The Journal of Immunology
RESEARCH ARTICLE | DECEMBER 01 1987

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T Yoshioka; ... et. al

J Immunol (1987) 139 (11): 3861–3867.
https://doi.org/10.4049/jimmunol.139.11.3861

0022-1767/87/13911-3861 $02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 139, 3861–3867, No. 11, December 1, 1987
Printed in U.S.A.

A TRANSGENIC CLASS I ANTIGEN IS RECOGNIZED AS SELF AND FUNCTIONS AS A RESTRICTION ELEMENT[1]

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The function of a transgenic D^d class I molecule in the induction of immunologic tolerance to major histocompatibility complex antigens and in directing major histocompatibility complex restriction in C57BL/6 mice were investigated. All of the transgenic D^d mouse strains were found to be tolerant for the D^d antigen. Spleen cells from transgenic mice were immunocompetent but consistently failed to generate an anti-D^d cytotoxic T lymphocyte response in vitro, and skin grafts between transgenic D^d mice were not rejected. These data suggests that the D^d antigen was recognized as a self molecule. In addition, the transgenic D^d mice generated antigen-specific D^d-restricted cytotoxic T lymphocyte, indicating that the D^d antigen also functioned as a restriction element for antigen recognition. These observations demonstrate the usefulness of the transgenic mouse system for studying class I antigen expression and function.

Our knowledge of the molecular biology of the mouse major histocompatibility complex (MHC) has advanced rapidly in recent years. The number of genes and organization of loci within the MHC have been defined for several inbred strains of mice (1, 2). Several MHC class I genes have been sequenced and specific DNA probes that detect transcription from individual class I loci regardless of allele have been identified (3). Together these findings have permitted the study of MHC gene expression at the mRNA level (3, 4). The sequences that render class I genes responsive to interferons have also been localized (5, 6). Several groups have transferred individual genes into cells in culture to study antigen recognition (7–9) or to examine the role of class I antigen expression in immune surveillance of neoplastic cells (10–12). Unfortunately, the scope of problems that can be addressed by gene transfer into cultured cells is severely limited by the availability of suitable recipient cell lines.

The development of technology that allows cloned genes to be introduced into the germ line of mice has broadened the range of questions that can be addressed through gene transfer (13, 14). The production of transgenic mice makes it possible to define the effects of a single gene or gene product on complex biologic processes that involve interactions between many different cell types. For example, transgenic mice carrying pre-rearranged immunoglobulin (Ig) genes have been successfully used to gain new insights into the processes that control Ig gene rearrangement and allelic exclusion (15). In addition, recent studies suggest that the presence of a single pre-rearranged Ig transgene in all tissues has a profound effect on the Ig repertoire of mice (16).

We have recently applied the transgenic mouse technology to the study of class I antigens (4). The gene coding for the D^d antigen cloned from a BALB/c mouse was microinjected into C57BL/6 (B6)[4] embryos. The resulting transgenic mice correctly expressed the introduced D^d gene in a tissue-dependent manner which paralleled that of the endogenous K^b gene. Furthermore, the D^d transgene was inducible in response to interferon and suppressible by transformation with the human adenovirus 12 (T. Yoshioka, C. Bieberich, G. Scangos, and G. Jay, unpublished results).

Transgenic mice constructed on an inbred background provide a novel approach to the study of class I gene expression and antigen function. These two areas of investigation have classically depended on the use of painstakingly developed congenic mouse strains and on the isolation of spontaneous class I mutant mice (17). In the transgenic system, a single well defined gene can be placed on an inbred background in one generation. If the transgene is properly expressed and the locus carrying the transgene can be bred to homozygosity, the resulting mouse strains would be analogous to congenic lines. However, there are clear differences between congenic and transgenic strains that should be noted. In transgenic strains, a discrete fragment of well characterized DNA is transferred and adds to the background complement of genes; in congenic strains, the donor's selected traits replace those of the background strain. In the production of congenic strains, the total contribution of the donor to the background strain is difficult to assess accurately; in transgenic mice in which a single gene is transferred the amount of donor DNA is known precisely. In transgenics, however, the number of copies of the transgene and the chromosomal site of integration are at present uncontrollable.

