Recombinant T Cell Receptor Molecules Can Prevent and Reverse Experimental Autoimmune Encephalomyelitis

Dose Effects and Involvement of Both CD4 and CD8 T Cells[1]

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Autoimmune diseases are often characterized by spontaneous remission followed by relapses. Although the mechanism(s) controlling pathogenic self-reactive T cells are not fully understood, recent data in experimental autoimmune encephalomyelitis (EAE), a prototype for CD4 T cell-mediated autoimmune diseases, indicate that spontaneous recovery is mediated by regulatory T cells (Treg) specific for peptides derived from the β-chain of the TCR. Here we have tested whether recombinant single-chain TCRs (scTCRs) containing Vβ domains can be used as vaccines for efficient priming of Treg. A single injection of mice with these recombinant proteins leads to efficient in vivo priming of Treg and almost complete protection from Ag-induced EAE. Significantly, administration of scTCRs during ongoing disease at a 10-fold lower dose than that required for prophylactic treatment also reverses established EAE. However, if a higher dose of scTCR is administered during ongoing disease, paralytic symptoms become exacerbated and the majority of treated animals die from severe and chronic EAE. Furthermore, we demonstrate that regulatory determinants are processed and presented from scTCRs resulting in the recruitment of both CD4 and CD8 regulatory T cells which are required for efficient regulation induced by scTCR. Reversal of established disease following an optimum dose of recombinant TCRs suggests that proteins expressing appropriate Vβ domains could be used for the treatment of a variety of T cell-mediated pathologic conditions. *The Journal of Immunology,* **1997, 159: 5150–5156.**

Experimental autoimmune encephalomyelitis (EAE)[3] is a prototypic CD4 T cell-mediated autoimmune disease, characterized by inflammation in the central nervous system accompanied by demyelination and clinical paralysis following immunization with myelin Ags, e.g., myelin basic protein (MBP) or proteolipid protein. EAE is an instructive model for the human demyelinating disease, multiple sclerosis (MS), because it shares many of its pathologic and immune dysfunctions (1). In several models of EAE, the TCRs of MBP-reactive, autoaggressive T cells are encoded by a limited set of variable (V)-region gene segments (2–4). Oligoclonality of TCR gene usage in MBP-specific T cells in some MS patients has also recently been reported (5–8). Furthermore, TCR Ag-binding motifs (V-D-(J) junctional sequences) associated with T cells in MS lesions are homologous to those in activated MBP-specific peripheral human T cells, as well as in CD4 T cells mediating EAE (8). The EAE model is also valuable for the testing of experimental therapies for autoimmune diseases (9, 10).

Accumulated evidence supports the idea that autoaggression of MBP-reactive T cells depends on concerted control exerted by regulatory T cells (11, 12). Both CD4 and CD8 subpopulations of T cells appear to contribute to the regulation (13–16) and in the process may recognize TCR peptide determinants in the context of class II and class I MHC molecules, respectively. In the models that we have explored, the B10.PL mouse strain or the (SJL × B10.PL)F_1, the T cell response to the immunodominant N-terminal determinant Ac1-9 of MBP primarily utilizes the TCR Vβ8.2 gene segment (3). Recent experiments have indicated that TCR-peptide-specific regulatory T cells (Treg) mediate spontaneous recovery from EAE in these mice (15). Accordingly, CD4 Treg, directed against a framework determinant of the Vβ8.2 chain (amino acids 76–101), become apparent during the recovery period and appear to participate in remission, as well as in resistance to reinduction of disease (15). Furthermore, CD8 regulatory T cells, which recognize a distinct Vβ8.2 determinant, are evidently recruited during this period, and in collaboration with CD4 Treg bring about down-regulation of autoaggressive MBP-reactive T cells (V. Kumar et al., unpublished observations). However, in other models of EAE, different determinants on the Vβ8.2 chain have been shown to play a role in controlling MBP-specific responses (13, 14). It would clearly be advantageous to present the regulatory system with an assemblage of determinants that appear on the Vβ8.2 chain itself, which would then permit the selection of dominant and functional regulatory determinants by individuals with different MHC molecules. An important goal of our work is the design of reagents capable not only of preventing but also of treating/reversing ongoing EAE through engagement of regulatory CD4 and CD8 cells, which may be present but quiescent.

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; MS, multiple sclerosis; Treg, TCR-peptide-specific regulatory T cells; scTCRs, single-chain T cell receptor molecules; PTx, pertussis toxin; scTCR, single-chain T cell receptor for antigen.

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Until very recently, it has been difficult to use TCR molecules for this purpose, due to a lack of suitable systems for the generation of soluble TCRs or surrogates. However, recombinant, soluble TCRs derived from MBP-reactive T cell clones have recently been expressed using *Escherichia coli* as a host and purified as single Vα or Vβ domains or single-chain TCR molecules (scTCRs) in which the Vα domain is linked to the Vβ domain by a synthetic peptide linker (17). These soluble V domains have proved to be useful in x-ray crystallographic studies of the TCR (18). The scTCRs appear to be correctly folded (17) and have the advantage of bearing all the potential Vα/Vβ-derived determinants that may be targets for both CD4 and CD8 regulatory T cells. In this study, we have determined whether different Treg populations can be activated in vivo to both prevent and reverse EAE following injection with these TCR proteins. The findings of this study have implications for the therapy of autoimmune disease.

