Commentary

Myelin–specific CD8 T Cells in the Pathogenesis of Experimental Allergic Encephalitis and Multiple Sclerosis

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Experimental autoimmune encephalomyelitis (EAE) serves as the prototypic model for T cell–mediated autoimmunity. EAE has striking similarities with the human disease acute disseminated encephalomyelitis, a complication seen with vaccination and after certain viral infections. EAE has been used as a model to help understand the pathogenesis of multiple sclerosis (MS) and to help identify potential therapeutic candidates for this disease (Table I), Until now, research has focused nearly exclusively on the role of CD4$^+$ T cells in EAE (1–3). In this issue and in a recent publication in the *Journal of Immunology* (4, 5), a pathogenic role of CD8 T cells in EAE has now been described. Lesions in inflammatory infiltrates in the brain and spinal cord of MS patients (6, 7), and in brain lesions in EAE (8), include both CD4 and CD8 T cells. Therefore, it is important to seriously consider both of these T cell subsets in the evolution of this mysterious disease which afflicts nearly a million individuals worldwide.

Over 4,500 papers have been published on the EAE model since it was first described (9, 10). *The Journal of Experimental Medicine* has published many of the landmark papers on EAE, dating back >60 yr to the publication of River's classic paper on the induction of encephalomyelitis in primates with extracts from rabbit brain (11). Kabat's description of the use of Freund's adjuvant in the induction of EAE appeared in *The Journal of Experimental Medicine* 54 yr ago (12). The use of Complete Freund's Adjuvant to induce EAE reduced the number of injections required in River's model from 85 over 1 yr to a single injection of brain extract (12)! The use of Freund's Adjuvant to initiate EAE probably induced biases in the types of cells that expanded after such immunization. T cells that could be expanded after injection of Complete Freund's Adjuvant were mostly Th1, probably due to the CpG motifs in the DNA from the killed mycobacteria tuberculosis. Thus, via history and peer selection *The Journal of Experimental Medicine* has traditionally been the forum for new developments in this model.

Until now almost all of the work done in the EAE model has focused on CD4 T cells and the resulting cascade of cytokines and chemokines involved in pathogenesis. Recently, again in this journal, LaFaille and Tonegawa showed that CD4$^+$, myelin-specific T cells induced EAE predominantly, but not always, via production of Th1 cytokines (13). Indeed they showed that Th2 T cells could trigger EAE (13). Such Th2 myelin-specific T cells can also cause anaphylaxis, creating a new version of "horror autotoxicus" with "allergy to self" (14). Thus, EAE has been a durable model, and may indeed come in at least two forms: experimental *autoimmune* encephalomyelitis and experimental *allergic* encephalomyelitis (9, 14). We now must dissect even deeper layers of our understanding of EAE. Not only are their roles for Th1 and Th2 T cells, and not only are there autoimmune and allergic forms of EAE, we now must deal with at least two versions of T cells, those bearing the CD4 molecule and those bearing the CD8 molecule, for both of them can induce this model disease.

Goverman and colleagues have shown that CD8$^+$ T cell clones, specific for a class I restricted–fragment of myelin basic protein, produce severe EAE with paralysis and other neurological deficits (4). The strategy employed by Goverman and colleagues is noteworthy. They first demonstrated the existence of cytotoxic T cells for myelin in C3H.*shi* mice. These mice have the "shiverer" mutation, rendering them deficient in one of the major constituents of the myelin sheath, myelin basic protein. Defective myelination causes these mice to have a tremor resembling shivering. The C3H.*shi* mice had cytotoxic T cells specific for the epitope MBPp79–87 restricted by H-2K. In normal C3H mice, cytotoxic T cell clones could be established after immunization with MBP79–87 in Complete Freund's Adjuvant. So, once the class I–restricted epitope was known, there was no intrinsic barrier for expansion of class I–restricted CD8 T cells that are cytotoxic for myelin, after immunization with MBPp79–87 in Complete Freund's Adjuvant. These cytotoxic T cells in Goverman's model have produced the newest version of EAE.

