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Immunology

Limitations in plasticity of the T-cell receptor repertoire

(hole in the repertoire/restricted variable gene usage/T-cell receptor V_β genes/truncated repertoire)

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ABSTRACT **How constrained is T-cell recognition? Is a truncated T-cell receptor (TCR) repertoire, missing half of its V_β components (where V indicates variable), still broad enough to produce an antigen-specific T-cell response to all determinants? These questions can be answered for certain T-cell antigenic determinants whose response in the wild type is limited to specific gene segments. Our results show that mice with such a deletion in their TCR V_β genes (V_β truncated haplotype, V_β^a) are unable to respond to two antigen determinants (sperm whale myoglobin 111–121/I-E^d and myelin basic protein 1–11/I-A^u) whose response in the wild type is restricted to the missing V_β (V_β8.2 in the case of 111–121/I-E^d and V_β8.2 and V_β13 in the case of 1–11/I-A^u) gene segments. Fundamentally, this restriction could have been attributed to another aspect of immunodominance—that a favored TCR with high affinity would dominate the response, but in its absence, a hierarchy of T cells with lesser efficiency and expressing alternate TCR V genes could take over. However, from our experiments it has become evident that there is an absolute limit to the flexibility inherent in the TCR repertoire. Since it is clear that mouse populations have many ambient deletion ligands (such as self-superantigens) that can result in the loss of multiple V_β gene segments during normal T-cell development, these deletions can have serious consequences, such as unresponsiveness to the antigen as a whole—a hole in the repertoire—if a dominant determinant of that antigen normally shows restricted TCR V_β gene usage.**

The T-cell repertoire has an almost unlimited potential available for diversification by rearrangement of variable (V), diversity (D), and joining (J) gene segments and introduction of junctional residues: approximately 10^{12} to 10^{18} unique heterodimeric structures can be constellated (1, 2). Four inbred strains and numerous wild mouse strains have recently been shown to have lost 50% of their V_β gene segments from the germ-line repertoire (3). These mice thus define a new genotype at the V_β locus—the T-cell receptor (TCR) V_β truncated (V_β^a) genotype. All members of the V_β8 and V_β5 gene families and four other V_β gene segments (V_β9, V_β11, V_β12, and V_β13) are missing from the germ line of these mice (3). Given the vast potential for diversification and the fact that V_β^a mice exist in the wild (4), it would have been predicted that, despite the absence of 10 TCR V_β gene segments from the germ-line repertoire of TCR V_β^a mice, their immune system would still be able to make a reasonable response to any antigenic challenge by using alternative gene segments or combinatorial strategies at the sites of rearrangement. We have used these V_β^a mice to ask how plastic the TCR repertoire is: Is the truncated repertoire (about half of the wild type) still broad enough to produce an antigen-specific T-cell response to all determinants? This question is of particular significance for those determinants, the response to which is limited to specific gene segments that are missing in the V_β^a mice (4–6). Using two such antigenic determinants, sperm whale myoglobin (SWM) 111–121/I-E^d and myelin basic protein (MBP) 1–11/I-A^u, which show extremely restricted V_β (V_β8.2 in the former and V_β8.2 and V_β13 in the latter) gene usage in the wild type (5–7), we show that mice with the truncated TCR V_β genotype show little or no response to these peptides. In these cases, the TCR repertoire is not flexible enough to recruit alternative gene segments for production of a recognition structure equivalent to the one produced with the wild-type V segment. Deletion or absence of a TCR gene segment due to either self-tolerance or mutation could thus result in unresponsiveness in some circumstances.

MATERIALS AND METHODS

Mice. Mice either were bred at our own colony or were obtained from The Jackson Laboratory.

Chimeras. Bone marrow chimeras were constructed as described in Gao *et al.* (8) after irradiating the hosts with 1000 rads (1 rad = 0.01 Gy).