Materials and Methods

Preparation of recombinant TCR proteins

T cell hybridomas were used as a source of Vα and Vβ domain genes; they are described along with their recognition specificity in Table I. MBP-specific T cell hybridomas 1934.4 (19) and 89-101 (20) were generous gifts from Dr. David Wraith; the qcII85.33 (21) and the 2B4 (22) T cell hybridomas were provided by Dr. Ed Rosloniec and Dr. David Karp, respectively (with permission from Dr. M. Davis for the 2B4 hybridoma).

The expression plasmids for the 1934.4 and qcII85.33 scTCRs have been described previously (17, 20). The scTCR derived from the 89-101 hybridoma was constructed by isolating the Vα and Vβ domain genes (Vα 11-Jα40; Vβ16-Jβ2.2) from the hybridoma using degenerate primers and inverse PCR, respectively. The Vα and Vβ genes were then tailored with suitable restriction sites for construction of a pUC19 derivative containing the pelB leader, Vα and Vβ genes linked by a $(Gly_4Ser)_3$ linker and a polyhistidine tag. For this, methodology analogous to that described previously (17) was used, except that a *Sma*I site was inserted at the 3′ end of the Jα coding sequence and a *Bam*HI site was inserted within the coding sequence of the $(Gly_4Ser)_3$ linker. To generate the hybrid scTCR containing the Vα from the 89-101 hybridoma and the Vβ (Vβ3-Jβ2.5) from the 2B4 hybridoma, the 2B4 Vβ domain gene was isolated from the hybridoma. The primers were designed to insert a 5′ *Bam*HI site and a 3′ *Bst*EII site and then used to replace the 89-101 Vβ domain gene in the 89-101 scTCR construct. Single Vα4, Vβ8.2, or Vβ8.3 domains were expressed and purified using the plasmids and methods described previously (17). For the production of the Vβ8.3 domain from the qcII85.33 hybridoma, a plasmid analogous to that described for the Vβ8.2 domain was constructed. *E. coli* cells harboring the Vα, Vβ domain or scTCR plasmids were grown and induced for expression, and recombinant TCRs were purified from periplasmic shock fractions as described previously (17).

Challenge with recombinant TCRs

SJL and B10.PL mice were purchased from The Jackson Laboratory, Bar Harbor, ME, and PL/J $CD8^{-/-}$ mice (11) were kindly provided by Dr. Tak Mak. (SJL × B10.PL)F_1 mice were bred under specific pathogen-free conditions in our own colony. Female mice were used at 8 to 16 wk of age. For lymph node proliferation assays, mice were challenged s.c. with 10 μg of scTCRs or individual V domains emulsified in CFA. Lymph node cells were recalled in vitro with varying concentrations of recombinant TCR proteins (50–60 μg/ml being the optimum concentration). For most vaccination experiments, mice were challenged i.p. twice (days −7 and +3 in relation to MBP or Ac1-9 injections) with 10 to 12.5 μg scTCR/injection in IFA (except in the dose titration experiment). In some experiments (see legends), mice were injected only once with 20 or 25 μg of recombinant TCRs.

Lymph node and splenic proliferation assay

Lymph nodes or spleens of mice were removed 10 or 30 days after immunization and single-cell suspensions were prepared. 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, or X-vivo 10, BioWhittaker, Walkersville, MD) supplemented with 2 mM glutamine; peptides were added at final concentrations ranging from 0.1 to 7 μM. Proliferation was assayed by addition of 1 μCi [^{3}H]TdR (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.

Table I. *Recombinant TCRs used in this study*

Vα, Vβ Domains, or, scTCR	T Cell Hybridoma	Specificity	Designation[a]
Vα4.2	1934.4	NA[b]	Vα4
Vβ8.2	1934.4	NA	Vβ8.2
Vβ8.3	qcII85.33	NA	Vβ8.3
Vα4.2-sc[c]-Vβ8.2	1934.4	MBP Ac1-9/A^u	scVβ8.2-Vα4
Vα11-sc-Vβ16	89-101	MBP 89-101/A^s	scVβ16-Vα11
Vα11-sc-Vβ3	89-101/2B4	NA	scVβ3-Vα11
Vα11-sc-Vβ8.3	qcII85.33	cII260-270/A^q	scVβ8.3-Vα11

[a] Nomenclature used in this study.
[b] Not applicable, because of the use of single Vα or Vβ domains (Vα4, Vβ 8.2, Vβ8.3) or because the scTCR is a Vα Vβ hybrid derived from TCRs of distinct specificities (sc Vβ3-Vα11). The qcII85.33 and 89-101 hybrids utilize Vα11.5 and Vα11.3 subfamilies (36).
[c] Single chain linker encoding $(Gly_4Ser)_3$.

Induction of EAE

Mice were immunized s.c. with 100 μg of guinea pig MBP or Ac1-9 emulsified in CFA; 0.15 μg of pertussis toxin (PTx) (List Biologic, Campbell, CA) was injected in 200 μl of saline i.v. 48 h later. Mice were observed daily for signs of EAE until >60 days after immunization. The average disease score for each group was calculated by averaging the maximum severity of all of the affected animals in the group. Disease severity was scored on a 5-point scale, as described earlier (15): 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, whole body paralysis; 5, death.

