INHIBITION OF MHC CLASS II-RESTRICTED T CELL RESPONSE BY Lyt-2 ALLOANTIGEN

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The TCR is a 90-kD heterodimeric glycoprotein composed of somatically rearranged α and β chains (1–7). This receptor recognizes a complex consisting of antigenic peptide bound to MHC glycoprotein expressed on APCs and/or target cells (8–10). The majority of mature T cells expressing TCR-α/β can be separated into two populations, based on their expression of CD4 and CD8 molecules (11, 12). In the mouse, the subset of T cells expressing the CD8 molecule (Lyt-2 and Lyt-3), but lacking the CD4 (L3T4) molecule, functions as CTL; their interaction with target cells is restricted by class I MHC antigen, whereas the $CD4^+$ $CD8^-$ subset contains helper T lymphocytes (HTL)[1] whose interaction with APCs and B cells is restricted by class II MHC antigen. It is generally assumed that the process of maturation and selection in the thymus is similar for class I and class II–restricted T cells (13–15).

Analysis of TCR V gene usage in various T cell clones and populations has demonstrated that the same V genes can be used by both class I–restricted or class II–restricted T cells (16, 17). These results do not provide a molecular basis for the differences between class I and class II MHC-restricted recognition of T cells.

Recently, it has been shown that the participation of certain Vβ segments in the TCR heterodimer predisposes that receptor to class II–restricted reactivity (e.g., Vβ17, Vβ6, and Vβ8.1 and Vβ3 for I-E alloantigen or for gene products of the minor lymphocyte stimulating locus (Mls) restricted by class II molecules) (18–21). This specificity assignment of certain $V\beta^+$ T cells to class II MHC molecules and Mls is supported by the general finding that T cells bearing such Vβ gene products are deleted from the peripheral lymphocyte pool in mouse strains expressing I-E or Mls.

Recently, we and others have shown that cells expressing Vβ6 and Vβ8.1 are eliminated from both mature T cells populations ($CD4^+8^-$ and $CD4^-8^+$) in mice expressing Mls^a (20, 21). This finding raises interesting questions. Since Mls responses are mediated exclusively by $CD4^+8^-$ T cells (23, 24), and since $CD4^-8^+$ cells, even those expressing the potentially reactive Vβ TCR elements, are unreactive to Mls in culture, how are such $CD8^+$ Vβ–bearing T cells deleted in vivo in Mls^+ mouse strains? Also, if Vβ6 and Vβ8.1 endow T cells with reactivity to Mls, why are $CD4^-8^+$ cells unreactive?

Two possibilities, not mutually exclusive, seem worth considering. First, T cells bearing Vβ6 or Vβ8 elements in their TCR might be eliminated as an early event

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in T cell maturation, either at the double-negative ($CD4^-8^-TCR^+$) stage or at the double-positive ($CD4^+8^+TCR^+$) stage. Secondly, the nonreactivity of mature $CD4^-8^+$ $V\beta6^+$ or $V\beta8.1^+$ T cells to Mls might reflect changes in the functional specificity of such T cells imposed by expression of CD8 molecules in the absence of CD4 markers.

To explore a possible role of differential expression of CD4 and CD8 molecules on the functional specificity of T cells, we have established several $V\beta8.1^+$ T cell hybridomas by fusion of an HTL clone OI6 ($V\beta$ 8.1^+ $CD4^+8^-$, reactive to H-Y/I-A^b and Mls^a) and a CTL line OH2 ($V\beta8.1^+$ $CD4^-8^+$, reactive to H-Y/H-$2D^b$ but not to Mls) to two different BW5147 thymoma cell lines (an TCR-α^-/β^- variant and the same variant cell line expressing transfected mouse Lyt-2 molecules). Additional variants of these hybridoma lines lacking CD4 molecules were created by selection with anti-CD4 antibodies and complement.

We report here the specificity analyses of these cell lines and show that (*a*) either the gain of CD8 or the loss of CD4 from the HTL ($CD4^+8^-$, H-Y/A^b, and Mls^a reactive) line results in the loss of class II-restricted specificity, and (*b*) the loss of CD8 from the CTL ($CD4^-8^+$, H-2 Y/D^b reactive, and Mls nonreactive) line causes both the loss of class I-restricted specificity and the gain of Mls reactivity by a cell line otherwise not reactive to this molecule. These data provide direct evidence that the CD8 molecule can have a dramatic effect on the specificity of T cells; with the cell lines reported here, it blocks class II MHC-restricted T cell responses, and it blocks responses to Mls by $CD4^-$ T cells.

