A public T cell clonotype within a heterogeneous autoreactive repertoire is dominant in driving EAE

Juscilene S. Menezes,[1] Peter van den Elzen,[2] Jordan Thornes,[1] Donald Huffman,[1] Nathalie M. Droin,[2] Emanual Maverakis,[2] and Eli E. Sercarz[1,2]

[1]Division of Immune Regulation, Torrey Pines Institute for Molecular Studies, San Diego, California, USA.
[2]La Jolla Institute for Allergy and Immunology, San Diego, California, USA.

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis. Immunization of B10.PL mice with the Ac1–9 peptide, the immunodominant determinant of myelin basic protein (MBP), produced a single episode of EAE followed by recovery and resistance to reinduction of disease. Using the CDR3 length spectratyping technique, we characterized the clonal composition of the Ac1–9–specific T cell repertoire from induction through onset and resolution of disease. Two clonally restricted subsets within a heterogeneous self-reactive repertoire were found in mouse lymph nodes, spleen, and spinal cord soon after immunization, before any sign of EAE. These clonotypes, designated BV8S2/BJ2S7 and BV16/BJ2S5, were present in all mice examined and thus considered public. BV8S2/BJ2S7 was found in far greater excess; was exclusively Th1 polarized; disappeared from the spinal cord, spleen, and lymph nodes concomitantly with recovery; and transferred disease to naive recipients. In contrast, BV16/BJ2S5 and numerous private clonotypes were either Th1 or Th2 and persisted following recovery. These results are consistent with the hypothesis that the public clonotype BV8S2/BJ2S7 is a driver of disease and necessary for its propagation.

Introduction

MS is a demyelinating disease of the CNS known to be mediated in part by autoreactive $CD4^+$ T cells. Interestingly, myelin-specific T cells are found both in healthy subjects and in patients with MS (1); thus the presence of myelin-reactive cells per se is not an indication of clinical disease. In fact, a protective role for autoreactive cells has been demonstrated in EAE in mice (2, 3). Several studies have attempted to track pathogenic cells during the course of EAE (4–7) and, through in vitro cloning and analysis of TCR transgenic mouse models, have provided much information about the behavior of individual clones. However, these clonally restricted studies have not yet provided the necessary insights required for understanding the dynamics of autoimmunity in intact individuals. In unperturbed systems, we have only limited ability to identify and characterize the particular cells of the self-directed T cell repertoire that have the capability of driving autoimmune disease. Yet this ability seems essential for our understanding of autoimmunity and for designing specific immunotherapies such as T cell vaccines (8, 9) or TCR-peptide vaccines (10).

In this study, we sought to describe the features of the pathogenic cells residing within the bulk population that drive EAE. Previous studies have suggested that T cell responses generally include a heterogeneous repertoire composed of both public and private components (11–16). An autoimmune T cell response should be no different. We reasoned that the driver T cells would emerge as public from an initially heterogeneous clonal array with a particular set of efficient effector signals and pathogenic characteristics that would distinguish them within the self-reactive repertoire. Therefore, we sought evidence for T cell clones exhibiting publicity, high frequency, early response, enhanced levels of costimulatory receptors, primary resistance to downregulation, and abundant output of proinflammatory factors (Th1). Most importantly, we looked for the concordant presence of these cells during the disease course. Conceptually, the clones that would follow these criteria in vivo are the ones most likely to drive autoimmunity within a bulk population. To test this hypothesis, we took advantage of CDR3 length analysis and cell isolation techniques to detect and track clonal expansions as well as to characterize such expansions within phenotype-based populations during a self-limiting autoimmune pathology, the EAE model in B10.PL mice, which is known to be driven by T cells specific for the immunodominant determinant of myelin basic protein (MBP), Ac1–9.

We characterized specific encephalitogenic clones directly ex vivo from mice using CDR3 length spectroscopy. Our results show that Ac1–9 selects a more heterogeneous response than was previously appreciated (17). The Ac1–9–specific repertoire is composed of a single public clonotype with characteristics of dominance, a second public but subdominant clonotype, and many private and semi-private clonotypes. We present evidence that the development of a dominant T cell clonotype among a heterogeneous population drives the course of EAE in B10.PL mice. The dominant clonotype largely disappeared following recovery, while a sizeable majority of other self-reactive clones were maintained. The capacity to identify such T cell drivers within a bulk population in autoimmunity could identify critical targets that would be crucial in the design of therapies for autoimmune diseases such as MS.

Results

MBP-specific T cells remain present throughout the course of EAE in B10.PL mice. The course of EAE in B10.PL mice is monophasic following the administration of Ac1–9 in CFA. The initial symp-

Nonstandard abbreviations used: CD62L, CD62 ligand; L9, 9-aa CDR3 length; MBP, myelin basic protein.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:2176–2185 (2007). doi:10.1172/JCI28277.

