

# A Truncated T Cell Receptor Repertoire Reveals Underlying Immunogenicity of an Antigenic Determinant

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## Summary

Induction of T cell responses to an antigenic peptide that is known to bind a major histocompatibility complex molecule is a function of either the T cell receptor (TCR) repertoire or regulatory influences by CD8 or CD4 regulatory T cells. We have tested the hypothesis that a lack of 10 TCR  $V_{\beta}$  gene segments in  $V_{\beta}^a$  mice may result in an incomplete repertoire of regulatory T cells involved in maintaining peripheral tolerance. Such a hole in the repertoire of regulatory cells could result in expression of T cell responses to antigenic determinants that normally remain undetected in mice with a wild-type repertoire of TCR  $V_{\beta}$  gene segments. We show here that H-2<sup>d</sup> mice respond to the peptide 74-96 of hen egg-white lysozyme (HEL) when they are of  $V_{\beta}^a$  haplotype at their TCR locus. The wild-type ( $V_{\beta}^b$ ) H-2<sup>d</sup> mice with their complete set of 20 TCR  $V_{\beta}$  gene segments fail to respond to HEL 74-96. The 74-96-specific T cell responsiveness was revealed in the wild-type ( $V_{\beta}^b$ ) mice when they were treated in vivo with anti-CD8 antibody, implicating the existence of regulatory cells that prevent expression of T cell responses specific for peptide 74-96. This is a demonstration that holes in the regulatory T cell repertoire can, in certain circumstances, become beneficial to the host, for example, in susceptibility against pathogens.

Due to the loss of 10 T cell receptor  $V_{\beta}$  gene segments from their germline repertoire,  $V_{\beta}^a$  mice possess a truncated TCR repertoire (1, 2). We have previously shown that mice with such a deletion in their TCR  $V_{\beta}$  genes ( $V_{\beta}$  truncated haplotype,  $V_{\beta}^a$ ) are unable to respond to two antigenic determinants (sperm whale myoglobin [SWM]<sup>1</sup> 110-121/ $E_{\alpha}^d A_{\beta}^d$  and myelin basic protein [MBP] 1-11/ $A^u$ ) (2). These experiments indicated that there is an absolute limit to the flexibility inherent in the TCR repertoire: the absence of specific TCR  $V_{\beta}$  gene segments resulted in holes in T cell responsiveness to the above-mentioned determinants (2, 3).

One mechanism for maintenance of peripheral tolerance to self- and foreign antigens involves regulatory cells that inhibit the function of antigen-specific T cells such that there is no response upon immunization with specific peptide or protein antigens despite the existence of T cells that can recognize their determinants. Peripheral tolerance to self-peptides exists to prevent autoreactivity by T cells that have escaped deletion during thymic selection (4-6). Peripheral tolerance to foreign antigens is likely to exist to prevent immune pathology caused by the persistence of

certain activated T cells, for example in several parasitic systems (7). Peripheral tolerance or suppression to foreign antigens could also exist as a fortuitous consequence of the cross-reactivity of self-directed regulatory cells. Thus, (a) many foreign antigen determinants associated with self-MHC molecules are structural mimics of MHC-restricted self-determinants and thus should be susceptible to the same regulation as self-antigens (8), and (b) certain foreign and self-determinants induce T cells bearing similar or identical TCR V region gene segments (3, 9). TCR-centered regulatory cells that recognize specific TCR determinants may therefore be able to regulate both foreign and self-antigens through TCR-targeted circuitry (for reviews see references 9 and 10).

We hypothesized that regulatory cells controlling the expression of immune responsiveness toward certain antigenic determinants might also have a limited T cell repertoire. Thus, a lack of 10 TCR  $V_{\beta}$  gene segments in  $V_{\beta}^a$  mice could possibly give rise to holes in the repertoire of the regulatory cells, analogous to the holes in the repertoire of SWM 110-121/ $E_{\alpha}^d A_{\beta}^d$ - and MBP 1-11/ $A^u$ -specific CD4 T cells that we had previously reported. The outcome of such a hole in the repertoire of regulatory cells would be the expression of T cell responsiveness to normally nonimmunogenic determinants.

