 rad irradiation or by anti-asialo GM_1 antibody treatment (Fig. 1). The same treatments are known to abrogate NK cell-mediated H-2-associated resistance to RBL-5 in conventional F_1 and backcrosses, while T cell depletion by thymectomy and irradiation has no effect (11–13). This suggests that NK cells are involved, which was confirmed here for the rejection in transgenic mice by treatment of the animals with the mAb anti-NK 1.1 (8, 14). The tumors then grew equally well in D8, (D8 × B6)F_1, and B6 mice (Table I). (D8 × B6)F_1 hybrids were also resistant to threshold doses of two other B6 lymphomas, the rad-LV-induced P52-127.166 and the benzpyrene-induced EL-4 (Table I). Our results with the D8 mice, and the association between resistance and D^d in the H-2-recombinant crosses lead to the conclusion that the lymphoma resistance is directly dependent on the *D^d* gene.

Apart from demonstrating that tumor rejection could be conveyed by a transgene, the results are pertinent for the interpretation of the MHC-linked control of F_1 hybrid resistance. Recessive Hh antigens have been postulated to explain hybrid resis-

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TABLE I

Tumor Growth after Subcutaneous Inoculation of RBL-5, EL-4, and P52-127. 166 Lymphomas

Tumors and strains	H-2 haplotype K	D	Transgene	Tumor takes* 10^3	10^4	10^5
RBL-5 MA						
B6	b/b	b/b	-/-	31/38	68/68	50/50
D8	b/b	b/b	d/d	0/8‡	7/23‡	14/16
D8 × B6	b/b	b/b	d/-	0/15‡	7/27‡	22/24
B10A(5R) × B6	b/b	b/d	-/-	0/7‡	2/23‡	10/13
B10D2 × B6	b/d	b/d	-/-	0/4§	1/8‡	3/3
B10A × B6	b/k	b/d	-/-	1/4	0/10‡	5/6
B6, anti-NK1.1	b/b	b/b	-/-	-	16/16	-
D8, anti-NK1.1	b/b	b/b	d/d	-	9/9§	-
D8 × B6, anti-NK1.1	b/b	b/b	d/-	-	8/8‡	-
EL-4						
B6	b/b	b/b	-/-	-	12/12	14/14
D8 × B6	b/b	b/b	d/-	-	3/10§	2/11‡
P52-127.166						
B6	b/b	b/b	-/-	-	16/18	-
D8 × B6	b/b	b/b	d/-	-	0/10‡	-

* Mice with tumors/inoculated mice.

‡ $p < 0.001$ by χ^2 tests, when compared with the relevant control, i.e., B6 in the case of transgenic and F_1 hybrids and untreated controls of the same genotype in the case of anti-NK 1.1-treated mice. No index means $p > 0.05$, i.e., NS.

§ $p < 0.005$.

tance to hematopoietic grafts (5). The *Hh* genes have been tentatively located to the D region of the H-2 complex, although rejection did not require expression of the D^b antigen on the graft (5, 15–17). It was suggested that the H-2^b-linked *Hh-1*^{b} allele is only expressed in the homozygous H-$2^{b/b}$ parent that would be tolerant to the corresponding antigen product. Heterozygous H-$2^{d/b}$ mice would not express Hh-1^b, with lack of tolerance and rejection of a Hh-1^{b+} lymphoma graft as the con-

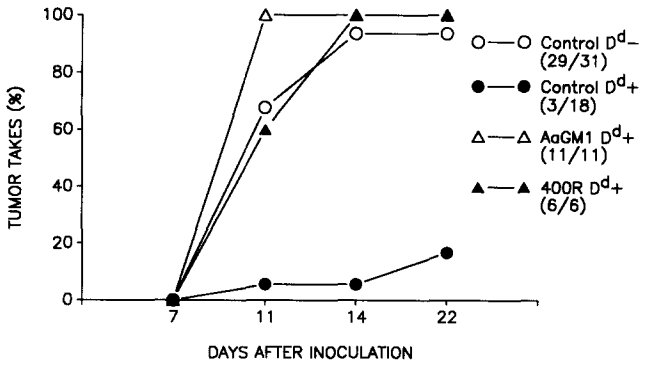

FIGURE 1. Effect of irradiation or anti-asialo GM_1 treatment on growth of RBL-5 lymphoma in D^{d+} and D^{b-} (D8 × B6) backcross mice. The figure shows percent tumor takes in D^{d-} (O), untreated D^{d+} (●), anti asialo GM_1-treated D^{d+} (△), and 400 rad-treated D^{d+} (▲) backcross mice at different days after inoculation. The number of mice that developed tumors over the total inoculated are shown in parentheses.