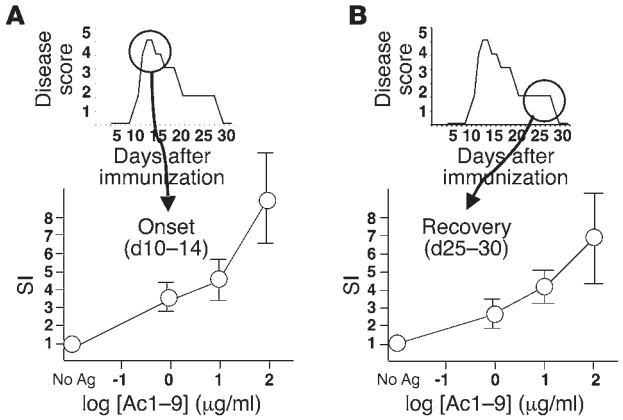

Figure 1
EAE in B10. PL mice follows a monophasic course. Splenocytes were harvested for measuring the dose-dependent proliferative response, as shown by the stimulation index (SI), to Ac1–9 at the onset of disease (**A**) and during recovery (**B**), as measured by ^{3}H-thymidine uptake. The mean ± SEM for 8 mice is shown.

toms appear at about day 10, the peak of disease is reached at approximately day 14, and the signs of paralysis disappear by day 30. However, in vitro stimulation of draining lymph node cells revealed comparable Ac1–9 proliferative activity both at EAE onset (Figure 1A) and during recovery (Figure 1B), indicating that remission is not the result of a loss of Ac1–9–reactive T cells from the circulating T cell repertoire.

Public and private repertoires. To analyze the T cell repertoire during the course of EAE, we used CDR3 length spectratyping analysis, referred to herein as immunoscope analysis (18). Immunoscope analysis of draining lymph nodes after immunization with Ac1–9 revealed expansion of a mean of 24 BV/BJ individual subfamilies per mouse, primarily in the BV4, BV8.2, BV8.3, BV10, and BV16 families (Figure 2A). Among them, 2 clonotypic expansions, BV8S2/BJ2S7 and BV16/BJ2S5, each with a 9-aa CDR3 length (L9), were found in 100% of the animals analyzed and therefore could be considered public (Figure 2A). The public expansions were found in lymph nodes, spleen, and spinal cord. BV8S2/BJ2S7 and