Herein, we present the results of studies undertaken to

Received for publication June 30, 1987.
Accepted for publication September 9, 1987.
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[1] This work was supported in part by a grant from the Eucaryotic Genetics Program of the National Science Foundation to George Scangos who is also a Leukemia Society of America Scholar.
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[4] Abbreviations used in this paper: B6, C57BL/6; CTL, cytotoxic T lymphocyte; Con A SN, concanavalin A culture supernatant; I-AED, *N*-iodoacetyl-*N'*-(5-sulfonic-1-napthyl)ethylene diamine.

assess the ability of a transgenic class I antigen to induce self-tolerance and to specify T-cell restriction. We show that the transgenic D^d antigen could be used to induce a D^d-specific cytotoxic T lymphocyte (CTL) response in the parental B6 mouse. The transgenic D^d mice failed to generate an anti-D^d CTL response, suggesting that the D^d antigen is recognized as a self molecule in these animals. Furthermore, T cells from the transgenic D^d mice can recognize the D^d antigen as a restriction element for antigen recognition.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, B10.D2(R107), B10.A, and B10.BR mouse strains were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.A(4R), B10.HTG, and BALB/cH-2dm2 mouse strains were gifts from Dr. D. Sachs, National Institutes of Health, Bethesda, MD. Derivation of the transgenic D^d mice has been described (4). All mice used in this study were 8 to 12 wk of age.

In vitro sensitization and cytotoxicity assay. CTL responses were induced in vitro by 5-day mixed lymphocyte culture (18). RPMI 1640 medium (Meloy, Springfield, VA) supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY), 2 mM L-glutamine (GIBCO), 5 mM HEPES, 1 mM sodium pyruvate (GIBCO), 0.1 mM nonessential amino acids (GIBCO), and 5×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) was used as a complete culture medium. Responding spleen cells (5×10^6) were co-cultured with mitomycin C (Sigma)-treated (100 μg/ml at 37°C for 60 min) stimulating spleen cells (4×10^6) in 24-well culture plates (Costar, Cambridge, MA) (2 ml/well) for 5 days at 37°C in a humidified incubator with 5% CO_2. CTL activity of effector cells was assayed in a 4-hr ^{51}Cr-release assay in round-bottom 96-well plates (Costar) using ^{51}Cr-labeled 2-day concanavalin A (Con A) (Sigma) blast cells (1×10^4 cells/well) as targets. CTL activity was assayed at four effector to target ratios, and each experimental point represents the mean of three replicate wells. Percent specific lysis was calculated using the formula $(Exp_{cpm} - Cont_{cpm})/(Max_{cpm} - Spont_{cpm}) \times 100$, where $Cont_{cpm}$ represents ^{51}Cr release from target cells incubated with effector cells from nonstimulated responding cells, Max_{cpm} represents release from target cells in a 0.5% Triton X-100 (Sigma) solution, $Spont_{cpm}$ represents the background release from target cells cultured for 4 hr in the absence of effector cells, and Exp_{cpm} represents release from target cells incubated with stimulated responding cells. The SEM for percent specific lysis were consistently less than 5%, and were excluded from the data presented.

Cold target inhibition test. Various numbers of nonradiolabeled Con A-blast cells (cold blocker) were mixed with a constant number of effector cells for 20 min. ^{51}Cr-labeled target cells were then added for a 4-hr ^{51}Cr-release assay.

Con A supernatant (Con A SN). An 18-hr culture supernatant of BALB/c spleen cells (1×10^7/ml) stimulated with 2.5 μg/ml Con A was used as a source of exogenous helper factors. The supernatant was supplemented with 0.2 M α-methyl-D-mannoside (Sigma) to neutralize the remaining Con A. The Con A SN was then filtered through a 0.45-μ pore membrane and stored at −20°C until use. The helper activity of the Con A SN was assayed for its ability to augment allospecific CTL activity from responding thymocytes.

Skin grafts. Skin grafts were performed using an adaptation of the method of Billingham and Medawar (21). Grafts were scored daily until rejection or until day 100.

