Induction of a type 1 regulatory CD4 T cell response following V\(_{\beta}\)8.2 DNA vaccination results in immune deviation and protection from experimental autoimmune encephalomyelitis

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Abstract

DNA vaccination has been used to generate effective cellular as well as humoral immunity against target antigens. Here we have investigated the induction and involvement of regulatory T cell (T\(_{reg}\)) responses in mediating prevention of experimental autoimmune encephalomyelitis (EAE), following vaccination with plasmid DNA encoding the TCR V\(_{\beta}\)8.2 chain predominantly displayed on disease-causing lymphocytes. Vaccination with DNA encoding the wild-type TCR results in priming of type 1 CD4 T\(_{reg}\) and skewing of the global response to myelin basic protein in a T\(_h\)2 direction, leading to significant protection from disease. In contrast, vaccination with mutant DNA encoding altered residues critically involved in recognition by the T\(_{reg}\) results in priming of a type 2 regulatory response which fails to mediate immune deviation or protection from EAE. Control mice immunized with DNA, encoding TCR with changes at an irrelevant site, were protected from antigen-induced disease. Furthermore, protection can be transferred into naive recipients with CD4 T\(_{reg}\) from wild-type DNA-immunized mice but not from animals vaccinated with the mutant DNA. These data suggest that vaccination with plasmid DNA encoding one or multiple V\(_{\beta}\) genes can be exploited to enhance natural regulatory responses for intervention in autoimmune conditions.

Introduction

Immunization with plasmid DNA encoding a protein antigen results in effective and long-lasting cellular and humoral immunity in several antigenic systems (1,2). Such DNA immunization can evoke both CD8 and CD4 T cell responses mediated by presentation of antigenic determinants in the context of class I and class II MHC molecules respectively (3–5). The intramuscular delivery of DNA generally induces an antigen-reactive, proinflammatory type 1 CD4 T cell response, through the activation and processing and presentation of antigen by bone marrow-derived professional antigen-presenting cells (APC) (6–8). The plasmid vector also contains immunostimulatory nucleotide sequences—unmethylated CpG motifs that activate APC, e.g. dendritic cells (DC), resulting in secretion of IL-12. The bone marrow-derived DC could potentially get transfected with the DNA, and directly process and present the encoded antigen (9–11). Alternatively, DC could cross-prime T cells after acquiring antigenic protein from other cell types; data supporting both routes of antigen presentation by DC have been reported. In rodents, DNA vaccines have been shown to induce protective, cell-mediated immunity against organisms such as Leishmania major, Mycobacterium tuberculosis, and conditions such as malaria, tumors and allergen-induced anaphylaxis (1,2). A recent report of the induction of cellular immune responses...
to a peptide of *Plasmodium falciparum* in humans by a DNA vaccine raises hopes for the clinical applicability of this method of immunization (12).

Experimental autoimmune encephalomyelitis (EAE) is a prototypic CD4 T cell-mediated autoimmune disease model for the human demyelinating disease multiple sclerosis (13). It is characterized by inflammation and demyelination in the central nervous system accompanied by paralysis following immunization with myelin antigens, e.g. myelin basic protein (MBP). A majority of the MBP-primed effector CD4 T cells which mediate EAE in H-2<sup>d</sup> mice recognize the N-terminal peptide MBP<sub>Ac1–9</sub> or Ac1–20 and predominantly use the TCR V<sub>β</sub>8.2 gene segment (14,15). Although the regulation and function of individual cytokines is complex, most experimental observations are consistent with the idea that myelin antigen-specific T<sub>H</sub>1 cells are encephalitogenic, whereas a T<sub>H</sub>2 response is protective (16,17).

Recently, vaccination with TCR V<sub>β</sub>8.2 plasmid DNA has been shown to result in significant protection from subsequent induction of antigen-induced EAE (18). However, the mechanism or the involvement of a regulatory T cell (T<sub>reg</sub>) response in the prevention of disease has not yet been defined. Furthermore, it has not been described how intramuscular V<sub>β</sub>8.2 DNA immunization that predominantly induces CD4 T<sub>H</sub>1 cells results in deviating the response to MBP in a protective T<sub>H</sub>2 direction. Here we have examined whether DNA vaccination can prime an appropriate T<sub>reg</sub> response that controls EAE (19). Using mutant V<sub>β</sub>8 DNA molecules and cell transfer strategies, we demonstrate that T<sub>reg</sub> reactive to the TCR peptide B5 (amino acids 76–101) containing the framework region (FR) 3 determinant are involved in mediating skewing of the anti-MBP response in a protective type 2 direction and prevention of disease. These findings have important implications for preventive or therapeutic vaccine approaches for T cell-mediated pathological conditions.