We show in this report that a strong T cell response, specific for peptide 74-96 of hen egg-white lysozyme (HEL),

<sup>1</sup>Abbreviations used in this paper: HEL, hen egg-white lysozyme; MBP, myelin basic protein; RI, recombinant inbred; SWM, sperm whale myoglobin.

can be induced in H-2<sup>d</sup> mice only when they lack 10 TCR gene segments, presumably encoding a TCR  $\beta$  chain on a potent regulatory T cell. None of the three strains of H-2<sup>d</sup> mice (BALB/c, B10.D2, and F<sub>1</sub>[BALB/c  $\times$  SJL]) with an intact repertoire of 20 TCR V $\beta$  gene segments responds to the peptide HEL 74-96. However, two different H-2<sup>d</sup> recombinant inbred mouse strains ([CxJ]9, [CxJ]8) with the V $\beta^a$ , truncated set of TCR V $\beta$  gene segments show very strong T cell responses to this peptide. Furthermore, although 74-96-specific long-term CD4<sup>+</sup> T cell lines and clones can be readily derived from V $\beta^a$  mice, it was not possible to obtain such a long-term T cell line from BALB/c (V $\beta^b$ ) mice (data not shown). We also show that in vivo treatment with anti-CD8 antibodies of nonresponsive, wild-type V $\beta^b$  mice, allows expression of T cell responsiveness to HEL 74-96. These results reveal a hole in the regulatory repertoire of CD8 T cells in V $\beta^a$  mice that usually controls the response to HEL 74-96.

## Materials and Methods

**Mice.** Mice either were bred at our own laboratory or were obtained from The Jackson Laboratory (Bar Harbor, ME).

**Antigens and Peptides.** Hen egg lysozyme was purified as described earlier (2). Peptide 74-96 was synthesized in bulk and HPLC purified in bulk by Macromolecular Resources (Ft. Collins, CO) in two batches. The sequence of HEL 74-96 (NLC-NIPCSALLSSDITASVNC AK) includes three cysteine residues. One batch of 74-96 was synthesized with the usual cysteine residues, whereas the other batch contained a replacement by  $\alpha$ -aminobutyric acid instead of cysteine in each of the three positions. The two batches of 74-96 showed identical responses in all assays (data not shown) and were used interchangeably. The pepscan series of truncated and extended peptides within the sequence 69-104 were made by synthesis on pins followed by cleavage into 96-well plates (11).

**Antibodies.** Anti-CD8 antibody (clone 53-6.7, a rat IgG2a antibody) and an isotype control antibody, rat IgG2a (rIgG2a), were obtained from PharMingen (San Diego, CA). FITC-conjugated murine anti-CD8 antibody (clone 53-6.7) and FITC-conjugated isotype control antibody were obtained from Becton Dickinson & Co. (San Jose, CA).

**In Vivo Depletion of CD8 T Cells.** 125  $\mu$ g of anti-CD8 antibody in PBS was injected intraperitoneally into BALB/c mice. The control groups were injected either with 125  $\mu$ g of rat IgG2a (PharMingen) in PBS or with PBS alone.

**Immunofluorescence.** Immunofluorescence on LN or splenic cells was performed and analyzed as described earlier (2).

**Immunization.** Mice were immunized with 7 nmol of peptide or protein antigen in the hind footpads with an emulsion of CFA (H37Ra; Difco Laboratories, Detroit, MI). On day 9, LN cells (LNC) from draining LN were harvested and used in antigen-induced proliferation assays or as a source of T cells to generate T cell hybridomas.

**Generation of T Cell Hybrids.** T cell hybridomas were generated from mice immunized with peptide 74-96 as described before (2) except that LNC were harvested and bulk cultured with the peptide 74-96 for only one cycle of stimulation before hybridization. Thus, LNC cultured for 6 d with peptide 74-96 were further cultured for 3 d with rIL-2. T cell blasts were then purified by Ficoll-Hypaque density centrifugation and fused with a

variant of BW5147, BW/ $\alpha^- \beta^-$  (2, 11) as a fusion partner, as described before. The resulting hybrids were seeded at less than one cell per well in 96-well plates; the positive wells were expanded and tested for their ability to produce IL-2 using HT-2 cells (IL-2 dependent) in response to peptide 74-96 and HEL. The antigen-reactive hybrids were further expanded and analyzed for function.