Estimation of CTL precursors by limiting dilution analysis. Spleen cells were cultured in round-bottom 96-well plates (Costar) under limiting dilution conditions as described (22). Groups of 24 round-bottom microwells containing various numbers of responding cells were cultured with 3×10^5 mitomycin C-treated B10.D2(R107)-stimulating cells. Cells were kept in complete culture medium supplemented with 12.5% Con A SN for 7 days. Individual wells were tested for CTL activity against 3×10^3 ^{51}Cr-labeled Con A-treated D8 target cells. Wells were considered positive when the amount of released ^{51}Cr was three SD above the mean of the base line group containing stimulating cells alone. CTL-precursor frequencies were calculated by the statistical method of χ^2-minimization as described (23).

Modification of spleen cells with hapten. The sulfhydryl-reactive hapten *N*-iodoacetyl-*N'*-(5-sulfonic-1-napthyl)ethylene diamine (I-AED) (Sigma) was used. Spleen cells (1×10^7) were resuspended in 2 mM I-AED in phosphate-buffered saline containing 0.01 M HEPES pH 8.0 and incubated at 37°C for 30 min with intermittent shaking. Cells were then washed three times with RPMI 1640 containing 5% fetal calf serum (19).

In vivo priming. Mice were inoculated s.c. with 5×10^7 I-AED modified syngeneic spleen cells either once or twice (20). Spleen cells from primed mice were used 1 to 2 wk after the last inoculation.

RESULTS

The results of recent gene mapping and DNA sequence analyses of the H-2 region of the MHC have suggested that the D^b gene from B6 mice and the L^d gene from BALB/c mice are allelic, and that B6 mice do not have a class I gene which is equivalent to the D^d gene of BALB/c (24–26). Based on this premise, we have derived several transgenic mouse lines by introducing the D^d gene under its own regulatory elements into B6 mice (4). Three of these lines (designated D8, D19, and D24) expressed the transgenic D^d antigen in a tissue-dependent manner analogous to endogenous H-2 class I antigens. In the present study, the MHC alleles of B6, BALB/c, and transgenic D^d mice at the K, I, D, and L loci are represented by (b, b,–, b), (d, d, d, d), and (b, b, d, b), respectively (see Table I).

Allorecognition of the D^d antigen on lymphoid cells from transgenic D^d mice. Expression of the D^d antigen on the surface of spleen cells from transgenic mice was examined in terms of allogeneic T cell recognition. An anti-D^dL^d CTL response was induced by MLC of B6 responders with B10.D2(R107) stimulator spleen cells (see Table I). Cytolytic activity was measured in a standard 4-hr ^{51}Cr release assay (Fig. 1, *a* to *d*). The B6 anti-B10.D2(R107) effector cell population showed significant cytolytic activity not only when assayed on B10.D2(R107) targets, but also on D8 and D24 cells. This observation suggests that spleen cells from both D8 and D24 mice expressed a D^d antigen that can be recognized by cytotoxic T cells. D24 cells were lysed more efficiently than D8 cells at all effector to target ratios (cf. Fig. 1 *b* and *c*). This effect, although not dramatic, was reproducible in all experiments and may be due to the higher level of expression of the transgenic D^d antigen observed in D24 cells (T. Yoshioka, C. Bieberich, G. Scangos, and G. Jay, unpublished results). B10.D2(R107) cells were also consistently lysed with a higher efficiency than D24 cells (cf. Fig. 1 *a* and *c*), which may reflect killing on the basis of an additional antigen (L^d) on B10.D2(R107) targets. Alternatively, L^d may induce a more vigorous CTL response than D^d in this system.

The ability of D8 cells to stimulate an anti-D^d CTL response in B6 spleen cells also was assessed (Fig. 1 *e* to *h*). B6 anti-D8 CTL lysed B10.D2(R107) and D8 cells with similar efficiencies whereas D24 cells were again consistently lysed with a higher efficiency at all effector to target ratios. This result is consistent with the higher

TABLE I

MHC alleles of mouse strains used in present study

Strains	MHC Alleles[a]			
	K	I	D	L
BALB/c	d	d	d	d
C57BL/6	b	b		b
B10.D2(R107)	b	b	d	d
B10.HTG	d	d		b
BALB/cH-2dm2	d	d	d	
B10.A	k	k	d	d
B10.A(4R)	k	k		b
B10.BR	k	k		k

[a] K, D, and L represent individual class I genes, and I represents collectively the class II loci.

