Koo, *ibid.* **138**, 4539 (1987).
22. C. Öhlén *et al.*, unpublished data.
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Vaccination Against Experimental Allergic Encephalomyelitis with T Cell Receptor Peptides

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system mediated by $CD4^+$ T cells reactive with myelin basic protein (MBP). Rats were rendered resistant to the induction of EAE by vaccination with synthetic peptides corresponding to idiotypic determinants of the β chain VDJ region and Jα regions of the T cell receptor (TCR) that are conserved among encephalitogenic T cells. These findings demonstrate the utility of TCR peptide vaccination for modulating the activity of autoreactive T cells and represent a general therapeutic approach for T cell–mediated pathogenesis.

ENCEPHALITOGENIC T CELLS (*1–3*) from mouse and rat models of EAE use common germline TCR genes (*4–8*), despite known differences in MBP-peptide specificity and presumed differences in major histocompatibility complex (MHC) restriction [reviewed in (*9*)]. The Vβ8.2 genes are preferentially utilized by encephalitogenic T cells in PL/J, (PL/J × SJL)F_1 (*5*), and B10.PL (*6*) mice. TCR α chain gene usage is similarly restricted, with Vα4 exclusively used in PL/J and (PL/J × SJL)F_1 mice and Vα2 and Vα4 used equally in B10.PL mice. In B10.PL mice, these α chain V region genes are rearranged only to the JαTA39 gene, while JαTA31 is primarily used in PL/J mice. This restricted TCR gene usage is consistent with the common MBP peptide specificity (acetylated residues 1 to 11) and MHC restriction (Ia^u) of encephalitogenic T cells in these mouse models (*6, 10*). Encephalitogenic T cells from Lewis rats also show restricted TCR gene usage, in accord with their common specificity for MBP residues 68 to 88 in the context of the rat RT-1^b MHC haplotype (*11*). TCR gene usage in Lewis rat EAE is identical to that in the mouse models described above. Vβ8.2 is used in conjunction with Vα2 that has been rearranged to JαTA39. Common TCR gene usage among mouse and rat encephalitogenic T cells is enigmatic, because different, noncrossreactive combinations of MHC and peptide antigen (*9*) are recognized in the respective models. The EAE induced by T cells in rats or mice is histologically identical, which supports a role for the reported TCRs in the pathogenesis of EAE.

To define EAE-associated idiotypic determinants, we analyzed published sequences of TCR genes from encephalitogenic T cells in mouse and rat models of EAE. Amino acid sequence was conserved in the region of TCR β chain VDJ joining and in the Jα elements of these T cells (Fig. 1). The B10.PL sequence (Fig. 1A) is that reported for four of five encephalitogenic T cell clones whose Vβ8 rearrangements were sequenced (*6*). Similar β chain VDJ sequences are reported for three independently isolated Lewis rat Vβ8 T cell clones (*7, 8*). In PL/J and (PL/J × SJL)F_1 mice, four different TCR β chain VDJ sequences were reported (*5*); only one had limited homology with these sequences.

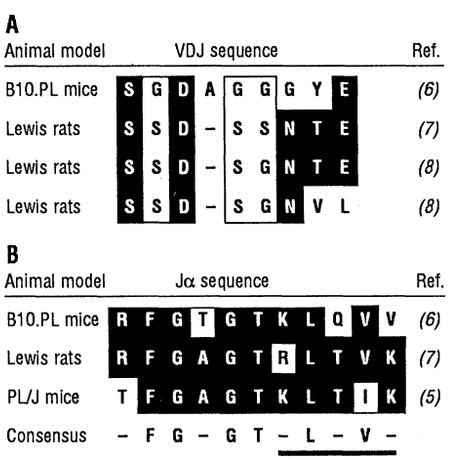

Fig. 1. T cell receptor β chain VDJ (**A**) and Jα (**B**) sequences in EAE. Literal homologies are boxed and shaded; substitutions acceptable according to a Dayhoff matrix are boxed but unshaded. The 75% consensus mouse Jα residues were taken from (*12*). The bold line in (B) denotes r-JαTA39 residues conforming to patterns characteristic of T cell epitopes.

