run were dried under vacuum and sequenced. One of the peptides (eluting at ~24% 1-propanol) gave the sequence [A]AEKEKTF[C]VNGGEXFM-VKDLXNP. Residues in brackets were uncertain and an X represents a cycle in which it was not possible to identify the amino acid. The initial yield was 8.5 pmol and the sequence did not correspond to any known protein. Residues 1, 9, 15, and 22 were later identified in the cDNA sequence as cysteine. Direct sequencing of the ~45-kD band from a gel that had been overloaded and blotted onto a PVDF membrane revealed a low abundance sequence XEXKE[G][R]GK[G]K[G]-KKKEXGXG[K] with a very low initial yield (0.2 pmol). This corresponded to residues 2 to 22 of proHRG-α (Fig. 2), suggesting that serine 2 is the NH_2-terminus of proHRG-α. Although the NH_2-terminus was blocked, we have observed that occasionally a small amount of a normally blocked protein may not be post-translationally modified. The NH_2-terminal assignment was confirmed by mass spectrometry of the protein after digestion with cyanogen bromide. The COOH-terminus of the isolated protein has not been definitely identified; however, by mixture sequencing of proteolytic digests, the mature sequence does not appear to extend past residue 241. Abbreviations for amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

14. An oligo(dT)-primed λgt10 [T. V. Huynn, R. A. Young, R. W. Davis, *λgt10 and λgt11 DNA Cloning Techniques: A Practical Approach*, D. Glover, Ed. (IRC Press, Oxford, 1984)] cDNA library was constructed [U. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983)] with mRNA purified [J. M. Chirwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)] from MDA-MB-231 cells. The following eightfold degenerate antisense deoxyoligonucleotide encoding the 13–amino acid sequence AEKEKTFCVNGGE (*13*) was designed on the basis of human codon frequency optima [R. Lathe, *J. Mol. Biol.* **183**, 1 (1985)] and chemically synthesized: 5′-CTCGCC(G or T)CC(A or G)TTCAC(A or G)CA-GAAGGTCTTCTCCTTCTCAGC-3′. For the purpose of probe design a cysteine was assigned to an unknown residue in the amino acid sequence (*13*). The probe was labeled by phosphorylation and hybridized under low-stringency conditions to the cDNA library. The proHRG-α protein was identified in this library. HRG-β1 cDNA was identified by probing a second oligo(dT)-primed λgt10 library made from MDA-MB-231 cell mRNA with sequences derived from both the 5′ and 3′ ends of proHRG-α. Clone 13 (Fig. 2A) was a product of screening a primed (5′-CCTCGCTCCTTCTTCT-TGCCCTTC-3′ primer; proHRG-α antisense nucleotides 33 to 56) MDA-MB-231 λgt10 library with 5′ HRG-α sequence. We used a sequence corresponding to the 5′ end of clone 13 as the probe, to identify proHRG-β2 and proHRG-β3 in a third oligo(dT)-primed λgt10 library derived from MDA-MB-231 cell mRNA. Two cDNA clones encoding each of the four HRGs were sequenced [F. Sanger, S. Milken, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)]. Another cDNA designated clone 84 has an amino acid sequence identical to proHRG-β2 through amino acid 420. A stop codon at position 421 is followed by a different 3′-untranslated sequence.
15. B. Roberts, *Biochim. Biophys. Acta.* **1008**, 263 (1989).
16. G. I. Bell *et al.*, *Nucleic Acids Res.* **14**, 8427 (1986).
17. S. Higashiyama, J. A. Abraham, J. Miller, J. C. Fiddes, M. Klagsbrun, *Science* **251**, 936 (1991).
18. G. D. Plowman *et al.*, *Mol. Cell. Biol.* **10**, 1969 (1990).
19. R. Derynck, A. B. Roberts, M. E. Winkler, E. Y. Chen, D. V. Goeddel, *Cell* **38**, 287 (1984).
20. Fourteen oligonucleotide probes to the conserved and divergent regions of the proHRGs were chemically synthesized. RNA blot hybridizations were done with individual radiolabeled probes on portions (5 μg) of MDA-MB-231 cell poly(A)$^+$ mRNA that were separated according to size by gel electrophoresis and transferred to nitrocellulose.
21. J. Lee and W. Wood, unpublished data.
22. Y. Yonemura *et al.*, *Cancer Res.* **51**, 1034 (1991); S. Jain *et al.*, *Int. J. Cancer* **48**, 668 (1991); N. R. Lemoine *et al.*, *Br. J. Cancer* **64**, 79 (1991); J. Yokota *et al.*, *Oncogene* **2**, 283 (1988).