Materials and Methods

Mice. C57BL/6, B6.C-H-2^{bm12}, B6.C-H-2^{bm14}, B10.D2, B10.BR, DBA/2, and AKR/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

T Cell Lines and mAbs. Two male antigen-specific T cell clones derived from B6 females, OH2 ($CD8^+$, cytolytic) and OI6 ($CD4^+$ noncyclic), were established and maintained in vitro as described (25). The TCR α and β chain loss variant of AKR-derived thymoma cell line BW5147 was kindly provided by Dr. W. Born (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). mAbs directed to the $V\beta8$ TCR (MR5-2) as well as to clonotypic determinants of OH2 TCR (MR2-6) and OI6 TCR (MR5-10) were established as described previously (24). mAbs directed to Thy-1 (AT.83), L3T4 (GK1.5), and Lyt-2 (3.155) were kindly provided by Dr. F. W. Fitch (University of Chicago, Chicago, IL) (26).

T Cell Stimulation Assay. For antigen-induced proliferation assays, T cells (2×10^4) were cultured with irradiated (2,000 rad) spleen cells (5×10^5) in flat-bottomed microtiter plates in a final volume of 200 μl in 5% FCS DMEM. After 72 h of culture and a 6-h pulse with 1 μCi of [^{3}H]thymidine, cultures were harvested onto filters and counted. T cell hybridomas were tested for their ability to produce IL-2 upon stimulation with various spleen cells as described previously (27). In brief, 10^5 hybridoma cells were cultured with 5×10^5 stimulator spleen cells (2,000 rad irradiated) in a final volume of 200 μl in 5% FCS DMEM in the presence or absence of mAb (1/8, vol/vol). For the stimulation of the hybridomas with mAbs directed to the TCR, culture plates were coated with mAb overnight and T cells were incubated without stimulator spleen cells. After 24 h of incubation, culture supernatants were harvested and tested for the presence of IL-2 using the IL-2-dependent T cell line CTLL. Results are presented as units of IL-2 per milliter of culture supernatant (28).

Lyt-2 Gene Transfection and the Establishment of T-T Hybridomas. A plasmid containing the Lyt-2 gene and bacterial *gpt* gene (pCA208) (29) was kindly provided by Dr. B. Malissen (INSERM-CNRS de Marseille-Luminy, France). The plasmid (20 μg) and 5×10^6 TCR-α^-/β^- BW5147 cells were suspended in 0.5 ml PBS (pH 7.4) and electroporated using a BTX 100 power supply (BTX, San Diego, CA). The cells were cultured in complete culture medium

in 96-well microtiter plates. After 48 h of incubation, cells were selected for the expression of the transfected gene using mycophenolic acid (2 μg/ml), xanthine (250 μg/ml), and hypoxanthine (15 μg/ml). Growing cells were expanded, selected for the expression of Lyt-2 gene expression and cloned. T-T cell hybridomas were established as described previously (30). For the selection of the BW Lyt-2 × T cell clone hybridomas, since the transfected *gpt* gene confers on the thymoma cell line resistance to HAT selection, hybridomas cultured in HAT medium were selected for the expression of both Thy-1.1 (BW thymoma origin) and Thy-1.2 (T cell clone origin) alloantigens by extensive panning, cloned, and tested for the expression of surface TCR and Lyt-2 alloantigen. $CD4^-$ variants of hybridomas were established by two cycles of anti-mouse CD4 mAb plus complement treatment and cloning.

Flow Cytometry Analysis. The analysis was performed as described previously (25). In brief, cells (10^6) were incubated with 100 μl of hybridoma culture supernatant for 30 min on ice followed by adding FITC-conjugated second reagent for an additional 30-min incubation. Stained samples were analyzed using FACScan analyzer. Control samples were prepared in the same manner but without the first mAb.

