the transcription start site are 5′ to 3′ as shown in Table 2. β-Galactosidase activity for mutant sites 9 to 25, wild-type Bicoid, and wild-type Antp sites are directly comparable because the number and orientation of binding sites were identical in each construct as confirmed by DNA sequencing. The general form for each pair of oligonucleotides is as follows:

Bicoid sites: upper strand, 5′-TCGAA(TCTAATNNNTA)$_5$(TCTAATNNN)T-3′; lower strand, 5′-TCGAA(NNNATTAGA)(TANNNATTAGA)$_5$T-3′.

Antp sites: upper strand, 5′-TCGAA(TCATTTAATNNN)$_6$T-3′; lower strand, 5′-TCGAA(NNNATTAAATGA)$_6$T-3′.

29. Asn^{10} → Ala; Arg^{12} → Ala; and Asn^{10} → Gln substitutions in the Bicoid recognition helix were created with an overlapping polymerase chain reaction (PCR) strategy as described [R. M. Horton *et al.*, *BioTechniques* **8**, 538 (1990)]. The *bicoid*-containing Eco RI fragment of pSH11-1 (*11*) was subcloned into pUC119 [J. Vieira and J. Messing, *Methods Enzymol.* **153**, 3 (1987)]. Two inner oligonucleotide primers were used to introduce the mutations in two separate PCR reactions extending in opposite directions from the site of the base changes. The purified products of the first sets of reactions were annealed and used for a second round of PCR with only outer primers to amplify an intact Sal I–Sal I fragment, which was then recloned into the *bicoid* backbone in pUC119. Vent polymerase was used for all PCR reactions (New England Biolabs). Inner primer oligonucleotides were as follows:

Asn^{10} → Ala: 5′-GGTGAAGATCTGGTTTAAGGCCCGTCGGCG-3′ and 5′-CGCCGACGGGCCTTAAACCAGATCTTCACC-3′.

Arg^{12} → Ala: 5′-GTGAAGATCTGGTTTAAGAACCGTGCGCGTCGTC-3′ and 5′-GACGACGCGCACGGTTCTTAAACCAGATCTTCACC-3′.

Asn^{10} → Gln: 5′-GGTGAAGATCTGGTTTAAGCAACGTCGGCGTC-3′ and 5′-CGACGCCGACGTTGCTTAAACCAGATCTTCACC-3′.

Outer primer oligonucleotides were: 5′-AGCGGATAACAATTTCACACAGGA-3′ (reverse primer for pUC series) and 5′-GGCCGCCATTGACATTGGTCGACCCAG-3′ [base pairs 746 to 772 of the *bicoid* cDNA, as in T. Berleth *et al.*, *EMBO J.* 7, 1749 (1988)]. Base changes that created the desired amino acid substitution and a silent mutation that created a diagnostic Bgl II site are underlined. After reconstruction of the mutant genes in pUC119, the Eco RI *bicoid* fragments were reinserted into yeast expression vector, pSH2-1(*11*). Base substitutions were confirmed by DNA sequencing. Independently derived clones for each mutant protein behaved identically in a yeast transcription assay.

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T Cell Receptor Peptide Therapy Triggers Autoregulation of Experimental Encephalomyelitis

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Encephalitogenic T cells specific for myelin basic protein share common $V_\beta8$ peptide sequences in their T cell receptor (TCR) that can induce autoregulatory T cells and antibodies that prevent clinical signs of experimental autoimmune encephalomyelitis (EAE). It is not known, however, if TCR peptides can treat established disease. To test its therapeutic value, TCR-$V_\beta8$-39-59 peptide was injected into rats with clinical signs of EAE. This treatment reduced disease severity and speeded recovery, apparently by boosting anti-$V_\beta8$ T cells and antibodies raised naturally in response to encephalitogenic $V_\beta8^+$ T cells. These results demonstrate that synthetic TCR peptides can be used therapeutically, and implicate the TCR-$V_\beta8$-39-59 sequence as a natural idiotope involved in EAE recovery. Similarly, human TCR peptides may be effective in enhancing natural regulation of autoreactive T cells that share common V genes.

