International Immunology, Vol. 13, No. 6, pp. 835–841

Induction of a type 1 regulatory CD4 T cell response following V_β8.2 DNA vaccination results in immune deviation and protection from experimental autoimmune encephalomyelitis

Vipin Kumar[1], Jeannie Maglione, Jayant Thatte[2], Brian Pederson, Eli Sercarz and **E. Sally Ward[2]**

Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90095, USA
[1]Division of Immune Regulation, La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121, USA
[2]Center for Immunology and Cancer Immunobiology Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Keywords: DNA vaccine, experimental autoimmune encephalomyelitis, regulatory T cells, TCR, T_h1/T_h2

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_β8.2 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_h2 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_β 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 *Leishmaniasis 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

Correspondence to: V. Kumar

Transmitting editor: L. Steinman

Received 28 December 2000, *accepted* 9 March 2001

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^u mice recognize the N-terminal peptide MBPAc1–9 or Ac1–20 and predominantly use the TCR V_β8.2 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_h1 cells are encephalitogenic, whereas a T_h2 response is protective (16,17).

Recently, vaccination with TCR V_β8.2 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_{reg}) response in the prevention of disease has not yet been defined. Furthermore, it has not been described how intramuscular V_β8.2 DNA immunization that predominantly induces CD4 T_h1 cells results in deviating the response to MBP in a protective T_h2 direction. Here we have examined whether DNA vaccination can prime an appropriate T_{reg} response that controls EAE (19). Using mutant V_β8 DNA molecules and cell transfer strategies, we demonstrate that T_{reg} 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_β8.2 construct, a plasmid containing the V_β domain gene (V_β–D_β–J_β) with a C-terminal c-*myc* epitope has been described previously (36). A 5′ *Eco*RI site followed by an in-frame methionine codon were appended to the 5′ end of the gene encoding the mature V_β 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 *Bam*HI site using the PCR and the primer: 5′-TGA TGG ATC CTT ATT AGA GAA CAG TCA GTC TGG T-3′. The gene was initially cloned into the vector pCMV5 as an *Eco*RI–*Bam*HI fragment and then subsequently ligated into pCMV8 (a derivative of pCMV5 with an additional leader intron which expresses higher levels of protein) as a *Sal*I–*Bam*HI fragment. Mutated variants of the V_β8.2 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, V12S and L107A). These mutated V_β genes were then used to replace the wild-type region of the V_β8.2 gene as *Pst*I (site overlapping codons 2–4 of the mature gene)–*Bst*EII (site 5′ to c-*myc* tag fragments). pCMV8 has a *Pst*I site in the polylinker proximal to the 5′ end of the V_β gene and this cloning therefore resulted in the loss of ~25 bases of coding sequence. Following *Pst*I digestion, this sequence was ligated into the construct as an oligonucleotide duplex to reconstitute the complete coding sequence. The V_β3 construct, containing the V_β 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_β8.2 construct. The inserts of all constructs were sequenced prior to use. All constructs were transiently transfected into COS cells and a similar level of expression of V_β-myc protein was verified by analysis of cell lysates on immunoblots using the anti-c-*myc* antibody 9E10 as described previously (36).

Plasmid DNA vaccination

The purified plasmid DNA samples were dissolved in PBS. DNA (100 μg) was injected 2–3 times intramuscularly at weekly intervals. One week following the last injection of DNA, mice were s.c. immunized with MBPAc1–9/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)F_1 mice were bred under specific pathogen-free conditions in our colony. Female B10.PL or (SJL×B10.PL)F_1 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_β8.2 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 MBPAc1–9 or Ac1–20 and TCR peptides were determined in lymph nodes or spleens essentially as described earlier (19). To monitor priming of CD4 T_{reg}, spleens were removed 7–10 days after the last plasmid DNA injection. Lymph node cells (4×10^5 cells/well) and splenocytes (8×10^5 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 [^{3}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