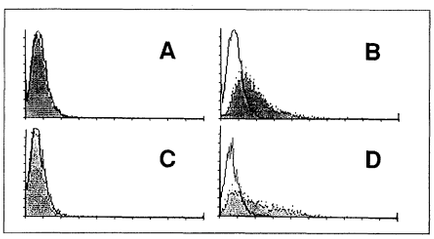

Fig. 2. Cytofluorometry of Lewis rat MBP-reactive T cells stained with antibodies to TCR peptides. A Lewis rat MBP-reactive T cell line in its fifth in vitro cycle of MBP stimulation (approximately one-third $Vβ8^+$) was incubated with protein A–purified immunoglobulin G (IgG) from pre-immune sera (**A**), affinity purified Abs to r-VDJ2 (**B**), pre-immune IgG (**C**), or affinity-purified Abs to JαTA39 (**D**). Antibody binding was revealed with fluorescein-conjugated goat Abs to rabbit IgG. Specific binding is indicated by shaded profiles and background staining of goat Abs to rabbit Ig by unshaded profiles.

The JαTA39 sequences used in B10.PL mice (*6*) and Lewis rats (*7*) (Fig. 1B) display homology at 9 of the 11 residues shown. The JαTA31 sequence from PL/J mice (*5*) shares ten residues with the Lewis rat JαTA39 sequence and eight residues with the B10.PL mouse JαTA39 sequence. Analysis of 27 additional mouse Jα sequences (*12*) identified only one other very similar sequence, JαTA37, which is consistent with the restricted Jα gene usage in EAE. The conservation of β chain VDJ and Jα sequences among encephalitogenic T cells, even in different animal models of EAE, implicates these TCR idiotopes in the pathogenesis of EAE and provides targets for immunotherapeutic intervention.

We vaccinated Lewis rats with synthetic peptides corresponding to these TCR idiotopes (Table 1). In initial experiments, the m-VDJ peptide was administered in complete Freund's adjuvant (CFA). Age- and gender-matched control rats received either CFA alone or were not vaccinated. Thirty days after vaccination, all animals were challenged with guinea pig MBP in CFA. Disease, characterized by severe paralysis and wasting, was observed in unvaccinated control animals by day 10 or 11 (Table 2), persisted for 5 to 6 days, and spontaneously remitted. Disease courses among CFA-vaccinated rats were not significantly different from unvaccinated controls. In contrast, disease in the rats vaccinated with m-VDJ (5,

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50, or 250 μg) was slightly decreased in severity and duration and delayed in onset. A slight dose effect was observed for the mean day of onset and duration of disease. Though one animal in each of these groups exhibited no clinical signs of disease, histologic evaluation (*13*) revealed a lymphocytic infiltration of the central nervous system in all animals. Although protection against histologic disease was not induced by this peptide, these initial observations prompted us to test other peptides.

Vaccinations with peptides that corresponded to the Lewis rat β chain VDJ (r-VDJ1, r-VDJ2, and r-VDJ3 in Table 1) gave similar results (Table 2). To determine if the low efficacy of the rat VDJ peptides was due to their length, we synthesized smaller peptides of the r-VDJ2 sequence (Table 1). Vaccination with r-VDJ2$_9$ (Table 2) induced significant resistance to EAE; none of the animals had clinical or histological signs of disease ($P = 0.003$). In contrast, r-VDJ2$_8$ was more effective than r-VDJ2, but did not induce complete protection; only two of four animals showed neither clinical nor histological signs of disease ($P = 0.3$). Although r-VDJ2$_9$ would not be predicted as a T cell epitope by any of the accepted methods (*14, 15*), the relationship observed between r-VDJ2 peptide length and immunogenicity (that is, vaccination efficacy) is typical of peptides specifying T cell epitopes (*16*).

Vaccination with the JαTA39 peptide (Table 1) also protected Lewis rats from EAE; only one of three animals developed disease, which was mild and had delayed onset ($P = 0.1$). The presence or absence of histological signs of disease corresponded with the clinical state of these animals (Table 2). The COOH-terminal five amino acids of r-JαTA39 (Fig. 1B) are predicted to contain significant amphipathic α-helix (*14*) and to conform to a linear-sequence motif common to T cell epitopes (*15*). Vaccination with a mixture of r-JαTA39 and the rat β chain VDJ peptides (r-VDJ1, r-VDJ2, and r-VDJ3) also induced complete resistance to EAE induction ($P = 0.01$), both clinically and histologically (Table 2). However, a claim of enhanced efficacy, compared to JαTA39 alone, cannot be statistically validated with experimental groups of three animals. Nonetheless, the protection induced by vaccinating with JαTA39 is highly significant ($P = 0.004$), if all animals that received JαTA39 ($n = 6$) are compared to controls ($n = 12$).

