Holes in the T Cell Repertoire to Myelin Basic Protein Owing to the Absence of the Dβ2-Jβ2 Gene Cluster: Implications for T Cell Receptor Recognition and Autoimmunity

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Summary

Models of T cell recognition suggest that amino acid residues in the CDR3 region of the T cell receptor (TCR) α or β chain directly contact the major histocompatibility complex–bound peptide, and thus are crucial for providing peptide specificity. T cells derived from B10.PL or PL/J mice of H-2^u haplotype, use only Dβ2 and Jβ2 gene segments in the recognition of the dominant determinant, Ac1-9/A^u, of myelin basic protein (MBP). New Zealand White (NZW) mice, with identical class II H-2^u genes (I-A and I-E), carry an 8.8-kb deletion in their TCR β chain locus encompassing Dβ2 and Jβ2 gene segments. How does this deletion of the crucial Dβ2-Jβ2 region in NZW mice influence specific responses to Ac1-9/A^u as well as to other known A^u or E^u determinants of MBP? We found that these mice respond very poorly to the dominant Ac1-9/A^u and to the subdominant 31-50/E^u determinant in in vitro proliferation assays as well as in their in vivo capacity to induce experimental autoimmune encephalomyelitis. This loss of response is apparently owing to the absence of high avidity TCRs with essential CDR3 residues contributed by Dβ2 or Jβ2 gene segments. These data reveal constraints in the recognition of certain antigenic structures, and further support a TCR-recognition model in which CDR3 residues of the TCR α and β chains constitute the antigenic peptide-binding sites on the TCR molecule. Implications for autoimmune manifestations contributed by NZW genes in (NZB × NZW)F_1 disease are also discussed.

T cells recognize peptide–MHC complexes using specific TCR heterodimers (1). In most peripheral T cells, this recognition is mediated by the TCR–CD3 complex, consisting of invariant CD3 polypeptides (2) in association with polymorphic α and β chains that provide specificity for the recognition of Ag-MHC. As with immunoglobulins, receptor diversity is generated using somatic recombination of variable (V), diversity (D), joining (J), and constant (C) gene segments that encode both chains of the TCR. The homology of Vα and Vβ sequences to those of VH and VL of Ig suggest that they might display a similar secondary and tertiary structure. X-ray crystallographic studies of Ig have shown that V region domains on each chain form a series of loops that constitute complementarity determining regions (CDR)[1] for antigen binding. Based on Ig structure, models for TCR-Ag-MHC recognition have been proposed in which the CDR3 region interacts with the MHC-bound peptide and CDR1 and CDR2 interact with the flanking MHC α-helices (3–5). It has been shown that junctional CDR3 sequences correlate with peptide recognition in T cells with similar specificity (6–10). Consistent with this model, mutations in conserved junctional residues result in altered recognition of I-E^k-cytC (11). Recently, direct CDR3-peptide contact has been suggested in a transgenic model for cytochrome C recognition, where substitution of charged residues on the peptide elicited reciprocal charges in CDR3 sequences upon peptide immunization (12).

New Zealand white (NZW) mice have generated interest because of their role in the autoimmunity of New Zealand black (NZB × NZW)F_1 hybrids, which display similar features to those observed in human systemic lupus erythematosus (SLE) (13). The exact basis of their genetic contribution to the F_1 disease is not yet understood. Both H-2–linked (14–16) as well as TCR genes (17) and other non-MHC background genes have been suggested to be important in disease development. Furthermore, it is clear that although SLE is a B cell–mediated disease, T cells are evidently involved at the initial stages of autoimmunity (18, 19).

[1] *Abbreviations used in this paper:* CDR, complementarity determining region; EAE, experimental autoimmune encephalomyelitis; Gp, guinea pig; m, mouse; MBP, myelin basic protein; NZB, New Zealand black; NZW, New Zealand white.