**Methods**

**Construction of plasmid DNA**

The plasmids for genetic vaccination were made as follows: for the V<sub>β</sub>8.2 construct, a plasmid containing the V<sub>β</sub> domain gene (V<sub>β</sub>-D<sub>β</sub>-J<sub>β</sub>) with a C-terminal c-myc epitope has been described previously (36). A 5' EcoRI site followed by an in-frame methionine codon were appended to the 5' end of the gene to encode the mature V<sub>β</sub> domain using the PCR and the following oligonucleotide primer: 5'-ATC AGA ATT CAT GGA GGC TGC AGT CAC CCA A-3'. The 3' end of the gene (i.e. the c-myc epitope tag) was tailored with a BamHI site using the PCR and the primer: 5'-TGA TGG ATC CTT AGA GAA CAG TCA GTC TGG T-3'. The gene was initially cloned into the vector pCMV5 as an EcoRI–BamHI fragment and then subsequently ligated into pCMV8 (a derivative of pCMV5 with an additional leader intron which expresses higher levels of protein) as a Sall–BamHI fragment. Mutated variants of the V<sub>β</sub>8.2 gene were generated by site-directed mutagenesis (37) (Q85A, V88A and F90A, where Q85A indicates mutation of Gln85 to alanine, etc.) or splicing by overlap extension (38) (V10A, V125 and L107A). These mutated V<sub>β</sub> genes were then used to replace the wild-type region of the V<sub>β</sub>8.2 gene as PstI (site overlapping codons 2–4 of the mature gene)–BstEII (site 5’ to c-myc tag fragments). pCMV8 has a PstI site in the polylinker proximal to the 5’ end of the V<sub>β</sub> gene and this cloning therefore resulted in the loss of ~25 bases of coding sequence. Following PstI digestion, this sequence was ligated into the construct as an oligonucleotide duplex to reconstitute the complete coding sequence. The V<sub>β</sub>3 construct, containing the V<sub>β</sub>3 domain gene derived from the 2B4 hybridoma (a generous gift of Dr Mark Davis, Stanford University), was made in an analogous way to the wild-type V<sub>β</sub>8.2 construct. The inserts of all constructs were sequenced prior to use. All mice were s.c. immunized with MBP<sub>Ac1–9</sub>/complete Freund's adjuvant (CFA) for the induction of EAE.

**Mice**

B10.PL and SJL/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). (SJL×B10.PL)<sub>F1</sub> mice were bred under specific pathogen-free conditions in our colony. Female B10.PL or (SJL×B10.PL)<sub>F1</sub> mice, as indicated in the text or in legends for the figures and tables, were generally used at 10–14 weeks of age, and were maintained on standard laboratory diet and water ad libitum in specific pathogen-free animal facilities at UCLA and LIAI.

**TCR peptides**

The TCR V<sub>β</sub>8.2 peptides used were the same as reported previously (19): B1, amino acids 1–30L (TCR FR1 region peptide); B4, amino acids 61–90; B5, amino acids 76–101 (TCR FR3 region peptide).

**Measurement of antigen-specific proliferative responses**

Proliferative responses to MBP<sub>Ac1–9</sub> or Ac1–20 and TCR peptides were determined in lymph nodes or spleens essentially as described earlier (19). To monitor priming of CD4 T<sub>reg</sub> spleens were removed 7–10 days after the last plasmid DNA injection. Lymph node cells (4×10<sup>5</sup> cells/well) and splenocytes (8×10<sup>5</sup> cells/well) were cultured in 96-well microtiter plates in 200 µl of serum-free medium (HL-1; Ventrex, Portland, ME/X-vivo 10; BioWhittaker, Walkersville, MD) 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 [<sup>3</sup>H]thymidine (ICN, Irvine, CA) for the last 18 h of a 5-day culture and incorporation of label was measured by liquid scintillation counting.