To insure that these TCR idiotopes were present and recognizable on the surface of encephalitogenic T cells, we performed cytofluorometry with affinity-purified rabbit antibodies (Abs) to the peptides (Fig. 2). The r-VDJ2 and r-JαTA39 Abs each stained a significant proportion of an encephalitogenic Lewis rat MBP-reactive T cell line (Fig. 2, B and D), but did not bind (r-VDJ2) or bound only weakly (JαTA39) to nonencephalitogenic Lewis rat T cells (*17*).

Our data suggest that TCR peptides, which themselves represent T cell epitopes, effectively induce resistance to EAE. This is of interest given reports of cytotoxic T cells that are specific for idiotypic determinants on encephalitogenic T cells and which adoptively transfer resistance to EAE (*18, 19*). We have preliminary evidence that one such T cell line [the anti-S1 line (*18*)] proliferates in vitro in response to autologous antigen-presenting cells (APCs) and r-VDJ2$_9$ (*20*). Anti-S1 was derived by regular in vitro stimulation with MBP-reactive T cell blasts; its stimulation by r-VDJ2$_9$ and APCs suggests that the ligand for anti-S1 TCRs involves endogenous TCR peptides, possibly cycling through MHC molecules on the surface of MBP-reactive T cells (*21*).

Successful vaccination with TCR idiotopes should be specific for only the disease-inducing T cells, yet broad enough to target all of the reactive clones. The most specific peptides, those derived from junctional sequences, have the greatest propensity for variation and may not target the entire pathogenic T cell population. Vaccination with peptide mixtures or with peptides derived from germline V and J elements could be used instead. Germline sequences are less heterogeneous than junctional sequences and, being larger, allow more flexibility in the selection of peptides with optimal immunogenic characteristics. Germline peptides, however, may be less selective than junctional peptides, because the immune response induced would influence the activity of all T cells with those germline elements.

Table 1. Synthetic T cell receptor idiotopes identified in EAE.

Peptide	Sequence	Reference
m-VDJ	S G D A G G G Y E	(*6*)
r-VDJ1	C A S S D – S S N T E V F F G K	(*7*)
r-VDJ2	C A S S D – S G N T E V F F G K	(*8*)
r-VDJ3	C A S S D – S G N – V L Y F G E G S R	(*8*)
r-VDJ2$_8$	S S D – S G N T E	(*8*)
r-VDJ2$_9$	A S S D – S G N T E	(*8*)
r-JαTA39	R F G A G T R L T V K	(*7*)

Table 2. Vaccination against EAE with T cell receptor peptides. Peptides shown in Table 1 were synthesized by solid-phase methods, desalted by Sephadex G-25 column chromatography in 0.1*M* acetic acid, and lyophilized. Peptides were resuspended in phosphate-buffered saline and emulsified with equal volumes of CFA, made by suspending heat-killed desiccated *Mycobacterium tuberculosis* H37ra (10 mg/ml; Difco Laboratories, Detroit, MI) in incomplete Freund's adjuvant. The emulsions (50 μl) were administered intradermally in each of the hind footpads of 8- to 12-week-old female Lewis rats at the indicated doses. At 30 days, each rat was challenged with guinea pig MBP (50 μg in CFA) in the front footpads. Animals were monitored daily beginning at day 9 for clinical signs of disease and were scored on a three-point scale: 1 = limp tail; 2 = hind leg weakness; and 3 = hind leg paralysis. After recovery, animals were sacrificed and histological evaluation of disease performed as previously described (*13*). *P* values were calculated with Fisher's exact test (*27*), corrected for multiple comparisons, by analyzing disease incidence in controls (unvaccinated plus CFA-vaccinated animals) and each individual experimental group. N.S. = not significant.