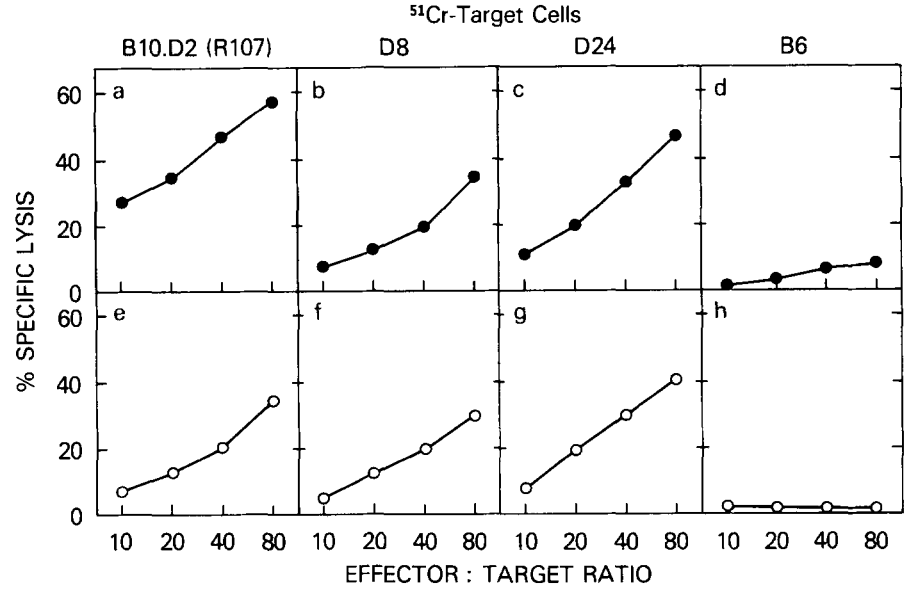

Figure 1. B6 spleen cells (5×10^6) were stimulated in vitro with either B10.D2(R107) (*a* to *d*) or D8 (*e* to *h*) spleen cells (4×10^6) for 5 days. Generated CTL activity was assayed on different ^{51}Cr-labeled target cells. The extent of specific lysis at various effector to target ratios are shown.

level of D^d expression in D24 cells. B6 target cells were not significantly lysed by either B6 anti-B10.D2(R107) or B6 anti-D8 CTL.

To demonstrate the specificity of the B6 anti-D8 CTL response, we tested the ability of spleen cells from several congenic lines to block killing of D8 targets. As shown in Figure 2, BALB/cH-2dm2 cells were as efficient as D8 cells in blocking B6 anti-D8 CTL activity. This observation established a *d* haplotype specificity in the B6 anti-D8 CTL response. Furthermore, cross-reactivity with anti-L^d CTL in lysis of D8 targets is unlikely because the BALB/cH-2dm2 strain lacks the L^d antigen (Table I) (27, 28). The failure of B10.HTG spleen cells to block lysis of D8 cells suggests that the K^d and I^d region antigens are not involved in the B6 anti-D8 CTL response. These data strongly suggest that the B6 anti-D8 response is specific for the D^d antigen.

To determine the ability of the transgenic D^d antigen to function as a determinant of transplant rejection, we performed skin graft experiments. Tail skin from D8, D24, and B10.D2(R107) mice was engrafted to B6 recipients. D8 and D24 grafts, presumably mismatched with B6 at the D locus only, were both rejected with a median survival time of approximately 20 days (Fig. 3, *top*). B10.D2(R107) grafts, mismatched with B6 at both the D and L loci were rejected even faster, with a median survival time of 12 to 13 days. Control B6 grafts were not rejected within the 100-day observation period. These results clearly demonstrated that tail skin from two independent D^d transgenic mouse strains functioned as an allograft in B6 recipients. We concluded that the transgenic D^d product was expressed in the tail skin and can induce immunologic rejection when engrafted to B6 mice.