Results

Immunodominance of the determinant(s) within TCR peptide B5

Among five overlapping peptides derived from the Vβ8.2 chain, three of them, B2 (amino acids 21–50), B4 (amino acids 61–90), and B5 (amino acids 76–101), induce strong proliferative responses after immunization of B10.PL or (SJL × B10.PL)F_1 mice (23). However, in mice recovering from Ag-induced EAE, peripheral splenic T cells proliferated in response to only a single TCR peptide, B5. This suggested that at least one TCR determinant is processed and presented efficiently from the Vβ8.2 chain. To directly test this possibility, mice were challenged with either a soluble recombinant scTCR containing the Vα4.2 and Vβ8.2 domains (Vβ8.2–Vα4), or a single Vα4.2 (Vα4) domain. Ten days later, draining lymph node cells were exposed in vitro to varying concentrations of different overlapping TCR peptides or to scTCRs. Clearly, a significant proliferative response was directed against B5 but not to any of the other TCR peptides (Fig. 1*A*). Furthermore, there was no in vitro recall response to B5 in lymph node cells isolated from mice challenged with the Vα4 domain alone (Fig. 1*B*). Next we determined whether T cells primed earlier with B5 could be stimulated in the presence of scTCR proteins containing the Vβ8.2 chain. Figure 2 demonstrates that lymph node cells isolated from B10.PL mice injected with B5 responded vigorously to B5 as well as to the scVβ8.2-Vα4 TCR or the Vβ8.2 domain, but not to the Vα4 domain. On the other hand, B2-primed lymph node cells were unable to proliferate in response to the scTCR (data not shown). Thus, a determinant within B5 appears to be the immunodominant proliferative determinant on the Vβ8.2 chain in B10.PL mice. Interestingly, in addition to the complementarity-determining region 2 determinant (13, 14), a second determinant within the framework 3 region of Vβ8.2 has recently been shown to also induce regulation in the Lewis rat model of EAE and seems to be immunodominant (24).

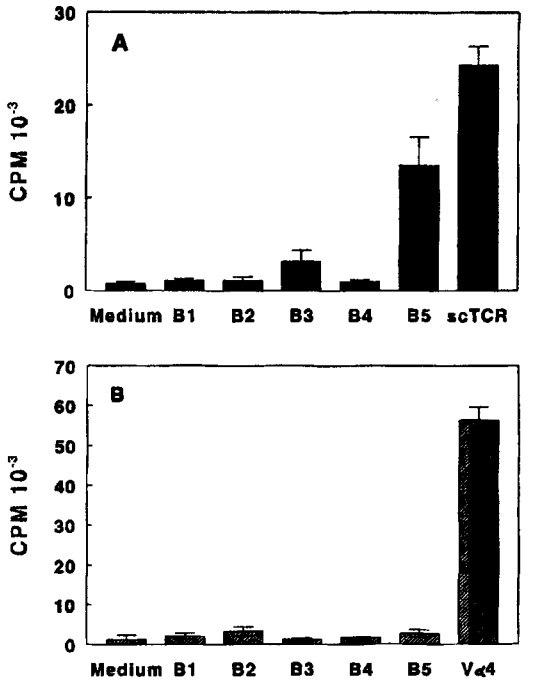

FIGURE 1. Determinant within TCR peptide B5 represents the immunodominant proliferating determinant on the Vβ8.2 chain. B10.PL mice were s.c. primed with scTCR Vβ8.2-Vα4 (*A*) or Vα4 (*B*), emulsified in CFA. Ten days later, draining lymph node cells were recalled in vitro with varying concentrations of the overlapping TCR peptides (B1 through B5) or with the respective TCR proteins. Responses to TCR peptides or recombinant TCR proteins are shown at an optimum concentration of 3 μM or 50 μg/ml, respectively. [^{3}H]TdR incorporation of pooled lymph node cells from two mice is shown as an arithmetic mean ± SD in triplicate cultures. These data are representative of three independent experiments. TCR Vβ8.2 chain peptides, as described earlier (15), correspond to the sequence predominantly used in the MBP-specific response in B10.PL mice (3) and are as follows: B1, 1–31; B2, 21–50; B3, 41–70; B4, 61–90; B5, 76–101.

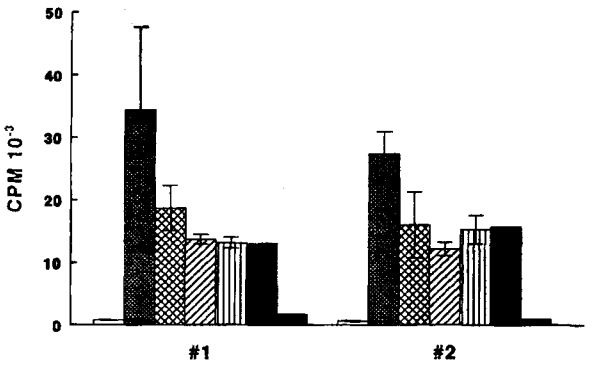

FIGURE 2. TCR peptide B5-primed T cells are able to recognize TCRs expressing the Vβ8 domain. B10.PL mice were challenged s.c. with 7 nmol of B5 emulsified in CFA. Ten days later, draining lymph node cells were recalled in vitro in the presence of varying concentration of Ags. Responses are shown at an optimum concentration (3.5 μM for B5 and 50 μg/ml for scTCR molecules) of Ags in two individual mice: medium only (□); B5 (▩); scVβ8.2-Vα4 (▨); scVβ8.2-Vα11 (▨); Vβ8.2 (▩); Vβ8.3 (▩), Vα4 (■). The data are expressed as arithmetic means ± SD of [^{3}H]TdR incorporation (cpm × 10^{-3}) in triplicate cultures. These data show the result of one of two similar experiments. Consistent with earlier findings (15), lymph node cells from unimmunized mice did not show any significant proliferative response to B5 (stimulation index, <2).