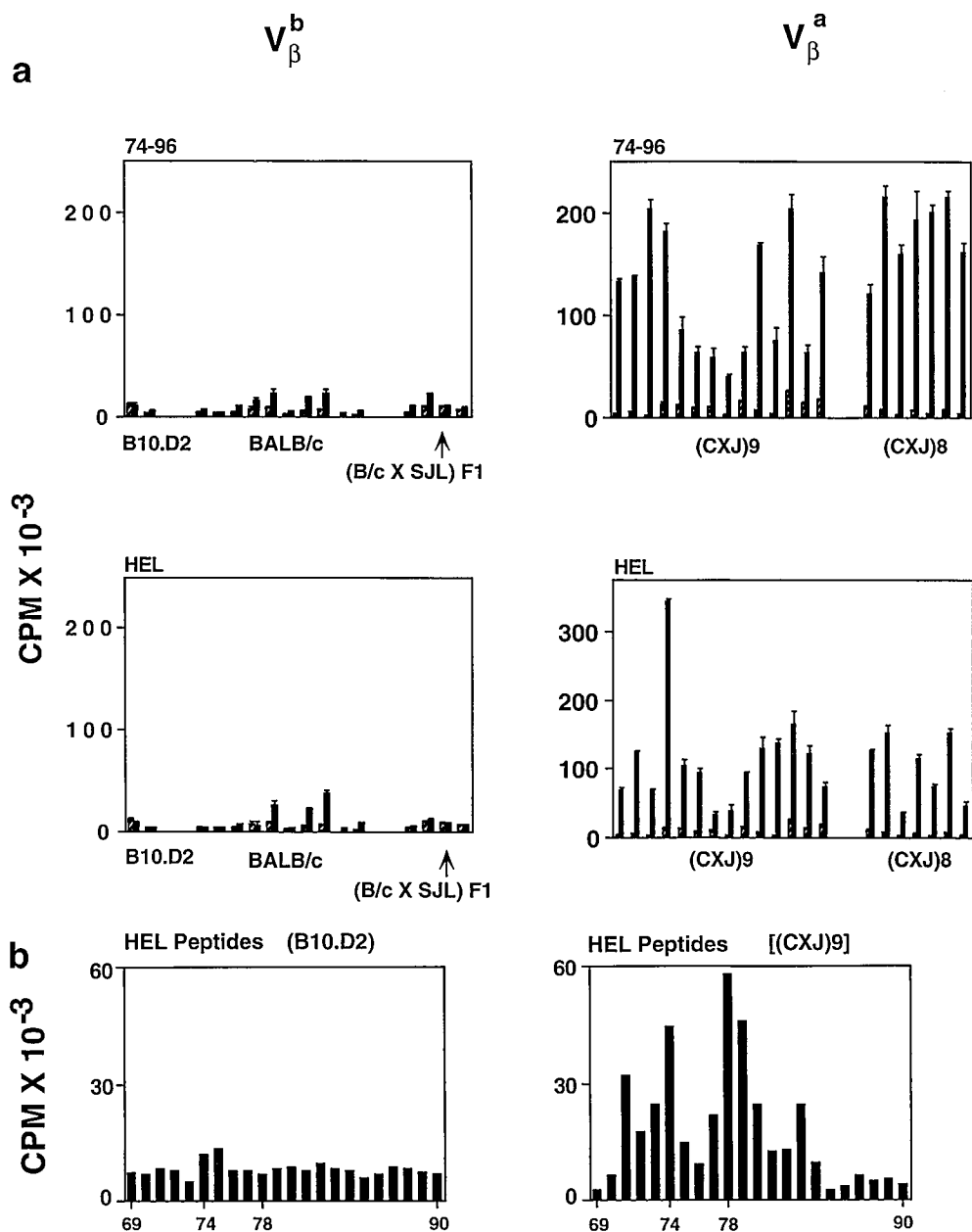
**Specificity Assays.** For antigen-primed LNC, LNC proliferation assays were performed as described earlier (2). Briefly,  $4 \times 10^5$  (unless otherwise indicated) LNC from individual mice were cultured with 14, 7, or 3.5  $\mu$ M concentrations of peptide 74-96 or HEL in 0.2 ml/well of HL-1 medium (Ventrex Laboratories Inc., Portland, ME), supplemented with 2 mM glutamine, 100 U/ml of penicillin, and 100  $\mu$ g of streptomycin. All cultures were done in triplicate. Proliferation was measured by addition of 1  $\mu$ Ci of [<sup>3</sup>H]thymidine for the last 18 h of a 5-d culture, and the incorporation was assayed by scintillation counting.

**T Cell Hybrids.**  $10^5$  T hybridoma cells were cultured with various concentrations of the peptide 74-96, or the pepscan peptides within the sequence 70-104, or HEL, with  $5 \times 10^5$  irradiated BALB/c spleen cells as APC in 0.2 ml of DMEM (Flow Laboratories, Irvine Ayrshire, Scotland) supplemented with 2 mM glutamine, 0.05 mM 2-ME, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin and 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT), as described (2, 11). All cultures were performed in triplicate. The supernatants collected 24 h later were assayed for IL-2 activity on the IL-2/IL-4-dependent cell line, HT-2.  $10^4$  HT-2 cells were cultured with medium alone or supernatants for 48 h. Proliferation was measured by adding 1  $\mu$ Ci [<sup>3</sup>H]thymidine during the last 18 h of culture and incorporation assayed by liquid scintillation counting.