**Induction and clinical evaluation of EAE**

Mice were immunized s.c. with 100 µg of guinea pig MBP or Ac1–9 or its high-affinity variant (9.4Met) emulsified in CFA; 0.15 µg pertussis toxin (PT) (List Biological, Campbell, CA) was injected in 200 µl saline i.v. 48 h later. Mice were observed...
daily for signs of EAE until 40–60 days after immunization. The average disease score for each group was calculated by averaging the maximum severity for all of the affected animals in the group. Disease severity was scored on a five-point scale, as described earlier (19): 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, hind and front limb or whole body paralysis; 5, moribund or death.

Measurement of lymphokine secretion

The frequency of antigen-induced IFN-γ- or IL-4-producing T cells was determined using the sensitive, single-cell ELISA-spot assay or ELISA, as described earlier (39). For ELISA-spot assays, after culture of lymph node or splenic cells with antigen for 48 h, live cells were recovered, washed and transferred by serial dilution (from 10^4 to 5 x 10^3 cells/well) to 96-well microtiter plates (Millipore, Bedford, MA) that had been precoated with the capturing mAb (anti-IFN-γ or anti-IL-4) at 2 mg/ml. After 24 h, the cells were removed, and spots were visualized using biotinylated detecting mAb and avidin peroxidase in conjunction with 3-aminooethylcarbazole (Sigma, St Louis, MO) substrate. Spots were counted under a dissecting microscope, and the frequency of antigen-specific cells was determined from the difference between the number of spots seen with and without antigen. All capturing and detecting antibody pairs were purchased from PharMingen (San Diego, CA).

Results

**Vβ8.2wt DNA vaccination protects B10.PL mice from antigen-induced EAE**

B10.PL mice were immunized 2–3 times at weekly intervals intramuscularly with plasmid DNA encoding the Vβ8.2wt gene in PBS. In parallel, mice were also vaccinated with an irrelevant plasmid DNA encoding the Vβ3 gene or with PBS. One week following the last DNA injection, animals were challenged with MBPac1–9/CFA/PT for the induction of EAE and monitored daily for clinical symptoms. As shown in Fig. 1, mice vaccin-ated with the Vβ8.2 DNA were significantly protected from disease. In the Vβ8-vaccinated group only three out of 12 mice developed mild paralysis and recovered quickly (duration of disease ranged from 4 to 8 days). In contrast, all animals in the PBS or the Vβ3 DNA vaccinated group contracted severe EAE (duration of disease ranged from 9 to 20 days). Data from two different experiments are summarized in Table 1. The levels of expression of different DNA constructs were found to be similar (see Methods).

Vaccination with mutant Vβ8.2 DNA encoding altered residues in the FR3 region recognized by Treγ does not prevent EAE

We have shown earlier that CD4 Treγ reactive to the dominant FR3 region of the Vβ8.2 chain (peptide ‘B5’, amino acids 76–101/Au, also referred to as the TCR FR3 peptide) are spontaneously primed in B10.PL mice following MBP injection and mediate recovery from EAE (19,20). We asked whether TCR peptide B5-reactive Treγ expanded following DNA vaccination. Ten days following the last DNA challenge, proliferative recall responses to B5 peptide in draining lymph node cells isolated from PBS-, Vβ3- or Vβ8.2 DNA-immunized mice were examined. Stimulation indices (SI) in the Vβ8.2 group ranged from 6.1 to 17.4 in comparison to 1.6 to 2.7 in the two control groups. There was no proliferative response to another Vβ8.2-derived peptide, B1 (amino acids 1–30L), in any of the vaccinated animals. To determine the cytokine phenotype of Treγ, secretion of IL-4 or IFN-γ was determined in the culture supernatants. IFN-γ production in response to B5 was ~4-fold higher (average 912 pg/ml) in the Vβ8.2 DNA-immunized group versus Vβ3- or PBS-treated controls (230 pg/ml). There was no detectable level of IL-4 in cultures from any of the groups. These data suggest that processing and presentation of the FR3 region determinant of the Vβ8.2 chain following DNA vaccination results in expansion of B5-reactive type 1 Treγ in vivo.