The sequences of I-Aα, I-Aβ, I-Eα, and I-Eβ from NZW mice, originally tissue-typed as the unique haplotype, H-2^z, are actually identical to the respective genes of mice of the H-2^u haplotype, such as B10.PL and PL/J (20, 21). Initially, most myelin basic protein (MBP)-reactive T cells from B10.PL or PL/J mice respond to the immunodominant NH_2-terminal fragment Ac1-9, and predominantly use the TCR Vβ8.2 gene segment (7, 8). Although, other Vβ gene segments (Vβ13 or Vβ4) are also used for the recognition of Ac1-9–specific T cells in both mouse strains, all T cells that have been analyzed only used the same Dβ and Jβ gene segment cluster, Dβ2-Jβ2 (see Table 1). Moreover, junctional amino acid residues across the third hypervariable region were conserved in spite of N-region variation at the nucleotide level (7, 22). Importantly, in spite of the different non-MHC genetic background in B10.PL and PL/J mice, TCR V-gene segment usage was highly similar and restricted in the recognition of Ac1-9/A^u. Since NZW mice also express identical MHC class II molecules, i.e., I-A^u and I-E^u (20, 21), we were interested in analyzing the TCR-recognition pattern of Ac1-9/A^u in this mouse strain because of its genomic deletion of an 8.8-kb segment of DNA containing the crucial Dβ2 and Jβ2 gene segments (23). Although, the relative importance of these gene segments in antigen recognition has been demonstrated in congenic strains such as BALB/c and the congenic partner BALB/c.Vβ^{NZW} that bears the Dβ2-Jβ2-deletion (24), responses to specific determinants whose recognition is biased towards the usage of Dβ2-Jβ2 gene segments have not been analyzed. Would NZW mice, in the absence of this gene cluster, be able to exploit an alternative gene segment in order to respond to Ac1-9/A^u?

We therefore have asked whether the T cell repertoire is highly constrained in its usage of the Dβ-Jβ2 region in the recognition of Ac1-9. We have shown that in the absence of genes coding for the appropriate CDR3 region, the NZW mouse simply fails to respond to this dominant determinant on MBP. Likewise, upon peptide immunization, NZW mice are almost nonresponsive to Ac1-9/A^u and 31-50/E^u, whereas response to other, subdominant/cryptic determinants of MBP, different determinants within Ac1-20 and MBP 121-140/A^u, remained unaffected. The loss of response to the dominant and subdominant self-determinants has important consequences for self-tolerance and autoimmunity.

Table 1. *The Dβ2 and Jβ2 Gene Segments Are Used for Recognition of Ac1-9/A^u Irrespective of the Non-MHC Background Genes*

Vβ gene segments	Dβ-Jβ gene segments	B10.PL mice	PL/J mice
		%	
8.2	2-2.6	79	50
8.2	2-2.3	–	25
8.2	2-2.5	–	12
13	2-2.2	21	–
4	2-2.5	–	12

See references 7, 8, 22.

Materials and Methods

Mice. B10.PL and NZW mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and bred under specific pathogen-free conditions in our own colony. Female mice were used at 8–20 wk of age.

TCR Peptides. Peptide Ac1-9 was synthesized by C. Miles (Macromolecular Resources, Fort Collins, CO), and MBP Ac1-20, MBP31-50, and MBP121-140 were synthesized by S. Horvath (Caltech, Pasadena, CA) using a solid phase technique on a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) and purified on a reversed phase column by high performance liquid chromatography (25).

Proliferation Assays. For lymph node proliferation assays, mice were immunized subcutaneously with 7 nmol of the mMBP peptides or 100 μg of GpMBP, emulsified (1:1) in CFA (Difco Laboratories, Detroit, MI). The draining popliteal and inguinal lymph nodes were removed 10 d after immunization and a single cell suspension was prepared. Lymph node cells (4×10^5 cells/well) were cultured in 96-well microtiter plates in 200 μl of serum-free medium (HL-1; Ventrex Laboratories, Portland, ME) supplemented with 2 mM glutamine; peptides were added at concentrations ranging from 0.1 to 7 μM, final concentration. Proliferation was assayed by addition of 1 μCi [^{3}H]thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 h of a 5-day culture, and incorporation of label was measured by liquid scintillation counting.

The antigen processing and presentation capacity of NZW APCs was determined by measuring antigen-specific proliferation of the Ac1-9–specific T cell clone, 2C6 (7). T cells (5×10^4–1×10^5 cells/well) were incubated with irradiated spleen cells ($1–5 \times 10^5$ cells/well) in the presence of MBP or Ac1-9 at different concentrations (1 nM to 14 μM). Proliferation of the T cell clone was measured by [^{3}H]thymidine incorporation for the last 18 h of a 3-day culture as described above.