Vaccination	Dose (μg)	Number with disease/ number tested	Mean max. severity	Mean onset* (days)	Mean duration (days)	Number with histology/ number tested
None		6/6	3.0	10.5	5.2	
CFA		6/6	2.8	11.5	5.2	
m-VDJ	5	2/3 N.S.	1.3	13.5	2.7	3/3
m-VDJ	50	2/3 N.S.	1.7	13.0	2.3	3/3
m-VDJ	250	2/3 N.S.	1.3	15.0	1.7	3/3
r-VDJ1	50	3/3	2.0	13.3	2.7	3/3
r-VDJ2	50	3/3	2.3	13.3	3.0	3/3
r-VDJ3	50	2/3 N.S.	2.0	11.0	2.7	3/3
r-VDJ2$_9$	50	0/4 ($P = 0.003$)	0.0	None	0.0	0/4 ($P = 0.003$)
r-VDJ2$_8$	50	2/4 ($P = 0.3$)	1.5	12.5	1.5	2/4 ($P = 0.3$)
r-JαTA39	50	1/3 ($P = 0.1$)	.7	15.0	1.7	1/3 ($P = 0.1$)
r-JαTA39 + r-VDJ1–3	50 each	0/3 ($P = 0.01$)	0.0	None	0.0	0/3 ($P = 0.01$)
All r-JαTA39		1/6 ($P = 0.004$)	0.3	15.0	0.8	1/6 ($P = 0.004$)

*Of those animals that developed disease.

Immunotherapeutic strategies for EAE have included infusion of monoclonal antibodies (MAbs) to Vβ8, which prevents disease in mice (*5, 6*) and reverses it if administered after onset (*5*). Similarly, infusion of a MAb to a clonotypic determinant on the surface of encephalitogenic T cells protects Lewis rats from EAE (*22*). Resistance to EAE induction has also been conferred by vaccination with attenuated, encephalitogenic T cell clones (*23, 24*) and by transfer of ex vivo–derived immunoregulatory T cells (*18, 19, 25*).

Vaccination with TCR peptides is preferable to MAb infusion because persistent, active immunity is induced. Whereas active immunity is also induced by vaccinating with attenuated encephalitogenic T cells, the efficacy of this procedure is reported to be variable (*26*). Further, whole T cell vaccination induces immunity to T cell determinants other than those that confer protection (*26*) and, in an outbred population, must be administered autologously to avoid alloreactive complications. Administration of ex vivo–derived regulatory T cells must be similarly individualized. Such labor-intensive strategies are unlikely to be employed on a significant scale. TCR peptide vaccination is a selective approach to the modulation of T cell–mediated autoimmunity. It also may be applicable to the helper T cells in antibody-mediated autoimmunity, T cell lymphomas, and other pathogenic conditions mediated by specific, oligoclonal T cell populations.

REFERENCES AND NOTES

1. A. Ben-Nun, H. Wekerle, I. R. Cohen, *Eur. J. Immunol.* **11**, 195 (1981).
2. A. Ben-Nun and I. R. Cohen, *J. Immunol.* **129**, 303 (1982).
3. S. Zamvil *et al.*, *Nature* **317**, 355 (1985).
4. S. S. Zamvil *et al.*, *J. Exp. Med.* **167**, 1586 (1988).
5. H. Acha-Orbea *et al.*, *Cell* **54**, 163 (1988).
6. J. L. Urban *et al.*, *ibid.*, p. 577.
7. R. R. Burns *et al.*, *J. Exp. Med.* **169**, 27 (1989).
8. J. Chluba, C. Steeg, A. Becker, H. Wekerle, J. Epplen, *Eur. J. Immunol.* **19**, 279 (1989).
9. E. Heber-Katz and H. Acha-Orbea, *Immunol. Today* **10**, 164 (1989).
10. S. S. Zamvil *et al.*, *Nature* **324**, 258 (1986).
11. C.-H. J. Chou, R. B. Fritz, F. C.-H. Chou, R. F. Kibler, *J. Immunol.* **123**, 1540 (1979).
12. R. K. Wilson, E. Lai, P. Concannon, R. K. Barth, L. E. Hood, *Immunol. Rev.* **101**, 149 (1988).
13. R. A. C. Hughes and H. C. Powell, *J. Neuropath. Exp. Neurol.* **43**, 154 (1984).
14. J. A. Berzofsky *et al.*, *Immunol. Rev.* **98**, 9 (1987).
15. J. B. Rothbard and W. R. Taylor, *EMBO J.* **7**, 93 (1988).
16. A. R. M. Townsend *et al.*, *Cell* **44**, 959 (1986).
17. M. D. Howell, unpublished data.
18. D. Sun, Y. Qin, J. Chluba, J. T. Epplen, H. Wekerle, *Nature* **332**, 843 (1988).
19. D. Sun, A. Ben-Nun, H. Wekerle, *Eur. J. Immunol.* **18**, 1993 (1988).
20. D. Sun, M. D. Howell, S. W. Brostoff, unpublished data.
21. S. Buus, A. Sette, S. M. Colon, H. M. Grey, *Science* **242**, 1045 (1988).
22. M. Owhashi and E. Heber-Katz, *J. Exp. Med.* **168**, 2153 (1988).
23. A. Ben-Nun, H. Wekerle, I. R. Cohen, *Nature* **292**, 60 (1981).
24. O. Lider, T. Reshef, E. Beraud, A. Ben-Nun, I. R. Cohen, *Science* **239**, 181 (1988).
25. K. E. Ellerman, J. M. Powers, S. W. Brostoff, *Nature* **331**, 265 (1988).
26. H. Offner, R. Jones, B. Celnik, A. A. Vandenbark, *J. Neuroimmunol.* **21**, 13 (1989).
27. W. L. Hays, *Statistics* (Holt, Rinehart & Winston, New York, 1981), pp. 552–555.