Single Vβ8 domains or scTCRs containing the Vβ8.2 chain protect mice from MBP or Ac1-9-induced EAE

Since B5 peptide-specific Treg could be primed following immunization of mice with scTCRs, we asked whether animals thus primed would be protected from Ag-induced EAE. B10.PL or (SJL × B10.PL)F_1 mice were each injected twice, i.p., with 10 μg of different scTCRs or Vβ domains emulsified in IFA. Recombinant TCRs containing the Vβ8.3 domain (25) were also used in these experiments. The Vβ8.3 chain has the same amino acid sequence as the Vβ8.2 chain in the B5 core region (23) with the exception of a conservative V88 to L88 change. The first injection was given 7 days before the MBP or Ac1-9 injection, followed by a second i.p. injection of scTCR 3 days following Ag injection. Table II demonstrates that mice vaccinated with scTCRs containing the Vβ8.2 or Vβ8.3 domain in combination with either the Vα4 or Vα11 domain, or single Vβ8.3 domain, were significantly protected from Ac1-9-induced EAE. In contrast, mice vaccinated with scTCRs containing homologous Vα domains but expressing Vβ domains other than Vβ8.2 or Vβ8.3 (scTCR Vβ 16-Vα11 or scTCR Vβ3-Vα11) were not protected from disease. Furthermore, Vβ- or scTCR-vaccinated mice were equally protected from whole MBP-induced EAE.

Table III shows a summary of data from eight independent experiments using vaccination with either single Vβ domains or scTCRs. Animals injected with PBS, scVβ16-Vα11, or scVβ 3-Vα11 proteins served as controls, whereas the Vβ8-vaccinated group includes mice injected with Vβ8.2, Vβ8.3 domains, or scVβ8.2-Vα4, ScVβ8.3-Vα11 scTCRs. Thus, 10 of 12 (84%) B10.PL mice contracted EAE in the control group, whereas only 1 of 17 mice (~6%) developed paralysis in the Vβ8-TCR-vaccinated group. Similarly, 30 of 34 (88%) (SJL × B10.PL)F_1 mice developed clinical symptoms following MBP or Ac1-9/CFA injection in the control group, whereas only 3 of 32 (9%) mice developed EAE in the Vβ8 or Vβ8-scTCR-vaccinated group. We have also monitored cachexia in two of the vaccination experiments and found that following Vβ or scTCR vaccination, the nonparalyzed animals did not lose weight and remained healthy, whereas control animals with EAE had lost 20 to 25% of their weight during the 20 days after MBP injection (data not shown). The protection following scTCR-vaccination is dose dependent. In two independent experiments, injection of 20 or 25 μg of scVβ 8.2-Vα4 before Ac1-9 challenge

Table II. *Prior injection with single Vβ8 domain or scTCRs containing Vβ8 domains, but not other Vβ domains, protects mice from antigen-induced EAE*[a]

Treatment in IFA	EAE Incidence (Animals with Disease/ Total Number ofAnimals (Individual Maximum Disease Score))
Expt. 1	
PBS	8/8 (4, 4, 4, 3, 3, 1, 1, 1)
scVβ8.2-Vα4	0/5
scVβ8.3-Vα11	0/5
Expt. 2	
PBS	7/8 (5, 4, 4, 4, 3, 2, 1)
Vβ8.3	0/4
scVβ16-Vα11	5/5 (4, 4, 3, 2, 1)
scVβ3-Vα11	4/4 (4, 4, 4, 3)

[a] For EAE induction, B10.PL (Expt. 1) or (SJL × B10.PL)F_1 (Expt. 2) mice were injected s.c. with guinea pig MBP/CFA/PTx or Ac1-9/CFA/PTx, respectively. Animals in each group were also injected i.p. twice (10–12.5 μg/injection) with PBS/IFA or scTCR proteins/IFA at days −7 and +3 with respect to the antigenic challenge.

Table III. *Protection from EAE following injection with recombinant TCRs*

Treatment Groups (Vaccinated with 20–25 μg TCRs in IFA)	EAE Incidence (Animals with Disease/ Total Number of Animals)
B10.PL mice	
Control (non-Vβ8-TCR)	10/12
Vβ8-TCR	1/17 ($p < 0.001$)[a]
(SJL × B10.PL)F_1 mice	
Control (non-Vβ8-TCR)	30/34
Vβ8-TCR	3/32 ($p < 0.001$)[a]

[a] p values between the control and vaccinated groups were calculated using the χ^2 test with Yates correction. This table summarizes the data obtained in eight different experiments using PBS, or non-Vβ8 scTCR-vaccinated mice (control group) or Vβ8 recombinant TCR-vaccinated mice (Vβ8-TCR-vaccinated group).

completely protected mice from EAE. However, mice vaccinated with 12.5 μg or less of scTCR were left unprotected or only partially protected from Ag-induced EAE.

Treatment of mice with low levels of scVβ8.2-Vα4 results in reversal of established EAE, while higher levels exacerbate the disease

We next sought to test whether mice with established disease could be treated with soluble scTCRs to reverse ongoing EAE. As described above, F_1 mice were almost completely protected from Ac1-9-induced EAE when vaccinated with 20 to 25 μg of a scTCR before antigenic challenge. Two experimental approaches were chosen to investigate whether postantigenic vaccination with soluble scTCR could affect ongoing disease.

