Analysis of the HLA-restricted Influenza-specific Cytotoxic T Lymphocyte Response in Transgenic Mice Carrying a Chimeric Human-Mouse Class I Major Histocompatibility Complex

By Antonella Vitiello,* Donata Marchesini,‡ Jillian Furze,* Linda A. Sherman,‡ and Robert W. Chesnut*

*From the *Department of Cellular Immunology, Cytel Corporation, and the ‡Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037*

Summary

Transgenic murine lines have been constructed that express a chimeric class I molecule composed of the α1 and α2 domains of HLA-A2.1 and the α3, transmembrane, and cytoplasmic domains of H-2K^b. Upon immunization with influenza virus, transgenic mice developed a strong A2.1K^b-restricted cytotoxic T lymphocyte (CTL) response specific for the same matrix protein epitope that serves as the dominant A2.1-restricted determinant in the equivalent human response. Fine specificity analysis of CTL clones using truncated peptides revealed strong similarity between the response repertoire of transgenic mice and that previously reported using influenza-specific A2.1-restricted CTL clones from humans. This suggests that even when considering T cell responses by different species, the α1 and α2 domains of the restriction element play a dominant role in determining the CTL specific repertoire. Thus, substituting the α3 domain of A2.1 with a murine counterpart has permitted development of a transgenic strain that should serve as an excellent model system in studies of HLA-restricted responses.

MHC-encoded molecules bind antigenic fragments of processed proteins and present these on the surface of APC for recognition by TCRs (1–3). Whereas class II MHC molecules function primarily to present antigen to CD4-bearing T cells of the helper/inducer phenotype, class I MHC presents antigen to CD8-bearing CTL.

Due to the central role of MHC in the generation of an immune response, transgenic mice carrying human MHC products (HLA) could provide an important model system in which to assess the induction, prevention, or alteration of an HLA-associated immune response. However, to be of value, it must be established that antigen processing and presentation by murine cells reveal the same set of HLA-restricted antigenic epitopes recognized by human T cells, and that murine transgenic T cells respond to these epitopes in the context of the HLA transgene product.

The demonstration by Gomard et al. (4) that HLA-A2- or HLA-A11-transfected murine P815 cells could serve as targets for human antiviral CTL indicated that at least some of the epitopes displayed by human APC are generated and presented by murine cells expressing HLA molecules. However, success in obtaining in vivo induced responses in transgenic mice expressing HLA molecules has been limited. Responses against influenza and Sendai virus have been obtained in transgenic mice carrying HLA-CW3 class I or both HLA-B27 and human β_2-microglobulin (β_2M)[1] (5–7). Other workers using HLA-A2 transgenic mice have reported poor in vivo priming (8, 9), despite the fact that the A2 molecule could serve as an alloantigen for recognition by human CTL (9).

The lack of detectable murine HLA-A2-restricted responses might be due to species-specific differences in MHC molecules that preclude efficient recognition by the murine TCR. It is possible that the variable region genes of the TCR have evolved to recognize the α1 and α2 domains of MHC molecules of the species. Alternatively, considering the significant role of accessory molecules such as CD8 in repertoire selection (10, 11), TCR capable of being restricted by xeno MHC might be present but not selected in the thymus due to poor interaction between the α3 domain of HLA-A2 and murine CD8 (12, 13).

To discriminate between these possibilities, we have produced murine transgenic lines that express a chimeric class I molecule composed of the α1 and α2 domains of HLA-A2.1 and the α3 domain of H-2K^b(A2.1/K^b). In such mice, a species-matched interaction between CD8 and its ligand on the α3 domain of class I molecules should occur, thus permitting assessment of the clonotypic TCR.

We show that A2.1/K^b transgenic mice can be primed in

[1] *Abbreviation used in this paper:* β_2M, β_2-microglobulin.

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vivo with influenza virus resulting in the generation of A2.1-restricted CTL. Moreover, both epitope recognition and fine specificity of murine A2.1-restricted CTL are similar to those defined for human T cells (14–16). These results indicate the utility of this system as a model for human T cell recognition of viral antigens and support the conclusion that even in xenogeneic combinations, determinant selection by MHC is a major factor in dictating the antigen-specific TCR repertoire.

Materials and Methods

Mice. C57BL/6, B10.D_2, and BALB/c mice were purchased from the breeding colony of Scripps Clinic and Research Foundation.