## Results

We compared T cell responsiveness in HEL-immunized H-2<sup>d</sup> mice with and without a wild-type repertoire, to test the hypothesis that the lack of 10 TCR V $\beta$  gene segments in V $\beta^a$  mice could result in a hole in the repertoire of regulatory T cells and lead to a manifestation of T cell responsiveness to certain determinants that are normally nonimmunogenic. For these initial experiments, we used the wild-type (V $\beta^b$ ), H-2<sup>d</sup> haplotype BALB/c mice and the RI, H-2<sup>d</sup> haplotype mice, (CxJ)8 and (CxJ)9, that have a truncated (V $\beta^a$ ) repertoire at their TCR locus. The (CxJ)9 and (CxJ)8 mice have been described earlier (2). A pepscan analysis (using a series of overlapping peptides that walk the whole antigen [HEL] in single amino acid steps) of LNC from HEL-primed (CxJ)9 (H-2<sup>d</sup>, V $\beta^a$ ) and BALB/c (H-2<sup>d</sup>, V $\beta^b$ ) mice revealed that only V $\beta^a$  mice were able to respond to peptides in the 74-96 region of HEL (data not shown). We therefore selected peptide 74-96 (a tryptic peptide of HEL), to further study the differential responsiveness of V $\beta^a$  and V $\beta^b$  mice in this haplotype.

**In the H-2<sup>d</sup> Haplotype, Peptide HEL 74-96 Induces T Cell Responses Exclusively in V $\beta^a$  Mice.** Fig. 1 shows the responses from individual mice of three different V $\beta^b$  strains (B10.D2, BALB/c, and F<sub>1</sub>[BALB/cxSJL], left), and two V $\beta^a$  strains ([CxJ]8 and [CxJ]9; right), H-2<sup>d</sup> mice primed with peptide HEL 74-96. As the RI (CxJ)8 and (CxJ)9 (V $\beta^a$ , H-2<sup>d</sup>) mice were originally derived from BALB/c (V $\beta^b$ ) and SJL/J (V $\beta^a$ ) parents, we chose to use F<sub>1</sub>(BALB/cxSJL) V $\beta^b$  mice

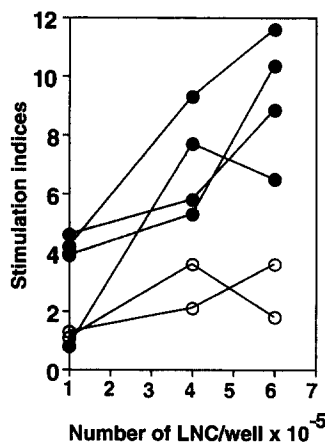


**Figure 1.**  $V_{\beta}^a$  (truncated TCR repertoire) but not  $V_{\beta}^b$  (wild-type TCR repertoire) mice of H-2<sup>d</sup> haplotype are able to respond to HEL 74-96. (a) 16  $V_{\beta}^b$  (B10.D2, BALB/c and [BALB/c × (Cxj)9]F<sub>1</sub>) mice and 21  $V_{\beta}^a$  [(Cxj)9 and (Cxj)8], immunized with HEL peptide 74-96 in foot pads, were tested individually for proliferative responses to p74-96 and HEL using LNC as described earlier (2). The data are represented as mean cpm ± SE ( $\times 10^{-3}$ ) from triplicate cultures of LNC from each mouse at the optimal concentration of 7  $\mu$ M of p74-96 (top) or HEL (bottom). LNC tested at concentrations of either 14 or 3.5  $\mu$ M of peptide or HEL gave similar results (data not shown). The mean ± SE values for medium controls (▨) are presented in front of the bars for p74-96 and HEL (■). The PPD values for the first six  $V_{\beta}^b$  mice were: (mean ± SE  $\times 10^{-3}$ ) 210.8 ± 12.2, 203.2 ± 3.6, 228.0 ± 2.2, 193.4 ± 8.0, 159.8 ± 6.5, 531.7 ± 7.8. The PPD values for the remaining 10  $V_{\beta}^b$  mice and the  $V_{\beta}^a$  mice were in the same range as those for  $V_{\beta}^b$  mice (not shown). (b) LNC from three B10.D2 and three (Cxj)9 mice immunized with p74-96 were pooled and tested for proliferative responses to 15-mer pepsan peptides (peptides covering the sequence HEL 69-104 in one amino acid step). The data are presented as the means of triplicate cultures as described in a.