Antigens. SWM and purified protein derivative of tuberculin *Bacillus* purified protein derivative (PPD) were obtained from Accurate Chemicals (Westbury, NY) and Evans Medical (Horsham, U.K.), respectively. Hen egg white lysozyme was purified as described (9). SWM peptides were synthesized (M. McMillan and L. Williams, University of Southern California) using a model 430 A peptide synthesizer (Applied Biosystems), and MBP1–11 was a kind gift from Cetus. The peptides were passed over a Sephadex G-10 column (Pharmacia) and the peptide fractions were further purified as a single peak by HPLC on a preparative C_8 column with a solvent system of 0.1% trifloroacetic acid and an increasing gradient of acetonitrile from nil to 60%.

Immunization. Mice were immunized with 7 nmol of peptide or protein antigen in the hind footpads with an emulsion of complete Freund's adjuvant (H37Ra; Difco). The chimeric mice were immunized as above, except that the chimeras simultaneously received 10^7 (B10.PL × B6)F_1 spleen cells i.v. as antigen-presenting cells (APCs). On day 9, lymph node cells (LNCs) from draining lymph nodes were harvested and used in antigen-induced proliferation assays or as a source of T cells to generate long-term T-cell lines.

T-Cell Lines and Hybrids. Continuously growing T-cell lines were generated from mice immunized with SWM. LNCs were harvested, bulk cultured, and maintained as cell lines as described (9) except that SWM was used as an antigen for the first two cycles and peptide 110–121 was used thereafter to maintain these lines. Irradiated (3000 rads) BALB/c spleen cells were used as APCs. T-cell blasts from these cell lines were fused with a variant of BW5147, BW/$\alpha^-\beta^-$ (10) as a

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Abbreviations: MHC, major histocompatibility complex; SWM, sperm whale myoglobin; TCR, T-cell receptor; MBP, myelin basic protein; APC, antigen-presenting cell; LNC, lymph node cell; V, variable; D, diversity; J, joining; PPD, purified protein derivative.

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fusion partner, as described by Bill *et al.* (11). The resulting hybrids were seeded at less than one cell per well in 96-well plates, and the positive wells were expanded and tested for their ability to produce interleukin 2 using HT-2 cells in response to SWM and peptide 110–121. The antigen-reactive hybrids were further expanded and analyzed for function and TCR V_β expression.

Antigen-Primed LNCs. LNCs (4×10^5) from individual mice were cultured with 14, 7, 3.5, or 1.75 μM SWM or SWM peptide 105–118, 110–121, or 111–121 in 0.2 ml of HL-1 medium per well (Ventrex Laboratories, Portland, ME) supplemented with 2 mM glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin. All cultures were done in triplicate. Proliferation was measured by addition of 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine for the last 18 hr of the 5-day culture, and the incorporation was assayed by scintillation spectroscopy.

Long-Term T-Cell Lines. T cells (10^4) were cultured with 5×10^5 irradiated (3000 rad) spleen cells as a source of APCs under conditions identical to those for LNCs. Cells were cultured for a total of 3 days and [^{3}H]thymidine incorporation was assessed during the last 18 hr.

T-Cell Hybrids. T-hybridoma cells (5×10^4) were cultured with various concentrations of peptide 110–121 with 5×10^5 irradiated BALB/c (I-A^d, I-E^d) or B10.GD (I-A^d) spleen cells as APCs in 0.2 ml of Dulbecco's modified Eagle's medium (Flow Laboratories) supplemented with 2 mM glutamine, 0.05 mM 2-mercaptoethanol, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 10% heat-inactivated fetal calf serum (HyClone). All cultures were performed in triplicate. The supernatants collected 24 hr later were assayed for interleukin 2 activity on the interleukin 2/interleukin 4-dependent cell line HT-2. HT-2 cells (10^4) were cultured with medium alone or supernatants for 48 hr. Proliferation was measured by adding 1 μCi of [^{3}H]thymidine during the last 18 hr of culture and incorporation was assayed by liquid scintillation spectroscopy.