In one experimental series, F_1 mice injected once with 20 μg of scVβ8.2-Vα4 TCRs, just 1 to 2 days before the onset of EAE (day 9 or 10 after Ag injection), were significantly protected from the disease (Table IV). In a second approach, similarly primed mice were injected i.v. with 20 μg of Vβ8.2-Vα4 or Vβ3-Vα11 scTCRs in 200 μl of saline on days 12 to 14, at a time when they were already clinically diseased with a score of 1 or 2 (Fig. 3*B*). Surprisingly, in the Vβ8.2-Vα4 scTCR-vaccinated group, all four of the paralyzed mice not only failed to recover from EAE but actually died with severe paralysis. However, in this group, a single animal that had not shown signs of EAE at the time of scTCR vaccination eventually developed mild disease at ~day 20 and recovered very quickly (Fig. 3*B*). In contrast, all four mice in the Vβ3-Vα11 scTCR-vaccinated group recovered normally (data not shown). These observations suggested that in the animals with EAE, TCR-peptide-reactive T cells had already been primed and probably needed only a low dose of scTCR to be engaged. Apparently, in such previously primed Treg populations, injection of 20 μg was excessive and possibly induced high dose tolerance. In a test of this hypothesis, mice were treated with 10-fold less of the Vβ8.2-Vα4 or Vβ3-Vα11 scTCRs, i.v., either when they already had grade 1 or 2 disease or just before the onset of the disease. As shown in Table IV, Expt. 2, 4 of 5 mice treated with only 3 μg of Vβ8.2-Vα4 scTCR on day 10, immediately before the onset of EAE, were completely protected from the disease. The fifth animal escaped from protection and displayed late onset and rapid recovery from grade 4 disease. Furthermore, the disease in mice in the low dose treatment group, treated with 2 μg of Vβ8.2-Vα4 scTCR at days 12 or 14 during ongoing disease (grade 1 or 2), did not progress further and in fact their clinical symptoms were quickly resolved (Fig. 3*C*). In contrast, injection of 2 μg of the irrelevant Vβ3-Vα11 scTCR (Fig. 3*D*) did not result in any significant change with clinical symptoms similar to those in mice in the control (saline-injected) group (Fig. 3*A*). Treatment of mice with 0.2 μg of scTCR during ongoing EAE did not lead to any change in the progression of disease (data not shown). In summary, a sharp dose response relationship was apparent in the attempts to treat mice with ongoing disease.

Table IV. *Postantigenic challenge with soluble scTCRs containing the Vβ 8 domain (days 9 or 10, just before the onset of disease) significantly protects mice from EAE*

Treatment in IFA	EAE Incidence in (SJL × B10.PL)F_1 Mice[a] (Animals with Disease/ Total Number of Animals (Individual Maximum Disease Score))
Expt. 1	
PBS	4/4 (4, 3, 3, 1)
scVβ3-Vα11 (20 μg)	3/3 (5, 3, 1)
scVβ8.2-Vα4 (20 μg)	0/4
Expt. 2	
PBS	5/5 (5, 3, 2, 2, 2)
scVβ8.2-Vα4 (3 μg)	1/5 (4)[b]

[a] F_1 mice were injected with Ac1-9/CFA/PTx for the induction of EAE. Animals were injected once i.p. with PBS/IFA or scTCR/IFA at days 9 or 10 following the antigenic challenge. At the time of vaccination, there were no signs of paralysis in mice in all groups. The onset of EAE in these experiments ranged from day 12 to day 14.

[b] A single mouse developed paralysis at day 18 and quickly recovered by day 24 from rather severe disease (grade 4).

Protection from EAE appears to correlate with the level of priming of B5-specific CD4 T cells

Does protection from Ag-induced EAE correlate with the level of priming of B5-specific CD4 Treg? We have examined the level of priming of B5-specific T cells in nonparalyzed mice vaccinated with scTCR (Fig. 4*A*), as well as spontaneous priming in control mice recovering from a normal course of EAE (Fig. 4*B*). Proliferative responses to B5 in splenic cells from individual mice are shown. It is evident that mice in the scTCR-vaccinated group showed relatively higher proliferative responses to B5 than did those in the nonvaccinated, recovered group. There was no response to another TCR peptide, B1 (data not shown). In the control (PBS/IFA) group, all mice contracted EAE. In contrast, in the scTCR-vaccinated group, 6 of 7 animals did not develop paralytic symptoms, and only a single mouse had a delayed onset of relatively mild clinical symptoms. Interestingly, the proliferative response to B5 in this partially protected mouse was the lowest among all the protected animals.

Mutant scTCRs containing conservative amino acid changes within the B5 region induce partial protection

To clarify the role of B5-specific CD4 Treg in scTCR-induced protection from EAE, we prepared mutated Vβ8.2-scTCR with substitutions at three key residues (Q85A, V88L, and F90L) necessary for T cell responsiveness to B5 (23) and tested the effect of the variant on the ability to induce regulation. To avoid any drastic alterations in the structure that might have resulted in difficulty in either expression or purification of the protein we did not modify the critical cysteine residue that forms the conserved —S—S— bridge in all Ig superfamily domains. The F_1 mice were vaccinated with 20 μg/animal of the wild-type scTCR or with the mutant protein, followed by immunization with Ac1-9/CFA/PTx to induce EAE. As shown in Table V (Expt. 1), some of the mice vaccinated with the mutant scTCR were still protected from disease, but only partially. Furthermore, in one experiment, injection with a higher amount of the mutant scTCR (50 μg/animal) still resulted in partial protection only (data not shown).