23. M. F. Press, C. Cordon-Cardo, D. J. Slamon, *Oncogene* **5**, 953 (1990).
24. The proHRG-α and proHRG-β1 cDNAs were spliced into Epstein Barr virus–derived expression vectors containing a cytomegalovirus promoter. The rHRGs were purified [essentially as described (*12*)] from the serum-free conditioned medium of stably transfected CEN4 cells [human kidney 293 cells (ATCC no. CRL 1573) expressing the Epstein Barr virus EBNA-1 transactivator]. Amino acid sequence analysis of proteolytic digestion products from rHRG-α and -β1 confirmed their similarity to naturally derived HRGs, which appear to be cleaved versions of proHRGs (W. Henzel, unpublished data). A factor causing phosphorylation of $p185^{erbB2}$ was derived from conditioned medium of transfected COS7 monkey kidney cells transiently transfected with constructs expressing full-length proHRG-α, -β1, or -β2 (*21*). However, similar constructs expressing proHRG-β3 failed to yield activity, suggesting that the hydrophobic domain missing in proHRG-β3 may be necessary for delivery of the precursor to the cell surface and cleavage to form the mature protein. Truncated versions of proHRG-α [63 amino acids, serine 177 to tyrosine 239, (rHRG-$\alpha_{177-239}$)] and proHRG-β1 [68 amino acids, serine 177 to tyrosine 241 (rHRG-$\beta1_{177-241}$)] (Fig. 2B), each encoding the EGF structural unit and immediate flanking regions, were expressed in *E. coli*. These proteins were purified from the periplasmic space and culture broth of cells transformed with expression vectors designed to secrete recombinant proteins [C. N. Chang, M. Rey, B. Bochner, H. Heyneker, G. Gray, *Gene* **55**, 189 (1987)] and stimulated tyrosine phosphorylation of $p185^{erbB2}$ with an EC_{50} of ~40 pM (*26*). Thus, the biological activity of HRG appears to reside in the EGF-like domain of the protein, and carbohydrate moieties are apparently not essential for activity in this assay. Purified rHRG-$\beta1_{177-241}$ was used for binding and cross-linking experiments.
25. G. Lewis and H. M. Shepard, unpublished data.
26. M. X. Sliwkowski, unpublished data.
27. N. E. Davidson, E. P. Gelman, M. E. Lippman, R. B. Dickson, *Mol. Endo.* **1**, 216 (1987).
28. Y. Yarden and J. Schlessinger, *Biochemistry* **26**, 1434 (1987).
29. R. Winzler, *Hormonal Proteins and Peptides*, C. H. Li, Ed. (Academic Press, New York, 1973), pp. 1–15.
30. R. D. Marshall, *Biochem. Soc. Symp.* **40**, 17 (1974).
31. L. A. Goldstein *et al.*, *Cell* **56**, 1063 (1989).
32. A. E. Bolton and W. M. Hunter, *Biochem. J.* **133**, 529 (1973).
33. P. Munson and D. Robard, *Anal. Biochem.* **107**, 220 (1980).
34. R. M. Hudziak, J. Schlessinger, A. Ullrich, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7159 (1987).

35. We thank S. Smigelski and G. Polastri for cell culture; J. Bourell and J. Stults for mass spectrometry; P. Jhurani, M. Vasser, and P. Ng for DNA synthesis; A. Lee for DNA sequencing; B. Fendly for antibodies; G. Bennett for labeling of HRG; D. Reilly for *E. coli* fermentations; and M. Carver, I. Figari, C. Kotts, and J. Lofgren for biological assays.

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Less Mortality but More Relapses in Experimental Allergic Encephalomyelitis in CD8$^{-/-}$ Mice

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Mice lacking in CD8 were generated from homologous recombination in embryonal stem cells at the CD8 locus and bred with the experimental allergic encephalomyelitis (EAE)-susceptible PL/J H-2^u through four backcross generations to investigate the role of CD8$^+$ T cells in this model of multiple sclerosis. The disease onset and susceptibility were similar to those of wild-type mice. However, the mutant mice had a milder acute EAE, reflected by fewer deaths, but more chronic EAE, reflected by a higher frequency of relapse. This suggests that CD8$^+$ T lymphocytes may participate as both effectors and regulators in this animal model.