Immunofluorescence. Cells (10^6) of each of the antigen-specific hybridomas were stained with anti-TCR V_β antibodies F23.1 (V_β8.1, V_β8.2, V_β8.3), F23.2 (V_β8.2), KJ16 (V_β8.1 and V_β8.2) (12), and 44-22 (V_β6) (13). The second layer reagent for F23.1 and F23.2 was fluorescein isothiocyanate (FITC)-coupled anti-mouse immunoglobulin and for KJ16 and 44-22 was FITC-conjugated anti-rat immunoglobulin. The immunofluorescence analysis was done on a FACScan (Becton Dickinson), and all positive hybridomas showed staining in >90% of cells (data not shown). The cells were considered positive for TCR V_β8.1 when they stained with F23.1 and KJ16 but not with F23.2.

RESULTS

As the TCR V^a_β genotype is expressed in nature only in a few laboratory strains—C57L (*H-2^b*), C57BR (*H-2^b*), SJL (*H-2^s*), and SWR (*H-2^q*) (3), but not in *H-2^d* and *H-2^u* haplotypes needed for studying the SWM 111–121/I-A^d and MBP 1–11/I-A^u responses—alternatives had to be devised for this study. Therefore, responses to myoglobin were investigated in two different V^a_β *H-2^d* strains of mice: (*i*) recombinant inbred strains of mice (C×J)3 and (C×J)8 (*H-2^d*, V^a_β) originally derived from BALB/c (V_β wild type) ($V_\beta$wt or V^b_β) and SJL/J (V^a_β) parents (14) and (*ii*) congenic strain B10.D2.βL (15), which has the TCR V^a_β haplotype, donated by C57L, on a B10.D2 background. To study *H-2^u*-restricted responses to MBP, radiation bone marrow chimeras (8) of the type V^a_β (C57L, *H-2^b*) → V^b_β [C57BL/6 × B10.PL)F_1, *H-2^{bxu}*] were constructed.

SWM. *Restricted TCR V_β gene usage in T cells recognizing SWM 110–121/I-E^d in BALB/c mice.* It has been previously shown that the large majority of DBA/2 T-cell clones (12 of 13) that recognize 110–121/I-E^d in response to SWM use the V_β8.2 gene segment (7). The DBA/2 strain of mice do not express TCR V_β8.1 in the periphery due to expression of the self-superantigen Mls-1^a, which induces deletion of V_β8.1 cells in the thymus during development of T cells (16). Therefore we first examined the T-cell V_β usage in response to this region of myoglobin in BALB/c mice, which are Mls-1^b (16) and have normal levels of T cells expressing TCR V_β8.1, V_β8.2, and V_β8.3. To examine T-cell V_β usage in response to peptide 110–121, T-cell hybridomas were prepared from a BALB/c T-cell line, CM8, originally induced by SWM and maintained on peptide 110–121. An unusual result was that this peptide could induce T cells with two different specificities, one restricted by I-A^d and the other restricted by I-E^d. Fig. 1 presents one T-cell hybrid, with a representative of each specificity type obtained from BALB/c mice. CM8-3 is an I-E^d-restricted hybrid, responding only with BALB/c APCs (I-A^d, I-E^d) but not B10.GD APCs (I-A^d I-E^0), whereas CM8-16 is an I-A^d-restricted hybrid that responds to either BALB/c or B10.GD APCs. All 16 T-cell hybrids recognizing the I-E^d-restricted specificity express V_β8.2, thus confirming previous results obtained using DBA/2 mice. Each of the 6 hybrids that respond to the I-A^d-restricted specificity expresses TCR V_β8.1.