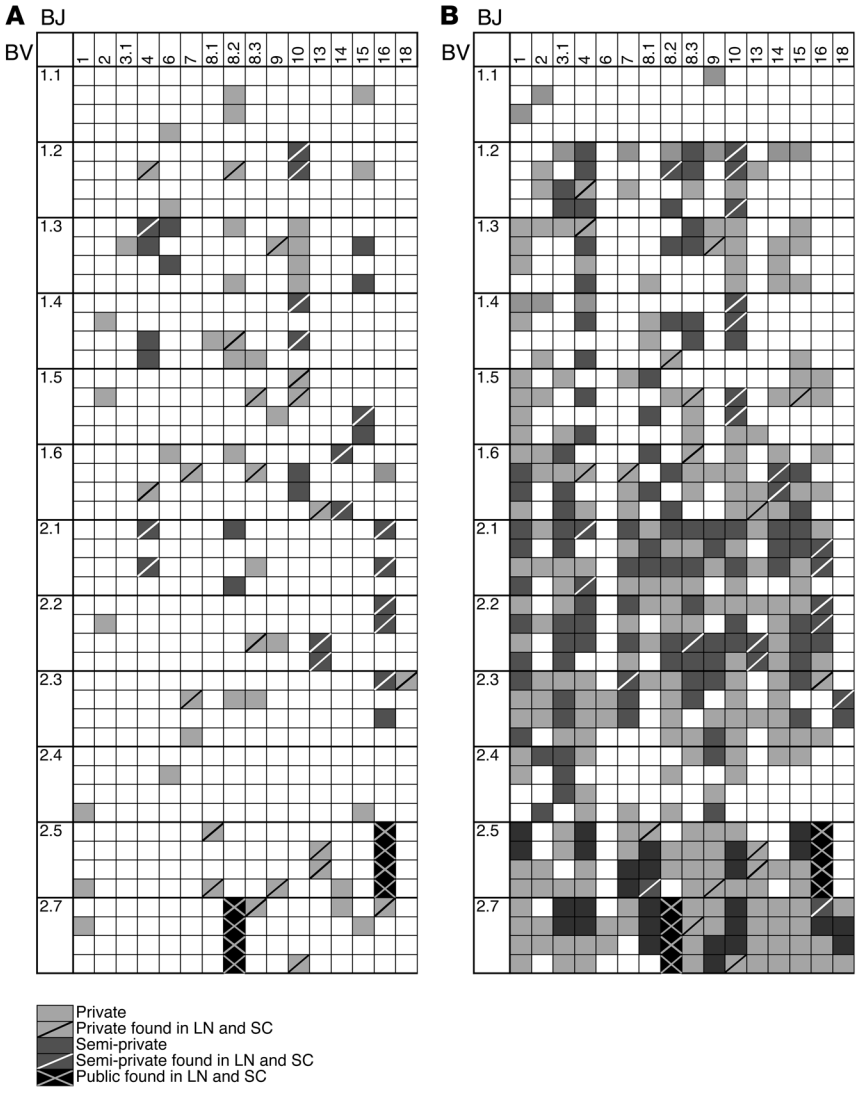

Figure 2
The BV/BJ usage of cells responding to Ac1–9 is diverse. Immunoscopic analysis of (**A**) draining lymph node cells 8 days after immunization followed by in vitro stimulation with Ac1–9 and (**B**) spinal cord–infiltrating cells without in vitro stimulation. cDNA was amplified in RT-PCR as described in Methods. Results represent clonotypic CDR3 expansions found among the BV/BJ families of 4 mice. Shown are expansions in which the same CDR3 length was found in 1 of 4 mice (Private), in 2–3 of 4 mice (Semi-private), and in all mice analyzed (Public). Results are representative of 3 independent experiments. SC, spinal cord.

Table 1
CDR3 aa sequences of the TCR β chain

BV	Junctional region (V-N-D-N-J)	BJ	Identical sequences per clones analyzed
BV8S2-CAS	G**DAGGGYEQ**YFGP	BJ2S7	36/44
BV8S2-CAS	G**DAGGSYEQ**YFGP	BJ2S7	8/44
BV16-CAS	S**SGTDQDTQ**YFGP	BJ2S5	24/32
BV16-CAS	S**LDWDNQDTQ**YFGP	BJ2S5	8/32

The CDR3 region of the TCR β chain is encoded within the VNDNJ junction, as shown in bold.

BV16/BJ2S5 were absent in mice immunized with PBS in CFA plus pertussis toxin but without peptide, although other expansions were observed, e.g., a public BV10/BJ1S3 expansion (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI28277DS1). These data support the interpretation that BV8S2/BJ2S7 and BV16/BJ2S5 are specific for Ac1–9.

The infiltrating repertoire in the spinal cord at disease onset was clearly more heterogeneous (Figure 2B) than that in the lymph node (Figure 2A). The expansions BV8S2/BJ2S7(L9) and BV16/BJ2S5(L9), together with several private and semi-private expansions, were found in every affected mouse. Expansions were observed in most BV families (Figure 2B). It has previously been shown that CNS-derived dendritic cells are able to migrate to the cervical lymph nodes and activate naive T cells (19), which can then gain access to the inflamed CNS, increasing the heterogeneity of the T cell response (20). Therefore, as shown previously (21), the heterogeneity observed within the CNS in Figure 2B is likely to represent a more diverse polyclonal set of specificities, distinct from T cells reactive with Ac1–9. Consistent with this, approximately 50% of the clonal expansions (mean of 12 per mouse) seen in the lymph nodes (Figure 2A) were also found in the spinal cord (Figure 2B). However, as only 2 clonotypes, BV8S2/BJ2S7(L9) and BV16/BJ2S5(L9), were public in lymph nodes, spleen, and spinal cord, it was important to determine whether these public clonotypes were causally related to pathogenicity.

Public expansions display a conserved CDR3 region. To ascertain whether the BV8S2/BJ2S7(L9) and BV16/BJ2S5(L9) public expansions were a result of clonal proliferation, we sequenced the PCR samples used for immunoscope analysis from 4 separate Ac1–9–primed mice. A conserved 9-aa sequence, GDAGGGYEQ and SSGTDQDTQ (or rarely GDAGGSYEQ and SLDWDNQDTQ) emerged at the V(D)J junctions of BV8S2/BJ2S7 and BV16/BJ2S7, respectively (Table 1). Notably, although the DNA sequences repeatedly translated into the same aa sequence, they differed at the nucleotide level, even within the same animal. This demonstrates that there is an antigen-based selection of nonidentical clones based on the same aa sequence of the CDR3 region. Therefore, these public expansions actually represent a clonal family with unique DNA sequences, encoding the same TCR β chain aa sequence.