Results

Mls Reactivity of $CD4^+8^-$ and $CD4^-8^+$ T Cell Clones. Clone OI6, $CD4^+8^-$, recognizes male antigen (H-Y) in the context of $I\text{-}A^b$ class II MHC, and clone OH2, $CD4^-8^+$, recognizes H-Y antigen in the context of D^b class I MHC antigen. Both clones express the Vβ8.1 TCR gene product as part of the surface receptor as determined by nucleotide sequencing of TCR cDNAs (Kanagawa, O., and Y. Takagaki, unpublished data) and staining with Vβ8-specific mAbs (Table I). Both clones exhibited strong proliferative responses upon stimulation with B6 ($H\text{-}2^b$) male spleen cells but not with B6 female spleen cells (Table I). The restriction specificity of both clones was more precisely defined by the finding that OI6 T cells failed to respond to bm12 ($I\text{-}A^b$ mutant) male stimulator cells, and the OH2 T cells did not respond to bm14 (D^b mutant) male spleen cells (Table I).

When OI6 and OH2 cells were stimulated with spleen cells from various strains of mice, it was found that OI6, but not OH2 cells, could respond to stimulator cells from Mls^a-positive strains, even those with different H-2 haplotypes, but not to stimulators from Mls^a-negative strains (Table I). The Mls^a specificity of the OI6 T cell clone was confirmed by using various stimulator cells expressing different MHC and Mls antigens (data not shown).

Antigen-specific proliferative responses of OH2 and OI6 T cells were inhibited by either mAb MR5-2 (anti-Vβ 8) or the anticlonotypic mAbs, MR2-6 (specific for OH2 TCR) and MR5-10 (specific for OI6 TCR) (Table I). These data clearly demonstrate that (*a*) the $CD4^+8^-$ clone OI6 is reactive to H-Y + A^b and Mls, while the $CD4^-8^+$ clone OH2 is reactive only to H-Y + D^b, and (*b*) TCR molecules identified by anti-Vβ8 and anticlonotypic mAbs are involved in these responses to Mls and H-Y.

Antigen Specificity of T-T Hybridomas. To better understand the basis for the failure of OH2 T cells to respond to Mls^a antigen, a panel of T cell hybridomas was generated with OH2 and OI6 T cells. The parent cell line used for the fusion was a variant of the BW5147 cell line ($\alpha^-\beta^-$BW) that lacks expression of both endogenous α and β chains of the TCR due to irradiation-induced deletions of these genes. Both OH2 and OI6 cell lines were fused with the $\alpha^-\beta^-$ BW cell line and selected in HAT medium. Growing hybridomas were cloned under limiting dilution conditions and selected for expression of either OH2 or OI6 derived TCR.

TABLE I

Proliferative Response of T Cell Clones

T cell clones	Stimulation with							
	B6 male ($H\text{-}2^b$,Mls^b)	B6 female ($H\text{-}2^b$,Mls^b)	bm12 male (bm12,Mls^b)	bm14 male (bm14,Mls^b)	B10.D2 male ($H\text{-}2^d$,Mls^b)	DBA/2 male ($H\text{-}2^d$,$Mls^{a,c}$)	B10.BR male ($H\text{-}2^k$,Mls^b)	AKR male ($H\text{-}2^k$,Mls^a)
	cpm × 10^{-3}							
Exp. I								
OH2	20.1*	0.8	14.4	0.6	0.5	0.9	ND	ND
OI6	100.3	7.5	5.2	164.1	8.2	160.6	ND	ND
Exp. II								
OH2	73.7	0.9	ND	ND	0.4	1.0	1.0	2.0
OI6	204.4	0.5	ND	ND	4.1	205.5	2.1	211.5
	B6 male	B6 male + MR5-2	B6 male + MR2-6	B6 male + MR5-10	AKR	AKR + MR5-2	AKR + MR2-6	AKR + MR5-10
OH2	24.1	4.1	2.2	20.4	0.3	ND	ND	ND
OI6	150.3	13.2	123.4	20.3	130.4	11.3	110.5	10.5

T cell clones OH2 and OI6 were tested for the proliferative response to the spleen cells from various strains of mice, as described in the Materials and Methods. The inhibition of the T cell proliferation by mAbs directed to the TCR was assayed by culturing T cells with stimulator spleen cells in the presence (1/8 vol/vol culture supernatant) or absence of mAb.

* Results are shown as mean cpm × 10^{-3} of triplicates (standard deviation did not exceed >20% of mean value).