TCR V^a_β mice do not respond to peptide SWM 111–121. Along with peptide 110–121, two other peptides were employed in the experiments described below, 105–118 and 111–121, the former because it was the most potent stimulator for both of the specificities (this manuscript; N.K.N. and E.S., unpublished data) and the latter because the loss of residue 110 has been shown to result in considerable loss of binding to I-A^d molecules (17). Peptide 111–121 was therefore used to probe for I-E^d-restricted responses.

Individual BALB/c (*H-2^d*, V^b_β) and (C×J)3 (*H-2^d*, V^a_β) mice were compared for their T-cell proliferative responses after peptide 110–121 priming. Draining LNCs from either strain respond very well to added native SWM or peptide 105–118, with mean stimulation indices in the range of 7.2–15.3 (Table 1). However, the (C×J)3 mice show a considerably reduced

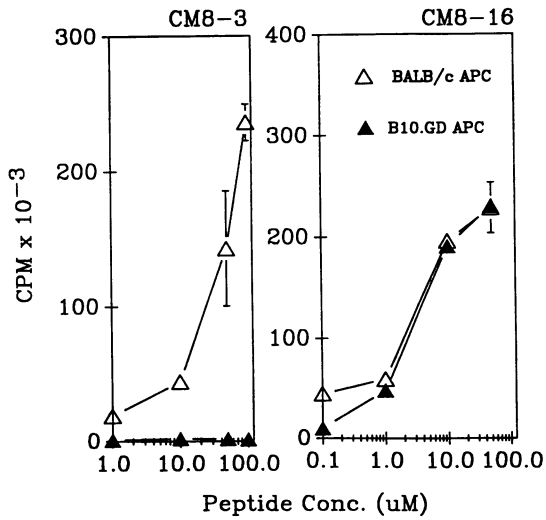

FIG. 1. Peptide 110–121 of SWM can be recognized by T cells in the context of either I-A^d or I-E^d molecules. T-hybridoma cells were cultured with various concentrations of peptide 110–121 with irradiated BALB/c (I-A^d, I-E^d) or B10.GD (I-A^d) spleen cells as APCs. The supernatants were assayed for interleukin 2 activity on HT-2 cells. Data are expressed as mean ± SD of each triplicate culture of HT-2 cells minus the medium alone background. The T-cell hybrid CM8-3, which responds only to BALB/c APCs but not to B10.GD APCs, is restricted to the I-E^d molecule, and the hybrid CM8-16, which responds to peptide 110–121 in the presence of either BALB/c or B10.GD APCs, is I-A^d-restricted.

Table 1. TCR V_β^a mutant mice show little activation of SWM 111–121-specific T cells

In vitro challenge	Mean stimulation index*		Stimulation index (cpm × 10^{-3})†	
	BALB/c (n = 10)	(C×J)3 (n = 10)	B10.D2	B10.D2.βL
SWM	12.2	7.2		
Peptide 105–118	15.3	11.6		
Peptide 110–121	8.4 (n = 7)	2.6	10.1 (41.0 ± 9)	6.1 (16.6 ± 0.6)
Peptide 111–121	7.8 (n = 8)	1.6	9.0 (37.2 ± 2)	0.48 (1.3 ± 0.18)
PPD	19.0	34.1		

All mice were primed with SWM 110–121.

*Data represent the mean of stimulation indices obtained from a pool of n number of mice tested individually, in three different experiments. LNCs were tested individually from each mouse and three mice of each strain were used in each group within the experiment. In one of the three experiments that formed part of the data, the mean cpm × 10^{-3} (±SD), obtained from three mice, after subtracting the medium only backgrounds were as follows. BALB/c: peptide 105–118, 75 ± 14; peptide 110–121, 67 ± 16; peptide 111–121, 67 ± 27; and PPD, *Mycobacterium tuberculosis*, 196 ± 41. (C×J)3: peptide 105–118, 87 ± 22; peptide 110–121, 8.6 ± 0.4; peptide 111–121, 1.8 ± 0.6; PPD, 299 ± 46. The average medium only controls in this experiment were 7.5 ± 0.4 and 6.1 ± 1 for the BALB/c and (C×J)3 strains, respectively. Data for SWM from another experiment, after subtracting the background values, were 168 ± 14 and 151 ± 8.2 for BALB/c and (C×J)3, respectively. The stimulation indices were calculated as the ratio of mean cpm of cells cultured with antigen versus cpm with medium alone. Results obtained with (C×J)8 recombinant inbred mice were similar to those obtained with (C×J)3 mice.