Production and Detection of Transgenic Mice. Transgenic mice were produced using a standard protocol (17) by injecting the A2.1/K^b chimeric gene (12) into fertilized eggs obtained by crossing (C57BL/6 × DBA/2)F_1 mice. Transgenic lines were established from mice carrying the transgene as detected by tail DNA dot blot analysis. Two transgenic lines, 66 and 372, were selected for these studies based on cell surface expression of the transgene product. To detect cell surface expression of A2.1/K^b, spleen cells or peripheral blood (~0.5 ml) collected from the tail vein of test mice were treated with Tris-buffered ammonium chloride (5 ml) to lyse red blood cells. Cells were washed and resuspended in RPMI 10% supplemented with 2.5 μg/ml Con A, 250 ng/ml ionomycin, 3 ng/ml PMA, and 5% culture supernatant of Con A–activated rat splenocytes. Samples were incubated at 3 × 10^6 cells/well in a volume of 2 ml for 3 d at 37°C in a humidified 5% CO_2 atmosphere.

A2.1 or A2.1/K^b cell surface expression was assessed by flow cytometry (FACS IV®; Becton Dickinson & Co., Mountain View, CA) using a biotinylated HLA-A2.1-specific mAb, MA2.1 (18), and PE-conjugated streptavidin (Biomeda, Foster City, CA). A2.1 expression was compared to H-2 expression on either Con A–activated or nonactivated spleen cells using the appropriate mAbs and a Fc fragment–specific, $F(ab')_2$ FITC–conjugated goat anti–mouse IgG (Pel-Freeze Biologicals, Rogers, AR). The class I–specific mAbs used were: MA2.1 (A2.1-specific) (18), 34.2.12S (H-2D^d-specific) (19), and 28.14.8S (H-2D^b-specific) (20). Cells were analyzed using a flow cytometer.

Line 372 was maintained by backcrossing to B10.D_2 and line 66 by backcrossing to C57BL/6.

Tumor Cell Lines. The following tumor cell lines were used both as stimulator cells or target cells in cytotoxicity assays: EL-4 murine thymoma cells originally derived from C57BL/6 mice; EL-4 cells stably transfected with the A2.1/K^b chimeric gene (12); Jurkat human T cell leukemia cells that are HLA A2.1 negative; and stable transfectants of Jurkat expressing A2.1 or A2.1/K^b (12); and a B10D_2-derived SV40-transformed cell line. All cell lines were grown in RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 50 μg/ml gentamicin, and 5 × 10^{-5} M 2-ME (RPMI 10%). Transfected lines were grown in RPMI 10%, containing 400 μg/ml of G418 sulfate (Gibco Laboratories, Grand Island, NY) and were periodically checked to ensure stability of the phenotype.

Peptides. Matrix peptide 55–73 (sequence: LTKGILGFVFTLTVPSERG) and a series of truncated peptides were synthesized on a peptide synthesizer (430A; Applied Biosystems, Foster, CA) as previously described (21). The peptides were then purified by reversed-phase HPLC. The purity of the peptides was substantiated by amino acid sequence and/or composition analysis. They were routinely >95% pure after HPLC. The index peptide 55–73 was substituted in the later experiments by the more active 57–68 peptide.

In Vitro Secondary Responses. Mice were primed intraperitoneally with 300 hemagglutinating units of A/PR/8/34 influenza virus (PR8) in the form of allantoic fluid. After 3 wk, cultures were established in 24-well plates as previously described (22). Briefly, spleen cells from primed mice (5 × 10^6/well) were cultured with syngeneic irradiated (3,000 rad), PR8-infected (3 × 10^6/well) spleen cells. After 6 d, effector cells were assayed for cytotoxicity.

CTL Lines and Clones. After two in vitro restimulations with PR8-infected spleen cells, transgenic CTL lines were maintained by weekly restimulations with either: (*a*) Jurkat A2.1/K^b cells that were irradiated (20,000 rad), pulsed for 1 h at 37°C in the presence of 55–73 matrix peptide (15 μM), and washed three times (JA2.1/K^b-M); or (*b*) EL-4 A2.1/K^b cells that were irradiated (20,000 rad), infected with 50 HAU of PR8/ml for 1 h at 37°C, and washed three times (EL-4A2.1/K^b-PR8). These stimulator cells were used at a concentration of 0.1–0.2 × 10^6/well in the presence of irradiated (3,000 rad) H-2 congenic feeder splenocytes (3 × 10^6/well) in RPMI 10% supplemented with 5% rat Con A supernatant. Clones were derived from the CTL line 66 by limiting dilution using EL-4 A2.1/K^b-PR8 as stimulators in the presence of irradiated C57BL/6 feeder splenocytes. Clones were derived from CTL lines 372-37-38 and 372-219 by limiting dilution using JA2.1/K^b-M stimulators in the presence of B10D_2 feeder splenocytes. Clones have been kept by restimulation with the stimulator cells used for their derivation.