Experimental allergic encephalomyelitis is a T cell–dependent, induced autoimmune disease and is considered an instructive experimental model for the human demyelinating disease multiple sclerosis because of the pathological and clinical similarities between the two. EAE can be induced in a variety of animals by injection of myelin basic protein (MBP), proteolipid protein (PLP), or peptide fragments of the proteins along with an adjuvant (*1–3*).

The role of T cells in EAE has been amply substantiated by immunological approaches such as neonatal thymectomy (*4*) and T lymphocyte depletion with antibodies (*5, 6*) as well as by the ability to transfer disease into a naïve mouse with activated CD4$^+$ T cells (*7–12*). Depletion of CD4$^+$ and CD8$^+$ T cell subsets implicates the CD4$^+$ cells as the main disease-initiating component (*5, 6, 13, 14*). Studies involving antibody depletion in rats (*13, 14*) have not implicated CD8$^+$ cells as having a

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significant role in the immune modulation of EAE, although in vitro work suggests that $CD8^+$ cells may modulate the disease by eliminating the $CD4^+$ effector T cells through direct cytotoxicity (*15*). Studies on oral tolerance (*16, 17*), as well as reinduction of disease in EAE (*18*) and an interstitial nephritis model (*19*), indicate that the $CD8^+$ cells may influence the pathogenicity of EAE. Hence, we undertook the study of the EAE disease course and immunological responses in H-2^u mice lacking $CD8^+$ T cells to evaluate the role of these cells in immunomodulation of EAE.

The $CD8^{-/-}$ mutant mouse lacks $CD8^+$ cytotoxic cells but has normal development and function of the $CD4^+$ helper cells (*20*). Recent studies with the lymphochoriomeningitis virus (LCMV) have shown that the $CD8^+$ T cells are responsible for the higher mortality in the control mice after intracranial injection, whereas mutant mice are protected (*21*). In vivo humoral responses to vesicular stomatitis virus (VSV) are normal in these mice (*20, 21*).

In this study, we immunized mutant and control mice with whole MBP (*2, 3*). The initial antigen-specific proliferation and antibody production of these mutant mice were normal (Table 1) and consistent with previous results with viral antigens (*20, 22*). However, the mutant mice appeared to be less reactive in MBP-stimulated proliferation and interleukin-2 (IL-2) production (*23*).

To determine the effects of $CD8^+$ cells on the clinical disease, we observed a group of 7 $CD8^{-/-}$ and 14 control mice after immunization with MBP (Fig. 1). The onset and severity of disease in these mice were similar, but there appeared to be more relapses in the $CD8^{-/-}$ group. We then immunized a large number of animals (n = 49 for each group) with MBP. Furthermore, we also increased the dose of the MBP and adjuvant given in order to examine the effects on the severity of the disease as well as on the mortality with respect to the $CD8^+$ cells (*24*).