Dominance of the public clonotypes. Qualities of the public clonotypes that might contribute to their pathogenicity and dominance were then characterized. Expression of the major public expansion, BV8S2/BJ2S7(L9), was approximately 10-fold higher than that of the minor public expansion, BV16/BJ2S5(L9), at both day 4 and day 8 (Figure 3A) following immunization. Both clonotypes expanded 2- to 3-fold from day 4 to day 8 in the lymph node (Figure 3A). In the spinal cord, both public clonotypes were detected, again with a 10-fold difference in expression, on day 8 after immunization (Figure 3B), before signs of disease are seen. From day 8 to day 14, during which time disease symptoms appear, BV8S2/BJ2S7(L9) increased at a higher rate than did BV16/

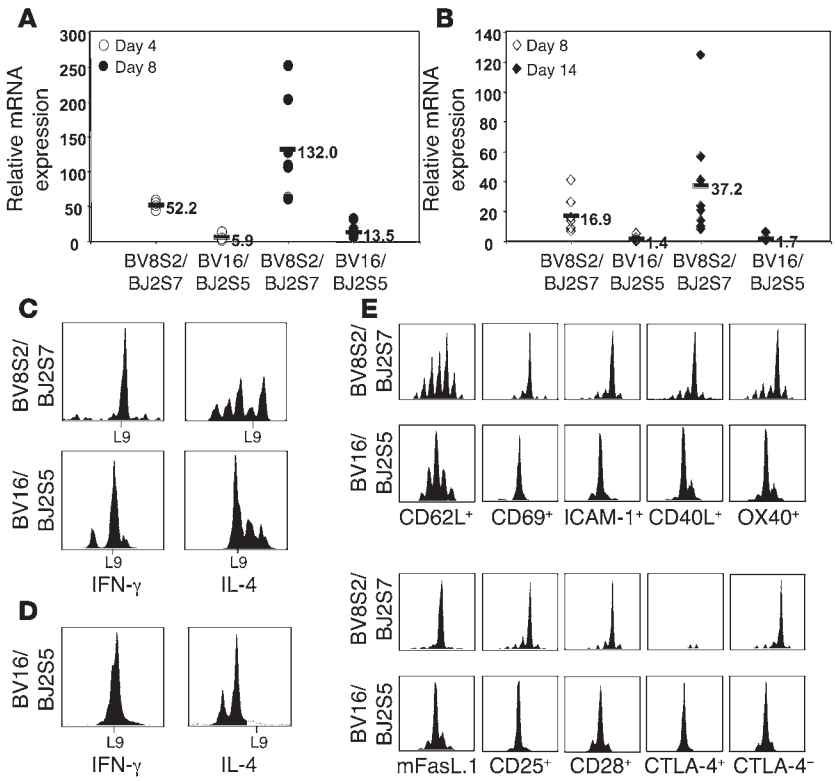

Figure 3
Immunologic features of public clonotypic expansions. Analysis of mRNA expression of the public expansions by real-time PCR was performed (**A**) from draining lymph node cells of individual mice collected 4 and 8 days after immunization, followed by restimulation in vitro with Ac1–9 for 3 days, or (**B**) from spinal cord cells of individual mice collected 8 and 14 days after immunization without in vitro stimulation. Symbols represent individual samples; mean expression is denoted by horizontal bars, and mean values are shown. After 6 (**C**) or 48 (**D** and **E**) hours in culture, pooled cells from the draining lymph nodes and spleen of individual mice, 8 days after immunization, were sorted with antibodies to different surface markers using magnetic beads and analyzed by immunoscope, as described in Methods. Results are representative of 4 independent experiments. mFasL.1, mouse FasL.1.