Cytotoxicity Assay. 1.2 × 10^6 target cells were incubated at 37°C in the presence of 150 μCi of sodium ^{51}Cr chromate for 90 min in the presence or absence of 65 HAU of PR8 during labeling. Cells were washed three times and resuspended in RPMI containing 10% FCS. Matrix peptide (10 μM) was added either during ^{51}Cr-labeling (pulsed cells) or during the lytic assay at the final concentrations indicated in the text. For the assay, 10^4 ^{51}Cr-labeled target cells were incubated with different concentrations of effector cells in a final volume of 200 μl in U-bottomed 96-well plates. Supernatants were removed after 6 h at 37°C, and the percent specific lysis was determined by the formula: percent specific release = 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release).

Results

Expression of A2.1/K^b on Transgenic Spleen Cells. Splenocytes isolated from line 66 and 372 transgenic mice were analyzed by comparative cytofluorometry for the surface expression of HLA-A2.1 and endogenous class I molecules (D^b for line 66 or D^d for line 372). Only 50% of line 66 spleen cells expressed A2.1/K^b at ~40% of the level of the endogenous H-2D^b (Fig. 1 *A*). Analysis of purified lymphocyte subsets from line 66 demonstrated that only 50% of either B or T lymphocytes were positive (data not shown). Although 100% of lymphocytes from line 372 expressed the transgene, it was present at only 10% the level of the endogenous H-2D^d (Fig. 1 *C*). However, after Con A stimulation, the expression of A2.1/K^b increased proportionally more than the expression of the endogenous H-2, reaching 70% of the H-2D^b level in line 66 and 30% of the H-2D^d level in line 372 transgene blast cells (Fig. 1, *B* and *D*). No binding of anti-HLA antibody was observed for control spleen cells derived from H-2 congenic animals not expressing the transgene product (data not shown).

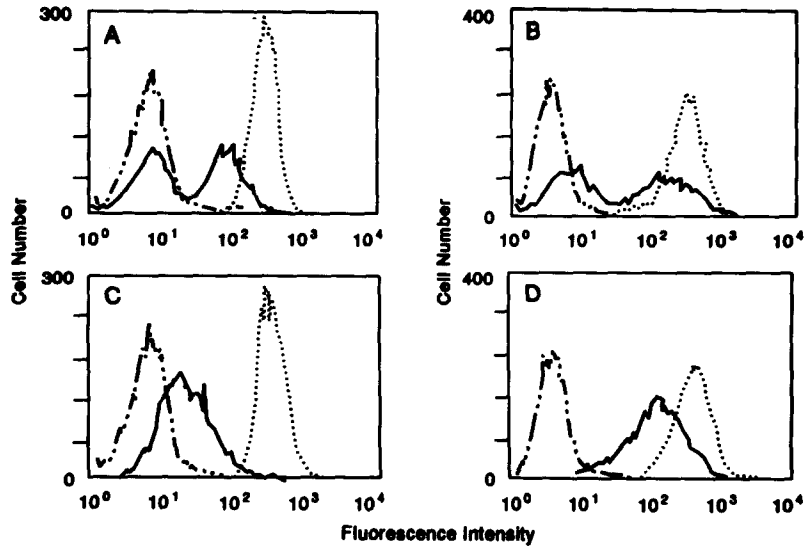

Figure 1. Comparison of cell surface expression of A2.1/K^b and endogenous H-2 on transgenic spleen cells. Resting (*A* and *C*) or Con A–activated (*B* and *D*) spleen cells from line 66 (*A* and *B*) or 372 (*C* and *D*) transgenic mice were analyzed by cytofluorometry using A2.1-specific antibody MA2.1 (——); H-2D^b-specific antibody 28-14-8S (· · · · ·); the H-2D^d-specific antibody 34-2-12S (· · · · ·); or no first antibody (– · · – · · –). The FITC-labeled second antibody was specific for the IgG Fc fragment.