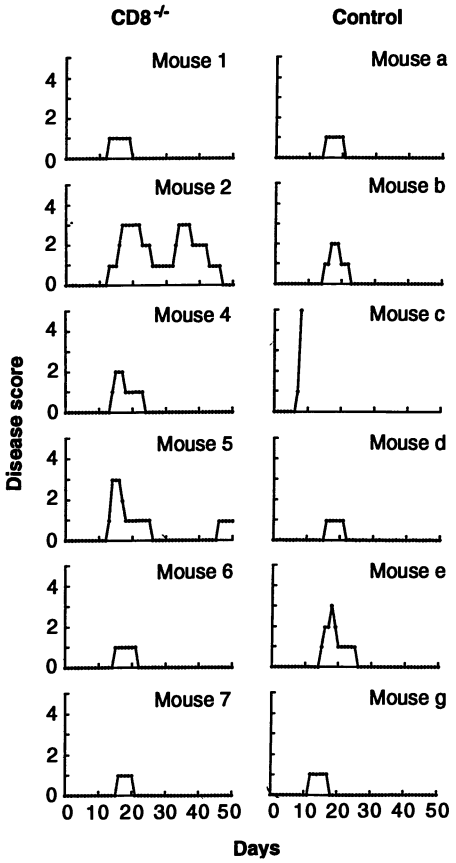

Fig. 1. $CD8^{-/-}$ mutant mice were bred with PL/J mice, and backcrosses from the F_1 generation were made with PL/J mice. A total of four backcrosses was made, and mutant PL/J H-$2^{u/u}$ mice were then expanded for our experiments. As heterozygotes for $CD8^{+/-}$ were used for the intercross, the controls used were littermates of $CD8^{+/+}$ and $CD8^{+/-}$ mice. Studies have shown that there is no difference in the immune response between +/+ and +/− to viruses (*19, 20*) and allogeneic graft rejection (*22*). Fourteen control and seven mutant mice were immunized with 100 μg of whole rabbit MBP in complete Freund's adjuvant (CFA), and immune augmentation was given with 100 ng of pertussis toxin on days 0 and 2. Mice were charted with respect to disease score and weighed daily in a blinded fashion. The scoring system: 0, no disease; 1, tail paralysis; 2, hind-limb weakness; 3, hind-limb paralysis; 4, hind-limb plus forelimb paralysis or weakness; and 5, moribund. Observations were performed for 50 days. Mice 3 and f were excluded from the figure as they did not have the disease. Mouse c had a fatal disease.

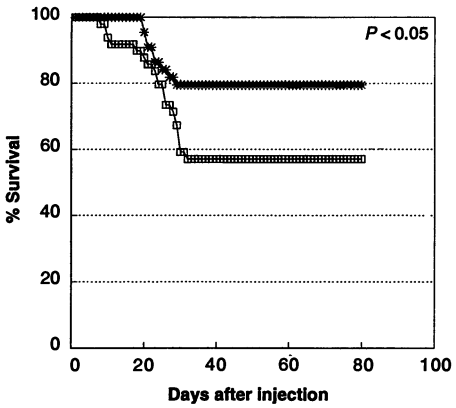

Fig. 2. EAE survival. Control (□) and mutant (✳) mice (n = 49 for each group) were immunized with 200 μg of whole rabbit MBP in CFA. Immune augmentation was given by way of intravenous pertussis toxin (400 ng) given on days 0 and 2. For ethical reasons, mice of grade 4 were killed if their weight loss was great or if they looked ill, and specimens of brain and spinal cord were sent for histological analysis. The mice were observed for 80 days. Statistical analyses of the survival curves were performed with the LIFETEST procedure (Kaplan Mier statistic) in the Statistical Analysis System (SAS).

The general course of the disease in this group is summarized in Table 2. There is no difference with regard to the time of onset and the incidence of disease in the two groups, in accord with other $CD8^+$ T cell–depletion studies (*13, 14*). However, the mutant mice had less mortality over the first 30 days but a higher incidence of relapse (Table 2 and Figs. 2 and 3). This became more significant when the deaths were studied within the same litter comprising mutant and control mice. This may reflect the higher doses of MBP and adjuvant used in our induction procedure (*24*). All of the deaths coincided with the progression of disease scores as well as with a decrease in body weight, suggesting a relation to EAE itself. Furthermore, histological analysis of a few of the mice showed lesions typical of EAE (*25, 26*).

Thus, the $CD8^+$ cells may have an effector (cytotoxic) function during the initial course of the disease. It is tempting to speculate that these $CD8^+$ cells are recruited during the initial inflammatory response and that they recognize endogenous antigen as well as major histocompatibility complex (MHC) class I. These cells may add to the destruction of the myelin sheath or may secrete specific lymphokines that enhance the response. Interferon-γ (IFN-γ) is involved in EAE (*9, 27, 28*), as are T_H1 helper cells, which secrete both IL-2 and IFN-γ (*9, 29*); $CD8^+$ effector T cells also secrete IFN-γ. Tumor necrosis factor–α (TNF-α) and lymphotoxin (LT) have also been noted as important in encephalogenicity (*30*). $CD4^+$ cells will most likely be the first cells to be primed as the immunization process is biased to the exogenous pathway of antigen presentation, but other cells are recruited once the inflammatory response commences. Even though $CD4^+$ cells alone can transfer disease in athymic mice (*31*), $CD8^+$ cells can be recruited and subserve an effector function when stimulated.