Cytoxic CD8 T cell clones injured brain and induced ataxia, spasticity, and hind limb paralysis in mice after intravenous injection. Neuropathology revealed perivascular cuffs in the vascular walls of the brain. Interestingly, CD4–

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induced EAE produces perivascular cuffs in the blood vessel walls in brain and in spinal cord. Demyelination was noted (4). In the paper by Sun and colleagues (5), CD8^{+} T cells reactive to myelin oligodendroglial glycoprotein peptide 35–55 induced massive inflammation and demyelination in the central nervous system. Thus there is strong evidence from two independent labs, with data emerging simultaneously, to support a major role for CD8 T cells in autoimmune demyelination.

The major differences between CD4-induced EAE and CD8-induced EAE appear in attempts at modulating disease with agents that block the cytokines TNF-α and IFN-γ. The roles for these cytokines in MS and EAE remain controversial. When EAE is induced by CD4^{+} T cells, disease is blocked with antibodies to TNF-α or fusion proteins that block TNF receptor. With one notable exception, described by Bernard and colleagues, EAE induced by CD4 T cells is usually ameliorated when TNF-α or lymphotoxin is inhibited (references 10 and 15, and Table II). In contrast, in the model of CD8-induced EAE devised by Goverman's group (4), TNFR:FC has no effect, while in MS TNFR:FC actually worsens disease (16). Further, a large number of studies in EAE show that EAE is blocked by administration of rIFN-γ (10). Moreover, in Goverman's study showing EAE induced with CD8 T cells (4), anti–IFN-γ antibody reduces disease, while in EAE induced by CD4^{+} T cells, anti–IFN-γ actually worsens disease. In MS a clinical trial of rIFN-γ was discontinued prematurely due to worsening of disease (17). Thus, when considering the potential pathologic roles of TNF and IFN-γ, there is more concordance between the model of CD8^{+}-induced EAE and MS than the corresponding CD4^{+} models of EAE and MS (Table II). Thus, to some extent the CD8 model of EAE reflects some of the results obtained in clinical trials of MS better than the CD4-induced models of EAE.

The role of CD8 T cells in MS and its experimental models has remained enigmatic. There have been bold attempts to delete CD4 T cells in MS with chimerized monoclonal antibodies that are cytotoxic. While it is certain that CD4^{+} T cells are critical in EAE induced with class II–restricted T cell clones or with Complete Freund's Adjuvant (18, 19), the role of CD4^{+} T cells in MS is far less clear. Extensive depletion of CD4^{+} T cells in MS made small but significant improvements in relapse rates and reduced the level of inflammatory activity in the brain, detected with magnetic resonance imaging (20, 21). A Phase II placebo–controlled double-masked study showed that "the degree of depletion of CD4^{+} cells was important with regard to treatment efficacy. Using CD4 counts as a covariate, there was a statistically significant effect on the number of active lesions >18 mo ($P = 0.04$). There was a statistically significant reduction of 41% in the number of clinical relapses (a secondary efficacy parameter) after 9 mo ($P = 0.02$), which was still present after 18 mo." (21) Perhaps similar depletion studies ought to be contemplated in MS with anti-CD8 antibodies, or even a combination of anti-CD4 and anti-CD8 antibodies. The new models proposed by J. Goverman and by D. Sun certainly provide a basis for considering this strategy.

The immunochemistry of T cell interactions with antigen and recent genetic studies on susceptibility to disease

Table I. *Comparisons Between MS and EAE, Clinical, Pathological, and Genetic*

Clinical Presentation	MS	EAE
Relapses and remissions	present	present
Paralysis	present	present
Ataxia	present	present
Visual impairment	present	present
Genetics	MS	EAE
MHC-linked susceptibility	yes	yes
Females more susceptible	yes	yes
Pathology in Lesions	MS	EAE
Clonal CD4^{+} T cells react to myelin	present	present
Clonal CD8^{+} T cells react to myelin	present	present
Perivascular cuffs	present	present
Antibodies to myelin	present	present
α4 integrin, complement	present	present
TNF-α, IFN-γ	present	present
Demyelination	present	present
Axonal dystrophy	present	present

Table II. *Comparisons of Therapeutic Approaches in MS, CD4-induced and CD8-induced EAE*