Induction of Experimental Autoimmune Encephalomyelitis (EAE). MBP was isolated from the brains of guinea pigs (Pel-Freez Biologicals, Rogers, AR) or mice as described (26). For induction of EAE, mice were immunized subcutaneously with 100 μg mMBP or its peptides in CFA. 0.1 μg pertussis toxin (List Biological Laboratories Inc., Campbell, CA) was injected in 200 μl of saline, intravenously, 24 and 72 h later. Mice were observed daily for signs of EAE as previously described (27, 28) and until >60 d after MBP immunization.

Flow Cytometry Analysis. To determine the expression of Vβ8.2$^+$ T cells, the following mAbs were used: anti-CD4-PE (GK1.5, Becton Dickinson & Co., Mountain View, CA) and anti-Vβ8.2 (F23.2) (29). F23.2 antibody was purified from hybridoma supernatants by protein A chromatography. Antibodies were used in PBS containing 1% fetal bovine serum. 10^6 splenocytes, after red blood cell lysis, were stained with 0.5 μg of antibody in a total volume of 50 μl at 4°C for 30 min. Cells were washed twice with PBS and then resuspended in 50 μl of a 1:50 dilution of goat anti-mouse FITC (Southern Biotechnology Associates, Birmingham, AL). After 20 min at 4°C, cells were washed, fixed with 1% paraformaldehyde in PBS, and analyzed using a cytofluorograph (Becton Dickinson & Co.).

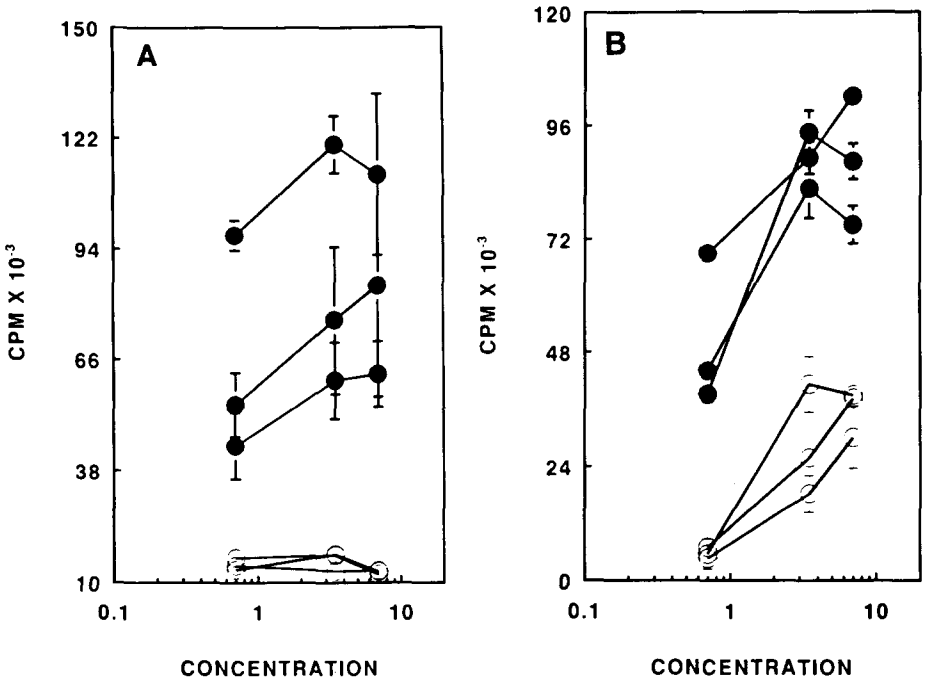

Figure 1. The loss of the dominant response to MBP Ac1-9/A^u in NZW mice. Proliferative responses of GpMBP-primed lymph node cells from NZW (*A*) and B10.PL (*B*) mice were recalled in vitro with various concentrations (μM) of Ac1-9 (○) or MBP (●). Responses from three individual mice are shown. The data are expressed as arithmetic means ± SD of [^{3}H]-thymidine incorporation (cpm × 10^{-3}) in triplicate cultures. The background incorporation in NZW (*A*) and B10.PL (*B*) mice ranged from 10,263 to 11,960 and 1,372 to 2,480 cpm, respectively. This experiment is one representative of three separate experiments.