During the latter part of the disease, no more deaths were observed, whereas 67% of

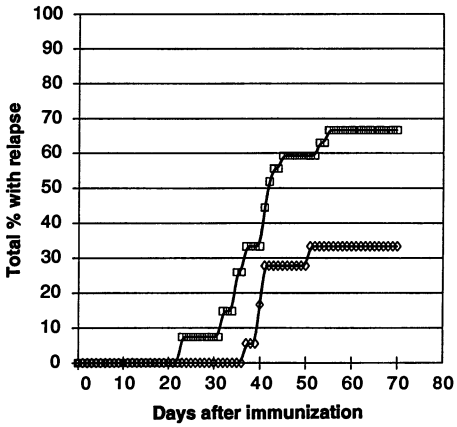

Fig. 3. Cumulative relapse frequency for mutant (□) and control (◇) mice.

Table 1. In vitro immune responses to MBP (±SEM). Four mutant and five control mice were killed on day 14, and splenocytes were obtained for a 5-day in vitro antigen-specific proliferation assay. Splenocytes (5×10^5) were cultured in triplicate on 96-well plates (round bottom NUNC) along with antigen rMBP at 50 μg/ml or MBP 1-11Nac at 20 μg/ml in HL-1 serum-free media (Ventrex) supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin, and streptomycin. The plates were pulsed for 18 hours with [^{3}H]thymidine just before harvesting. Cells were harvested and counted. The means of the values were computed and shown as above. The stimulation index was computed as follows: (experimental − background)/background counts. The background counts were 4122 ± 3272 and 2679 ± 1373 cpm for mutant and control mice, respectively. IL-2 production was assessed on the splenocyte supernatant 72 hours after activation with MBP. The supernatant was diluted 1 in 3, and 5×10^3 CTLL cells that responded to IL-2 only were added to each well. Cells were incubated for 24 hours and pulsed for 8 hours with [^{3}H]thymidine. Background counts for the mutant and control mice were 137.5 ± 30.4 and 153 ± 30.4 cpm, respectively. The serum obtained was diluted 1 in 200 and assayed in an enzyme-linked immunosorbent assay (ELISA) in triplicate as described (*10*). In brief, 96-well Maxisorp (NUNC) plates were coated with 1 μg/ml rabbit MBP dissolved in carbonate buffer (pH 9.0) overnight at 4°C. Plates were rinsed with phosphate-buffered saline containing 0.05% Tween (PBS-Tw). The plates were then blocked with 1% bovine serum albumin (BSA) in PBS for 2 hours at room temperature. Test samples diluted 1 in 200 were introduced along with positive and negative controls. The plates were incubated for 2 hours at 37°C and washed in PBS-Tw. Horseradish peroxidase conjugated goat anti-mouse immunoglobulin G (IgG) (Tago) or goat anti-mouse IgM (Sigma) antibody was then introduced and incubated for 2 hours at 37°C. After rinsing with PBS-Tw, the plates were developed with diaminobenzidine (DAB), and the reaction was stopped with 3 M HCl. Absorbance was then read at 495 nm (A_{495}) with a Dynatech ELISA reader.

Mice	[^{3}H]Thymidine incorporation (10^3 cpm)			Stimulation index			A_{495}	
	rMBP	MBP 1-11Nac	IL-2 (CTLL)	rMBP	MBP 1-11Nac	IL-2 (CTLL)	IgG	IgM
$CD8^{-/-}$	69.67 ± 18.75	15.23 ± 6.4	1.68 ± 0.026	20.81 ± 4.1	2.60 ± 0.1	12.59 ± 2.2	0.235 ± 0.043	0.211 ± 0.085
Control	91.26 ± 13.57	13.52 ± 4.5	3.79 ± 0.69	36.05 ± 8.0	4.55 ± 1.4	24.46 ± 2.0	0.348 ± 0.078	0.110 ± 0.020

the mutant mice had relapsed compared to 33% in the control arm (Table 2). The mean score and proportion relapsed in the mutant mice are consistently higher throughout the experiments (Fig. 3). Thus, the $CD8^+$ cells are influencing the down-modulation of the inflammatory response and spontaneous relapses during this phase of the disease, either directly on indirectly.