When these hybridomas were tested for antigen specificity by measuring IL-2 production upon stimulation with spleen cells, hybridoma KR3, established by fusing OI6 T cells with the $\alpha^-\beta^-$ BW cell line, was found to exhibit the same regimen specificity as the original OI6 T cell clone, namely to H-Y + I-A^b and Mls (Table II A). In contrast, the hybridoma KV24, established by fusing the CTL clone OH2 with BW, was incapable of producing IL-2 upon stimulation with B6 male spleen cells, but surprisingly it showed response to Mls (Table II A). This was confirmed in another experiment where KV24 failed to show alloreactivity to B10.BR (H-2^k, Mls^b), but showed good responses to AKR/J, RF/J, and CBA/J strains expressing Mls^a antigen and the same H-2^k haplotype (result not shown).

To determine whether this Mls-specific response of the KV24 hybridoma was mediated by the TCR of the original OH2 CTL clone, hybridomas were stimulated by AKR spleen cells in the presence or absence of the anti-TCR mAbs (MR5-2, MR2-6 and MR5-10). As shown in the Table II B, the Mls^a-specific responses of both KV24 and KR3 were inhibited by mAb MR5-2 specific for the Vβ8 TCR. The finding that the anticlonotypic mAb MR2-6 that is directed to the OH2 TCR was also capable of inhibiting the Mls^a-specific response of the KV24 hybridoma, but not KR3, indicated that the TCR of the CTL clone OH2 was responsible for the Mls^a specificity of the KV24 hybridoma.

The phenotype of hybridomas KV24 and KR3 were analyzed by surface immunofluorescent staining (Table II C). Hybridoma KR3 was found to express the CD4 molecule; however, KV24, derived from the $CD8^+$ OH2 cell line, was found to be negative for the expression of the CD8 molecule. A requirement for CD8 mol-

TABLE II

Antigen Specificity and Surface Phenotype of the Hybridomas

	Hybridomas	Parental cell lines	Stimulation with B6 male*	B6 female	B10.BR	AKR
A	KV24	OH2 × BW $\alpha^-\beta^-$	<1‡	<1	<1	18
	KR3	OI6 × BW $\alpha^-\beta^-$	22	<1	<1	142

		Stimulation with AKR	AKR + MR5-2	AKR + MR2-6	AKR + MR5-10
B	KV24	13	<1	<1	11
	KR3	110	4	98	7

		Surface phenotype Thy-1.2	Lyt-2	L3T4	MR5-2	MR2-6	MR5-10
C	KV24	+	−	−	+	+	−
	KR3	+	−	+	+	−	+

The antigen-specific IL-2 production by the T cell hybridomas and inhibition of antigen-specific T cell response with mAbs was measured as described in Materials and Methods. Surface phenotype of the hybridomas was analyzed by immunofluorescence staining using the mAbs described in Materials and Methods.

* Irradiated spleen cells.

‡ IL-2 units per milliliter of culture supernatant. The minimum detectable unit in our assay was 1 U/ml.

ecules in class I-restricted antigen recognition by CTL has been well documented, thus the failure of KV24 to respond to B6 male spleen cells may be due to the lack of expression of CD8.

Establishment of Vβ8.1⁺ Hybridomas Expressing Different CD4/CD8 Phenotypes. Given the special circumstances of the KV24 hybridoma, lacking CD8 and the concomitant appearance of Mls reactivity, we decided to assess the role of CD8 molecule on the functional antigen specificity of the hybridomas. First the CD8 gene was introduced into an $\alpha^-\beta^-$ BW cell line by gene transfection. $CD8^+$ BW5147 cells (BW CD8) were then used to establish hybridomas with the OH2 CTL (NA3 hybridomas) and OI6 HTL (NB hybridoma). The NA3 hybridoma (OH2 CTL × BW CD8) cells were cloned and shown to express both the CD8 molecule and TCR derived from OH2 (Fig. 1 *A*). The NB hybridoma (OI6 × BW CD8) NB were tested in a similar manner and shown to be $CD8^+$ and TCR^+ (Fig. 1 *B*).