Results and Discussion

MBP Does Not Induce the Dominant Ac1-9–specific T Cells. To determine the consequences for the dominance of response to Ac1-9 in the absence of critical Dβ-Jβ genes, both NZW (Fig. 1 *A*) and B10.PL (Fig. 1 *B*) mice were immunized with MBP and the T cell proliferative recall to MBP or Ac1-9 was followed in the draining lymph nodes. Comparable responses to MBP were found in both mouse strains. However, the proliferative recall to Ac1-9, present in B10.PL mice, was absent in NZW mice immunized with MBP (Fig. 1 *A*). This suggests that these animals either are nonresponsive to Ac1-9, or they are able to respond to Ac1-9, but in the absence of certain critical CDR3-bearing T cells, Ac1-9 behaves as a cryptic determinant and these T cells can not be recalled after MBP immunization (30).

Peptide Immunization Fails to Stimulate Ac1-9/A^u–specific T Cells. Proliferative responses to Ac1-9 were then tested in mice immunized with the peptide itself emulsified in CFA. Fig. 2 indicates that lymph node cells from NZW mice showed almost no proliferation to Ac1-9 (stimulation indices <3), whereas peptide-reactive T cells were readily primed in B10.PL mice. Indeed, the primed T cells could be recalled with MBP in B10.PL mice, indicating dominance of the Ac1-9/A^u–specific response. Furthermore, immunization as well as in vitro recall with an almost 100-fold higher concentration (700 nmol) of Ac1-9 did not result in any significant increase in lymph node proliferation in NZW mice (Fig. 3 *B*; stimulation indices <3). These observations clearly demonstrate the absence of the T cell proliferative response to Ac1-9 in NZW mice and further suggest an essential role for particular amino acid residues in the CDR3 region in the recognition of Ac1-9/A^u. Other T cells, directed against a cryptic determinant within MBP Ac1-20 (region 6-20 of MBP), remain unaffected as peptide-immunization resulted in a comparable proliferative response to that in B10.PL mice (Fig. 2). Similarly, re-

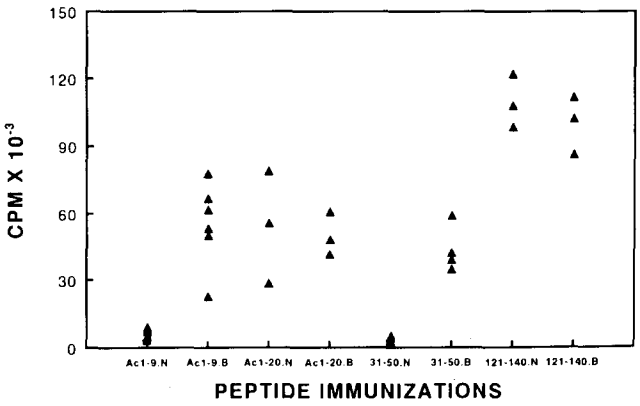

Figure 2. NZW mice respond very poorly to the dominant Ac1-9/A^u as well as to the subdominant 35-47/E^u. Groups of mice (N, NZW; B, B10.PL) were immunized subcutaneously with 7 nmol of each peptide in CFA. 10 d later, lymph node proliferative responses to respective peptides were measured at varying concentrations of antigen. Response of 3–6 individual mice to each peptide at the optimal concentration of 7 μM is shown. Background incorporation with medium alone ranged from 658 to 4,251 cpm. The data are expressed as arithmetic means of [^{3}H]thymidine incorporation (cpm × 10^{-3}) in triplicate cultures. The proliferative recall responses to purified protein derivative in both B10.PL and NZW mice were comparable and ranged from 65,000 to 140,000 cpm. These data are representative of three separate experiments.

sponses to other downstream determinants of MBP remain unaltered (Kumar, V., K. Stellrecht, M. Meyer, and E. Sercarz, manuscript in preparation).