The mechanisms of spontaneous relapses in EAE and other autoimmune disorders remain enigmatic. Susceptibility to EAE in rats might relate to glucocorticoids released during stress and thus would be genetically determined (*32*). Cytokines are involved in this response, and it is possible that $CD8^+$ cells modulate the response directly or indirectly through cytokines. Transforming growth factor–β (TGF-β) (*27, 33, 34*) and suppression of IFN-γ production (*27, 35*) may modulate disease, and $CD8^+$ T cells generated by oral tolerance suppress disease through TGF-β (*34*). Conversely, $CD8^+$ cells may modulate disease by directly killing effector cells or preventing reinduction of disease (*4, 16, 17, 19*).

Even though studies that used antibody depletion have discounted the modulatory effect of $CD8^+$ cells on the course of EAE, the effect may have been missed because the antibody may cause cytokine release and because depletion may have been insufficient to have a complete effect. Moreover, $CD8^+$ T cells may have subtle effects on the disease course, and it is not necessary to remove or alter $CD8^+$ T cells to cause relapse, as in adoptive transfers of activated $CD4^+$ clones or lines (*8*). $CD8^+$ T cells can function as "veto" cells and can suppress an in vitro and in vivo allogeneic response (*36*). This would not explain the lower mortality in the mutant mice earlier on in the disease but may account for the higher relapse rate later on. Our data indicate that a lack of $CD8^+$ cells in the mutant mice predisposes them to relapses that are like "spontaneous reinductions," and this is corroborated by antibody depletion of $CD8^+$ T cells, in which mice that have recovered from EAE are susceptible to reinduction of EAE, whereas normal mice are resistant (*18*). The deaths are lacking in that system most likely because the immunogen is the NH_2-terminal peptide of MBP (MBP 1-9Nac) instead of whole MBP. The whole MBP can potentially give rise to more peptides and could, therefore, activate more T cells than a fixed peptide. Thus, a more intense response with recruitment of more cells can be anticipated with an immune response to whole MBP. Furthermore, different mouse strains are involved, B10.PL and PL/J backcrosses, and in our case the $CD8^+$ T cells are eliminated during development, allowing for the possibility of a CD8 salvage pathway.

In the initial immune response to MBP, $CD4^+$ effector cells are probably stimulated and expanded, and they home to the central nervous system by an unknown mechanism where an inflammatory response is elicited and various other immune cells are recruited, including $CD8^+$ T cells. The $CD8^+$ cells then subserve an effector function or mediate one that augments the damage of myelin. These cells may also eliminate $CD4^+$ effector cells and dampen the immune system after some time. Differences in $CD8^+$ lymphokine production (*29*) can relate to effector or suppressive effects of these cells. Furthermore, both immunomodulatory $CD8^+$ and $CD4^+$ T cells are probably generated that have different lymphokine profiles. Thus, a lack of $CD8^+$ cells would not abrogate a particular

Table 2. Summary of disease course of mice. The incidence of disease is the number of mice that have been sick during the period of observation. The mean day of onset is the mean of all of the days of onset of the sick mice. The mean peak score is the average of the peak initial disease scores of all mice with disease, excluding relapses. The fatalities are the number of mice that have died during observation, including the three that were killed for ethical reasons. The incidence of relapse is the number of mice with relapses in the group of mice that were still alive at the end of the observation period. The mean relapse rate is the total number of relapses per the number of mice alive, giving the mean relapse rate per mouse. The mean relapse peak is the average of the peak scores during relapse. The mean day of relapse is the mean of the days of onset for the first relapse. Statistical significance has been calculated with the Student's *t* test or the chi-square test, comparing the $CD8^{-/-}$ with the controls (ns, not significant).

	$CD8^{-/-}$	Control	Statistics
Disease incidence	39/49 (79.6%)	38/49 (77.6%)	ns
Onset day ± SEM	19.77 ± 0.89	19.84 ± 1.2	ns
Peak score ± SEM	3.22 ± 0.2	3.71 ± 0.21	ns
Fatalities	12/39 (30.8%)	20/38 (52.6%)	$P < 0.05$
Relapse incidence	18/27 (66.67%)	6/18 (33.3%)	$P < 0.03$
Relapse rate ± SEM	0.74 ± 0.1	0.38 ± 0.11	$P < 0.04$
Relapse peak ± SEM	2.50 ± 0.19	2.91 ± 0.15	ns
Day of relapse ± SEM	37.90 ± 1.8	41.60 ± 2.0	ns

function but would rather alter the intensity of part of the immune response.