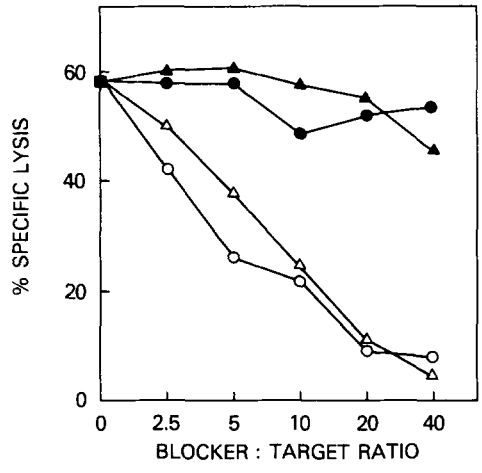

Figure 2. CTL effector cells were generated by in vitro stimulation of B6 spleen cells with D8 spleen cells. CTL activity directed against D8 target cells was assayed at an effector to target ratio of 80:1 in the presence of B6 (●), B10.HTG (▲), D8 (○), and BALB/cH-2dm2 (△) cold-blocking cells.

Immunologic tolerance to the D^d antigen in transgenic D^d mice. Northern blot analysis of poly(A)$^+$ RNA from tissues of the transgenic D^d mice demonstrated that the foreign D^d gene was subject to tissue-dependent regulation similar to that which controls the endogenous K^b gene (4). The D^d transgene was also inducible by interferon and suppressible by Ad12 transformation (T. Yoshioka, C. Bieberich, G. Scangos, and G. Jay, unpublished results). Although these observations strongly suggest that the D^d transgene can be expressed in a regulated fashion, they do not address the problems of developmental timing of expression or of antigen function. As an initial approach to these questions, we chose to investigate whether T cells from these transgenic mice were reactive to, or tolerant of, the D^d antigen.

B6 anti-B10.D2(R107) CTL showed significant cytolytic activity when assayed on B10.D2(R107), D8 and D24 targets (Table II, *experiment 1*). In contrast, D8 anti-B10.D2(R107) CTL lysed only B10.D2(R107) target and did so somewhat less efficiently than B6 anti-B10.D2(R107) CTL. This suggests that D8 spleen cells were immunocompetent. To determine whether the failure to respond to D^d was common in other transgenic strains, D19- and D24-anti B10.D2(R107) CTL were generated and were also found to lyse B10.D2(R107) but not D8, D19, or D24 targets (Table III, *experiment 2*). Similar results were also observed when B10.A cells were substituted as stimulators in an analogous experiment. To assess the specificity of the D8-anti B10.D2(R107) CTL activity, we performed mixed lymphocyte culture using mutant and congenic mice as sources of stimulator and target cells. D8 spleen cells stimulated with

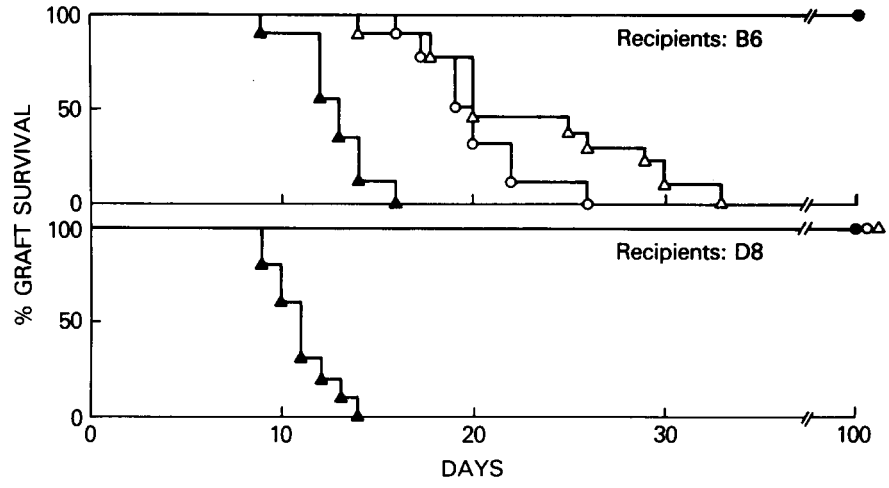

Figure 3. Tail skin from B6 (●), B10.D2(R107) (▲), D8 (○), and D24 (△) strains was grafted onto either B6 or B8 mice. The number of recipients were 10/group. Grafts were scored daily until rejection or for 100 days.