The Subdominant 31-50/E^u–reactive T Cells Fail to Be Primed by Peptide Immunization. Interestingly, a proliferative response to the subdominant determinant MBP 31-50/E^u was also not detected after peptide immunization (Fig. 2). As with Ac1-9/A^u, challenge and in vitro recall at a 100-fold higher concentration (700 nmol) of MBP 31-50/E^u did not show any proliferation (data not shown). It has been shown previously that T cells specific for MBP 35-47 (or 31-50) do not use Vβ8 gene segments (31). However, Dβ-Jβ gene segments used by 31-50–reactive T cells have not been identified. The absence of a proliferative response to this subdominant determinant of MBP in NZW mice is a strong indication that 31-50/E^u–specific T cells also predominantly express Dβ2 or Jβ2 gene segments.

Response to the 121-140/A^u Determinant Is Unaffected by the Absence of the TCR Dβ2 or Jβ2 Gene Segments. We then asked whether the response to other known A^u-restricted determinants on MBP, for example MBP121-140 (32), was compromised due to deletion of the Dβ2-Jβ2 cluster in NZW mice. Mice were immunized subcutaneously with MBP 121-140 and 10 d later, the proliferative response was checked in the draining lymph nodes (Fig. 2). All mice responded well to this subdominant/cryptic determinant when peptide was used for immunization. Presumably, MBP121-140–reactive T cells use other Dβ and Jβ genes and therefore, response to this determinant was not affected.

Failure to Respond to Ac1-9 Is Not Linked to the Peripheral Expression of the Vβ8.2 Gene Segment. To determine whether there might be an overall decrease in Vβ8.2$^+$ T cells in NZW mice, we next examined their expression in peripheral T cells by flow cytometry. Actually, expression of the Vβ8.2 gene segment was significantly increased in NZW mice (CD4$^+$-Vβ8.2$^+$ = 9.8%) compared with B10.PL mice (CD4$^+$-Vβ8.2$^+$ = 5%). The loss of the dominant Ac1-9/A^u response in these mice is therefore not due to lower expression of the Vβ8.2 gene segment among mature T cells.

APC from NZW Mice Are Not Defective in Processing MBP or in Presenting Ac1-9. One possibility is that NZW mice have an appropriate MHC molecule (A^u) as well as a T cell repertoire capable of recognizing Ac1-9, but a processing defect exists preventing formation of the Ac1–9/A^u complex. Several factors determine the binding of a given peptide including its availability after antigen processing. A processing defect resulting in genetic unresponsiveness to a hen egg lysozyme determinant by failure to remove a single amino acid hindering peptide binding to MHC has recently been shown (Grewal, I., K. Moudgil, E. E. Sercarz, manuscript submitted for publication). Accordingly, we tested whether NZW splenic APC pulsed with MBP or Ac1-9 were able to stimulate an Ac1-9–specific T cell clone derived from B10.PL mice. Fig. 4 clearly demonstrates that APCs from NZW mice were able to present Ac1-9 to specific T cells in vitro. Also, since NZW APCs were able to stimulate Ac1-9–reactive T cells when pulsed with whole MBP, there seemed to be no defect evident in antigen processing.