A.2/K^b Can Act as a Restriction Element. Splenocytes from line 372 transgenic mice (H-2^d; A2.1/K^b) primed with PR8 influenza virus were restimulated in vitro and tested for their ability to recognize viral antigens presented by the transgene product. Since it has been shown that the influenza matrix peptide 55–73 is the major determinant recognized in conjunction with A2.1 in the human response (15), target cells included both PR8-infected and matrix peptide–pulsed target cells. Fig. 2 shows the results obtained using splenocytes from 372 mice. Specific lysis of PR8-infected EL-4-A2.1/K^b (H-2b; A2.1/K^b) cells (Fig. 2 *A*) indicated that 372 transgenic mice were able to generate an A2.1/K^b-restricted, anti-PR8 CTL response; moreover, EL-4-A2.1/K^b cells pulsed with the influenza matrix peptide were recognized by the effector cells as efficiently as the PR8-infected targets, suggesting that at least some of the CTL were specific for the same antigenic determinants as detected after influenza infection in humans (14, 15). Similar analysis using Jurkat cells transfected with A2.1/K^b as targets (Fig. 2 *B*) revealed a high level of specific lysis that required expression of the A2.1/K^b molecule as well as the matrix peptide. The influenza-primed transgenic spleen cells were also able to lyse PR8-infected Jurkat A2.1/K^b cells (data not shown).

Analysis of the specific lytic activity of splenocytes from each of three additional PR8-primed transgenic 372 mice consistently revealed matrix peptide–specific lysis of Jurkat A2.1/K^b target cells, albeit to a lesser extent than the specific lysis obtained on H-2-bearing target cells (Table 1). Indeed, 3–10 times more effector cells were required to obtain lysis of matrix-coated Jurkat A2.1/K^b targets equivalent to the level obtained using PR8-infected $B10D_2$ targets. However, considering that PR8 infection of H-2^d cells generates several CTL epitopes (23), the magnitude of the CTL response restricted by the transgene appears to be in the same range as that restricted by endogenous H-2. Unprimed 372 mice did not yield specific effectors, ruling out the possibility that

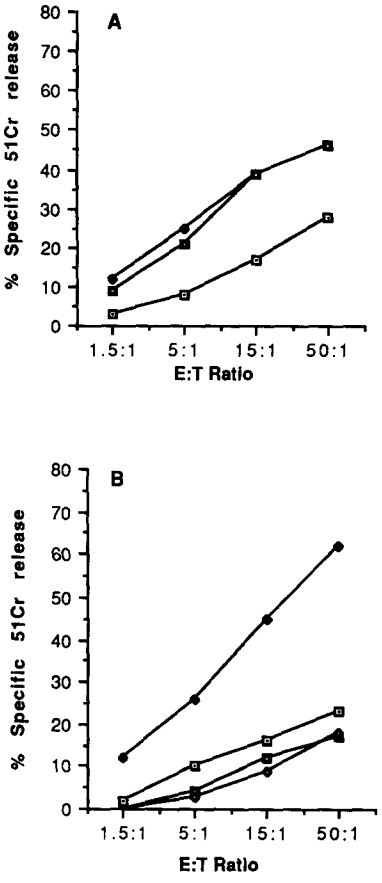

Figure 2. In vivo priming of influenza-specific HLA-A2.1/K^b-restricted transgenic CTL. Pooled splenocytes from two PR8-immunized 372 transgenic mice were restimulated in vitro with irradiated PR8-infected line 372 spleen cells. After 6 d, effector cells were assayed for lytic activity in a 6-h ^{51}Cr release assay. (*A*) EL-4 A2.1/K^b cells, untreated (⊡), PR8-infected (◆), or pulsed with matrix peptide 55-73 (□). (*B*) Jurkat A2.1/K^b cells, untreated (⊡) or pulsed with matrix peptide 55-73 (◆), or Jurkat cells untreated (□) or pulsed with matrix peptide 55-73 (◇).

Table 1. *Lytic Activity of In Vivo Primed, HLA A2.1/K^b-restricted CTL from Individual Mice*

Line 372 mice (animal no.)	E/T	Percent specific ^{51}Cr release from target cells* Jurkat A2.1/K^b‡ –	Jurkat A2.1/K^b‡ Matrix peptide	B10 D$_2$§ –	B10 D$_2$§ PR8
213	50:1	15	45	26	72
	15:1	8	29	11	80
	5:1	5	13	2	38
214	50:1	20	57	13	60
	15:1	13	40	9	70
	5:1	5	21	1	32
215	50:1	27	66	22	73
	15:1	14	57	8	71
	5:1	4	32	2	55

Spleen cells from three PR8-primed line 372 transgenic mice were restimulated in vitro with irradiated PR8-infected line 372 spleen cells, and 6 d later, CTL effector cells were assayed for lytic activity as described in Materials and Methods.

* As described in Materials and Methods.

‡ Target cells were either untreated or pulsed with matrix peptide 55–73 as described in Materials and Methods.

§ Target cells were either untreated or infected with PR8 virus as described in Materials and Methods.