TABLE II

Failure of D8 mice to generate D^d-specific CTL responses

Expt.	Responding Cells	Stimulating Cells	% Specific Lysis of Target Cells (E/T)[a]							
			B10.D2(R107)		D8		D24		B6	
			80	40	80	40	80	40	80	40
1	B6	B10.D2(R107)	66	58	37	26	46	36	9	7
	D8	B10.D2(R107)	45	40	0	2	5	1	6	6
2	B6	B10.A	57	48	31	23	43	31	0	0
	D8	B10.A	43	42	2	7	7	2	8	3

Expt.	Responding Cells	Stimulating Cells	BALB/c		BALB/cH-2dm2		B10.HTG	
			40	20	40	20	40	20
3	B6	B10.D2(R107)	42	32	34	18	11	7
	D8	B10.D2(R107)	34	30	8	8	10	6

Expt.	Responding Cells	Stimulating Cells	B10.HTG		B10.D2(R107)		B6	
			80	40	80	40	80	40
4	B6	BALB/cH-2dm2	58	43	40	25	0	6
	D8	BALB/cH-2dm2	52	40	9	7	4	0

[a] B6 or D8 spleen cells (5×10^6) were stimulated in vitro with either B10.D2(R107), B10.A, or BALB/cH-2dm2 cells (4×10^6) for 5 days. Generated CTL activity was assayed on ^{51}Cr-labeled target cells listed. E/T, effector to target cell ratio.

TABLE III

Failure of transgenic D^d mice to generate D^d-specific CTL responses

Expt.	Responding Cells	Stimulating Cells	Con A SN (12.5%)	% Specific Lysis of Target Cells (E/T)[a]							
				B10.D2(R107)		D8		D19		D24	
				80	40	80	40	80	40	80	40
1	B6	B10.D2(R107)	−	59	52	31	34	ND[b]		47	33
	D8	B10.D2(R107)	−	55	47	0	0	ND		5	1
	D8	B10.D2(R107)	+	59	43	5	1	ND		11	6
2	B6	B10.D2(R107)	−	79	54	28	26	35	30	57	37
	D8	B10.D2(R107)	−	68	53	0	0	4	0	2	6
	D19	B10.D2(R107)	−	58	48	0	0	0	0	11	0
	D24	B10.D2(R107)	−	59	52	1	7	0	4	9	9

[a] Responding spleen cells (5×10^6) were stimulated in vitro with B10.D2(R107) cells (4×10^6) for 5 days. Generated CTL activity was assayed on ^{51}Cr-labeled target cells listed. E/T, effector to target cell ratio.
[b] ND, not done.

B10.D2(R107) cells lysed BALB/c target cells but failed to lyse BALB/cH-2dm2 target cells which expressed all H-2^d antigens except L^d (Table II, experiment 3). No activity directed against K^d or I^d antigens could be detected since no significant lysis of B10.HTG targets occurred. These data strongly suggest that CTL activity directed against L^d but not D^d antigens can be generated by D8 spleen cells.

D8 spleen cells stimulated with BALB/cH-2dm2 cells efficiently lysed B10.HTG target cells but failed to lyse B10.D2(R107) cells. These results indicate that D8 CTL can respond against K^d and I^d antigens, but not against D^d. The inability of D8 spleen cells to generate CTL activity against the D^d antigen could not be overcome by the addition of Con A SN as an exogenous helper factor in the CTL assay (Table III, experiment 1) or by in vivo priming of responding mice with D^d antigen-expressing cells before in vitro sensitization (data not shown).

The failure of spleen cells from D8 mice to generate an anti-D^d CTL response strongly suggests that a state of

immunologic tolerance for the product of the transgene exists in these animals. To test this hypothesis further, we engrafted D8 mice with tail skin from D8, D24, and B10.D2(R107) mice. As shown in Figure 3 (*bottom*) B10.D2(R107) grafts were rapidly rejected, with a median survival time of 11 days, indicating that D8 mice were competent to carry out allograft rejection. In contrast, D8 isografts and D24 grafts were not rejected within the 100-day observation period. These results provide further evidence that a state of tolerance for the D^d antigen exists in D8 mice.