in order to have comparable strain backgrounds in the  $V_{\beta}^a$  and  $V_{\beta}^b$  groups. The results clearly show that none of the 16 different  $V_{\beta}^b$  mice primed with peptide 74-96 could respond to the HEL peptide 74-96 when recalled with peptide 74-96 (Fig. 1 a, top left). The mean delta counts per minute (cpm of LNC incubated with the peptide 74-96 minus the cpm of cells incubated medium alone) of 21  $V_{\beta}^a$  mice tested individually is 128,000 and that of 16  $V_{\beta}^b$  mice is 5,850. With HEL (Fig. 1 a, bottom left) as the in vitro recall antigen, 3 of 10 BALB/c mice showed a low level of responsiveness. Surprisingly, each of the 21  $V_{\beta}^a$  mice, primed with 74-96, showed strong T cell proliferation to both peptide 74-96 and HEL used as in vitro antigens (Fig. 1 a, right). Similar results were obtained from an additional 10

$V_{\beta}^b$  and 10  $V_{\beta}^a$  mice (data not shown). HEL was used as a recall antigen to ensure that T cells induced by the peptide 74-96 could be stimulated by the determinant derived from natural, intracellular processing of HEL. A pepsan of HEL 74-96-primed  $V_{\beta}^b$  and  $V_{\beta}^a$  mice, recalled by sequential 15-mer peptides within HEL 69-104, is shown in Fig. 1 b. It seems clear that (a) only T cells from  $V_{\beta}^a$  mice recognize the peptide 74-96 and (b) several determinant cores are recognized by T cells from  $V_{\beta}^a$  mice, with peaks at 71-85, 74-88, and 79-93 (Fig. 1 b).

The responsiveness of (Cxj)8 and (Cxj)9 mice might have been owing to contribution of portions of the SJL genome found in each of these strains, other than within the  $V_{\beta}^a$  region. However, this is unlikely because of the lack of



**Figure 2.** Lack of antigen-specific responses of LNC from primed  $V_{\beta}^b$  mice is independent of cell numbers used per well. LNC from four (CxB)9  $V_{\beta}^a$  (●) and 2 BALB/c [ $V_{\beta}^b$ ] (○) mice were tested individually using  $10^5$ ,  $4 \times 10^5$ , and  $6 \times 10^5$  cells per well with or without peptide 74-96 (at  $7 \mu\text{M}$ ). The data are shown as stimulation indices (cpm of LNC cultured with peptide divided by cpm of LNC cultured with medium alone).  $3 \times 10^5$  normal (CxB)9 or BALB/c mice splenic cells were added to wells containing  $10^5$  respective LNC in order to have a minimal cell number of  $4 \times 10^5$  cells per well. The PPD responses of these mice were in the same range as in Fig. 1.

74-96-specific T cell responses found in primed  $F_1$  (BALB/c  $\times$  SJL) mice (Fig. 1 a, left).

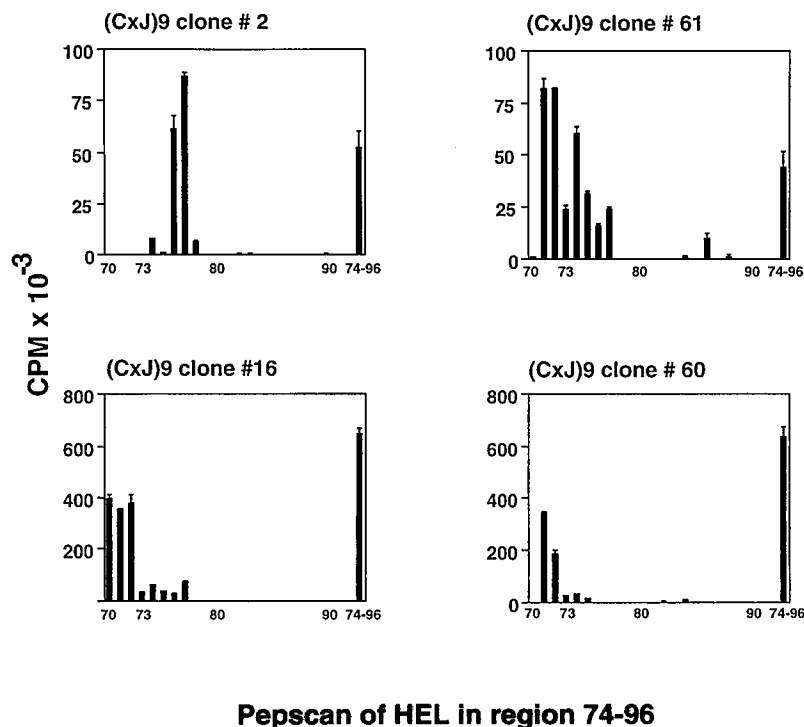
Fig. 2 shows a titration of LNC from individual  $V_{\beta}^a$  or  $V_{\beta}^b$  mice primed with peptide 74-96. It is clear that at  $10^5$ ,  $4 \times 10^5$  and  $6 \times 10^5$  cells per well, LNC from  $V_{\beta}^a$  (CxB)9 mice showed markedly greater proliferative responses than  $V_{\beta}^b$  (BALB/c) mice.