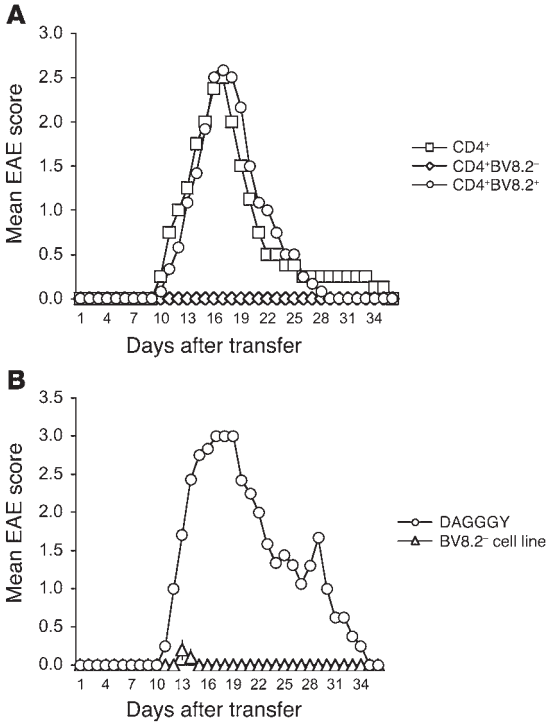

Figure 4
CD4+ BV8S2/BJ2S7-DAGGGY clonotypic T cells induce EAE. (**A**) Cells from mice immunized with Ac1–9 8 days earlier were stimulated in vitro with the peptide and IL-12; sorted into CD4+, CD4+BV8.2+, and CD4+BV8.2− populations; and adoptively transferred into naive mice. (**B**) The BV8.2+ T cell clone bearing the DAGGGY motif in the CDR3 region and the Ac1–9–specific T cell line depleted of BV8.2+ T cells were stimulated in vitro with the peptide and IL-12, washed, and adoptively transferred into naive mice. The numerical range of cells transferred was adjusted for different populations as described in Methods. Mice were monitored daily for clinical signs of EAE.

BJ2S5(L9) in the spinal cord (Figure 3B). Together, these results indicate that BV8S2/BJ2S7(L9) is the dominant clonotype found in the lymph node and spinal cord in the B10.PL EAE model.

Th1 polarization of public clonotypes. It has been difficult to attribute a particular cytokine profile to a single T cell clone within a bulk population of T cells. It remains unclear whether Th1 and Th2 cytokine profiles can be ascribed to different members of the T cell repertoire or whether a given TCR clonotype is represented in both populations. We took advantage of cytokine capture reagents, which allow sorting of cells according to their cytokine profiles. By performing immunoscope analysis following magnetic separation of IFN-γ– and IL-4–secreting cells, we could determine which cells of the Ac1–9–reactive repertoire were polarized to Th1- or Th2-like cytokine profiles. The BV8S2/BJ2S7(L9) public expansion was found in the IFN-γ–sorted population, but not in the IL-4 population, by 6 hours after in vitro stimulation with Ac1–9 (Figure 3C). However, BV16/BJ2S5(L9) cells were still in a Th0 state at this early time, producing both IL-4 and IFN-γ (Figure 3C). After 48 hours of stimulation, the BV16/BJ2S5(L9) cells had also polarized toward a Th1 cytokine profile, suggesting that the early secretion of IFN-γ by BV8S2/BJ2S7(L9) cells could play a role in the polarization of other cells (Figure 3D). It is also possible that BV16/BJ2S5(L9) cells have a slower developmental program. Similar analyses of private clonotypes revealed that expansions were randomly found in either IL-4– or IFN-γ– producing populations (data not shown).

Effector characteristics of the public populations. To characterize the effector response of the public clonotypes infiltrating and driving EAE, we further analyzed the phenotype of clonotypes upon Ac1–9 activation. By isolating T cells with antibodies to different surface markers using magnetic beads, followed by spectratyping to test for a signature peak, it is possible to conveniently identify the expression phenotype of a particular cell within a bulk population. Cells within the BV8S2/BJ2S7(L9) clonotype were found among all positively selected populations analyzed except for the CD62 ligand–positive (CD62L+) or CTLA-4+ populations (Figure 3E). Thus, the predominant phenotype of the BV8S2/BJ2S7(L9) clonotype following Ac1–9 stimulation is as follows: CD62L−, FasL.1+, ICAM-1+, OX40+, CD40L+, CD28+, CD25+, and CTLA-4−, which is a phenotype known to be essential for inducing EAE (22). On the other hand, the BV16/BJ2S5(L9) clonotype revealed minor expansions within the CD62L+ and CTLA-4+ populations (Figure 3E), suggesting that this clonotype is less responsive to Ac1–9 activation or is more susceptible to regulation than is BV8S2/BJ2S7(L9). Similar analysis of some private clonotypes, such as BV4/BJ2S1 and BV10/BJ1S6, revealed expansions in random positive populations (data not shown).

Pathogenicity of the BV8S2/BJ2S7 clone bearing the DAGGGY motif. We next examined whether a BV8S2/BJ2S7+ clone bearing the CDR3 motif DAGGGY, the core sequence of L9 (the BV8S2/BJ2S7-DAGGGY clonotype), would induce disease when adoptively transferred into naive mice. Figure 4 shows that total CD4+ T cells as well as the CD4+BV8S2+ population induced disease in naive recipients. Most importantly, depletion of BV8S2+ T cells from the CD4+ population removed the ability of the transferred cells to induce disease (Figure 4A). In addition, the BV8S2/BJ2S7-DAGGGY clonotype induced EAE in recipient mice (Figure 4B). However, neither the Ac1–9–specific T cell line generated in the absence of BV8S2 (Figure 4B) nor another BV population, such as BV10 isolated from immunized mice (data not shown), adoptively transferred disease in recipient mice. These results show that, as predicted, the dominant public clonotype is also pathogenic in B10.PL mice. Compellingly, although other public and many private clonotypes are expanded by Ac1–9 immunization and comprise a majority of the Ac1–9–specific repertoire, they lack the ability to adoptively transfer EAE.