†Data were obtained from pooled LNCs from three mice. The response of peptide 110–121-primed B10.D2 and B10.D2.βL mice to peptides 110–121 and 111–121 was assayed as in footnote *. The mean cpm × 10^{-3} (±SD) for the medium alone backgrounds for B10.D2 and B10.D2.βL were 4.0 ± 0.31 and 2.7 ± 0.3, respectively. The stimulation indices were calculated as in footnote *.

response to peptide 110–121 compared to BALB/c mice (Table 1) and only minimal stimulation by peptide 111–121. Table 1 shows analogous data from congenic V_β^a B10.D2.βL mice and V_β^b B10.D2 mice. After priming with peptide 110–121, there was no detectable response to peptide 111–121 in the B10.D2.βL mice. The lack of response to peptide 111–121 in the two V_β^a strains can be attributed to a loss in the I-E^d-restricted response, as most of the proliferative response induced by peptides 110–121 and 111–121 in BALB/c mice is inhibited by anti-I-E^d antibodies (Table 2).

Absence of the I-E^d-restricted specificity in cell lines and cloned T-cell hybridomas derived from V_β^a mice. Even though two different strains of the V_β^a haplotype, (C×J)3 and B10.D2.βL, showed little response to the I-E^d-restricted determinant, it could still have been reasoned that T cells capable of recognizing this determinant were present in these mice, but at a much lower frequency and/or were of low affinity. To address this issue, we raised several T-cell lines from individual BALB/c and (C×J)3 mice on peptide 110–121 under identical conditions. BALB/c (I-A^d, I-E^d) and B10.GD (I-A^d) spleen cells were used as APCs to distinguish between I-A^d- and I-E^d-restricted specificities of these cell lines. Table 3 shows the restriction pattern of one representative T-cell line obtained from each strain. In agreement with the results obtained using fresh LNCs (Tables 1 and 2), it is clear that the dominant response in BALB/c lines was directed toward the I-E^d-restricted specificity. On the contrary, cell lines from (C×J)3 mice were directed exclusively to the I-A^d-restricted determinant.

Table 4 shows a summary of hybridomas obtained from each of these lines. Two conclusions emerge. (*i*) The larger proportion of BALB/c hybrids (16 of 22, expressing TCR V_β8.2) showed reactivity to the I-E^d-restricted specificity. The remaining 6 hybrids (all expressing TCR V_β8.1) were I-A^d-restricted. That hybrids within both groups were derived from distinct clones was shown by their distinct patterns of fine specificity (data not shown). (*ii*) All hybrids obtained from the (C×J)3 T-cell line CJM4 respond to peptides 110–121 or 111–121 in the context of the I-A^d molecule. No hybrid could be found with the I-E^d-restricted specificity from the (C×J)3 cell line, consistent with the bulk cell line that also displayed no I-E^d-restricted reactivity.

Table 2. Most of the SWM 111–121 recall response in SWM 110–121-primed BALB/c mice is I-E^d-restricted

Recall response	Mean stimulation index	
	No antibody	Anti-I-E^d antibody
105–118	14.6 ± 3.3	5.8 ± 2.2
110–121	5.1 ± 2.01	1.3 ± 0.2
111–121	4.7 ± 1.3	1.0 ± 0.3

Data represent mean ± SD of stimulation indices obtained from three mice tested individually. LNCs were tested individually from three peptide 110–121-primed BALB/c mice (see Table 1) with or without anti-I-E^d antibody 14-4-4S (18).