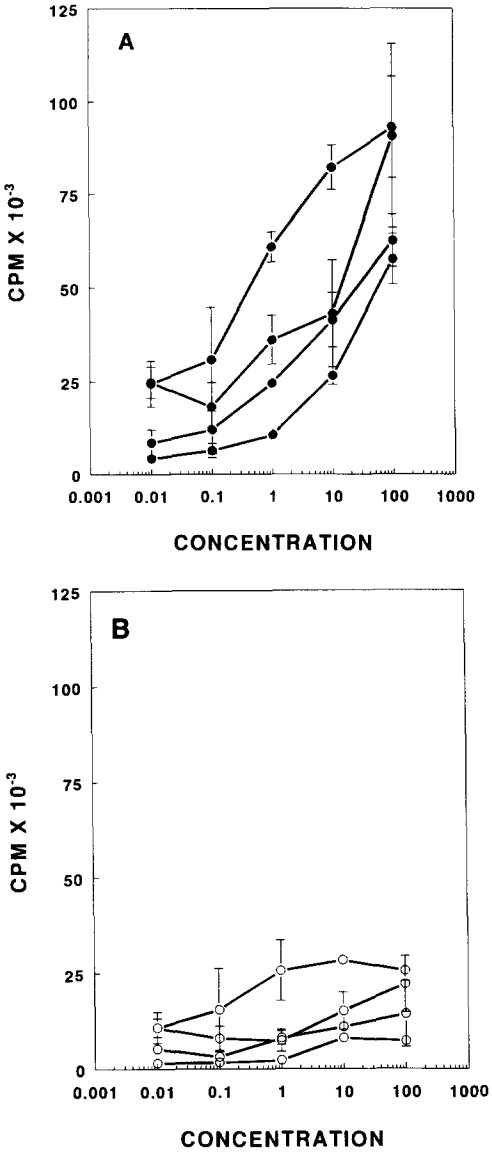

Figure 3. Challenge of NZW mice with increased antigenic dose does not overcome proliferative unresponsiveness to Ac1-9. Both B10.PL (*A*) and NZW (*B*) mice were immunized with Ac1-9 (700 nmol) in CFA. 10 d later, lymph node proliferative responses to various concentrations (μM) of the antigen were recalled in vitro, as in Fig. 1. Responses from four individual mice are shown. The data are expressed as arithmetic means ± SD of [^{3}H]thymidine incorporation (cpm × 10^{-3}) in triplicate cultures. This experiment is one representative of two separate experiments.

Peptide Immunization Also Fails to Induce a Significant Incidence of EAE. The poor proliferative responses to Ac1-9 and 31-50 in NZW mice shown by in vitro assays were further confirmed by monitoring induction of Ac1-9 or 31-50-induced EAE as a measure of in vivo response. Table 2 shows that the incidence of disease is extremely low when mice were

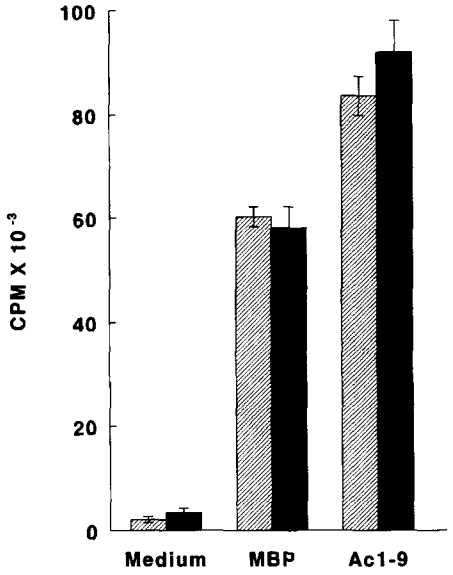

Figure 4. NZW splenic APCs are able to process MBP and present Ac1-9. MBP or Ac1-9–specific proliferation of clone 2C6, derived from B10.PL mice after challenge with MBP (7), was measured in the presence of irradiated splenic cells from NZW (■) or B10.PL (▨) mice. ^{3}H-Incorporation in response to an optimum concentration of antigen (7 μM) is shown. The data are expressed as arithmetic mean ± SD of [^{3}H]thymidine incorporation (cpm × 10^{-3}) in triplicate cultures. Clone 2C6 is specific for Ac1-9 and does not recognize any of the other determinants of MBP, for example, MBP 31-50, 121-140, or 131-150.

injected with either of these two determinants with adjuvant and pertussis toxin. However, immunization with whole MBP as well as with other determinants, Ac1-20 and MBP 121-140, resulted in a significant incidence of EAE in NZW mice, although, the onset of disease in NZW mice was later (19–21 d) than in B10.PL mice (10–13 d) after antigenic challenge. Thus, disease induction with whole MBP in NZW mice must be due to the presence of other dominant and cryptic encephalitogenic determinants on MBP, for example, 121-140 (Kumar, V., K. Stellrecht, M. Meyer, and E. E. Sercarz, manuscript in preparation). Similar findings regarding the induction of EAE with Ac1-11 and 35-47 have recently been reported (33).