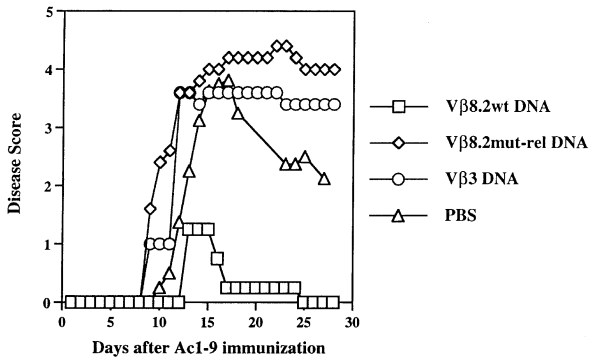

Fig. 1. Mice vaccinated with the V_β8.2wt DNA but not with V_β8.2mut-rel DNA are protected from antigen-induced EAE. Groups of mice (four or five each) were immunized intramuscularly with 100 μg of plasmid DNAs (V_β8.2wt, V_β8.2mut-rel, V_β3 or PBS) 3 times at weekly intervals. One week after the last DNA injection, animals were injected with MBPAc1–9/CFA/PT for the induction of EAE. The clinical symptoms of EAE were monitored and scored daily, as described in Methods.

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 5×10^5 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, cells were removed, and spots were visualized using biotinylated detecting mAb and avidin D-peroxidase in conjunction with 3-amino-9-ethylcarbazole (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

V_β8.2wt 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 V_β8.2wt gene in PBS. In parallel, mice were also vaccinated with an irrelevant plasmid DNA encoding the V_β3 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 vaccinated with the V_β8.2 DNA were significantly protected from disease. In the V_β8-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 V_β3 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).

Table 1. Mice vaccinated with the V_β8.2wt DNA but not with V_β8.2mut-rel DNA encoding altered residues in the critical FR3 region are protected from antigen-induced EAE

Treatment	EAE incidence [no. animals with disease/total mice (maximum clinical score)]	Duration of EAE (days)
PBS	5/5 (3,2,2,1,1)	10–14
V_β3 DNA	9/10 (4,3,3,3,2,2,1,1,1)	9–20
V_β8.2wt DNA	3/12 (2,1,1,0,0,0,0,0,0,0,0,0)	4–8
V_β8.2mut-rel	DNA 10/10 (5,5,5,5,5,5,5,4,3,1)	15–23
V_β8.2mut-irr DNA	2/9 (4,4,0,0,0,0,0,0,0)	7–14

Mutated variants of the V_β8.2 gene were generated by site-directed mutagenesis as described in Methods. V_β8.2mut-rel and V_β8.2mut-irr DNA encode for alterations in the FR3 region (Q85A, V88A and F90A) and an irrelevant region (V10A, V12S and L107A) of the V_β8.2 chain respectively.

Vaccination with mutant V_β8.2 DNA encoding altered residues in the FR3 region recognized by T_{reg} does not prevent EAE

We have shown earlier that CD4 T_{reg} reactive to the dominant FR3 region of the V_β8.2 chain (peptide 'B5', amino acids 76–101/A^u, 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 T_{reg} 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-, V_β3- or V_β8.2 DNA-immunized mice were examined. Stimulation indices (SI) in the V_β8.2 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 V_β8.2-derived peptide, B1 (amino acids 1–30L), in any of the vaccinated animals. To determine the cytokine phenotype of T_{reg}, 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 V_β8.2 DNA-immunized group versus V_β3- 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 V_β8.2 chain following DNA vaccination results in expansion of B5-reactive type 1 T_{reg} *in vivo*.