To explore the effect of CD8 expression in the absence of the CD4 molecule on

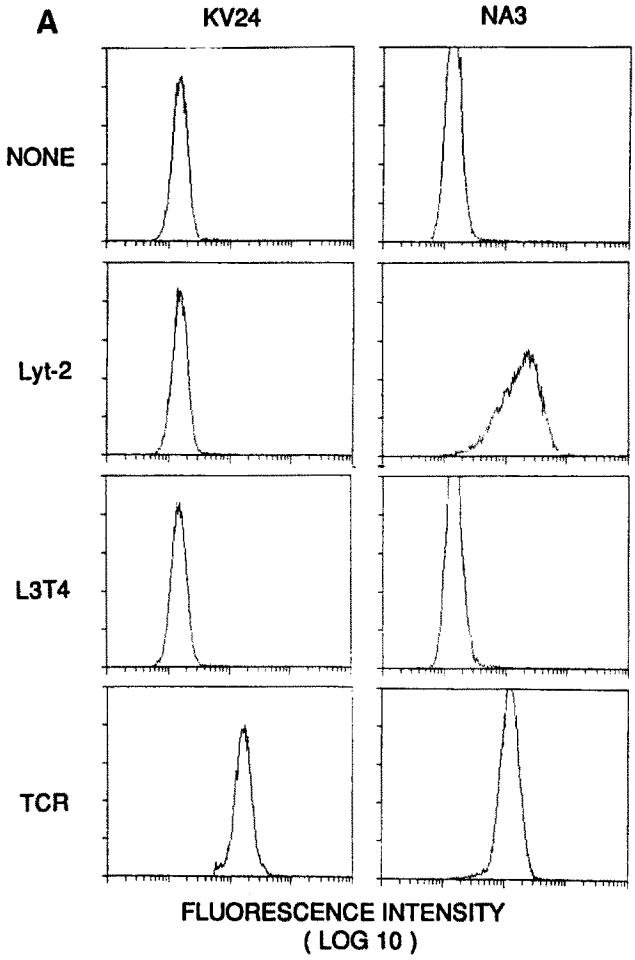

FIGURE 1. Flow cytometry analysis of the hybridomas and their immunoselected variants. (*A*) OH2 × BW5147 (KV24) and OH2 × BW Lyt-2 (NA3) were analyzed for the surface phenotypes using anti-Lyt-2 (3.155), anti-L3T4 (GK 1.5), and anti-TCR (MR2-6) antibodies. (*B*) See facing page. OI6 × BW5147 (KR3) and OI6 × BW Lyt-2 (NB9.1) and their immunoselected $L3T4^-$ variants, KR3N and NB7.3, were analyzed in the same manner but using MR5-10 as an anti-TCR antibody.

HTL × BW hybridomas, the KR3 ($CD8^-$, $CD4^+$, TCR^+) and NB hybridomas were treated with anti-CD4 mAb and complement. Clones KR3N ($CD4^-$, $CD8^-$, TCR^+) and NZB7.3 ($CD8^+$, $CD4^-$, TCR^+) were selected (Fig. 1 *B*). The amount of TCR expressed on these selected hybridomas was found to be significantly less.

The functional antigen specificity of these various manipulated Vβ8.1 hybridomas expressing the HTL OI6 and CTL OH2 TCR was determined in IL-2 assays by stimulation with splenic cells from several different strains (Table III). The data can be summarized as follows: First, for hybridomas derived from OH2 ($CD4^-8^+$, H-Y/D^b reactive, and Mls nonreactive), the loss of CD8 (KV24, $CD4^-8^-$) is accompanied by the loss of class I-restricted reactivity and the gain of Mls reactivity. Thus, as expected, loss of CD8 causes diminished responses to class I-restricted antigen, but it also causes the appearance of a new reactivity to Mls. The introduction of CD8 molecule into hybridoma (NA3) restored the class I-restricted H-Y response, but resulted in the loss of Mls reactivity.

Second, for hybridomas derived from the dual reactive OI6 clone ($CD4^+8^-$, H-Y/I-A^b and Mls reactive), either the loss of CD4 (KR3N, $CD4^-8^-$), or the gain of CD8 (NB9.1, $CD4^+8^+$) causes nonreactivity to a class II MHC-restricted antigen. In addition, for cells bearing this TCR, the presence of CD8 in the absence of CD4 blocks Mls responses.