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The Neuron-Specific Protein PGP 9.5 Is a Ubiquitin Carboxyl-Terminal Hydrolase

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A complementary DNA (cDNA) for ubiquitin carboxyl-terminal hydrolase isozyme L3 was cloned from human B cells. The cDNA encodes a protein of 230 amino acids with a molecular mass of 26,182 daltons. The human protein is very similar to the bovine homolog, with only three amino acids differing in over 100 residues compared. The amino acid sequence deduced from the cDNA was 54% identical to that of the neuron-specific protein PGP 9.5. Purification of bovine PGP 9.5 confirmed that it is also a ubiquitin carboxyl-terminal hydrolase. These results suggest that a family of such related proteins exists and that their expression is tissue-specific.

COVALENT ATTACHMENT OF UBIQUITIN to a number of cellular proteins has been postulated to play a role in a variety of cellular processes (*1–3*). This widespread posttranslational modification of proteins is thought to target the attached protein for various metabolic fates. In the case of proteolysis, polyubiquitination of substrate proteins is observed, and these conjugates are recognized and degraded by specific proteases in the cell (*2*). In contrast, the reversible ubiquitination of histones is limited to one or two ubiquitin molecules per histone molecule, does not lead to degradation, and may be important for chromatin condensation or cell cycle progression (*4–6*). Covalent attachment of ubiquitin to the T cell homing receptor (*7*) and the platelet-derived growth factor receptor (*8*) has been demonstrated, but the consequences of this modification are unknown. Three eukaryotic genes code for proproteins that consist of an NH_2-terminal ubiquitin and a COOH-terminal ribosomal protein (*9, 10*); the ubiquitin is proteolytically processed from these proteins at a poorly defined point in the maturation of the ribosome. Finally, whereas a fourth ubiquitin gene codes for a polyubiquitin precursor that is a heat shock protein (*11, 12*), its role in the stress response is undefined. These diverse functions have one common feature; that is, they all require that ubiquitin derivatives be recognized by specific proteases that are able to hydrolyze a peptide bond at the COOH-terminal glycine of ubiquitin. Thus, the ubiquitinyl-*N*ε amide bond is enzymatically hydrolyzed during the removal of ubiquitin from histones that occurs before mitosis (*13, 14*), cytoplasmic conjugates are disassembled by activities present in most tissues (*15*), and proteolytic intermediates must be released from the COOH-terminus

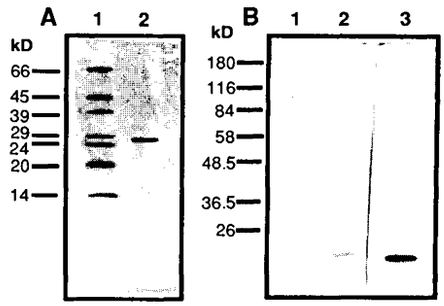

Fig. 1. Specificity of affinity-purified antibodies to homogeneous UCH-L3. Antibodies were affinity-purified with antigen immobilized on nitrocellulose paper. (**A**) Silver-stained SDS-polyacrylamide gel showing the purified UCH-L3 used to elicit antibodies in rabbits. Lane 1, molecular mass standards; lane 2, purified UCH-L3. (**B**) Protein immunoblots of an SDS-polyacrylamide gel obtained using the affinity-purified antibody. Lane 1, molecular mass standards; lane 2, bovine thymus homogenate; lane 3, purified bovine UCH-L3.

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