Having established the expansion of T_{reg}, we next examined whether these cells directly participate in the DNA-induced protection from disease. To test this notion, mutated V_β8.2 plasmid DNA was created and used for vaccination. In the first instance, mutations were introduced in a relevant region, V_β8.2mut-rel, to change three critical residues (Q85, V88 and

Table 2. Adoptive transfer of CD4 T_{reg}, isolated from animals vaccinated with the V_β8.2 DNA, but not from mice immunized with the V_β8.2mut-rel DNA prevents EAE in recipient B10.PL mice

Cell transfer from mice	EAE Incidence (as in Table 1)[a]	Duration of EAE vaccinated with DNA (days)
V_β3	4/5 (4,3,2,1,0)	8–12
V_β8.2wt	1/6 (4,0,0,0,0,0)	9
V_β8.2mut-rel	4/6 (5,4,3,3,0,0)	7–14
V_β8.2mut-irr	2/8 (3,2,0,0,0,0,0,0)	10

[a]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 T_{reg} (2×10^6/animal) isolated from spleens of syngeneic mice previously immunized 3 times with the indicated V_β plasmid DNAs. Following culture with TCR peptide B5, CD4 populations were isolated and purified, as described before (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 T_{reg} (21). In parallel, another mutant DNA (V_β8.2mut-irr) was made encoding three altered residues (A10, S12 and L107) in an irrelevant portion of the V_β8.2 chain that is not involved in recognition by T_{reg} but juxtaposed on the TCR. As shown in Fig. 1 and Table 1, mice vaccinated with the V_β8.2mut-rel DNA encoding changes in the FR3 determinant region are not protected from EAE. In contrast, animals vaccinated with the V_β8.2mut-irr DNA are significantly protected from disease (see Table 1). Interestingly, mice vaccinated with V_β8.2-mut-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 T_{reg} reactive to the FR3 region of the V_β8.2 chain mediate protection from EAE

Although data presented above suggest that FR3 peptide-reactive T_{reg} are involved in regulation, it was still possible that mutation in the FR3 region of the V_β8.2 chain altered both the T_{reg} 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, V_β3 or V_β8.2 immunized) contained anti-V_β8.2 antibodies. Contrary to the earlier suggestion (18), using flow cytometry analysis, we did not detect significant staining of $V_\beta8.2^+$ T cells with any of the sera tested (data not shown). To further examine the involvement of FR3 peptide-reactive T_{reg}, CD4 T cells were isolated from mice vaccinated with V_β8.2wt, V_β8.2mut-rel, V_β8.2mut-irr or V_β3 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 V_β8.2wt or V_β8.2mut-irr DNA molecules were significantly protected from disease. In contrast, recipients of T cells from control mice vaccinated with either the V_β8.2mut-rel or V_β3 DNA are not protected from EAE. These data clearly establish that CD4 T_{reg} are crucially involved in V_β8.2 DNA-mediated prevention of EAE in B10.PL mice.

Type 1 CD4 T_{reg} are involved in the V_β8.2 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 splenic 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 V_β8.2-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 V_β8.2wt, V_β8.2mut-rel or V_β8.2mut-irr DNA plasmids (Fig. 2). In contrast, there was no significant response (SI $<$ 2) to B5 in the PBS- or the V_β3-immunized animals. Interestingly, while the T_{reg} response was T_h1-like in animals immunized with either the V_β8.2wt or the V_β8.2mut-irr, mice vaccinated with the V_β8.2mut-rel DNA showed a predominantly T_h2-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 T_h1-predominant response correlated with susceptibility to EAE, whereas T_h2-like responses were dominant in protected mice. Thus, following V_β8.2wt or V_β8.2mut-irr, the response to Ac1–20 was deviated in a T_h2 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 T_h1-like response. The frequency of IFN-γ- or IL-4-secreting cells in response to PPD did not vary significantly in any of the groups (data not shown). These data indicate that type 1 T_{reg} are required to deviate the MBP-reactive response in a T_h2 direction and are consistent with our recent experiments using mucosal priming with TCR peptide B5 (17).