MBP. Most T-cell clones obtained after immunization of B10.PL mice with MBP recognize the N-terminal region (Ac1–9 or Ac1–11/I-A^u) of MBP and, among these T cells, either the V_β8.2 or V_β13 gene segments are used (5, 6). The V_β8 and V_β13 gene segments are absent from the TCR V_β^a genotype (3). Table 5 compares the T-cell proliferative responses of sets of mice immunized with peptide Ac1–11 of MBP. The critical comparison is between chimeric bone marrow donors having a complete set of V_β genes (B6) and those donors with a V_β^a repertoire (C57L); the (B6 × B10.PL)F_1 recipients are the same in both cases. Whereas *H-2^{bxu}* F_1 mice and the control chimeras with V_β^b respond to Ac1–11 with mean stimulation indices ranging from 5.4 to 5.9, respectively, chimeras with a V_β^a repertoire do not respond to

Table 3. SWM 110–121 response in (C×J)3 cell lines is I-A^d-restricted

Exp.	Origin	Cell		Stimulation index	
		Line	Antigen	BALB/c A^d, E^d	B10.GD A^d
1	BALB/c	CM8	110–121	24.5	0.5
			111–121	14.3	1.0
			105–118	48.8	2.8
	(C×J)3	CJM4	110–121	2.7	2.3
			111–121	ND	1.25
			105–118	5.4	5.68
2	(C×J)3	CJM4	110–121	4.7	4.1
			111–121	2.1	2.2
			105–118	6.0	6.4

T cells (1×10^4) from cell lines, obtained as described in the legend to Fig. 1, were cultured with 5×10^5, 3000-rad-irradiated BALB/c or B10.GD spleen cells (see Fig. 1). The peptide antigens were included at an optimal concentration of 7 μM. Background incorporations in medium alone were 0.4–1.9 × 10^{-3} cpm and stimulation indices were calculated as in Table 1. ND, not done.

Table 4. Characteristics of SWM 110–121-induced T-cell hybridomas in BALB/c and (C×J)3 mice

Strain	No. of hybridomas	Restriction specificity	TcR V_β usage			
			V_β8.2	V_β8.1	V_β6	Unknown
BALB/c	16	I-E^d	16	—	—	—
	6	I-A^d	—	6	—	—
(C×J)3	6	I-A^d	—	—	4	2
	0	I-E^d	—	—	—	—

Cloned T-cell hybrids reactive to SWM peptide 110–121 from a BALB/c cell line, CM8 (see Fig. 1), and another cell line, CJM4, derived from (C×J)3 mice, were tested as described in the legend to Fig. 1, for restriction to either I-A^d or I-E^d molecules, using either BALB/c or B10.GD APCs. The hybridoma cells were stained with various antibodies (see *Materials and Methods*).

the dominant determinant of MBP at various antigen doses. All animals, however, respond equally well to PPD. The LNCs from hen egg white lysozyme-primed chimeras responded normally in the two types of chimeras when recalled with the immunogen *in vitro* (data not shown).

DISCUSSION

We have shown that two different strains of *H-2*^{d} V_β^a mice, recombinant inbred (C×J)3 and congenic B10.D2.βL, show no detectable response to the I-E^d-restricted specificity (probed by myoglobin peptide 111–121) either in fresh LNCs or in long-term cell lines, even though they can make perfectly normal responses to the I-A^d-restricted specificity. We have also shown that chimeras with a V_β^a haplotype, lacking the TCR V_β8 and V_β13 gene segments, fail to respond to the dominant I-A^u-restricted determinant of MBP Ac1–11, although chimeras with the wild-type repertoire do respond. These results support the observation that *in vivo* administration of anti-V_β8 and -V_β13 antibodies can block all T-cell responses to MBP 1–11, the dominant determinant in induction of experimental autoimmune encephalomyelitis (EAE), as well as the induction of EAE (19). The TCR repertoire is thus not infinitely flexible: it cannot devise alternative TCR structures in the absence of those V gene segments that are preferentially used by T cells for response to some peptide–MHC complexes. Although the presence of extremely low-affinity/frequency T cells of this specificity in our systems cannot be ruled out, their activity is not detectable in our assays, and thus a hole in the repertoire in the face of certain antigen challenge would still exist. We would like to point out that a test of plasticity of the T-cell repertoire requires that almost all T-cell clones to that determinant use the same V gene segments in the normal animal; when a reasonable number of alternatives are known to exist, deletion of one of the predominant genes will not reduce responsiveness (20).