Table 2. *Susceptibility of B10.PL and NZW Mice to EAE Induction with the Dominant or the Subdominant Determinant of MBP*

Antigen	B10.PL mice	NZW mice
MBP	8/11	6/12
Ac1-9 (Expt. 1)	8/10	1/13*
Ac1-9 (Expt. 2)	5/5	0/5
Ac1-20	5/5	4/5
MBP 121-140	4/5	3/5
MBP 31-50 (Expt. 1)	6/10	0/9
MBP 31-50 (Expt. 2)	3/5	0/5

* A single mouse appeared to have developed transient tail paralysis (for 3 d) after almost 30 d. Although the severity of EAE in both mouse strains induced with mMBP as well as with Ac1-20 and 121-140 was comparable, the onset of disease was 5–8 d later in NZW mice (15–20 d after antigenic challange).

Absence of Critical Residues within CDR3 Constrains T Cell Recognition Specificity. These data demonstrate constraints in the recognition of certain antigenic structures in spite of the myriad possibilities for TCR diversity. Presumably, the lack of particular dominant T cells with high avidity TCRs results in nonresponsiveness even to strong antigenic determinants, or "holes in the repertoire." Genetic unresponsiveness to protein antigens has generally been attributed to three different mechanisms: the failure to bind to MHC molecules (34–36); or to holes in the T cell repertoire, even when the peptide binds to MHC (37, 38); or to regulatory effects (39). The absence of an appropriate T cell repertoire capable of recognizing the antigen has been attributed primarily to clonal deletion during thymic tolerance induction (40) or to peripheral clonal exhaustion (41) after a vigorous response. It has been previously demonstrated that the germline deletion of appropriate Vβ gene segments compromises the immune responsiveness to certain antigens (42). In these studies, chimeric mice with a truncated Vβ^a repertoire missing the TCR Vβ8 and Vβ13 gene segments and expressing H-2^u MHC genes were unable to devise a response to Ac1-9/A^u. Here, we have demonstrated that the lack of appropriate genes within the CDR3 region results in the loss of a proliferative response to a dominant and a subdominant self-determinant, probably owing to the absence of high avidity TCRs capable of recognizing the MBP Ac1-9/A^u or the 31-50/E^u complexes, respectively.

As in the comparison of the response to Ac1-9/A^u in B10.PL and PL/J mice, it has been shown that remarkably similar junctional sequences among TCR β chains are employed to respond to a given determinant of sperm whale myoglobin by animals that differ in their non-MHC background genes, including Mls (43). Thus, the lack of response to Ac1-9 in NZW mice suggests that the TCR structures capable of recognizing this determinant are similar with respect to the usage of Dβ-Jβ gene segments in all three H-2^u haplotypes. Although the presence of extremely low affinity/frequency T cells of Ac1-9/A^u or 35-47/E^u-specificity in NZW mice cannot be ruled out, their activity is not detectable with in vitro or in vivo assays. It is possible that low affinity T cells specific for these determinants still exist and are of the Th2 type. These cells usually do not proliferate well and are likely to be poorly encephalitogenic. Thus, holes in the repertoire exist, probably due to the absence of TCRs employing appropriate Dβ-Jβ gene segments. It seems that the interaction between the TCR structures and their ligands, Ac1-9/A^u or 31-50/E^u complexes, is stringent enough to de-

mand not only specific Vβ gene segments but also specific residues in the CDR3 region (Dβ2-Jβ2), despite the availability of closely related gene segments within the Dβ1-Jβ1 region. These data favor proposed models of TCR recognition (4, 5, 12) that stress involvement of the region in the neighborhood of the V, (D), and J junctions of the α and β TCR chains in constituting the antigenic peptide-specific sites on the TCR molecule.

We would like to suggest a hypothesis that NZW genes have two functions in promoting autoimmunity in the (NZB × NZW)F_1 mouse. First, they provide the H-2^u haplotype which permits immune responsiveness to certain self-determinants; second, they mediate autoimmunity by failing to provide an adequate regulatory repertoire. The deletion of Dβ2 and Jβ2 gene segments in NZW mice may not only affect the emergence of effector T cells, for example, specific for Ac1-9/A^u, but may also affect the appearance of a regulatory T cell repertoire in the F_1. In this connection, it is intriguing that (NZB × B10.PL)F_1 or (NZB × PL/J)F_1 do not develop characteristic anti-DNA antibodies. The lack of this protective regulatory T cell subset (for example, in reference 44) could result in the loss of peripheral tolerance to a self-antigen, leading to autoimmunity (28, 45).

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