THROUGH MECHANISMS THAT ARE not yet fully understood, rat and mouse T cells that arise in response to immunization with guinea pig or rat basic protein (Gp-BP; Rt-BP) preferentially utilize the $V_\beta8.2$ gene and to a lesser extent the $V_\alpha2$ gene in their TCR (*1–6*). The presence of common V region sequences on encephalitogenic T cells allowed us (*7*) and others (*8*) to pre-immunize Lewis rats with synthetic TCR peptides to induce anti-receptor immunity. Our use of the synthetic $V_\beta8$ peptide corresponding to residues 39 to 59 completely protected the animals from the subsequent induction of clinical EAE. Similarly, most signs of EAE could be suppressed if the TCR-$V_\beta8$-39-59 peptide was given during the induction phase but prior to onset of clinical disease (*9*). The protective mechanisms involved both major histocompatibility complex (MHC) class I–restricted T cells (*7*) and antibodies (*9*) specific for the TCR-$V_\beta8$-39-59 peptide that appeared to be directed at a "processed" MHC-associated fragment of the natural TCR V_β chain expressed on the surface of $V_\beta8^+$ T cells. Presumably, interaction of the regulatory T cell or antibody with the $V_\beta8^+$ encephalitogenic T cell perturbed membrane signaling pathways, thus altering effector cell functions.

Although many approaches have been described to prevent encephalomyelitis (EAE), effective treatment of established clinical signs has been much more difficult. To test its therapeutic potential, the TCR-$V_\beta8$-39-59 peptide was injected by several different routes into Lewis rats with moderate signs of EAE. As controls, rats with EAE were injected in parallel with a synthetic peptide corresponding to the TCR-$V_\beta14$-39-59 sequence (not utilized by encephalitogenic T cells), or saline.

Intradermal (id) injection of 50 μg of the TCR-$V_\beta8$-39-59 peptide in saline reduced the clinical severity of EAE from grade 3.2 (paralysis of hind limbs) in the control rats, to grade 1.5 (wobbly gait) within 48 hours, and to grade 0.2 (limp tail in some rats) within 72 hours (Fig. 1A). The TCR-$V_\beta8$-39-59 peptide treatment speeded overall recovery time from 6.3–6.6 days in control rats to 3.1 days (Fig. 1A). A lower dose (10 μg) of the id-injected peptide was only slightly less effective, with a recovery time of 4 days (Fig. 1A). The second route tested, subcutaneous injection of 100 to 500 μg of the TCR-$V_\beta8$-39-59 peptide in saline, produced a nearly identical resolution of clinical EAE, with a recovery time of 3.5 and 4 days, respectively (Fig. 1B). A third regime, injection of the TCR-$V_\beta8$-39-59 peptide in complete Freund's adjuvant (CFA), also arrested disease progression within 24 hours and caused a rapid resolution of the remaining clinical signs from 6.5–6.6 days (controls) to 3.5 days (Fig. 1C).

The rapid clinical resolution of EAE after injection of the TCR-$V_\beta8$-39-59 peptide suggested a recall response similar to that induced in man by tetanus or rabies booster shots. Such a recall response would imply the presence of a preexisting immunity to the TCR-$V_\beta8$-39-59 peptide. Indeed, one might rationalize that the induction of $V_\beta8^+$ encephalitogenic T cells during EAE could stimulate regulatory T cells and antibodies directed at the $V_\beta8$ molecule and more specifically at the TCR-$V_\beta8$-39-59 peptide.

One simple method of assessing preexisting T cell responses in vivo is to measure ear swelling [delayed hypersensitivity (DH)] 24 to 48 hours after an id injection of antigen.

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The rats treated in the ear (by id injection) with the TCR-V_β8-39-59 peptide (Fig. 1A) provided an ideal test group to determine if specific responses were already present in vivo when treatment began. Rats treated with TCR-V_β8-39-59 peptide on the first day of clinical EAE (Fig. 1A) developed significant DH responses to the TCR-V_β8-39-59 peptide (11 ± 2 mm/100 versus 5 ± 2 mm/100 in naïve rats or 6 ± 2 mm/100 in CFA-injected rats) [$P < 0.01$ by analysis of variance (ANOVA)], but did not develop increased DH responses to the control V_β14-39-59 peptide ($P > 0.5$).