Table 2. *Matrix Peptide 57–68 Is the Major Epitope Recognized by A2.1/K^b-restricted Virus-specific CTL Clones from Transgenic Mice*

Line 372 mice (animal no.)	Clone no.	Percent specific ^{51}Cr release from EL-4 A2.1/K^b target cells* –	PR8	Matrix peptide
350	1	0	33	24
	2	3	48	41
	3	6	35	29
	4	4	51	40
	5	1	21	9
	6	2	35	34
	7	5	47	37
	8	10	61	48
	9	4	61	51
	10	3	28	21
346	11	2	21	12
	12	5	40	24
	13	3	29	5
	14	4	40	14
	15	3	47	12
	16	8	57	35
	17	7	29	8
	18	6	40	16
	19	7	40	27

Spleen cells derived from two PR8-primed 372 mice were restimulated in vitro with irradiated PR8-infected syngeneic splenocytes for 7 d and then plated by limiting dilution onto EL-4 A2.1/K^b-PR8 and B10 D2 feeder cells. After 7 d, cultures were split three ways and assayed for their specificity. Wells were considered positive for the presence of an A2.1/K^b-restricted PR8-specific CTL clone if lysis was 10% or less on uninfected cells and 20% or more on infected cells. Wells were considered to contain clonally distributed cells if 20% or fewer wells plated at a given cell concentration were positive.

* As described in Materials and Methods. Target cells were untreated, PR8 infected, or in the presence of matrix peptide 57–68 at 1.3 μM.

the CTL were being primed in vitro instead of in vivo (data not shown).

These results indicate that the 372 transgenic line can be primed in vivo with PR8 influenza virus, leading to the generation of influenza-specific A2.1/K^b-restricted CTL, and that a portion of the response is directed toward the major human A2.1-restricted influenza epitope, i.e., matrix peptide 55–73. Studies carried out using the A2.1/K^b-expressing 66 transgenic line gave similar results (data not shown).

Matrix Peptide Is the Major Epitope Recognized by A2.1/K^b-restricted Virus-specific CTL Clones from Transgenic Mice. To determine what proportion of PR8-specific, A2.1-restricted murine CTL recognized the matrix peptide, lytic assays were carried out after clonally distributing spleen cells from PR8-primed 372 transgenic mice. A total of 19 A2.1-restricted, PR8-specific clones derived from two individual 372 mice were analyzed. 17 of these clones were specific for the matrix peptide suggesting that the dominant A2.1-restricted response in the transgenic mice is directed towards the matrix peptide. Only two clones (nos. 13 and 17) gave a response on PR8-infected targets that was sufficiently low when tested on matrix peptide targets to suggest recognition of a different influenza antigen (Table 2).

Priming for A2.1-restricted Murine CTL Does Not Occur on H-2. To ensure that murine A2.1-restricted CTL did not derive from the murine H-2-restricted PR8-specific response due to a fortuitous crossreaction, A2.1-restricted matrix-specific CTL clone (372-37-38-3), or CTL lines obtained by selective restimulation with the matrix peptide (372-219 and 66), were tested on A2.1-negative H2-congenic infected target cells. As seen in Table 3, all lines tested specifically killed the A2.1-bearing target only. No lysis was obtained on PR8-infected H-2 congenic target cells.

Fine Specificity Analysis of A2.1/K^b-restricted CTL Clones. The fine specificities of human T cells that respond to the influenza matrix peptide 55–73 have been investigated extensively by others (16). To determine whether the fine

Table 3. *A2.1-restricted Transgenic CTL Do Not Recognize Influenza Antigens in the Context of Endogenous H-2*

		Percent specific ^{51}Cr release from target cells*					
		Jurkat A2.1/K^b		$B10D_2$		EL-4	
Cell lines	E/T	UN	PR8	UN	PR8	UN	PR8
372-37-38-3‡	1.25:1	1	21	0	0	ND	
(H-2d)§	20:1	6	43	1	0		
372-219‡	1.5:1	3	30	0	0	ND	
(H-2d)	24:1	12	46	0	3		
BALB/c-PR8‖	2.4:1	ND		3	16	ND	
(H-2d)	22:1			15	44		
66‡	1.6:1	0	42			0	1
(H-2b)	26:1	6	49			6	5
C57BL/6-PR8‖	3.3:1					1	45
(H-2b)	30:1					12	65

* As described in Materials and Methods.
‡ CTL lines and clones were derived and maintained as described in Materials and Methods.
§ Murine H-2 haplotype.
‖ BALB/c-PR8 and C57BL/6-PR8 effector cells were obtained from immune spleen cells after two in vitro restimulations with syngeneic PR8-infected spleen cells as described in Materials and Methods.