Discussion

The data presented here clearly demonstrate that vaccination of B10.PL mice with plasmid DNA encoding the TCR V_β8.2 gene segment, predominantly expressed on MBP-reactive encephalitogenic T cells, results in significant protection from antigen-induced EAE. The protection is specific in that vaccination with DNA encoding the TCR V_β3 gene segment, not displayed on disease-causing T cells, does not influence the course of disease. Furthermore, the V_β8.2 DNA-mediated protection involves type 1 CD4 T_{reg} reactive with the dominant determinant from the FR3 region of the V_β8.2 chain. Thus, vaccination with V_β8.2mut-rel DNA encoding point mutations in the FR3 region, critical for recognition by T_{reg}, does not prevent EAE, whereas vaccination with the V_β8.2mut-irr DNA encoding alterations in an irrelevant region of the TCR chain

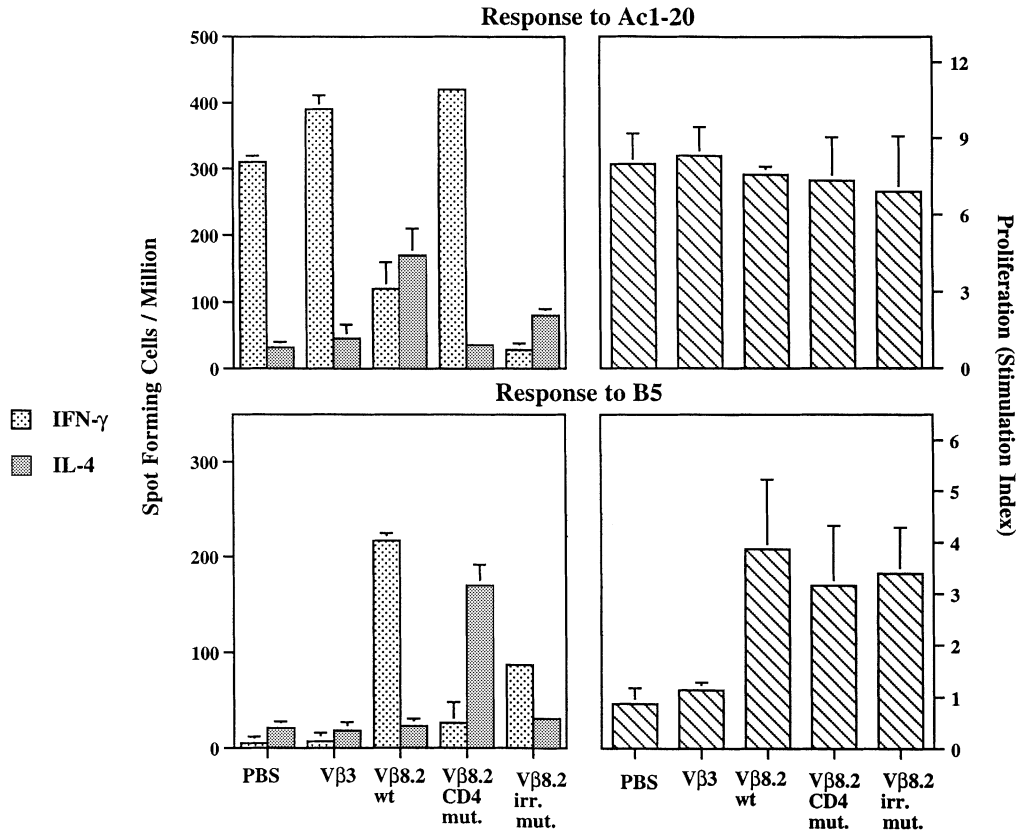

Fig. 2. Immune deviation of the MBPAc1–20 response following V_β 8.2 plasmid DNA vaccination. Groups of B10.PL mice (three each) were given 100 μg of plasmid DNAs intramuscularly 3 times at weekly intervals. One week after the last injection, these mice were s.c. challenged with 100 μg of Ac1–20. Nine days later lymph node or spleen cells were harvested, pooled and subjected to proliferation and ELISA-spot analysis in response to Ac1–20 or TCR peptide B5. The frequencies of IL-4- or IFN-γ-producing T cells are given as spot forming cells/million. The background in proliferation assay ranged from 1731 ± 243 to 3340 ± 510 c.p.m. These data are from one representative of three independent experiments.

is protective. Prevention of EAE is accompanied by deviation of the anti-MBPAc1–20 response in a T_h2 direction.