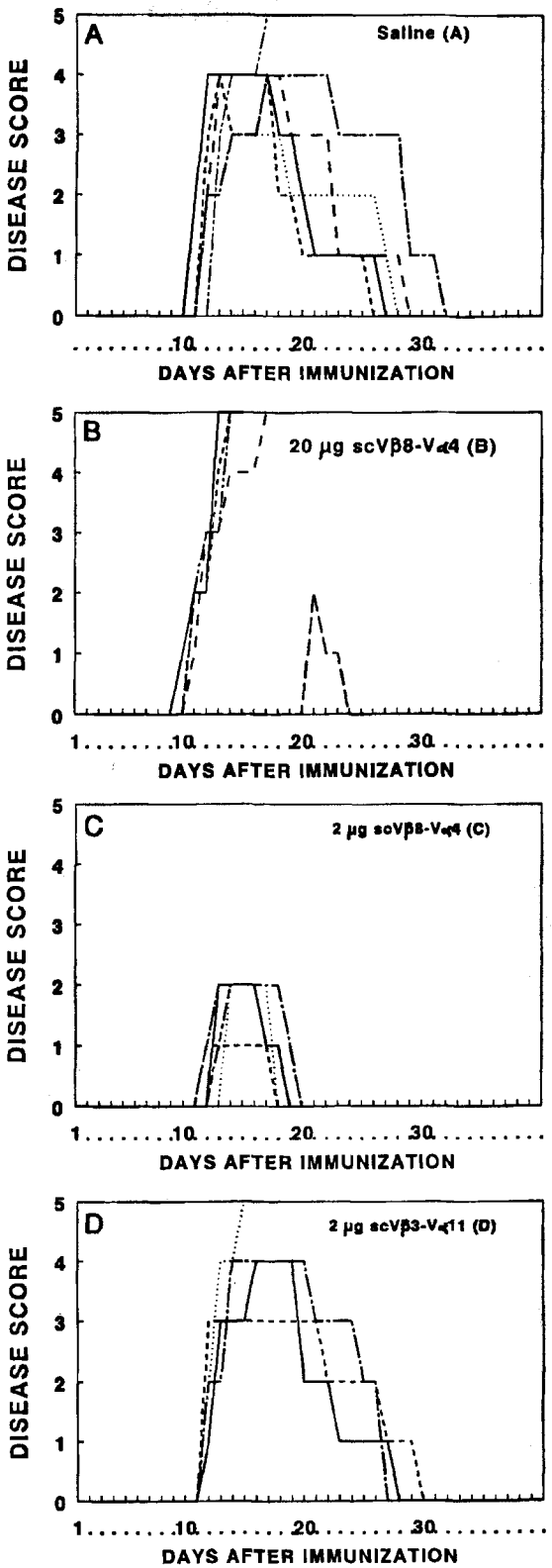

FIGURE 3. Reversal of ongoing EAE following injection of soluble recombinant scTCRs. (SJL × B10.PL)F_1 mice were s.c. injected with Ac1-9/CFA/PTx for the induction of EAE. Following the onset of EAE when animals had paralysis (grade 1 or 2, around days 12–14 after Ac1-9 injection), 4 to 7 individual mice in each group were injected i.v. with saline only (*A*), 20 μg of scVβ8.2-Vα4 (*B*), 2 μg of scVβ8.2-Vα4 (*C*), or 2 μg of scVβ3-Vα11 (*D*). The disease profile of individual mice in each group is shown.

Vβ8.2-Vα4 scTCR fails to prevent EAE in mice lacking CD8 T cells

To examine the requirement for CD8 regulatory T cells in control of EAE, PL/J mice either harboring CD8 T cells ($CD8^{+/+}$) or lacking CD8 T cells ($CD8^{-/-}$) were vaccinated with 20 μg of Vβ 8.2-Vα4 scTCR and subsequently challenged with Ac1-9 for the induction of EAE. As shown in Table V (Expts. 2i and 2ii), mice lacking CD8 T cells were not significantly protected from disease, whereas 9/13 (69%) mice with CD8 T cells were prevented from contracting EAE.

Both CD4 and CD8 regulatory T cells are involved in scTCR-mediated control of EAE

The experiments above suggest that both CD4 and CD8 T cells are involved in the regulation of EAE in B10.PL mice. To directly address this issue, we have performed adoptive transfer experiments with purified CD4 or CD8 T cells isolated from mice vaccinated with either Vβ8.2-Vα4 scTCR or Vβ16-Vα11 scTCR (Table VI). Mice transferred with either the CD4 or the CD8 subpopulation isolated from mice vaccinated with Vβ8.2-Vα4 scTCR were significantly protected from Ag-induced EAE. In contrast, mice transferred with either CD4 or CD8 T cells isolated from Vβ16-Vα11 scTCR-injected mice were not protected from disease. Interestingly, mice in the group that received serum isolated from either Vβ8.2-Vα4 or Vβ16-Vα11 scTCR-injected animals contracted EAE. Similarly, in experiment 2 (see Table VI), mice adoptively transferred with the T cell fraction, but not CD3-depleted splenic cell fraction, isolated from mice injected with Vβ8.2-Vα4 scTCR were significantly protected from Ag-induced EAE.