The increased DH responses suggested an underlying T cell recognition of the TCR-V_β8-39-59 peptide and prompted further analysis of TCR-specific T cell and antibody responses in rats developing EAE that had never before been purposefully immunized with this TCR peptide. Indeed, T cells selected from the lymph nodes of rat immunized previously with Gp-BP/CFA just prior to the onset of clinical EAE responded vigorously to the TCR-V_β8-39-59 peptide, in contrast to absent or much weaker T cell responses observed in naïve or CFA-immunized control rats (Fig. 2A). Conversely, there was no increase in response to the TCR-V_β14-39-59 peptide (Fig. 2A). Similar to the cellular response, antibody response to the TCR-V_β8-39-59 peptide was also increased selectively in rats immunized for 14 days with Gp-BP in CFA (Fig. 2B).

These data provide evidence that T cells and antibodies specific for the TCR-V_β8-39-59 peptide, both with potent autoregulatory activities (*7, 9*), are induced as a consequence of EAE. However, untreated rats still developed clinical EAE, indicating that the level of anti-TCR-V_β8 immunity was insufficient to prevent the encephalitogenic process from developing. If immunoregulation directed at the TCR-V_β8-39-59 peptide were important in successful treatment of EAE, one might expect that reexposure to the TCR-V_β8-39-59 peptide given as therapy would trigger a recall response to boost antibody to TCR-V_β8-39-59 responses in the treated rats versus that observed in untreated rats. To evaluate this possibility, we tested T cells and antisera from untreated and TCR-V_β8-39-59 peptide–treated rats after recovery from EAE, for response to the TCR peptides. T cells from rats treated successfully with the TCR-V_β8-39-59 peptide given id (as in Fig. 1A) responded vigorously (100,000 ± 6,000 cpm) to the V_β8 peptide, but not at all to the V_β14 peptide. In contrast, the response of T cells from untreated rats (Fig. 2A) that were not reexposed to the synthetic TCR-V_β8-39-59 peptide in vivo went from 40,000 ± 3,000 cpm to 20,000 ± 3,000 cpm after recovery from EAE. As a control for simple exposure to the TCR peptide, naïve rats given a single dose of TCR-V_β8-39-59 peptide (50 μg) id in saline had a relatively low T cell response (4,400 ± 300 cpm) to the immunizing peptide.

Similar to the T cell responses, specific antibody responses were also boosted after treatment, with antisera from TCR-V_β8-39-59 peptide–treated rats producing 0.22 ± 0.04 optical density (OD) units at 450 nm by enzyme-linked immunosorbent assay (ELISA), compared to 0.06 ± 0.01 units in untreated controls and 0.04 ± 0.01 units in singly immunized rats. Consistent with previous results (7), responses to the encephalitogenic peptide of Gp-BP were still present in both treated and untreated (naturally recovered) rats, indicating that the encephalitogenic T cell specificities were being regulated, not deleted.