Having established the expansion of Treγ, we next examined whether these cells directly participate in the DNA-induced protection from disease. To test this notion, mutated Vβ8.2 plasmid DNA was created and used for vaccination. In the first instance, mutations were introduced in a relevant region, Vβ8.2mut-rel, to change three critical residues (Q85, V88 and F90A) and an irrelevant region (V10A, V88A and F90A) and an irrelevant region (V10A, V88A and F90A) and an irrelevant region (V10A, V88A and F90A). The clinical symptoms of EAE were monitored and scored daily, as described in Methods.

### Table 1. Mice vaccinated with the Vβ8.2wt DNA but not with Vβ8.2mut-rel DNA encoding altered residues in the critical FR3 region are protected from antigen-induced EAE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EAE incidence [no. animals with disease/total mice] (maximum clinical score)</th>
<th>Duration of EAE (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>5/5 (3,2,2,1,1)</td>
<td>10–14</td>
</tr>
<tr>
<td>Vβ3 DNA</td>
<td>9/10 (4,3,3,3,2,1,1,1,1)</td>
<td>9–20</td>
</tr>
<tr>
<td>Vβ8.2wt DNA</td>
<td>3/12 (2,1,1,0,0,0,0,0,0,0,0,0)</td>
<td>4–8</td>
</tr>
<tr>
<td>Vβ8.2mut-rel DNA</td>
<td>10/10 (4,3,3,3,2,1,1,1,1,1,1,1,1,1,1)</td>
<td>15–23</td>
</tr>
<tr>
<td>Vβ8.2mut-irr DNA</td>
<td>2/9 (4,4,0,0,0,0,0,0,0,0)</td>
<td>7–14</td>
</tr>
</tbody>
</table>

Mutated variants of the Vβ8.2 gene were generated by site-directed mutagenesis as described in Methods. Vβ8.2mut-rel and Vβ8.2mut-irr DNA encode for alterations in the FR3 region (Q85A, V88A and F90A) and an irrelevant region (V10A, V88A and L107A) of the Vβ8.2 chain respectively.
Table 2. Adoptive transfer of CD4 Treg isolated from animals vaccinated with the Vβ8.2 DNA, but not from mice immunized with the Vβ8.2mut-rel DNA prevents EAE in recipient B10.PL mice

<table>
<thead>
<tr>
<th>Cell transfer from mice</th>
<th>EAE Incidence (as in Table 1)</th>
<th>Duration of EAE vaccinated with DNA (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ3</td>
<td>4/5 (4.3, 2.1, 0)</td>
<td>8–12</td>
</tr>
<tr>
<td>Vβ8 2wt</td>
<td>1/6 (4.0, 0.0, 0.0)</td>
<td>9</td>
</tr>
<tr>
<td>Vβ8 2mut-rel</td>
<td>4/6 (5.4, 3.3, 0.0)</td>
<td>7–14</td>
</tr>
<tr>
<td>Vβ8 2mut-irr</td>
<td>2/8 (3.2, 0.0, 0.0, 0.0)</td>
<td>10</td>
</tr>
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</table>

*For the induction of EAE, B10.PL mice were injected with MBPac1–9/CFA/PT. One day prior to Ac1–9 challenge, these mice were injected i.p. with CD4 Treg (2 × 10⁶/animal) isolated from spleens of syngeneic mice previously immunized 3 times with the indicated Vβ plasmid DNAs. Following culture with TCR peptide B5, CD4 populations were isolated and purified, as described above (17). Purity of the CD4 population was examined by flow cytometry and ranged from 95 to 98%.

F90) involved in the recognition of FR3 peptide by Treg (21). In parallel, another mutant DNA (Vβ8.2mut-irr) was made encoding three altered residues (A10, S12 and L107) in an irrelevant portion of the Vβ8.2 chain that is not involved in recognition by Treg but juxtaposed on the TCR. As shown in Fig. 1 and Table 1, mice vaccinated with the Vβ8.2mut-rel DNA encoding changes in the FR3 determinant region are not protected from EAE. In contrast, animals vaccinated with the Vβ8.2mut-irr DNA are significantly protected from disease (see Table 1). Interestingly, mice vaccinated with Vβ8.2mut-rel DNA contracted more severe disease in comparison to mice in the other control groups: seven out of 10 animals died following severe paralysis in this group. These data suggest that the FR3 region determinant represents a crucial target for the induction of regulation of EAE following DNA vaccination.