Life history of the dominant public clonotype parallels the course of disease. Previous studies have shown that the earliest responders to an encounter with antigen are likely to become dominant members of the subsequent T cell response (23). The BV8S2/BJ2S7(L9) clonotypic expansion was detected in the lymph node as early as 2.5 days after immunization (Figure 5), in agreement with Sakuma et al. (7). By day 4, all animals presented BV8S2/BJ2S7(L9) expansions, and by day 8 after immunization, each of these expansions was absolutely public in the lymph nodes (Figure 5A). During recovery from EAE, BV8S2/BJ2S7(L9) cells decreased substantially in the lymph nodes (Figure 4A), suggesting that this is a crucial clonotype for EAE induction and maintenance. T cell analysis at recovery was also performed by sequencing the predominant BV8S2/BJ2S7 clone. As disease resolution progressed, the CDR3 motif DAGGGY began to disappear (Figure 6, A and B). Few DAGGGY-bearing clonotypes remained in early remission, and they were

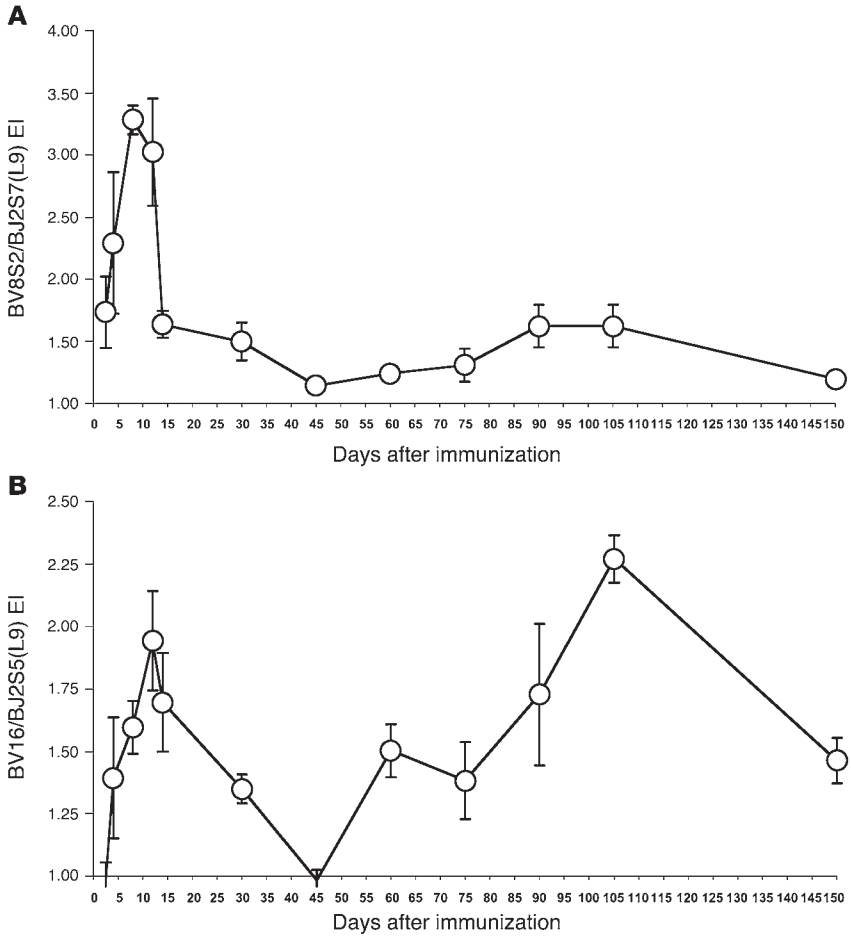

Figure 5
BV8S2/BJ2S7 expansion parallels disease. At the indicated time points after immunization, draining lymph node cells were harvested and stimulated in vitro with Ac1–9. cDNA was amplified by RT-PCR as described in Methods. Shown are expansion indexes (EIs) of the single CDR3 expansions, (**A**) BV8S2/BJ2S7(L9) and (**B**) BV16/BJ2S5(L9). An EI of 1 represents the particular expansion peak compared with its expansion within the Gaussian distribution; values above or below this limit show positive or negative expansions of the peak, respectively.

absent from the spinal cord by late remission 30 days after immunization (Figure 6C). The other public clonotype, BV16/BJ2S7(L9), also disappeared from lymph nodes just after recovery, but strikingly, these cells expanded vigorously in the absence of disease a short while after remission (Figure 5B). These results suggest that efficient regulatory control is exerted on the dominant clonotype during disease resolution. After recovery, BV8S2/BJ2S7(L9) is no longer detectable in lymph nodes. The minor public clonotype, BV16/BJ2S7(L9), persists but is unable to induce EAE.