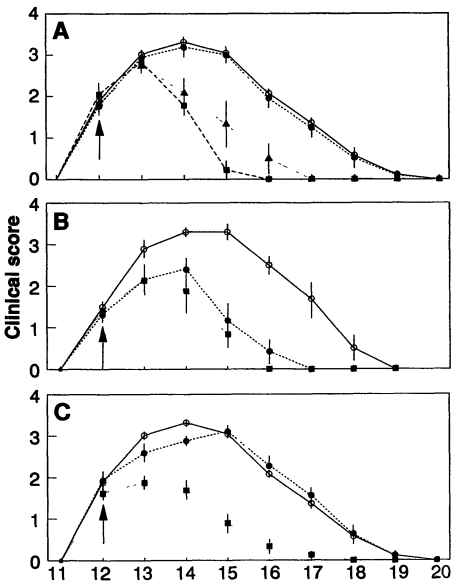

Fig. 1. Treatment of established EAE with TCR peptides. Groups of Lewis rats were injected with 50 μg of Gp-BP/CFA to induce clinical EAE. On the day of onset of clinical signs (day 12), rats were given the indicated doses of TCR-V_β8-39-59 peptide (DMGHGLRLIHYSYDVNSTEKG), TCR-V_β14-39-59 peptide (APGGTLQQLFYSFNVGQSELV) (as is indicated by the arrow), or were left untreated. The rats were scored daily for changes in clinical signs according to the following clinical rating scale: 0, no signs; 1, limp tail; 2, hind leg weakness, ataxia; 3, hind quarters paralysis; and 4, front and hind leg paralysis, moribund condition. Values indicate mean clinical score ± SEM on each day of clinical disease. (**A**) TCR peptides were given intradermally in the ear in 0.1 ml of saline. Differences were significant ($P < 0.01$ as analyzed by ANOVA) between groups treated with TCR-V_β8-39-59 peptide (both doses) and untreated or TCR-V_β14-39-59 peptide–treated control groups on days 14 to 18. Data represent composite of three separate experiments. ○: No treatment, n = 23, disease duration (dd) = 6.5 days; ▲: V_β8-39-59 (10 μg) treatment, n = 6, dd = 4 days; ■: V_β8-39-59 (50 μg), n = 15, dd = 3.1 days; ●: V_β14-39-59 (50 μg), n = 15, dd = 6.3 days. (**B**) TCR peptides were given subcutaneously over the flank in 0.1 ml of saline. Differences were significant ($P < 0.01$ by ANOVA) between both treatment groups and untreated control group on days 13 to 18. ○: No treatment, n = 6, dd = 6.5 days; ■: V_β8-39-59 (500 μg), n = 6, 4.0 days; ●: V_β8-39-59 (100 μg), n = 6, dd = 3.5 days. (**C**) TCR peptides were given subcutaneously over the flank in CFA. Differences were significant ($P < 0.01$) between groups treated with TCR-V_β8-39-59 peptide/CFA and those that were untreated or that were treated with TCR-V_β14-39-59 peptide/CFA on days 13 to 18. Data represent composite of four separate experiments. ○: No treatment, n = 23, dd = 6.5 days; ●: V_β14-39-59 (50 μg), n = 9, dd = 6.6 days; ■: V_β8-39-59 (50 μg), n = 20, dd = 3.5 days.

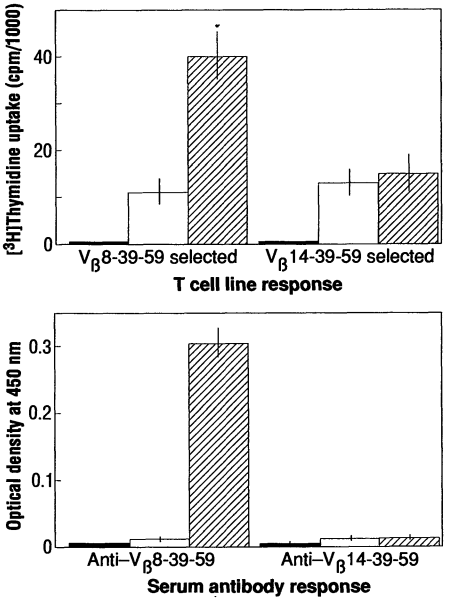

Fig. 2. Cellular and humoral responses to TCR peptides during the induction of EAE in rats with no prior exposure to synthetic TCR peptides. (**A**) Proliferation response to TCR peptides of 2 × 10^4 short-term T line cells selected with TCR-V_β8-39-59 peptide or TCR-V_β14-39-59 peptide from LNC of naïve rats or from rats immunized 12 days earlier with CFA or Gp-BP/CFA. Proliferation response to the selecting peptide was measured by uptake of [^{3}H]thymidine over the last 18 hours of culture and are expressed as average counts per minute of triplicate wells ± SD. (**B**) Serum antibody response to TCR peptides of a final 1:160 dilution of serum collected from naïve rats or rats immunized 14 days earlier with CFA or Gp-BP in CFA. Antibody reactivity was measured by ELISA, with 0.1 ml of diluted antisera in triplicate wells previously plated with 0.025 μg of TCR peptides. Peroxidase-labeled rabbit antibody to rat immunoglobulin (affinity-purified H and L chains) was used together with O-phenylenediamine for enzyme substrate. Normal rat serum was used as a negative control and gave an average optical density reading of 0.005 at a 1:160 dilution. Black bars, naïve rats; white bars, CFA-immunized rats; hatched bars, Gp-BP/CFA–immunized rats.