CD4 Treg reactive to the FR3 region of the Vβ8.2 chain mediate protection from EAE

Although data presented above suggest that FR3 peptide-reactive Treg are involved in regulation, it was still possible that mutation in the FR3 region of the Vβ8.2 chain altered both the Treg response as well as an anti-TCR humoral response. To examine this possibility, we determined whether sera collected from DNA vaccinated mice from any of the groups (PBS, Vβ3 or Vβ8.2mut-rel) contained anti-Vβ8.2 antibodies. Contrary to the earlier suggestion (18), using flow cytometry analysis, we did not detect significant staining of Vβ8.2⁺ T cells with any of the sera tested (data not shown).

To further examine the involvement of FR3 peptide-reactive Treg, CD4 T cells were isolated from mice vaccinated with Vβ8.2wt, Vβ8.2mut-rel, Vβ8.2mut-irr or Vβ3 DNA molecules. Following in vitro stimulation with the TCR peptide B5, purified CD4 T cell populations were transferred into naive B10.PL mice. Recipients were challenged with MBPac1–9/CFA/PT for the induction of EAE. As shown in Table 2, recipient animals which received T cells from mice vaccinated with Vβ8.2wt or Vβ8.2mut-irr DNA molecules were significantly protected from disease. In contrast, recipients of T cells from control mice vaccinated with either the Vβ8.2mut-rel or Vβ3 DNA are not protected from EAE. These data clearly establish that CD4 Treg are crucially involved in Vβ8.2 DNA-mediated prevention of EAE in B10.PL mice.

Type 1 CD4 Treg are involved in the Vβ8.2 DNA-mediated immune deviation of the MBPac1–20 response

What is the effect of TCR DNA immunization on the cytokine pattern of the anti-MBP response? One week following the last DNA challenge, mice (three in each group) were s.c. immunized with the dominant encephalitogenic determinant of MBP, Ac1–20. Ten days later, lymph node or spleenic cells were isolated and used for in vitro recall assays for proliferation and cytokine ELISA-spot analysis (IFN-γ and IL-4) in response to MBPac1–20 or TCR peptide B5 respectively. In parallel, responses to another Vβ8.2-derived peptide, B1 (amino acids 1–30L), and to the purified protein derivative of Mycobacterium (PPD) were determined and served as controls. There was no significant proliferative response (SI < 2) to TCR peptide B1 in any of the groups of mice. A similar proliferative response (SI = 2.6–5.3) to peptide B5 was detected in all three groups of mice immunized with the Vβ8.2wt, Vβ8.2mut-rel or Vβ8.2mut-irr DNA plasmids (Fig. 2). In contrast, there was no significant response (SI < 2) to B5 in the PBS- or the Vβ3-immunized animals. Interestingly, while the Treg response was T1-like in animals immunized with either the Vβ8.2wt or the Vβ8.2mut-irr, mice vaccinated with the Vβ8.2mut-rel DNA showed a predominance of T2-like response (Fig. 2). In all groups proliferative responses to Ac1–20 were similar, with SI = 6.3 to 9.1. However, as shown in Fig. 2, the frequencies of IL-4- or IFN-γ-secreting Ac1–20-reactive T cells were quite different: a T1-predominant response correlated with susceptibility to EAE, whereas T2-like responses were dominant in protected mice. Thus, following Vβ8.2wt or Vβ8.2mut-irr, the response to Ac1–20 was deviated in a T2 direction. In contrast, in other groups the frequency of Ac1–20-reactive T cells secreting IFN-γ was much higher than the cells secreting IL-4, representing a T1-like response. In our recent experiments using mucosal priming with TCR peptide B5 (17).
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is protective. Prevention of EAE is accompanied by deviation of the anti-MBPac1–20 response in a Th2 direction.

It is interesting that the in vivo processing and presentation of the TCR Vβ8.2 chain following DNA vaccination leads to priming/expansion of type 1 CD4 T cells reactive to the naturally processed, dominant determinant from the FR3 region. As proposed earlier (2,9–11), it is likely that professional APC, e.g. DC, process and present the TCR peptide to CD4 Treg resulting in their expansion. This is consistent with the finding that Treg, primed following DNA vaccination, predominantly secrete inflammatory cytokines, such as IFN-γ. Indeed, we have recently demonstrated that type 1 Treg are required to deviate the anti-MBPac1–20 response in a Th2 direction (17,22). It is noteworthy that vaccination with the Vβ8.2mut-rel DNA encoding relevant changes in the FR3 region results in activation of type 2 T cells specific for TCR peptide B5 and exacerbation of disease. Thus the relevant mutant DNA apparently encodes an altered peptide ligand for the CD4 Treg resulting in a state of dysregulation leading to exacerbation of disease and the death of most animals following paralysis. Thus, the encephalitogenic potential of the MBP-reactive effector population is crucially and dominantly influenced by the cytokine secretion phenotype of CD4 Treg.