*BALB/c (H-2<sup>d</sup>) Mice Are Able to Process and Present Peptide 74-96 to T Cell Hybrids Derived from (CxB)9 Mice and Specific for Various Determinants within the Peptide 74-96.* Fig. 3 shows the stimulation of four representative 74-96-specific T cell hybridomas derived from (CxB)9 mice using pepscan analy-

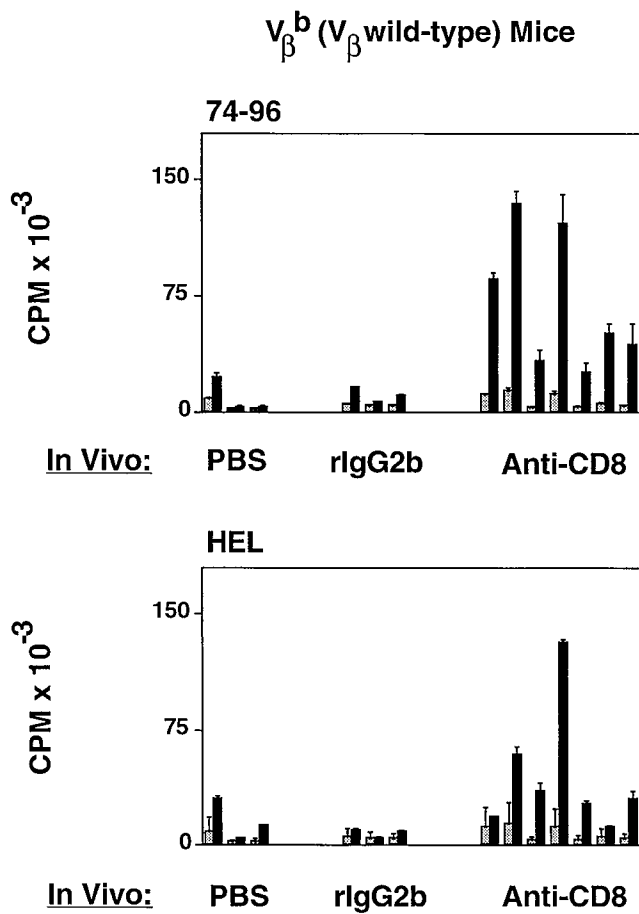
sis within the sequence 70-104. The minimal cores recognized by these T hybrids are (clockwise from top left): 77-90, 72-85, 71-84, and 72-84, respectively. It is surprising that 72-85, 71-84, and 72-84 are minimal cores for the T hybrids derived from mice immunized with peptide 74-96, which lacks residues 71 to 73. This result, however, is reminiscent of and is consistent with our recent report that a single TCR can be stimulated by multiple peptide cores (12). Thus, it is likely that 72-85, 71-84, and 72-84 each contain one of the peptide cores recognized by the latter three T hybrids, respectively.

As BALB/c splenic cells were used as APC in these experiments, it is evident that BALB/c APC are able to process and present each of these determinants to 74-96-specific T cells. Furthermore, each of these T cell hybridomas can be activated by HEL (data not shown), indicating the ability of BALB/c mice to generate all the determinants within peptide 74-96, from the intact protein or from the peptide. We would like to point out that even though it was possible to isolate 74-96-specific CD4 T cell hybrids from  $V_{\beta}^b$  BALB/c mice, their cloning efficiency was more than 10,000 times lower than that of the cloning efficiency of 74-96-specific T cell hybrids from (CxB)9 mice under equivalent conditions (Nanda, N.K., unpublished data). Cloned T hybrids derived from BALB/c mice nevertheless showed a similar diversity of recognition of different minimal determinants on the peptide 74-96.