The results presented above support the idea that expansion of TCR $V_\beta 8^+$ encephalitogenic T cells during EAE induces a regulatory network directed in part at the TCR-V_β8-39-59 peptide. The ability of this TCR peptide to trigger a natural regulatory network and to produce a beneficial therapeutic effect is a step forward in the quest to manipulate selective T cell responses to autoantigens in established disease. The rapid therapeutic effect of the TCR peptide is reminiscent of passive TCR antibody therapy reported in the mouse and rat models of EAE (*1, 12, 13*). However, the TCR peptide would have several advantages over passive antibody therapy, including its lack of foreign antigenic determinants and its ability to trigger long-lasting natural regulatory mechanisms. In addition, the ability to treat EAE with an id or subcutaneous injection of peptide in saline (without adjuvants) provides a feasible route for the eventual treatment of humans.

Of equal importance is the demonstration that the regulatory network directed at the synthetic TCR peptide is induced as a consequence of the autoimmune disease itself. By implication, the mere presence of a given anti-TCR response in patients with autoimmune disease would indicate its importance as a target of the immune network that potentially could be triggered with clinical benefit by injecting small doses of the appropriate soluble TCR peptide. T cells from patients with multiple sclerosis, in which BP may be a relevant target autoantigen, use a limited set of V region genes in plaque tissue (*14*) and in response to human myelin basic protein (*15, 16*). Our data would predict increased anti-idiotypic responses to the overexpressed TCR V genes if the T cells were involved in the pathogenesis of the disease, but no detectable or amplifiable responses if the T cells represent a baseline frequency. The presence of anti-TCR responses in vitro would thus provide the impetus for periodic TCR peptide therapy to boost and maintain specific anti-idiotypic responses in patients with established autoimmune disease.

The V_β8-39-59 peptide may not be the only important determinant on T cells (*17–21*) or the TCR (*8*), however, because other sequences within the TCR α or β chains may also induce regulatory T cells and antibodies. Our data do not distinguish the relative importance of immunity directed at this TCR peptide with respect to other well-described regulatory mechanisms, such as suppressor circuits (*22–26*) or cytotoxic T cells (*27–28*) directed at determinants on encephalitogenic effector cells. However, it is clear from its protective, suppressive, and therapeutic effects that the TCR-V_β8-39-59 sequence constitutes an important determinant for the idiotypic regulation of EAE.

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Intrinsic Oscillations of Neocortex Generated by Layer 5 Pyramidal Neurons

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Rhythmic activity in the neocortex varies with different behavioral and pathological states and in some cases may encode sensory information. However, the neural mechanisms of these oscillations are largely unknown. Many pyramidal neurons in layer 5 of the neocortex showed prolonged, 5- to 12-hertz rhythmic firing patterns at threshold. Rhythmic firing was due to intrinsic membrane properties, sodium conductances were essential for rhythmicity, and calcium-dependent conductances strongly modified rhythmicity. Isolated slices of neocortex generated epochs of 4- to 10-hertz synchronized activity when *N*-methyl-D-aspartate receptor–mediated channels were facilitated. Layer 5 was both necessary and sufficient to produce these synchronized oscillations. Thus, synaptic networks of intrinsically rhythmic neurons in layer 5 may generate or promote certain synchronized oscillations of the neocortex.

SYNCHRONIZED OSCILLATIONS ARE pervasive in the cerebral cortex. Cortical rhythms, as revealed by the electroencephalogram (EEG), vary with behavioral state; their frequencies range from the 4- to 7-Hz theta waves of sleep to the 14- to 60-Hz waves dominant during alertness (*1*). Neuropathological conditions such as epilepsy and coma can elicit distinctive EEG rhythms. Cortical oscillations may encode sensory information (*2, 3*). Despite the prevalence of rhythmic neocortical activity, little is known about its mechanisms. Some cortical oscillations are clearly driven by periodic input from the thalamus (*4*); however, others may arise within the cortex itself, independent of the thalamus (*5, 6*).

Neurons generate rhythms in a variety of ways. Some have an intrinsic propensity to oscillate (*7*), and groups of these may interact synaptically to produce synchronous patterns (*4, 8*). Synchronized rhythms can also arise as an emergent property of a network of neurons that, as individuals, are nonrhythmic (*9*). Neurons in the middle layers of neocortex can initiate some nonrhythmic forms of synchronized activity (*10*). We show here that neurons of layer 5 alone have the intrinsic properties and synaptic connections necessary to generate synchronized oscillations.

Recordings were made from neurons in

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