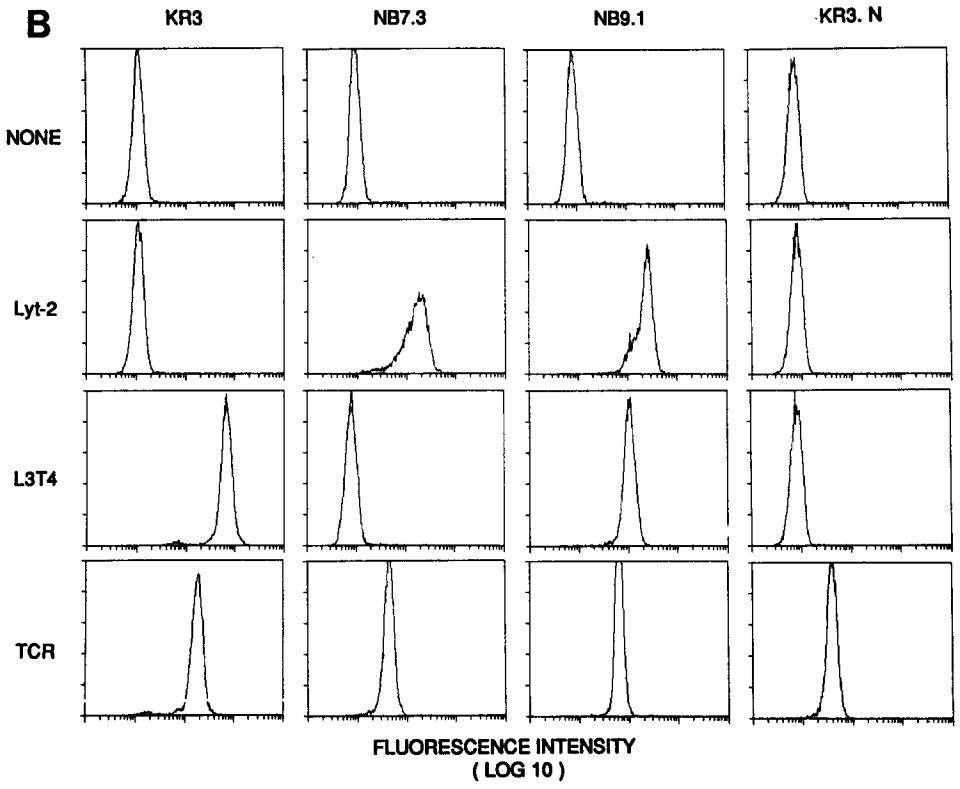

FIGURE 1. (*B*) See facing page for legend.

TABLE III

Effect of CD4 and CD8 Phenotype on the Antigen Specificity of the Hybridomas

Hybridomas	CD4/CD8 phenotype	Stimulation with				
		Anti-TCR Ab	B6 male	B6 female	B10.BR	AKR
OH2 hybridomas						
KV24	$CD4^-CD8^-$	48*	<1	<1	<1	54
NA3	$CD4^-CD8^+$	17	3	<1	<1	<1
OI6 hybridomas						
KR3	$CD4^+CD8^-$	227	38	<1	<1	178
NB7.3	$CD4^-CD8^+$	54	<1	<1	<1	1
NB9.1	$CD4^+CD8^+$	127	<1	<1	<1	137
KR3N	$CD4^-CD8^-$	114	<1	<1	<1	76

Hybridomas were stimulated with either irradiated spleen cells or anti-TCR mAb (MR2-6 and MR5-10 for OH2 and OI6 hybridomas, respectively) fixed onto the culture plates and amount of IL-2 produced was measured as described in the Materials and Methods.

* IL-2 U/ml of culture supernatant.

Discussion

The experiments reported here explore the effects on the functional specificity of two different Vβ8.1 C57BL/6 T cell clones caused by manipulating their expression of CD4 and CD8 molecules. One of these Vβ8.1 clones, a HTL (OI6), is $CD4^+8^-$ and is reactive to H-Y restricted by MHC class II molecules (I-A^b) and is also Mls^a reactive; the second Vβ8.1 clone, a CTL line (OH2), is $CD4^-8^+$ and reacts only to H-Y restricted by MHC class I (D^b). These clones were fused to the TCR-α^-/β^- variant of BW5147 that extinguishes CD8 expression, or to a re-engineered TCR-α^-/β^-, $CD8^+$ variant of BW5147, in which case resulting hybridomas continued to express CD8. Loss of CD4 expression was caused by selection in culture in the presence of anti-CD4 antibodies and complement. At each step in the manipulation of the CD4/CD8 phenotype, the resulting hybridomas were assessed for reactivity to H-Y/I-A^b, H-Y/D^b, Mls, and to triggering by immobilized mAbs specific for Vβ8 and clonotypic markers of the TCR α,β chains.