Therapy in CD4-induced EAE	MS	EAE
IFN-γ, systemic	worsens	cures
Anti–IFN-γ	not done	worsens
Anti-TNF	worsens	cures[a]
TNF, systemic	not done	cures[b]
TNF receptor blockade	worsens	improves
Anti-α4 integrin	beneficial	cures
Copaxone	beneficial	improves
Anti-CD4	beneficial	cures
Therapy in CD8-induced EAE	MS	EAE
IFN-γ, systemic	worsens	not done
Anti–IFN-γ	not done	cures
TNF receptor blockade	worsens	no effect
Anti-α4 integrin	beneficial	not yet done
Copaxone	beneficial	not yet done
Anti-CD8	not yet done	cures

[a]Reference 10.
[b]Reference 15.

have revealed new evidence supporting the importance of both CD4 and CD8 T cells in autoimmune disease. T cells recognize antigen via a complex interaction with antigen embedded in a binding cleft on MHC class I or class II molecules. CD4 anchors the T cell to MHC class II, while CD8 anchors the T cell to MHC class I. Both MHC class I and class II genes are associated with susceptibility to MS (22–24). Moreover, the MHC class I and II HLA genes are in strong linkage disequilibrium, suggesting that they commonly segregate together on the chromosome, giving broad genotypes involving a constellation of alleles from Class I HLA-A and -B genes, along with MHC class II HLA-DR and -DQ genes. There are common extended genotypes for susceptibility to MS involving HLA-A, -B, -DR, and -DQ. Thus there are cogent reasons to look not only for the pathogenic roles of CD4 T cells, which bind HLA class II–loaded with antigen, but also for a pathogenic role for CD8 T cells binding HLA class I plus antigen. Finally, HLA class I and II gene products are elevated on inflamed oligodendroglial cells and these may further serve as targets for both CD4 and CD8 T cells. Thus, the immunogenetics of MS provide a basis for understanding why both CD4 and CD8 T cells may play critical roles in the pathogenesis of demyelinating disease.

The CD8 T cell deserves at least as much attention as the CD4 T cell, as a prime suspect as one of the T cells causing demyelination, and therefore potentially a major culprit in MS. There are clonal expansions of both CD4 and CD8 T cells in the brains of MS patients. Some of these clones of T cells have specificity for myelin proteins. Therefore, it is a valid expectation that some of the clonally expanded CD4^{+} and CD8^{+} T cells in MS brain may turn out to be rogues and villains (25, 26). Their containment may be beneficial for those suffering from MS.