specificities of murine A2.1-restricted, virus-specific CTL clones were similar to that observed for human T cells, three clones (two originating from transgenic line 372 mice and one from a line 66 mouse) were tested for their lytic activity on Jurkat A2.1/K^b target cells in the presence of a series of truncated matrix peptides (56–68, 57–68, 57–73, 58–73, and 59–73). As shown in Fig. 3, analysis of these clones allowed us to detect two fine specificities distinguishable on the basis of recognition of peptide 59–73. Although this peptide was the one most efficiently recognized by clone 372-219-11 (inducing optimal lysis at <0.01 μM), it was not recognized by the other two clones. Despite the clear-cut difference in the recognition of peptide 59–73, all three clones recognized peptide 57–68 comparably with lysis obtained at ~0.1 μM peptide. The other peptides were recognized less well and clones differed by an order of magnitude in the concentration of peptide (0.1–1 μM) required for target cell recognition. Thus, murine A2.1/K^b-restricted CTL clones displayed clonal heterogeneity.

Recognition of A2.1 as Compared with A2.1/K^b-transfected Cells. It was of interest to determine whether the A2.1/K^b-restricted T cells generated in virus-primed A2.1/K^b transgenic mice were also able to recognize target cells expressing the native HLA-A2.1 molecule. CTL lines and clones isolated from both the 372 and the 66 transgenic A2.1/K^b murine lines were tested for their capacity to specifically lyse

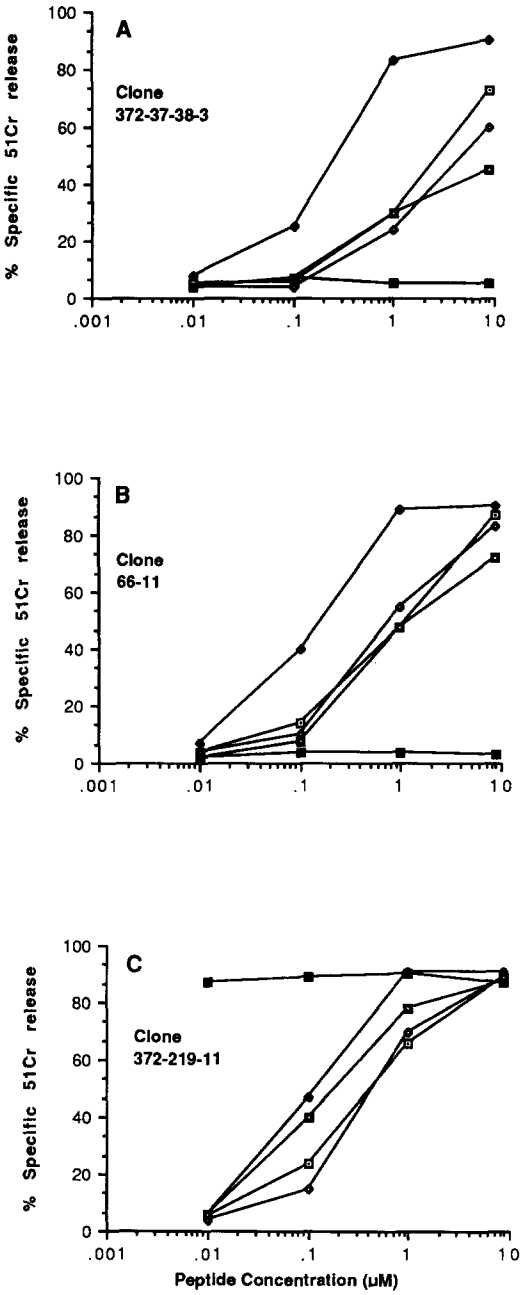

Figure 3. Fine specificity analysis of A2.1-restricted, virus-specific murine CTL clones. Lysis of Jurkat A2.1/K^b cells was measured in the presence of truncated matrix peptide 56–68 (⊡), 57–68 (◆), 57–73 (□), 58–73 (◇), and 59–73 (■) at the concentrations indicated. Clones 372-37-38-3, 66-11, and 372-219-11 were derived as described in Materials and Methods. The E/T was 5:1.

Jurkat cells that express either A2.1 or A2.1/K^b in the presence or absence of matrix peptide antigen.