Lack of anti-D^d CTL precursors in transgenic D^d mice. In an effort to determine the nature of tolerance for the D^d antigen in D8 mice, a limiting dilution analysis was performed to measure the frequency of anti-D^d CTL precursors. As shown in Figure 4, the frequency of anti-D^d CTL precursors among D8 spleen cells was $<1/1 \times 10^7$, whereas among B6 spleen cells, anti-D^d CTL precursors were present at a frequency of $1/5.5 \times 10^4$. Because D8 target cells were used, a direct comparison of frequencies of anti-D^d CTL precursors in control B6 and experimental D8 mice was possible. It appears that the failure of D8 mice to respond against the D^d antigen is due to a lack of CTL precursors with specificity for the D^d molecule.

Generation of an antigen-specific D^d-restricted CTL response in transgenic D^d mice. The ability of the D^d antigen in transgenic mice to act as a restriction element for antigen recognition was investigated. The sulfhydryl reactive hapten I-AED (19) was used to generate antigen-specific, MHC-restricted CTL responses. B6, BALB/c, and D8 mice were primed in vivo with I-AED-modified syngeneic spleen cells (I-AED-self). Spleen cells from primed mice were then stimulated in vitro with I-AED-self and assayed for their ability to lyse a panel of I-AED-modified or -unmodified target cells (Table IV, experiment 1).

B6 responder cells showed a strong cytolytic activity when assayed on I-AED-modified, L^b-matched B10.A(4R) target cells, but not on I-AED-modified D^d-bearing B10.A- or I-AED-modified B10.BR target cells. BALB/c responder cells exhibited significant cytolytic activity only against I-AED-modified, D^d-bearing B10.A target cells. In contrast, D8 spleen cells generated CTL activity against both I-AED-modified B10.A(4R) and B10.A cells, but not against B10.BR cells. These results indicate that D8 spleen cells can generate an antigen-specific, D^d-restricted CTL response. These observations were extended using other transgenic D^d mice (Table IV, experiment 2). It is unlikely that the cell-mediated lysis of I-AED-modified B10.A targets was due to cross-reactivity of L^b-restricted CTL, because cross-reactivity was not observed using B6 effectors on B10.A targets.

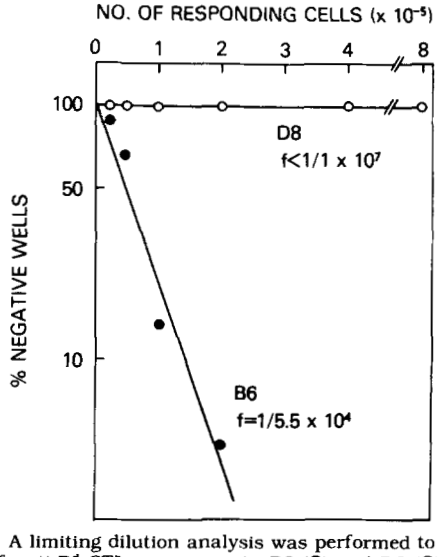

Figure 4. A limiting dilution analysis was performed to measure the frequency of anti-D^d CTL precursors in B6 (●) and D8 (○) spleen cells stimulated with B10.D2(R107) spleen cells. Con A-treated D8 spleen cells were used as targets. The calculated frequencies of CTL precursors are shown.

DISCUSSION

The transgenic mouse system provides an attractive model for studying the biology of class I antigens. Genes encoding class I molecules can be readily introduced into the germ line of inbred mice by microinjection and new strains can be established by interbreeding offspring of a founder transgenic mouse. The usefulness of this system, however, depends on the ability of the antigen encoded by the transgene to function in a manner analogous to endogenous class I molecules. Herein, we demonstrated that transgenic B6 mice (D8, D19, D24) carrying a heterologous D^d gene expressed the D^d antigen that was recognized by anti-D^d CTL generated in B6 parental mice. The specificity of the response was shown by the ability of only D^d expressing cells to block lysis of D8 targets.