*In Vivo Deletion of CD8 T Cells Reveals the Expression of Responsiveness to Peptide 74-96 in  $V_{\beta}^b$  Mice.* To explore the possibility that a TCR  $V_{\beta}$  gene required by a regulatory population and missing in  $V_{\beta}^a$  mice was the cause of the responsiveness of (CxB)9 mice to 74-96, we sought proof for



**Figure 3.** Different minimal determinants within HEL 74-96 can be presented by BALB/c APC. Data from one representative of two to three experiments are shown at the optimal dose of  $7 \mu\text{M}$  of peptides. T hybridoma cells were cultured with irradiated BALB/c (H-2<sup>d</sup>) spleen cells as APC and different concentrations of indicated 15-amino acid-long pepscan peptides (69-83 to 90-104) and p74-96 as described earlier and in Materials and Methods. The supernatants were assayed for IL-2 activity on HT-2 cells. The data are expressed as mean cpm  $\pm$  SE of each triplicate culture of HT-2 cells, minus the medium alone background.



**Figure 4.**  $V_{\beta}^b$  mice depleted of CD8 T cells are able to respond to p74-96. BALB/c (shown) and (C $\times$ J)9 (not shown) mice were injected intraperitoneally with either PBS alone or rat IgG2a in PBS, or anti-CD8 antibody in PBS, 4 d before immunization with p74-96. LNC from individual mice were tested in vitro for proliferation in response to p74-96 or HEL, 9 d after immunization as described in Fig. 1. LNC were simultaneously analyzed for presence of CD8 T cells by immunofluorescence (see Results). The data are expressed as described in Fig. 1. In vivo depletion of CD8 T cells in (C $\times$ J)9 mice resulted in a slight decrease (10–29%) in 74-96-specific responses of their LNC (data not shown). The decrease in proliferative responses in (C $\times$ J)9 mice could possibly be due to bystander stimulation of CD8 T cells and its effects on proliferative T cell responses.

the existence of this regulatory CD8 population in wild-type  $V_{\beta}^b$  mice. BALB/c ( $V_{\beta}^b$ ) mice were treated in vivo with anti-CD8 antibody 4 d before immunization with peptide 74-96. Mice treated with anti-CD8 antibody showed a marked depletion of CD8<sup>+</sup> T cells: on day 4 after anti-CD8 treatment (at the time mice were immunized with the peptide), treated mice had <1% CD8 T cells. By day 13 after the treatment with anti-CD8 antibody (9 d after immunization), anti-CD8-treated mice had regained 2.8–4% of their CD8<sup>+</sup> T cells, whereas mice treated with isotype control antibody rIgG2a had 13–15% CD8<sup>+</sup> T cells (data not shown) in their LNC.

It is evident from Fig. 4 that each of the seven anti-CD8 antibody-treated BALB/c mice showed a considerable responsiveness to peptide 74-96, whereas those treated with

PBS or an isotype control antibody remained unresponsive to this determinant. The mean delta counts per minute of three BALB/c mice treated with PBS is 5,040, that of three BALB/c mice treated with control antibody (rIgG2b) is 6,270, and that of seven BALB/c mice treated with anti-CD8 antibody is 63,140. Anti-CD8-treated, peptide 74-96-immunized BALB/c ( $V_{\beta}^b$ ) mice showed generally lower in vitro recall responses to the whole protein HEL than  $V_{\beta}^a$  mice (see Fig. 1 a).

## Discussion

It is evident that constraints in T cell recognition would create gaps not only in the direct repertoire of responsive cells but also in the repertoire of regulatory cells (2, 3). Thus, the absence of 10 TCR  $V_{\beta}$  gene segments in  $V_{\beta}^a$  mice resulted in a hole in the repertoire of CD4 T cell immune responses to two antigenic determinants, SWM 110-121/ $E_{\alpha}^d A_{\beta}^d$  and MBP 1-11/ $A^u$  (2). We now show that  $V_{\beta}^a$  mice with a truncated repertoire of TCR  $V_{\beta}$  gene segments also have a hole in the repertoire of their regulatory CD8 T cells that results in disclosing an underlying T cell immune responsiveness to HEL 74-96/ $A^d$ . The response to this determinant is absent in the wild-type mice displaying a complete repertoire of TCR  $V_{\beta}$  gene segments. CD8 T cell ablation in these wild-type mice, however, results in expression of 74-96-specific T cell responses. Thus, lack of responsiveness in the  $V_{\beta}^b$  mice cannot be attributed to the absence of a 74-96-specific CD4 T cell repertoire or to a defect in antigen processing.