The effector cells used in this experiment represented long-term cell lines or T cell clones. As documented by the results presented in Table 4, both Jurkat A2.1 and Jurkat A2.1/K^b targets were able to present peptide for CTL recognition. Of interest however, consistently lower levels of killing were obtained with Jurkat A2.1 targets. This was not due to a

Table 4. *Recognition of HLA A2.1: as compared to A2.1/K^b-transfected Jurkat Cells*

		Percent specific ^{51}Cr release from target cells*					
		Jurkat Untransfected		Jurkat A2.1		Jurkat A2.1/K^b	
Clones	E/T	–	M	–	M	–	M
372-37-38-3‡	0.3:1	3	1	0	7	1	37
	1:1	4	2	2	21	2	68
	3:1	5	7	3	44	5	85
372-37-38-5‡	0.3:1	3	3	1	4	1	26
	1:1	9	5	1	15	4	53
	3:1	15	11	4	40	9	88
372-219-11‡	0.3:1	1	1	0	27	2	42
	1:1	1	2	2	52	0	66
	3:1	2	2	2	81	2	88
66-11‡	0.3:1	0	0	0	36	0	58
	1:1	0	3	0	72	0	92
	3:1	7	13	2	93	3	98
372-219‡	0.3:1	2	1	1	35	1	52
	1:1	3	3	2	57	5	78
	3:1	6	6	4	78	9	90
66‡	0.3:1	1	0	2	21	1	50
	1:1	4	0	2	43	0	72
	3:1	3	7	1	68	2	89

* As described in Materials and Methods. Target cells were either untreated or in the presence of matrix peptide 57–68 at a concentration of 6.7 μM.
‡ CTL lines and clones were derived and maintained as described in Materials and Methods.

lower level of A2.1 expression, as FACS® analysis revealed slightly higher levels of A2.1 than A2.1/K^b molecules on these transfected lines (data not shown).

Discussion

The two major goals of the present study were: (*a*) to determine whether substitution of the α3 domain of A2.1 with its murine counterpart would override some of the difficulty in generating antigen-specific murine CTL restricted by HLA-A2.1; and (*b*) if a response was obtained, to determine whether the murine response entailed recognition of the same antigenic epitopes previously defined in the corresponding HLA-A2.1-restricted response in humans.

Our findings demonstrate that virus infection of A2.1/K^b transgenic mice does lead to generation of A2.1-restricted, influenza-specific CTL. That the actual priming in vivo resulted from antigen recognition in the context of A2.1/K^b and not the murine class I molecules was confirmed by the fact that none of the A2.1-restricted lines recognized PR8-infected nontransgenic H-2^d or H-2^b targets. Within both the 66 and 372 transgenic lines, the capacity to respond to influenza virus in the context of A2.1/K^b was consistent from mouse to mouse, suggesting differences in the basal level of expression of the transgene, as exists in these lines, did not substantially affect responsiveness.

These results are in contrast to previous findings by several groups who have found only weak (9) or no (8) A2.1-restricted virus-specific responses in transgenic mice. Thus, it is likely that the presence of the murine α3 domain greatly facilitated A2.1-restricted recognition of antigen. This is in agreement with previously published results, which have demonstrated that unlike the native A2.1 molecule, the chimeric A2.1/K^b molecule was effective in stimulating a primary in vitro response by murine CTL (12, 13).

The mechanism by which the murine α3 domain increases responsiveness in the context of A2.1 may involve several different components. Most prominent is the potential role of the α3 domain of K^b in facilitating interaction with murine CD8. Class I–CD8 interaction is important for efficient repertoire selection in the thymus (10, 11) for activation of CTL precursors (24), and for CTL recognition of target cells

(25–28). The residues in class I critical for CD8 binding have been mapped within the $\alpha 3$ domain. It is known that slight sequence variation in the $\alpha 3$ domain can greatly influence interaction with CD8 (26–28, 30). Studies that demonstrated the importance of a murine $\alpha 3$ domain in enhancing recognition by murine A2.1-specific CTL clones (12) also provided evidence that those clones most affected were the ones most dependent on CD8–class I interaction in their recognition of target cells. Thus, it is likely that in vivo utilization of the A2.1 molecule has been facilitated by providing it with a murine $\alpha 3$ domain, thereby permitting optimal interaction with the murine CD8 molecule. Interestingly, murine HLA-B27 molecules have been shown to function in both a primary xenogeneic MLC (31) and as a restriction element in transgenic mice (6, 7). It is possible that results obtained using different HLA class I molecules may vary due to sequence differences in the $\alpha 3$ domains that affect the efficiency of interaction with the murine CD8 molecule.