Discussion

This study demonstrates that dominant regulatory TCR determinants can be processed from the Vβ8 domains or Vβ8-scTCRs and presented in the appropriate MHC context in vivo to B10.PL or (SJL × B10.PL)F_1 T cells. Thus, injection with recombinant TCR molecules results in efficient priming/activation of TCR-peptide-specific regulatory T cells that modulate EAE. Recombinant TCR-induced protection is mediated by TCR peptide-specific CD4 and CD8 Treg cells: 1) there seems to be a correlation between the level of priming of B5-specific T cells and prevention of the disease; 2) vaccination with a mutant scTCR protein containing alterations in critical T cell recognition residues within the B5 region induced only partial protection; 3) vaccination with Vβ8-scTCR fails to prevent EAE in mice that lack CD8 T cells; 4) in adoptive transfer experiments, both CD4 and CD8 T cells isolated from the mice vaccinated with Vβ8-scTCR, but not with Vβ16-ScTCR, are able to prevent disease; 5) serum isolated from scTCR-injected mice does not protect mice from EAE, and consistent with this, the serum does not significantly stain Vβ8.2 T cells in flow cytometry experiments.

Experiments demonstrating rapid reversal of EAE following injection of soluble scTCR suggest activation/expansion of memory Treg populations that are otherwise primed slowly during the course of the disease. The intriguing feature of the dose response is that the amount required for treating ongoing disease (2 μg of scTCR) is about 10-fold lower than the dose required for prophylactic treatment (20–25 μg of scTCR). Injection of animals with an amount of scTCR most effective for prevention (20 to 25 μg), if administered during ongoing EAE, leads to exacerbation of the disease, implying that the regulatory T cells progress through a phase of high sensitivity to ligand dose and become susceptible to tolerance induction (26). As has been proposed earlier for B cells,

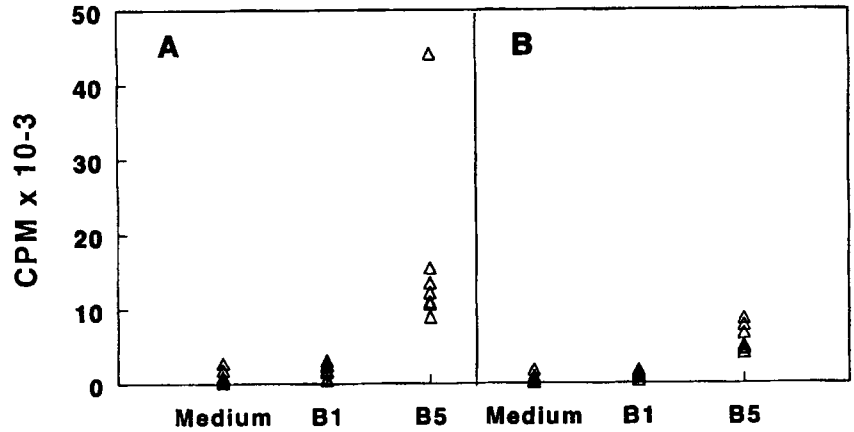

FIGURE 4. Proliferative response to TCR peptide B5 is relatively higher in mice protected from EAE following injection with the scTCR molecules. (SJL × B10.PL)F_1 mice were immunized with Ac1-9/CFA and PTx (48 h later). Thirty-five days later, the proliferative recall responses in the presence of varying concentrations of TCR peptides B1 and B5 from the Vβ8.2 chain were tested in the splenic populations isolated from mice vaccinated with scVβ8.2-Vα4 (*A*) or the control group (*B*). Mice in the control group had recovered from whole body and hind limb paralysis by the time of the assay. Responses to TCR peptides (none/medium only, B1, and B5) at an optimum concentration (3 μM) in individual mice is shown. The data are expressed as arithmetic means ± SD of [^{3}H]TdR incorporation (cpm × 10^{-3}) in triplicate cultures. These data are representative of two independent experiments.

Table V. *The wild-type scTCR in mice lacking CD8 T cells or a mutant scTCR, containing amino acid changes within the B5 region, do not significantly prevent Ac1-9-induced EAE*

Treatment in IFA	CD8 Status	EAE Incidence (Animals with Disease/ Total Number of Animals (Individual Maximum Disease Score))
Expt. 1		
PBS	+/+	7/8 (4, 4, 4, 4, 4, 3, 2, 0)
scVβ8.2-Vα4 (wild type) (Q T S V Y F C A S)[a]	+/+	0/5
scVβ8.2 (Q85A, V88L, F90L)-Vα 4 (mutant) (A . . L . L . . .)	+/+	3/5 (3, 2, 2, 0, 0)
Expt. 2i		
scVβ8.2-Vα4	+/+	1/7 (3, 0, 0, 0, 0, 0, 0)
scVβ8.2-Vα4	−/−	8/10 (5, 4, 4, 3, 3, 3, 2, 2, 0, 0)
Expt. 2ii		
scVβ8.2-Vα4	+/+	3/6 (2, 1, 1, 0, 0, 0)
scVβ8.2-Vα4	−/−	5/6 (5, 3, 1, 1, 1, 0)

[a] The bold residues were those changed in the mutant. (SJL × B10.PL)F_1 mice (Expt. 1) or PL/J mice (Expt. 2) harboring ($CD8^{+/+}$) or lacking ($CD8^{-/-}$) CD8 T cells were injected with 20 μg of Vβ8-scTCR or the mutant, as in Table II.