It is interesting that the *in vivo* processing and presentation of the TCR V_β8.2 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 T_{reg} resulting in their expansion. This is consistent with the finding that T_{reg}, primed following DNA vaccination, predominantly secrete inflammatory cytokines, such as IFN-γ. Indeed, we have recently demonstrated that type 1 T_{reg} are required to deviate the anti-MBPAc1–20 response in a T_h2 direction (17,22). It is noteworthy that vaccination with the V_β8.2mut-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 T_{reg} resulting in their priming in a T_h2 direction (23,24). In our preliminary experiments, in contrast to the wild-type FR3 region peptide, a mutated peptide containing such alterations does not prevent MBP-induced EAE. This is consistent with recent findings (17), which demonstrated that priming of type 2 T_{reg} results 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 T_{reg}.

Although, a precise molecular mechanism of the eventual deviation of the anti-MBP response following the action of type 1 T_{reg} is not yet clear, our data suggest that T_{reg} indirectly influence cytokine predominance in the MBP-reactive T cell population (25). The secretion of proinflammatory cytokines by CD4 T_{reg} is required for efficient recruitment/activation of CD8 T_{reg} reactive to another determinant, from the FR2/CDR2 region of the V_β8.2 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 T_{reg} in this regulation, in preliminary experiments we found that mice vaccinated with the mutant V_β8.2 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 T_{reg} cells may induce apoptosis or anergy (27,28) of the initially

rapidly expanding, high-avidity, MBP-reactive V_β8.2 T_h1 cell population. Since T_h2 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 V_β8.2) 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_h2 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 V_β chain is able to also control disease-inducing, MBPAc1–9-specific T cells that use other TCR V_β chains, e.g. V_β13 or V_β4 (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 V_β 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 V_β 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 V_β repertoire is involved in pathogenesis (34). Furthermore, V_β DNA along with DNA encoding appropriate co-stimulatory, cytokine or chemokine molecules could be used to render regulatory responses more effective (35).

Acknowledgements

This work was supported by grants from the National Multiple Sclerosis Society (V. K. and E. S. W.), NIH (E. S. and E. S. W.) and the Yellow Rose Foundation (E. S. W.). This is publication no. 375 from the La Jolla Institute for Allergy and Immunology. We would like to thank Dr Sergei Popov for help in making DNA constructs, Drs Randle Ware and Susanne Schneider for critically reading the manuscript, and Alex Jahng for help in producing the figures.

Abbreviations

APC	antigen-presenting cell
B5	TCR peptide containing FR3 region (amino acid 76–101) from the V_β8.2 chain
CFA	complete Freund's adjuvant
EAE	experimental autoimmune encephalomyelitis
DC	dendritic cell
FR3	framework 3 region
MBP	myelin basic protein
PPD	purified protein derivative
PT	pertussis toxin
SI	stimulation index
T_{reg}	regulatory T cell
V_β8.2wt DNA	TCR DNA encoding the wild-type V_β8.2 domain
V_β8.2mut-rel	mutant TCR DNA encoding changes in three residues critical for the recognition of FR3 peptide by T_{reg}, also referred to as V_β8.2CD4 Mut.
V_β8.2mut-irr	mutant TCR DNA encoding three altered residues in an irrelevant portion of the V_β8.2 domain