Peripheral hideouts for the aggressive driver clonotype in EAE. Next we investigated whether the BV8S2/BJ2S7(L9) clonotypic T cells would be maintained in nonlymphoid sites following recovery from EAE. As shown in Figure 7, this clonotype was recovered from lung, liver, bone marrow, and blood until 75 days after recovery (Figure 7). A much lower frequency of BV8S2/BJ2S7(L9) clonotypic T cells was observed in draining lymph nodes, spleen, thymus, and mesenteric lymph nodes. At 120 days after recovery, no BV8S2/BJ2S7(L9) clonotypic T cells were recovered from any site analyzed. These results show that specific autoreactive T cells generated in B10.PL mice after immunization with Ac1–9 persist in peripheral organs such as lung, liver, and bone marrow, as do other memory T cells. Interestingly, these persistent cells produced low to undetectable levels of IFN-γ (Figure 8), suggesting that they had lost pathogenic potential.

Finally, we asked whether the loss of the autoreactive BV8S2/BJ2S7(L9) clonotype was followed by the loss of autoreactivity among the other clonotypes. Interestingly, although the public clonotype BV8S2/BJ2S7(L9) was not detected between 75 and 120 days after recovery, many other Ac1–9–specific T cell clonotypes persisted (Figure 9). However, mice immunized at this point or even at 1 year after recovery were resistant to reinduction of EAE (data not shown). Therefore, recovery from EAE in B10.PL mice involves an apparent repertoire loss in which the pathogenic public BV8S2/BJ2S7(L9) clonotype is selectively purged or regulated although many other self-reactive clonotypes are maintained.

Discussion

By genotyping the CDR3 region of the TCR β chain, we followed the T cell response to the immunodominant determinant of MBP, Ac1–9, during the onset and through spontaneous recovery from EAE in mice. A diverse set of clonotypes arose, mostly within the BV4, BV8S2, BV8S3, BV10, and BV16 families, but only 2 clonotypes among these families were recruited in every animal studied. One of these presented features of dominance and was able to drive the pathology in B10.PL mice. The results presented in this study revealed that BV8S2/BJ2S7-DAGGGY was highly overrepresented in every animal during the onset of disease. This component of the Ac1–9 response displayed a highly polarized Th1 cytokine profile and was an early and highly activated member of the Ac1–9 repertoire, which was detected in lymph nodes and spinal cord well before any sign of disease. The ability of the BV8S2/BJ2S7(L9) clonotype to transfer disease to recipient mice, unlike many other Ac1–9–specific cells, confirmed the encephalitogenicity of these