Table VI. *Both CD4 and CD8 T cell populations are involved in scTCR-mediated protection from EAE*[a]

Adoptive Transfer	EAE Incidence (Animals with Disease/ Total Number of Animals (Individual Maximum Disease Score))
Expt. 1A (from SC-Vβ8-vaccinated)	
Serum	4/4 (5, 4, 3, 3)
CD4 T cells	1/6 (2, 0, 0, 0, 0, 0)
CD8 T cells	3/7 (4, 1, 1, 0, 0, 0, 0)
Expt. 1B (from SC-Vβ16-vaccinated)	
Serum	4/4 (4, 3, 3, 1)
CD4 T cells	4/5 (5, 3, 3, 3, 0)
CD8 T cells	4/4 (4, 4, 2, 2)
Expt. 2	
PBS	4/5 (4, 4, 3, 3, 0)
Splenic cells	1/5 (3, 0, 0, 0, 0)
CD3-depleted splenic cells	5/5 (3, 3, 3, 1, 1)

[a] For EAE induction, (SJL × B10.PL)F_1 (Expt. 1) or B10.PL (Expt. 2) mice were injected s.c. with Ac1-9/CFA/PTx. Animals in each group were also injected i.p. with purified CD4 (1.4×10^6 cells/mouse) or CD8 (1.1×10^6) subpopulations, or serum (0.4 ml), isolated from mice vaccinated (10 days earlier) with either Vβ8- (Expt. II 1A) or Vβ16- (1B) scTCRs, at day −1 with respect to the antigenic challenge. The proliferative response to B5 peptide among purified CD4 populations isolated from Vβ8- or Vβ16-scTCR-vaccinated mice resulted in stimulation indices of 8.3 or 2.1, respectively.

tolerance induction may result most readily when cells are in a very recently activated state (27). These critical dose-response relationships, along with the involvement of putative CD8 T cell-inducing determinants, may explain some of the earlier contradictory results achieved with a single prophylactic dose of TCR peptide (28). Clearly, with respect to the potential usage of TCR-based therapy for patients with ongoing disease, it becomes crucial to ascertain the optimum dosage and timing for a given individual, working upward from a low starting dose: it is likely that this will depend on the level of priming and the state of activation of the Treg populations.

The partial protective effect of the mutant scVβ8.2-Vα4 TCR implied the presence of another TCR determinant, distinct from B5, which is also capable of inducing regulation. Consistent with this idea, together with data (Tables V and VI) indicating involvement of CD8 T cells in protection, we have recently identified a putative CD8 determinant on the Vβ8.2 chain, upstream of the B5 region. Mice vaccinated with this 10-mer peptide are significantly protected from MBP/Ac1-9-induced EAE (V. Kumar, N. Purohit, and E. Sercarz, unpublished observations). Accordingly, injection with scTCRs containing an entire Vβ8.2 domain has advantages over the use of single peptides, given that it can result in simultaneous priming/activation of both CD4 and CD8 regulatory T cells, leading to effective down-regulation of the encephalitogenic T cells. Also, synthetic peptides are cleared very rapidly from the circulation (29), making delivery at an appropriate dose a potential problem. It is likely that professional APCs such as macrophages or dendritic cells take up the Vβ8-TCR protein and process and

present the relevant determinants of appropriate length in a class I or class II MHC context (30). Therefore, efficient development of CD8 effectors may result from collaboration with CD4 T cells activated in close proximity, perhaps by the same APC. Furthermore, it is possible that activated CD4 Treg cells provide a locally appropriate cytokine milieu for optimal activation of $CD8^+$ Treg. Although not shown directly, consistent with the role of CD8 T cells in TCR-based regulation, it has been demonstrated that mice vaccinated with vaccinia virus recombinants expressing the Vβ8.2 domain are significantly protected from Ag-induced EAE (31).

Because Ag-specific T cells constitute only a minor population in vivo, the exact fate of Ac1-9-specific T cells following scTCR vaccination is not yet fully understood. Preliminary experimental evidence suggests that a majority of the Vβ8.2 T cells are neither deleted nor anergized following scTCR treatment but rather deviated in cytokine secretion pattern. However, it is possible that Ag-specific $V\beta8.2^+$ T cells, which initially expand, are down-modulated following deletion or anergy (32). Eventually, a late responding population specific for the Ag can proliferate and perhaps secrete noninflammatory cytokines, such as IL-4, as shown recently (33). Experiments are currently in progress to determine the fate of Ag-specific Vβ8.2 T cells following treatment with scTCRs.

The prevention and reversal of MBP-induced EAE in F1 mice following injection with Vβ8-scTCRs or single Vβ8 domains indicate that a key participant in EAE is the Vβ8-expressing, Ac1-9-reactive T effector cell. By selectively targeting the initial encephalitogenic T cell for down-regulation, concomitant and subsequent recruitment of T cells specific for other subdominant determinants of MBP (34) does not necessarily result in EAE. Thus, by silencing the initial trigger for inflammation, the secondary infiltrate in the target organ can be controlled (35). This is of direct consequence for treatment of human autoimmune conditions, where diversification/spreading following an initiating event need not imply that therapy will be impossible. These studies have implications regarding the potential for highly selective immune therapy of autoimmune disease where the disease-causing TCR repertoire is oligoclonal.

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