References

1 Donnelly, J. J., Ulmer, J. B., Shiver, J. W. and Liu, M. A. 1997. DNA vaccines. *Annu. Rev. Immunol.* 15:617.
2 Gurunathan, S., Klinman, D. M. and Seder, R. A. 2000. DNA vaccines: immunology, application, and optimization. *Annu. Rev. Immunol.* 18:927.
3 Manickan, E., Rouse, R. J., Yu, Z., Wire, W. S. and Rouse, B. T. 1995. Genetic immunization against herpes simplex virus. Protection is mediated by $CD4^+$ T lymphocytes. *J. Immunol.* 155:259.
4 Ulmer, J. B., Fu, T. M., Deck, R. R., Friedman, A., Guan, L., DeWitt, C., Liu, X., Wang, S., Liu, M. A., Donnelly, J. J. and Caulfield, M. J. 1998. Protective $CD4^+$ and $CD8^+$ T cells against influenza virus induced by vaccination with nucleoprotein DNA. *J. Virol.* 72:5648.
5 Maecker, H. T., Umetsu, D. T., DeKruyff, R. H. and Levy, S. 1998. Cytotoxic T cell responses to DNA vaccination: dependence on antigen presentation via class II MHC. *J. Immunol.* 161:6532.
6 Carson, D. A. and Raz, E. 1997. Oligonucleotide adjuvants for T helper 1 (T_h1)-specific vaccination. *J. Exp. Med.* 186:1621.
7 Chu, R. S., Targoni, O. S., Krieg, A. M., Lehmann, P. V. and Harding, C. V. 1997. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (T_h1) immunity. *J. Exp. Med.* 186:1623.
8 Krieg, A. M., Hartmann, G. and Yi, A. K. 2000. Mechanism of action of CpG DNA. *Curr. Topics Microbiol. Immunol.* 247:1.
9 Akbari, O., Panjwani, N., Garcia, S., Tascon, R., Lowrie, D. and Stockinger, B. 1999. DNA vaccination: transfection and activation of dendritic cells as key events for immunity. *J. Exp. Med.* 189:169.
10 Casares, S., Inaba, K., Brumeanu, T. D., Steinman, R. M. and Bona, C. A. 1997. Antigen presentation by dendritic cells after immunization with DNA encoding a major histocompatibility complex class II-restricted viral epitope. *J. Exp. Med.* 186:1481.
11 Porgador, A., Irvine, K. R., Iwasaki, A., Barber, B. H., Restifo, N. P. and Germain, R. N. 1998. Predominant role for directly transfected dendritic cells in antigen presentation to $CD8^+$ T cells after gene gun immunization. *J. Exp. Med.* 188:1075.
12 Wang, R., Doolan, D. L., Le, T. P., Hedstrom, R. C., Coonan, K. M., Charoenvit, Y., Jones, T. R., Hobart, P., Margalith, M., Ng, J., Weiss, W. R., Sedegah, M., de Taisne, C., Norman, J. A. and Hoffman, S. L. 1998. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science* 282:476.
13 Paterson, P. Y. 1980. Autoimmune diseases of myelin. *Prog. Clin. Biol. Res.* 49:19.
14 Urban, J. L., Kumar, V., Kono, D. H., Gomez, C., Horvath, S. J., Clayton, J., Ando, D. G., Sercarz, E. E. and Hood, L. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell* 54:577.
15 Acha-Orbea, H., Mitchell, D. J., Timmermann, L., Wraith, D. C., Tausch, G. S., Waldor, M. K., Zamvil, S. S., McDevitt, H. O. and Steinman, L. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* 54:263.
16 Zamvil, S. S. and Steinman, L. 1990. The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* 8:579.
17 Kumar, V. and Sercarz, E. 1998. Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. *J. Immunol.* 161:6585.