Although, a precise molecular mechanism of the eventual deviation of the anti-MBP response following the action of type 1 Treg is not yet clear, our data suggest that Treg indirectly influence cytokine predominance in the MBP-reactive T cell population (25). The secretion of proinflammatory cytokines by CD4 Treg is required for efficient recruitment/activation of CD8 Treg reactive to another determinant, from the FR2/CDR2 region of the Vβ8.2 chain. For example, secretion of these cytokines may result in up-regulation of co-stimulatory or adhesion molecules on APC for an efficient induction of the CD8 population. Consistent with the involvement of a distinct CD8 Treg in this regulation, in preliminary experiments we found that mice vaccinated with the mutant Vβ8.2 DNA encoding alterations in the FR2/CDR2 region were not protected from EAE. Recently, two different groups have demonstrated that CD4 T cell help via a class II MHC-dependent pathway is required for the efficient generation of an effective CTL response following DNA immunization (5,26). CD8 Treg cells may induce apoptosis or anergy (27,28) of the initially
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rapidly expanding, high-avidity, MBP-reactive V8.2 T,1 cell population. Since T,2 cells are less susceptible to apoptosis (29), this would enable a relatively slower reacting compartment of low-avidity, MBP-specific type 2 cells (which may or may not express V8.2) to expand in the absence of cross-regulatory IFN-γ secreting cells, resulting in an apparent shift of the population as a whole in a T,2 direction. Our recent preliminary data (V. Kumar, unpublished data) using mice lacking a functional IFN-γ gene suggest that this cytokine is critically involved in TCR-based regulation. Immune deviation of antigen-specific T cells at the population level may explain how TCR-based regulation directed to a single Vβ chain is able to also control disease-inducing, MBPAC1-9-specific T cells that use other TCR Vα chains, e.g. Vα13 or Vα4 (14). Such modulation of T cell responsiveness to one target antigenic determinant may suppress bystander responses to other antigenic determinants (30,31), from the same protein, as well as from other myelin components that may arise as a result of determinant spreading during chronic demyelination (32). Furthermore, all newly primed T cell responses in the B10.PL mouse model of EAE resulting from determinant spreading are not necessarily pathogenic; some of them could rather be protective (33). Consistent with this, it has been shown that deviation of a dominant disease-causing T cell population using an altered peptide ligand or DNA vaccination can prevent EAE or diabetes respectively (23,31). Overall these findings suggest that vaccination with plasmid DNA encoding one or multiple Vβ genes could represent a powerful approach for intervention in T cell-mediated pathological conditions. In disease conditions where T cells using multiple V genes are involved, it is likely that plasmid DNA encoding multiple TCR V genes may be used to intervene. In a recent report, DNA vaccination using two diverse Vβ TCR chains of cardiac myosin-restricted T cells regulated autoimmune myocarditis demonstrating that T cell-centered regulation can be achieved when more than a single Vβ repertoire is involved in pathogenesis (34). Furthermore, Vβ DNA along with DNA encoding appropriate co-stimulatory, cytokine or chemokine molecules could be used to render regulatory responses more effective (35).

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>BS</td>
<td>TCR peptide containing FR3 region (amino acid 76–101) from the V8.2 chain</td>
</tr>
<tr>
<td>CFA</td>
<td>complete Freund’s adjuvant</td>
</tr>
<tr>
<td>EAE</td>
<td>experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>FR3</td>
<td>framework 3 region</td>
</tr>
<tr>
<td>MBP</td>
<td>myelin basic protein</td>
</tr>
<tr>
<td>PPD</td>
<td>purified protein derivative</td>
</tr>
<tr>
<td>PT</td>
<td>pertussis toxin</td>
</tr>
<tr>
<td>SI</td>
<td>stimulation index</td>
</tr>
<tr>
<td>Treg</td>
<td>regulatory T cell</td>
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References

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