In summary, with the use of mice lacking cytotoxic T cells we have generated data that support a dual role for $CD8^+$ T cells as effector and modulatory cells in the EAE model. The results indicate that the $CD8^+$ cells are not critical to, but play an adjunctive role in, disease induction and maintenance in the EAE model. This observation, however, may also reflect the bias on $CD4^+$ cells from exogenous antigen priming, and $CD8^+$ T cells may be more dominant in a spontaneous demyelinating disease, such as that from murine Theiler's virus infection or multiple sclerosis.

REFERENCES AND NOTES

1. R. Arnon, *Immunol. Rev.* **55**, 5 (1981).
2. S. S. Zamvil *et al.*, *Nature* **324**, 258 (1986).
3. H. Acha-Orbea, L. Steinman, H. McDevitt, *Annu. Rev. Immunol.* **7**, 371 (1989).
4. B. G. Arnason, B. D. Jankovic, B. H. Waksman, L. Wennerstein, *J. Exp. Med.* **116**, 177 (1962).
5. S. Sriram, L. Carrol, S. Fortin, S. Cooper, G. Ranges, *J. Immunol.* **141**, 464 (1988).
6. S. W. Brostoff and D. W. Mason, *ibid.* **133**, 1938 (1984).
7. C. B. Pettinelli and D. E. McFarlin, *ibid.* **127**, 1420 (1982).
8. S. Zamvil *et al.*, *Nature* **317**, 355 (1985).
9. D. G. Ando, J. Clayton, D. Kono, J. L. Urban, E. E. Sercarz, *Cell. Immunol.* **124**, 132 (1989).
10. J. M. Lemire and W. O. Weigle, *J. Immunol.* **137**, 3169 (1986).
11. D. M. Bitar and O. C. Whitacre, *Cell. Immunol.* **112**, 364 (1988).
12. J. D. Sedgwick *et al.*, *J. Immunol.* **145**, 2474 (1990).
13. J. D. Sedgwick, *Eur. J. Immunol.* **18**, 495 (1988).
14. S. Sriram and L. Carrol, *J. Neuroimmunol.* **17**, 147 (1988).
15. D. Sun, F. Qin, J. Chluba, T. J. Epplen, H. Wekerle, *Nature* **332**, 843 (1988).
16. O. Lider, L. M. B. Santos, C. Y. Lee, P. J. Higgins, H. L. Weiner, *J. Immunol.* **142**, 748 (1989).
17. P. J. Higgins and H. L. Weiner, *ibid.* **140**, 440 (1988).
18. H. Jiang *et al.*, *Science* **256**, 1213 (1992). In the accompanying report, the authors observed that mice depleted of $CD8^+$ T cells with antibodies after induction of primary EAE were susceptible to reinduction of disease, whereas untreated mice were resistant to or protected from further disease from reinduction.
19. P. W. Mathieson, K. J. Stapleton, D. B. G. Oliviera, C. M. Lockwood, *Eur. J. Immunol.* **21**, 2105 (1991).
20. W.-P. Fung-Leung *et al.*, *Cell* **65**, 443 (1991).
21. W.-P. Fung-Leung, T. M. Kundig, R. M. Zinkernagel, T.-W. Mak, *J. Exp. Med.* **174**, 1425 (1991).
22. W.-P. Fung-Leung *et al.*, unpublished data.
23. No statistical differences ($P < 0.05$) were found between the mutant and control mice with the Student's *t* test. The approximately twofold difference may not be significant but suggests that there may be a trend toward the mutant mice having less in vitro antigen-specific proliferation and IL-2 production compared with controls. The only other antigen responses known to date for the $CD8^{-/-}$ mutant are those toward alloantigens and viral antigens. Allo responses show no significant difference between mutants and controls in in vitro responses for $CD4^+$ MHC class II restricted T cells.
24. The amounts of MBP and adjuvant (pertussis toxin) used are about two to three times the amounts used in most protocols for inducing EAE in mice. Previous experiments have shown that a higher dose of MBP and adjuvant leads to a more severe and acute disease.
25. A. M. Brown, D. E. McFarlin, C. S. Raine, *Lab. Invest.* **46**, 171 (1982).
26. Histological analysis of brain and spinal cord sections of three mice that were moribund and killed showed typical demyelination and cellular infiltrates.
27. W. J. Karpus and R. H. Swanborg, *J. Immunol.* **143**, 3492 (1989).
28. ———, *ibid.* **146**, 1163 (1991).
29. T. R. Mossman and R. L. Coffman, *Adv. Immunol.* **46**, 111 (1989).
30. M. B. Powell *et al.*, *Int. Immunol.* **2**, 539 (1990).
31. K. Sakai *et al.*, *J. Immunol.* **137**, 1527 (1988).
32. D. Mason, *Immunol. Today* **12**, 57 (1991).
33. K. E. Ellerman, J. M. Powers, S. W. Brostoff, *Nature* **331**, 265 (1988).
34. A. Miller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 421 (1992).
35. M. K. Racke *et al.*, *J. Immunol.* **146**, 3012 (1991).
36. S. R. Sambhara and R. G. Miller, *Science* **252**, 1424 (1991).