18 Waisman, A., Ruiz, P. J., Hirschberg, D. L., Gelman, A., Oksenberg, J. R., Brocke, S., Mor, F., Cohen, I. R. and Steinman, L. 1996. Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates T_h2 immunity. *Nat. Med.* 2:899.
19 Kumar, V. and Sercarz, E. E. 1993. The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med.* 178:909.
20 Kumar, V., Stellrecht, K. and Sercarz, E. 1996. Inactivation of T cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). *J. Exp. Med.* 184:1609.
21 Kumar, V., Tabibiazar, R., Geysen, H. M. and Sercarz, E. 1995. Immunodominant framework region 3 peptide from TCR V beta 8.2 chain controls murine experimental autoimmune encephalomyelitis. *J. Immunol.* 154:1941.
22 Kumar, V. 1998. TCR peptide-reactive T cells and peripheral tolerance to myelin basic protein. *Res. Immunol.* 149:827.
23 Nicholson, L. B., Greer, J. M., Sobel, R. A., Lees, M. B. and Kuchroo, V. K. 1995. An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. *Immunity* 3:397.
24 Brocke, S., Gijbels, K., Allegretta, M., Ferber, I., Piercy, C., Blankenstein, T., Martin, R., Utz, U., Karin, N., Mitchell, D., *et al.* 1996. Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. *Nature* 379:343.
25 Kumar, V. and Sercarz, E. 1996. Genetic vaccination: the advantages of going naked. *Nat. Med.* 2:857.
26 Wild, J., Grusby, M. J., Schirmbeck, R. and Reimann, J. 1999. Priming MHC-I-restricted cytotoxic T lymphocyte responses to exogenous hepatitis B surface antigen is $CD4^+$ T cell dependent. *J. Immunol.* 163:1880.
27 Gaur, A., Ruberti, G., Haspel, R., Mayer, J. P. and Fathman, C. G. 1993. Requirement for $CD8^+$ cells in T cell receptor peptide-induced clonal unresponsiveness. *Science* 259:91.
28 Jiang, H., Zhang, S. I. and Pernis, B. 1992. Role of $CD8^+$ T cells in murine experimental allergic encephalomyelitis. *Science* 256:1213.
29 Zhang, X., Brunner, T., Carter, L., Dutton, R. W., Rogers, P., Bradley, L., Sato, T., Reed, J. C., Green, D. and Swain, S. L. 1997. Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J. Exp. Med.* 185:1837.
30 Weiner, H. L., Friedman, A., Miller, A., Khoury, S. J., al-Sabbagh, A., Santos, L., Sayegh, M., Nussenblatt, R. B., Trentham, D. E. and Hafler, D. A. 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol.* 12:809.
31 Coon, B., An, L. L., Whitton, J. L. and von Herrath, M. G. 1999. DNA immunization to prevent autoimmune diabetes. *J. Clin. Invest.* 104:189.
32 Vanderlugt, C. J. and Miller, S. D. 1996. Epitope spreading. *Curr. Opin. Immunol.* 8:831.
33 Kumar, V. 1998. Determinant spreading during experimental autoimmune encephalomyelitis: is it potentiating, protecting or participating in the disease? *Immunol. Rev.* 164:73.
34 Matsumoto, Y., Jee, Y. and Sugisaki, M. 2000. Successful TCR-based immunotherapy for autoimmune myocarditis with DNA vaccines after rapid identification of pathogenic TCR. *J. Immunol.* 164:2248.
35 Cohen, A. D., Boyer, J. D. and Weiner, D. B. 1998. Modulating the immune response to genetic immunization. *FASEB J.* 12:1611.
36 Ward, E. S. 1991. Expression and secretion of T-cell receptor V alpha and V beta domains using *Escherichia coli* as a host. *Scand J. Immunol.* 34:215.
37 Kunkel, T. A., Roberts, J. D. and Zakour, R. A. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367.
38 Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. and Pease, L. R. 1989. Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. *Gene* 77:61.
39 Kumar, V., Bhardwaj, V., Soares, L., Alexander, J., Sette, A. and Sercarz, E. 1995. Major histocompatibility complex binding affinity of an antigenic determinant is crucial for the differential secretion of interleukin 4/5 or interferon gamma by T cells. *Proc. Natl Acad. Sci. USA* 92:9510.