sequence (5). Our data do not address the role of the putative *Hh* gene products as target antigens, and they do not exclude a role of such genes at the host level. They could be under *trans*-acting control (16), regulated by sequences included in the transgene. However, no transcripts have been detected from the flanking sequences of the D^d gene carried by the construct (G. Jay, unpublished observation). It is therefore most likely that the D^d gene itself is responsible for the resistance of the D8 mice and of conventional F_1 hybrids (10–12). Hybrid resistance models based on MHC class I genes therefore deserve increased attention (18–23). Snell (20) has proposed, long before NK cells were recognized as effector cells in hybrid resistance, that MHC antigens of the effector cells could recognize an MHC mismatch on the graft. This could lead to effector cell triggering and killing. To explain hybrid resistance and certain in vitro observations on NK lysis, we have suggested a model that can be regarded as a development of Snell's idea (22, 23). Subsequent to their binding to the target cell, NK cells would sense the quantity of syngeneic MHC products expressed by the target. This could occur via an indirect mechanism, involving recognition of epitopes modulated by syngeneic MHC products or via a direct recognition of the latter. Adequate concentration of class I products would inhibit the triggering of NK lysis. This ability would be impaired in MHC-deficient variants (23), leading to lysis. Under conditions of incomplete host MHC expression (allogeneic graft, parental graft into F_1 or transgenic host), the inactivation of the NK cell would be only partial, leading to the rejection of the graft. The present results are consistent with the predictions of this model. Introduction of a new class I allele into the host was sufficient to turn graft acceptance into rejection, mediated by asialo GM_1 and NK 1.1 positive cells. It is not clear whether these cells recognized the absence of D^d themselves (22, 23), or acted less specifically, e. g., after the graft had been mispositioned in the transgenic mice by the action of other host cells (24). It must be stressed that NK cells may have multiple recognition strategies and surveillance functions. Other NK-mediated host-graft or host-virus (25) interactions may therefore be H-2 independent. Furthermore, a system scanning for absence of self must not necessarily distinguish class I epitopes by the same criteria as T cells, which means that not every new H-2 allele introduced by an F_1 cross or a transgene must lead to an altered picture of self from the point of view of NK cells.

H-2-associated natural resistance as well as NK cells are effective in mice with severe combined immunodeficiency (26, 27). These mice are defective with regard to Ig and TCR gene rearrangement (28). With the direct evidence that class I genes are involved in natural resistance, one may ask if the MHC products have other immunological functions than presentation of peptides to receptors assembled from the α, β, γ, and δ TCR families. By the use of bone marrow chimera and exon-shuffled genes, it may be possible to delineate the part of *H-2D*^{d} required, and the time and site of its expression necessary to switch off "tolerance" in an NK-dependent system.

Summary

The *H-2D*^{d} transgenic strain D8 on C57BL background was more resistant to subcutaneous challenge of RBL-5 lymphoma cells than B6 controls. The direct role of the H-2D^d antigen was investigated by the use of (D8 × B6)F_1 crosses and (D8 *B6)* × B6 backcrosses. The latter showed cosegregation with regard to D^d antigen

expression and lymphoma resistance, both of which were inherited in a pattern consistent with control by a single dominant gene. The rejection potential in (D8 × B6)F_1 mice appeared as strong as that seen in crosses between B6 and MHC congenic mice (on B10 background) carrying *H-2D^d*. The lymphoma resistance could be abrogated by treatment with anti-asialo GM_1 antiserum or anti-NK 1.1 mAb, indicating a role for NK cells.

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