Table 5. The T-cell repertoire to MBP 1–11 is not plastic

Strain	Stimulation index			
	MBP 1–11		PPD	
	PR	*n*	PR	*n*
Chimeric mice				
C57L (V_β^a) → (B10.PL × B6)F_1	1.3	10	14.5	10
C57BL/6 (V_β^b) → (B10.PL × B6)F_1	5.9	3	31.0	3
Recipient/donor mice				
(B10.PL × B6)F_1 (V_β^b)	5.4	10	17.5	
C57L (V_β^a)	1.5	4	—	

Data represent mean stimulation indices of *n* number of chimeric or normal mice tested individually as part of four different experiments. Shown is the proliferative response (PR) of MBP 1–11-primed LNCs to MBP 1–11 and PPD. The mean cpm × 10^{-3} (±SD), less medium backgrounds, at the optimal antigen dose of 7 μM in one experiment were as follows. C57L → F_1: peptide 1–11, 1.6 ± 0; PPD, 117 ± 4.0. B6 → F_1: peptide 1–11, 19.4 ± 1; PPD, 208 ± 2. (B10.PL × B6)F_1: peptide 1–11, 33.5 ± 2.5; PPD, 231 ± 32. C57L: peptide 1–11, 8.5 ± 3.5. The medium alone backgrounds in each of the four groups were 20.0 ± 0, 2.5 ± 1.0, 13.5 ± 5.5, and 9.0 ± 3.0, respectively. Data expressed in stimulation indices were calculated as in Table 1.

As we have shown here, there is a complexity in the response to the determinant 110–121 of SWM, allowing it to be seen in the context of I-A^d and I-E^d. However, each specificity context is seen by a TCR with a unique V_β chain in the wild-type mouse, and the response to only one of the specificities, the I-E^d-restricted specificity, is absent in mice with the V_β truncated genotype. In fact, most peptide determinants are like the determinant MBP 1–11, seen in the context of a single major histocompatibility complex (MHC) molecule in a given haplotype; structural constraints in their recognition can lead to unresponsiveness to the whole antigen, if they are dominant determinants. Only under this set of circumstances will the "rigidity" of the T-cell repertoire become evident. In the unusual example of myoglobin, the response to this circumscribed area of the molecule would appear to be intact.

Why do T-cell clones responding to some determinants show restricted V gene usage? It would seem that the interaction between the TCR structure and the MHC–antigen ligand may, at least in some cases, be stringent enough to demand one specific gene segment (8.2) despite the presence of closely related other genes (8.1, 8.3) of the same V_β gene family. In the case of peptide 110–121, T cells recognizing the I-E^d-restricted specificity of this peptide choose to express TCR V_β8.2, in widely different genetic backgrounds, such as DBA/2 (Mls 1^a, 2^a) (7), BALB/c (Mls 1^b, 2^a) (Table 4), and B10.D2 (Mls 1^b, 2^b) (N.K.N. and E.S., unpublished data), which might be another indication of structural constraints in recognition of this determinant.