This report is a first demonstration of expression of a CD4 T cell response to a peptide determinant in wild-type mice by simple in vivo ablation of CD8 T cells. CD8 T cells have been implicated in downregulation of immune responses in several systems over the last two decades (for reviews see references 13 and 14). Most of these studies involved complicated in vitro cell reconstitution experiments (13, 14) or adoptive transfer systems (15). Moreover, these studies focussed on experimental inhibition of either T or B cell function, which is distinct from our system in which the  $V_{\beta}^b$ , H-2<sup>d</sup> mice do not respond upon immunization with peptide 74-96 of HEL with CFA, a traditionally powerful immunization regimen. In addition, the expression of CD4 T cell responses in  $V_{\beta}^a$  mice, without having to deplete T cells, involved no experimental manipulation. Our results are aligned with more recent observations where the direct approach of in vivo CD8 T cell depletion was used in the murine experimental autoimmune encephalomyelitis (EAE) model (B10.PL, H-2<sup>u</sup> mice), and shown to result in inducing chronicity of disease as well as a lack of resistance to a second induction of disease in these mice (16). Similar results were obtained using mutant (CD8<sup>-/-</sup>) mice that lack the CD8 gene and therefore CD8 T cells. CD8<sup>-/-</sup> mice undergo more relapses of EAE than wild-type mice immunized by MBP (17). These experiments showed that CD8 T cells, under normal circumstances, downregulate the function of autoimmune CD4 T cells and thus prevent relapses or reinduction of EAE.

Why do  $V_{\beta}^a$ , H-2<sup>d</sup> mice respond to immunization with peptide HEL 74-96 without having been depleted of CD8 T cells? As discussed above, one reason could be the contribution of some genes (other than the TCR genes) from the SJL parent of the RI(CxJ)9 and (CxJ)8 mice (as these mice were derived from BALB/c and SJL grandparents). This appears unlikely as (BALB/c × SJL)<sub>F1</sub>, H-2<sup>dxs</sup> mice (expressing all genes contributed by the SJL parent) are unable to respond to peptide 74-96. Another reason could be that the (CxJ)9 and (CxJ)8 mice express quantitatively smaller amounts of a self-superantigen-like gene product which, when expressed in larger amounts in BALB/c mice, results in the deletion of HEL 74-96-specific CD4 T cells. The absence of 74-96-specific T cell deletion in (CxJ)9 and (CxJ)8 mice could conceivably enable them to respond to HEL 74-96. This is very unlikely because BALB/c mice are able to respond to HEL 74-96 after having been depleted of CD8 T cells. Our results favor the explanation that the lack of T cell responses to HEL 74-96 in wild-type  $V_{\beta}^b$  mice and their expression in  $V_{\beta}^a$  mice is solely determined by the TCR  $V_{\beta}$  locus: it is the lack of 10 TCR  $V_{\beta}$  gene segments underlying the existence of a regulatory contingent, that leads to responsiveness in  $V_{\beta}^a$  mice without having to deplete them of CD8 T cells. These CD8 T cells able to downregulate 74-96-specific T cell responses are present in the wild-type mice and are missing in (CxJ)9 and (CxJ)8 mice because of the absence of a critical TCR  $V_{\beta}$  gene segment in these mice. It is likely that further examples of neo-responses to determinants on other antigens will be detected in such mice.

Our results present evidence that deletion of multiple TCR  $V_{\beta}$  gene segments in an individual can not only result in a lack of immunogenicity, as shown earlier, but also in a gain of immunogenicity to an antigenic determinant, as shown in the current report (also discussed in 2). During evolution, a balance between these opposite repercussions from a loss of TCR  $V_{\beta}$  gene segments may have resulted in expression of different numbers of deletion ligands (self-superantigens) in different strains of mice, leading to a spectrum of possibilities of loss of TCR  $V_{\beta}$  segments across the species (18, 19), which in turn results in gain or loss of immunogenicity to different antigenic determinants in different strains. In addition, these results provide direct evidence that peripheral tolerance to specific antigenic determinants can be mediated by CD8 T cells and can be overcome by in vivo depletion of CD8 T cells.

What is the mechanism by which CD8 T cells prevent the expression of HEL 74-96-specific CD4 T cell responses in wild-type mice? The answer is unclear. We have now succeeded in isolating cloned CD8 T cell hybridomas specific for HEL 74-96 from wild-type BALB/c mice (Nanda, K. et al., unpublished observations). Our preliminary results have revealed that these CD8 T cells are unique in possessing a distinct set of requirements for activation as compared with conventional, cytotoxic CD8 T cells. For example, irradiated splenic APC are unable to activate these CD8 T cells. Whether or not, and how these cells downregulate HEL 74-96-specific CD4 T cells, remain to be learned.

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