The results presented in Table III demonstrate the effect of altering CD4/CD8 phenotypes on the functional specificity of these cells. First, the results show that either the loss of CD4 (KR3N, $CD4^-8^-$) or the gain of CD8 (NB9.1, $CD4^+8^+$) by HTL $CD4^+8^-$, H-Y/I-A^b, Mls reactive cells with the OI6 TCR is accompanied by the loss of reactivity to H-Y/I-A^b while Mls responses are retained. Subsequent loss of CD4 from NB9.1 ($CD4^+8^+$) in the NB7.3 line ($CD4^-8^+$) loses reactivity to both antigens. From these findings we conclude that (*a*) the presence of CD4 is required for class II-restricted reactivity, but not for Mls responses; (*b*) responses to class II MHC-restricted antigens, but not to Mls, are inhibited in $CD4^+$ cells by the presence of CD8 molecules; and (*c*) the presence of CD8 in a $CD4^-$ cell extinguishes responses both to class II-restricted antigens and Mls.

Second, with Vβ8.1 cells of the $CD4^-8^+$ phenotype, bearing the OH2 CTL receptor, reactive to H-Y/D^b only, the loss of CD8 is accompanied, as expected, by the loss of class I MHC-restricted specificity, but what may not have been expected is that loss of CD8 in this otherwise Mls nonreactive cell line also endowed it with a new reactivity to Mls. From this finding we conclude, as have others, that class I-

restricted responses rely heavily on CD8, and that CD8 blocks Mls responses in $CD4^-$ T cells, as discussed above for OI6 TCR.

The results of the present analyses correlating specificity of $V\beta8.1^+$ T cells with their CD4/CD8 phenotypes involve only a limited number of different $V\beta8.1^+$ TCR. Consequently, it is not yet certain whether they apply to all T cells using $V\beta8.1$ TCR. Nevertheless, we have recently examined three additional CTL $CD4^-8^+$ clones and hybridomas derived from them; all expressed $V\beta6$ or $V\beta8.1$, and all were reactive to class I-restricted antigens but not to Mls. By contrast, of several HTL $CD4^+8^-$, class II MHC-restricted clones expressing $V\beta6$ or $V\beta8.1$, all were reactive to Mls (Kanagawa, O., unpublished data).

In this report, we have found that the TCR density on the hybridomas expressing different CD4, CD8 phenotypes differs significantly and this TCR density difference might affect the antigen reactivity of the hybridomas. However, $CD4^-8^-$ hybridomas expressing varying degree of OH2 TCR exhibited similar Mls reactivity (Kanagawa, O., unpublished data), suggesting the minor effect of TCR density on Mls reactivity of OH2 TCR.

If $V\beta8.1^+$ $CD4^-8^+$ TCR cells are not reactive to Mls, how are they deleted in Mls^a positive mice? Studies of MacDonald and his colleagues (31) have shown that treatment of neonatal Mls^a-positive mice with anti-CD4 mAb prevents deletion of $V\beta6^+$ T cells, including those of the $CD8^+$ phenotype. They concluded that $V\beta6$ $CD8^+$ T cells might derive from immature $CD4^+8^+$ precursors, and they suggested that Mls-reactive cells could be deleted at this double-positive stage during thymic development in Mls-positive mouse strains. The results presented in this paper, showing that $V\beta8.1^+$ cells of the $CD4^+8^+$ and $CD4^-8^-$ phenotypes, but not $CD4^-8^+$ T cells, are Mls reactive, indicate that negative selection of such cells might occur during the double-positive stage, as suggested, or at an even earlier double-negative stage, but probably could not occur in the more mature $CD4^-8^+$ single-positive stage.