References

1. Ben Nun, A., H. Wekerle, and I.R. Cohen. 1981. The rapid isolation of clonable, antigen specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol.* 111:195–199.
2. Zamvil, S., P. Nelson, J. Trotter, D. Mitchell, R. Knobler, R. Fritz, and L. Steinman. 1985. T cell clones specific for myelin basic protein induce chronic relapsing EAE and demyelination. *Nature.* 317:355–358.
3. Zamvil, S., P. Nelson, D. Mitchell, R. Knobler, R. Fritzm, and L. Steinman. 1985. Encephalitogenic T cell clones specific for myelin basic protein: an unusual bias in antigen presentation. *J. Exp. Med.* 162:2107–2124.
4. Huseby, E., D. Liggitt, T. Brabb, B. Schnabel, C. Ohlen, and J. Goverman. 2001. A pathogenic role for CD8^{+} T cells in a model for multiple sclerosis. *J. Exp. Med.* 194:669–676.
5. Sun, D., J.N. Whitaker, Z. Huang, D. Liu, C. Coleclough, H. Wekerle, and C.S. Raine. 2001. Myelin antigen-specific CD8 T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J. Immunol.* 166:7579–7587.
6. Traugott, U., E. Reinherz, and C.S. Raine. 1983. Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science.* 219:308–310.
7. Hauser, S.L., A.K. Bhan, M. Gilles, M. Kemp, C. Kerr, and H.L. Weiner. 1986. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol.* 19:578–587.
8. Sriram, S., D. Solomon, R.V. Rouse, and L. Steinman. 1982. Identification of T cell subsets and B lymphocytes in mouse brain EAE lesions. *J. Immunol.* 129:1649–1654.
9. Weiner, H.L. 2001. The fine line between autoimmune and allergic encephalomyelitis. *Nat. Immunol.* 2:193–194.
10. Steinman, L. 1999. Assessment of the utility of animal models for multiple sclerosis and demyelinating disease in the design of rational therapy. *Neuron.* 24:511–514.
11. Rivers, T.M., and F.F. Schwentker. 1935. Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. *J. Exp. Med.* 61:689–702.
12. Kabat, E.A., A. Wolf, and A.L. Bezer. 1947. The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J. Exp. Med.* 85:117–129.
13. Lafaille, J.J., F.V. Keere, A.L. Hsu, J. Baron, W. Hass, C.S. Raine, and S. Tonegawa. 1997. Myelin basic protein T helper 2 cells cause experimental autoimmune encephalomyelitis in immunodeficient mice rather than protect them from disease. *J. Exp. Med.* 186:307–312.
14. Pedotti, R., D. Mitchell, J. Wedemeyer, M. Karpuj, D. Chabas, E. Hattab, M. Tsai, S.J. Galli, and L. Steinman. 2001. An unexpected version of horror autotoxicus: anaphylactic shock to a self-peptide. *Nat. Immunol.* 2:216–222.
15. Liu, J., M.W. Marino, G. Wong, D. Grail, A. Dunn, J. Bettadapura, A. Slavin, L. Old, and C.C.A. Bernard. 1998. TNF is a potent anti-inflammatory cytokine in autoimmune mediated demyelination. *Nat. Med.* 4:78–83.
16. Lenercept MS Study Group and the University of British Columbia MS/MRI Analysis Group. 1999. TNF neutralization in MS: results of a randomized placebo-controlled multicenter study. *Neurology.* 53:457–465.
17. Panitch, H.S., R.L. Hirsch, J. Schindler, and K.P. Johnson. 1987. Treatment of MS with γ interferon: exacerbations associated with activation of the immune system. *Neurology.* 37:1097–1103.
18. Brostoff, S.W., and D.W. Mason. 1984. Experimental allergic encephalomyelitis: successful treatment in vivo with a monoclonal antibody that recognizes T helper cells. *J. Immunol.* 133:1938–1942.
19. Waldor, M.K., R. Hardy, L.A. Herzenberg, L. Lanier, S. Sriram, M. Lim, and L. Steinman. 1985. Reversal of EAE with monoclonal antibody to a T cell subset marker (L3T4). *Science.* 227:415–417.
20. Lindsey, J.W., S. Hodgkinson, R. Mehta, D. Mitchell, D. Enzmann, and L. Steinman. 1994. Repeated treatment with chimeric anti-CD4 antibody in multiple sclerosis. *Ann. Neurol.* 36:183–189.
21. van Oosten, B.W., M. Lai, S. Hodgkinson, F. Barkhof, D.H. Miller, I.F. Moseley, A.J. Thompson, P. Rudge, A. McDougall, J.G. McLeod, and H.J. Adèr. 1997. Polman CH treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology.* 49:351–357.
22. Haines, J., L.M. Ter Minisan, A. Bazak, J. Gusella, D. Kim,

H. Terwedow, M. Pericak Vance, J. Rimmler, C. Haynes, A. Roses, et al. 1996. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. The multiple sclerosis genetics group. *Nat. Genet.* 13:469–471.

23. Ebers, G.C., K. Kukay, D.E. Bulman, A.D. Sadovnick, G. Rice, C. Anderson, H. Armstrong, K. Cousin, R.B. Bell, W. Hader, et al. 1996. A full genome search in multiple sclerosis. *Nat. Genet.* 13:472–476.

24. Sawcer, S., H.B. Jones, R. Feakes, J. Gray, N. Smaldon, J. Chataway, N. Robertson, D. Clayton, P. Goodfellow, and A. Compston. 1996. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat. Genet.* 13:464–468.

25. Oksenberg, J.R., M.A. Panzara, A.B. Begovich, D. Mitchell, H.A. Erlich, R.S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C.C.A. Bernard, and L. Steinman. 1993. Selection for T cell receptor Vβ-Dβ-Jβ gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature.* 362:68–70.

26. Babbe, H., A. Roers, A. Waisman, H. Lassmann, N. Goebels, R. Hohlfeld, M. Friese, R. Schroder, M. Deckert, S. Schmnidt, R. Ravid, and K. Rajewsky. 2000. Clonal expansions of CD8+ T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med.* 192: 393–404.