In addition to its role in CD8 binding, the $\alpha 3$ domain may also influence class I MHC function through its binding to β_2M. Association with β_2M is required for efficient transport (32, 33), folding (34, 35), and peptide binding (36) by class I. Moreover, heterologous β_2M induces conformational changes in class I that can be detected by mAbs (37). Recent data also suggest that in some cases β_2M may participate in the selection of peptides for association with class I (38). However, interaction of murine β_2M with the $\alpha 3$ domain of HLA-A2.1 does not appear to grossly affect the ability of HLA-A2.1 to serve as a restriction element for influenza virus–derived determinants since murine cells transfected with A2.1 serve as targets for influenza-specific, A2.1-restricted murine or human CTL clones (4; and A. Vitiello's, unpublished observations). However, it is difficult to rule out quantitative effects that may be important for CTL priming in vivo.

The A2.1/K^b transgene contained the transmembrane and cytoplasmic portions of K^b. Although hypothetical at this time, it is possible these regions may also play an important role during T cell maturation or activation, perhaps by mediating signals required for production of interleukins, as has been reported for class II molecules (39). If this were the case, then the presence of K^b as opposed to A2.1 sequence may have facilitated such signaling in murine cells.

The potential usefulness of HLA transgenic mice for vaccine and drug development depends on the ability of the murine TCR repertoire to recognize the same antigenic determinants recognized by human T cells. The matrix protein is the dominant influenza virus molecule recognized by A2.1-restricted human CTL (14). Recent work has demonstrated that the majority of the A2.1-restricted CTL recognize determinants within the matrix peptide represented by amino acids 55–73 (15). In the present studies, an epitope, or epitopes, present in matrix peptide 55–73 dominated the PR8-specific A2.1/K^b-restricted response. Analysis at the clonal level revealed this peptide specificity by the majority of the influenza-reactive clones (17/19). Several clones (nos. 5, 12, 14, and 15) appeared to recognize the matrix peptide less well than viral-infected cells. Since neither the quantity nor the size of the antigenic peptide generated upon PR8 infection of target cells is known, strict qualitative or quantitative comparisons between targets sensitized with PR8 vs. synthetic peptide are difficult. Clones 13 and 17 clearly did not recognize the 55–73 matrix peptide, suggesting that a different viral epitope was recognized by some clones.

As with influenza-specific A2.1-restricted human CTL (16), the transgenic CTL response contained clones with different fine specificities, as detected using truncated matrix peptides. The peptide most efficiently recognized by the murine and human T cell clones was 57–68. This may reflect higher binding affinity by the peptide for A2.1 than either the longer or truncated versions. One of the murine T cell clones (clone 372-219.11) lysed target cells sensitized with peptide 59–73 much more effectively (>100-fold) than targets sensitized with the other peptides. This phenotype cannot be explained simply on the basis of enhanced peptide binding since two of the clones did not recognize this peptide at all. Rather, it more likely reflects a true difference in fine specificity by TCRs. It is noteworthy that in the studies of the fine specificity of human T cells (16), one of the clones displayed a similar recognition pattern.

While the binding of antigenic peptides to MHC molecules has been recognized as a critical step in antigen presentation, several important differences between species could have skewed the response away from the matrix 55–73 determinant. Both the positive and negative T cell selection events that occur during ontogeny may have produced distinct T cell repertoires in humans and transgenic mice. Also, since it is likely that peptides derived from "self" as well as "non-self" proteins can compete for MHC binding (40–42), the presence of a different array of peptides in the mouse (as compared with human) could have prevented the association of the matrix peptide with the A2.1 molecule in vivo. Indeed, in light of these potentially important differences, it is surprising to find such a strong similarity between the murine and human specificity repertoires.

The ability of transgenic mice to mount a vigorous A2.1/K^b-restricted response argues that there is sufficient diversity in the murine TCR repertoire to supply a variety of receptors specific for antigen in the context of an HLA molecule. Nevertheless, it would be of interest to compare the actual number of different TCR genes used in the murine response with the repertoire of human TCR specific for the same antigen.

In conclusion, our demonstration that A2.1/K^b transgenic mice can be primed in vivo with influenza virus and that the response generated is specific for virtually the same epitopes as those recognized by human influenza-specific CTL points to the potential of HLA transgenic mice as an important model for the study of determinants recognized by human T cells. Although additional antigens restricted by HLA-A.2 as well as different MHC molecules must be examined in order to determine the limitations of this model system, transgenics in which HLA molecules appear to function efficiently as restricting elements represent an important resource for the identification of HLA-restricted determinants and for the development of molecules that enhance or decrease antigen-specific, HLA-restricted immune responses.

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Address correspondence to Robert Chesnut, Department of Cellular Immunology, Cytel Corporation, 11099 North Torrey Pines Road, La Jolla, CA 92037.

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