Similar conclusions were reached by von Boehmer and his colleagues using transgenic mice expressing TCR α and β chains (32). The majority of T cells in these mice were potentially reactive to H-Y/D^b. Three important observations were made in comparing male and female H-2^b mice expressing these transgenes: males showed (*a*) a marked reduction (~90%) in numbers of $CD4^+8^+$ thymocytes, (*b*) reduced numbers of $CD4^+8^-$ cells, and (*c*) the presence of transgene-expressing T cells with very low levels of CD8 expression. The common feature of both these examples is that $CD4^-8^+$ $V\beta6$ T cells are deleted in Mls^+ strains, and $CD4^+8^-$ $V\beta8^+$ T cells are deleted in the male transgenic mice, but neither cell population shows any reactivity to its respective antigen.

The point to be emphasized here is that the functional specificity of a given TCR-α/β^+ cell, consequently its selectivity in the thymus, depends on its CD4/CD8 phenotype, and its reactivity to a particular ligand can be changed by altering expression of CD4 or CD8 molecules without directly changing its receptor molecules. Several examples of this are presented in this paper: (*a*) starting from a $CD4^+8^-$ clone, loss of CD4 or the gain of CD8 extinguishes class II MHC reactivity, but Mls responses remain intact; (*b*) subsequent loss of CD4 from a $CD4^+8^+$ clone causes loss of Mls reactivity; and (*c*) loss of CD8 from a $CD4^-8^+$ clone blocks class I reactivity but it also causes the appearance of Mls reactivity.

The involvement of CD8 molecules in class I-restricted recognition has been demon-

strated in the past by experiments involving blocking with anti-CD8 antibodies (33, 34), transfection with CD8 genes (29, 35), and direct cell adhesion studies (36). The current study shows yet another effect of the presence of CD8 molecules on T cell specificity: in double-positive $CD4^+8^+$ T cells they inhibit class II-restricted responses while allowing recognition of Mls, and in $CD4^-8^+$ T cells, they block Mls recognition.

Just how this occurs is not clear, but the following scenario seems worth considering. At least with the pair of Vβ8.1 TCR used in this study, the requirement for CD4 in TCR-class II interactions might be for avidity sufficient to cause triggering by this ligand, and the presence of CD8 molecules interferes with this interaction, perhaps by competing with CD4 for a common interaction site. The avidity of TCR-Mls interactions seems to override CD8 inhibition in the presence of CD4 molecules, but not in their absence.

We do not know whether the double specificity of a single TCR is true for every CTL TCR using the class II or Mls reactive Vβ chains (Vβ 3, 6, 8.1, and 17a) as part of the surface receptor for antigen. Manipulation of CD4/8 phenotypes of T cell clones and hybridomas by methods described in this report and further analysis of antigen specificity of these manipulated hybridomas is now underway to resolve these puzzling and fundamenal issues of T cell recognition.

Summary

T cell hybridomas were established by fusing a $CD8^+$ V$\beta8.1^+$ CTL clone and a $CD4^+$ V$\beta8.1^+$ helper T lymphocyte (HTL) clone to the thymoma cell line BW5147. In contrast to the HTL × BW hybridomas, which retain the same antigen specificity as the original T cell clone, the CTL × BW hybridomas lost the class I MHC-restricted antigen response but acquired a new specificity to Mls^a antigen. Mls^a reactivity of CTL × BW hybridomas was shown to be mediated by the CTL TCR as assayed by inhibition using an anticlonotypic antibody to the CTL clone. Since hybridomas established with BW5147 lose CD8 expression, we have introduced the CD8 molecule into CTL × BW5147 hybridomas by gene transfection. The $CD8^+$ V$\beta8.1^+$ hybridoma was no longer capable of reacting to Mls^a antigen but exhibited the same antigen specificity as the parental CTL clone. Furthermore, the presence of the transfected CD8 molecule in the HTL × BW hybridomas was found to be inhibitory to class II MHC-restricted antigen reactivity. These results demonstrate that, besides its role in increasing the overall avidity of T cell-class I MHC/antigen interaction, the CD8 molecule inhibits T cell-class II MHC gene product/antigen interaction. This negative effect of the CD8 molecule on a class II MHC-restricted response may account for the failure of $CD8^+$ T cells using either Vβ8.1 or Vβ6, which impart reactivity to the Mls^a antigen on $CD4^+$ T cells, to respond to the Mls^a antigen.

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