The concept of lack of plasticity of TCR repertoire, as shown by our results, would have implications for proposed models of TCR recognition of its ligand, the antigen–MHC complex. According to the Davis and Bjorkman model of TCR structure (2), the V regions of TCR α and β chains form the first and second hypervariable regions of the molecule, which only interact with nonpolymorphic regions of the MHC molecule. The third hypervariable region, formed by the junctional region between V, (D), and J gene segments of the two chains, is largely responsible for the nominal antigen specificity of the TCR molecules, according to their model. This model, if strictly applied, would fail to explain the constraints we have reported, as all V_βs, especially closely related members of the V_β8 gene family, should be able to bind the nonpolymorphic regions of I-E^d molecules. We can explain our results by two alternatives: the first considers that the final form of the MHC molecule after peptide binding has subtle changes in conformation in the region that interacts with TCR molecules, such that in some cases it favors combination with one V_β and precludes combination with other V_βs. In our system, TCR containing V_β8.2 rather than V_β8.1 or V_β8.3 may present the correct first and second hypervariable regions necessary for interaction with the topography acquired by a nonpolymorphic region of MHC after having bound peptide 110–121. The second view is that V_β and V_α genes contribute to the overall shape of the TCR, and the presence of V_β8.2 prescribes a certain conformation to the entire receptor that is required to fit the unique geometry offered by given MHC–peptide complexes.

The absence of response to these determinants in V_β^a mice can also be considered an immune response (Ir) gene defect, where the Ir gene maps to the TCR locus. Collagen-induced arthritis is the only other example where an Ir gene has also been mapped, although indirectly, to the TCR locus (21, 22). The influence of the TCR repertoire on immune responsive-

ness of the host would have consequences for wild-type mouse strains expressing a number of self-superantigens, which have recently been defined to be mouse mammary tumor virus proteins. A mouse expressing Mtv-7 and Mtv-13, for example (DBA/2), would delete TCR $V_\beta 6$, $V_\beta 7$, $V_\beta 8.1$, $V_\beta 9$, and $V_\beta 3$ genes from its peripheral repertoire in order to be self-tolerant to Mtv-7 and Mtv-13 (23, 24). Our results indicate that loss of five TCR V_β genes would influence the pattern of immune response, especially for antigenic determinants exhibiting constrained recognition.

With respect to autoimmunity, the failure of $V_\beta 8^{-ve}$ and $V_\beta 13^{-ve}$ chimeras in our experiments to respond to MBP 1–11, the dominant disease-inducing determinant, shows that failure to respond to a determinant due to a hole in the repertoire may be advantageous. This was also the case for SWR and AU/ssJ, the V_β^a strains of the *H-2*^{q} haplotype, which were not susceptible to collagen-induced arthritis, unlike the V_β^b strains DBA/1 and B10.Q (22, 23). On the other hand, if the determinant were a dominant one on a pathogen, this constraint could be fatal. As V_β^a haplotype mice are frequently found in the wild, it is not clear whether the absence of 10 gene segments has any effect on their susceptibility to known pathogens. It is possible that association of certain disease susceptibilities (for example, susceptibility to *Trichinella spiralis*) with the presence of I-E molecules (25), on a closer look, may be related to the absence of crucial TCR gene segments deleted due to expression of self-superantigens in the relevant strains (24). Evidently, during evolution, a balance was reached between these two extremes, resulting in a moderately, but not infinitely, flexible immune system.

Note Added in Proof. Since submission of this manuscript, we found that seven of the I-E^d-restricted T cells that were tested are restricted to an isotype hybrid between I-E^d and I-A^d molecules (most likely $E_\alpha^d A_\beta^d$ molecule), as was also reported (since submission of this manuscript) by Ruberti *et al.* (26). This does not change our conclusions about lack of plasticity in response to a specificity of SWM 110–121 as T-cell response in V_β^b haplotype mice to this hybrid class II molecule-restricted determinant of SWM 110–121 shows a restricted TCR V_β (8.2) gene usage. The TCR V_β^a haplotype mice (lacking TCR $V_\beta 8.2$) do not show any detectable response to the isotype hybrid-restricted specificity.

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