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Role of $CD8^+$ T Cells in Murine Experimental Allergic Encephalomyelitis

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The course of experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis, is affected by immunoregulatory T lymphocytes. When animals are immunized with encephalitogenic peptide of myelin basic protein and recover from the first episode of EAE, they become resistant to a second induction of this disease. Animals depleted of $CD8^+$ T cells by antibody-mediated clearance were used to examine the role of $CD8^+$ T cells in EAE. These cells were found to be major participants in the resistance to a second induction of EAE but were not essential for spontaneous recovery from the first episode of the disease.

We have studied EAE, induced in B10.PL ($H\text{-}2^u$) mice by immunizing (*1*) the mice with 1-9Nac, the encephalitogenic NH_2-terminal–acetylated peptide of the first nine amino acids of mouse myelin basic protein (MBP) (*2*, *3*). This disease was reproducible: in 102 mice, 94% of the animals developed EAE, with mean severity of 3.5 and maximum severity of 5.0; the mean onset day was ~20 days after the first immunization; the disease lasted ~13 days; and recovery was spontaneous. The first episode induced resistance; after a second immunization, most animals did not show any symptoms, and in those that did (only 28% of the total animals) the disease was less severe and of shorter duration (mean severity was 0.4, maximum severity was 2.5, and the duration was ~9 days). Spontaneous relapses were rare in our experiments: only 3 of 96 mice receiving the first immunization relapsed (2 weeks after recovery).

We then depleted the $CD8^+$ T cells from B10.PL mice with monoclonal antibodies (MAbs) to mouse CD8 (*4*, *5*). We tested two MAbs to CD8: HO-2.2 [murine immunoglobulin M (IgM)] and 53-6.72 (rat IgG2a). Only the latter completely depleted $CD8^+$ T cells (Fig. 1 and Table 1). This depletion was evident on day 3 after the MAb injection and lasted for ~2 weeks, which suggests that the $CD8^+$ T cells were eliminated and not merely prevented from being detected with the same labeled MAb by the unlabeled MAb that was injected in vivo. In fact, an overnight culture of spleen cells from day 10 of 53-6.72–injected mice still showed almost a complete absence of $CD8^+$ T cells. The $CD8^+$ T cells were probably eliminated by antibody-dependent cytotoxicity (ADCC), because MAb 53-6.72 could bind to the Fc receptors of mouse monocytes. This $CD8^+$ T cell depletion was followed by the slow appearance of new $CD8^+$ T cells.

Depletion of $CD8^+$ T cells had no effect on the induction of EAE in B10.PL mice, as measured by the incidence and the severity of the disease (Table 2). The spontaneous recovery was similar in $CD8^+$ T cell–depleted mice and in control mice; however, $CD8^+$ T cells began to reappear ~2 weeks after depletion, and it is possible that these $CD8^+$ T cells contributed to the final stage of the spontaneous recovery in $CD8^+$ T cell–depleted mice. Furthermore, $CD8^+$ T cell depletion performed after recovery in 15 mice did not by itself induce any relapses. But $CD8^+$ T cell depletion did affect the resistance to a second induction of EAE. In three separate experiments, each with controls, $CD8^+$ T cell depletion eliminated the resistance to a second induction of the disease in mice that had recovered from the first episode of EAE; there was also a significant effect on the severity

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