Although the D^d antigen expressed on the surface of spleen cells from D8 mice was recognized by D^d-stimulated B6 effector cells, and must then be immunologically functional, D8 spleen cells consistently failed to generate an anti-D^d response. These observations suggest that D8 mice were tolerant for the transgenic antigen. Although the mice used in these experiments were 8 to 12 wk of age, we have obtained the same results using 18-mo-old transgenic D^d mice. The failure of D8 cells to respond to D^d-antigenic determinants could not be overcome by in vivo priming or by the addition of helper factors in vitro. The results of limiting dilution assays strongly suggest that the observed tolerance for the D^d antigen in D8 mice is due to a lack of anti-D^d CTL precursors. The tolerance for the D^d antigen in these transgenic mice is not entirely surprising because these animals express the transgene in many tissues in a manner that closely parallels expression of the endogenous K^b gene. However, it is an important observation in light of recent evidence that suggests that not all transgenic mice are tolerant for their transgene products (29, 30).

In addition to being seen as a self molecule in transgenic D^d mice, the D^d antigen can also serve as a functional restriction element. Spleen cells from these mice were able to generate a D^d-restricted anti-I-AED response when presented with D^d-expressing I-AED-modified stimulating cells.

The development of normal self-tolerance and MHC restriction are influenced by the MHC phenotype of the thymus in which T cells develop. Recent studies indicate that induction of tolerance and restriction are controlled by different populations of cells in the thymus. Intrathymic macrophages and dendritic cells are thought to play an important role in tolerization (31–33), and thymic epithelial cells are responsible for imprinting restriction specificity (34–36). Although the transgenic D8 strain is apparently tolerant for D^d and can utilize this class I molecule as a restriction element, it is not clear whether

TABLE IV

Induction of I-AED-specific, D^d-restricted CTL response in transgenic D^d mice

Expt.	Responding Cells	Stimulating Cells	% Specific Lysis of Target Cells (E/T)[a]								
			B10.A(4R)-AED		B10.A(4R)	B10.A-AED		B10.A	B10.BR-AED		B10.BR
			40	20	40	40	20	40	40	20	40
1	B6	B6-AED	31	22	0	5	3	0	1	3	0
	BALB/c	BALB/c-AED	9	0	5	38	23	2	1	1	3
	D8	D8-AED	47	31	4	38	28	6	1	5	4
2	B6	B6-AED	28	15	0	5	0	2	4	1	0
	BALB/c	BALB/c-AED	4	3	0	30	19	2	4	3	2
	D8	D8-AED	32	19	7	33	19	10	8	2	9
	D19	D19-AED	47	30	4	45	23	6	10	8	0
	D24	D24-AED	38	20	0	34	25	0	3	4	7

[a] Primed responding spleen cells (5×10^6) were stimulated in vitro with I-AED-modified self (2 mM) for 5 days. Generated CTL activity was assayed on ^{51}Cr-labeled, I-AED-modified (1 mM) or -unmodified target cells listed. E/T, effector to target cell ratio.

these two conditions arise by the same mechanisms that ensure tolerance and restriction of endogenous class I antigens. We currently are characterizing the expression of the transgenic D^d antigen more extensively in the thymus and during fetal development in D8 mice.

The results of these experiments demonstrate the usefulness of applying transgenic mouse technology to the study of tolerance and MHC restriction. Class I genes can now be altered in vitro, and the effects of the induced mutations assessed in vivo. Heterologous transcriptional control sequences can also be fused to class I coding regions and the hybrid genes can be returned to mice to create strains that express class I genes in aberrant patterns. We have recently generated transgenic mice carrying the D^d gene under the control of the enhancer/promoter region of the mouse metallothionein gene. These mice express the D^d gene in an inducible, tissue-restricted fashion that is markedly different from the expression of endogenous class I genes. It will be important to determine whether the transgenic D^d antigen in these mice is recognized as self and whether it can act as a restriction element.

Acknowledgments. We thank Dr. Gene Shearer and Dr. Hiromi Fujiwara for helpful suggestions and Dr. J. Barbosa for critically reviewing the manuscript.

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