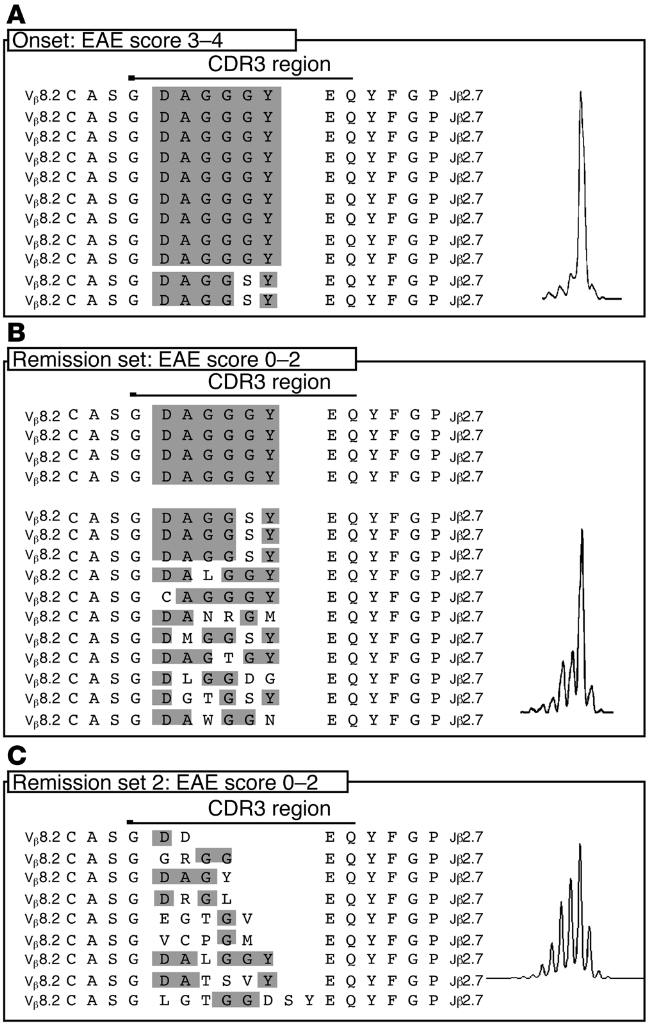

Figure 6

The CDR3 region of the public expansion is conserved at onset of EAE, but not during recovery. BV8S2/BJ2S7 PCR samples from individual mice were directly cloned and sequenced (**A**) at the onset of EAE on day 14, (**B**) in remission on day 25, and (**C**) later in the remission phase on day 30. Concordances with the DAGGGY motif are shaded gray. The figure is representative of the results obtained from restimulated splenocytes and directly from the spinal cord because both samples showed similar profiles.

cells. The pattern of immune response of these T cells, unlike other components of the Ac1–9–specific repertoire, was congruent with the pattern of disease. The results demonstrate that this clonotype is dominant in driving EAE in the B10.PL mouse model, apparently having the propensity to infiltrate, expand locally, and establish an inflammatory milieu in the CNS.

In different experimental systems, dominant responses have been documented from hybridoma and/or clonal evidence showing shared specificities and containing unique rearrangements or sequences. However, often these isolated clones have not been found in vivo and, because in vitro cloning imposes selective pressures different from those seen in vivo, could correspond to private specificities arising in vitro during the isolation process (24–28). According to previous views, the Ac1–9–specific response was essentially restricted to BV8S2 (17). However, our analysis of the entire Ac1–9–specific repertoire, expanded after immunization, revealed extensive heterogeneity and included the pathogenic clonotype as well as several other clonotypes able to expand in the in vivo environment.

Previously published data have suggested that T cell responses generally include both public and private components (11–15), and EAE in B10.PL mice has proved to be similar. Both public and private clonotypes expand after peptide immunization, with one clonotype becoming dominant, correlating with the course of EAE. The basis for selection of dominant public clonotypes is unknown. A public repertoire might be influenced by genetic (i.e., intrinsic) factors within the natural repertoire, while the appearance of distinct private expansions in particular animals may depend on unique factors that impinge on individual T cell repertoires.

Our results show that the dominant public clonotype was also pathogenic in this model of EAE, displaying a mixture of high frequency and enhanced effector qualities, including high levels of costimulatory molecules, an apparently unregulated period of expansion, a proinflammatory profile, and a rather fast activation time, which might lead to competitive advantage and encephalitogenicity. It has previously been shown that certain effector molecules expressed on MBP-specific lymphocytes such as FasL, OX40, CD40L, and ICAM correlate with the pathogenesis of EAE (6, 29–36). Furthermore, CD62L-deficient encephalitogenic $CD4^+$ T cells were able to transfer EAE to wild-type recipients (37, 38). CTLA-4 blockade during in vivo priming or in vitro stimulation has been shown to exacerbate EAE or accelerate disease relapse in susceptible and resistant strains of mice (39–41). Accordingly, such markers have been associated with encephalitogenesis. Their presence on a public and high-frequency self-reactive clonotype such as BV8S2/BJ2S7(L9), in addition to Th1 polarization, might explain the nature of these cells as encephalitogenic agents of EAE in B10.PL mice.

Publicity in this repertoire study appeared to be a crucial criterion for pathogenesis. However, our results also suggested that publicity is not the only entity defining a driver, because the other public clonotype, BV16/BJ2S5(L9), persisted, while BV8S2/BJ2S7(L9) disappeared at remission. Although BV16 T cells expressed many of the encephalitogenic markers described above, its 10-fold lower frequency when compared with BV8S2/BJ2S7(L9) as well as its partial expression of CTLA-4 might explain the lack of association of this expansion in vivo with the occurrence of disease.

Upon adoptive transfer, BV8S2/BJ2S7 cells carrying the DAGGGY CDR3 motif were clearly pathogenic. However, other members of the Ac1–9 specific repertoire (including BV16 T cells and all other BV clonotypes), even though capable of expansion in vivo, were unable to transfer disease. These results suggest that other private or public clonotypes are not, in and of themselves, pathogenic in this system. It is conceivable that such cells could contribute to disease, and indeed, many of them were also found in the spinal cord, but they might not expand aggressively enough in vivo to drive EAE.

The loss of a clonal family following expansion may be a common feature of T cell responses. Although individual clones may undergo activation-induced cell death following expansion, a portion of the family bearing a particular TCR usually persists. Repertoire studies to model foreign antigens have found that there is maintenance of and/or focus on certain dominant clonotypes in

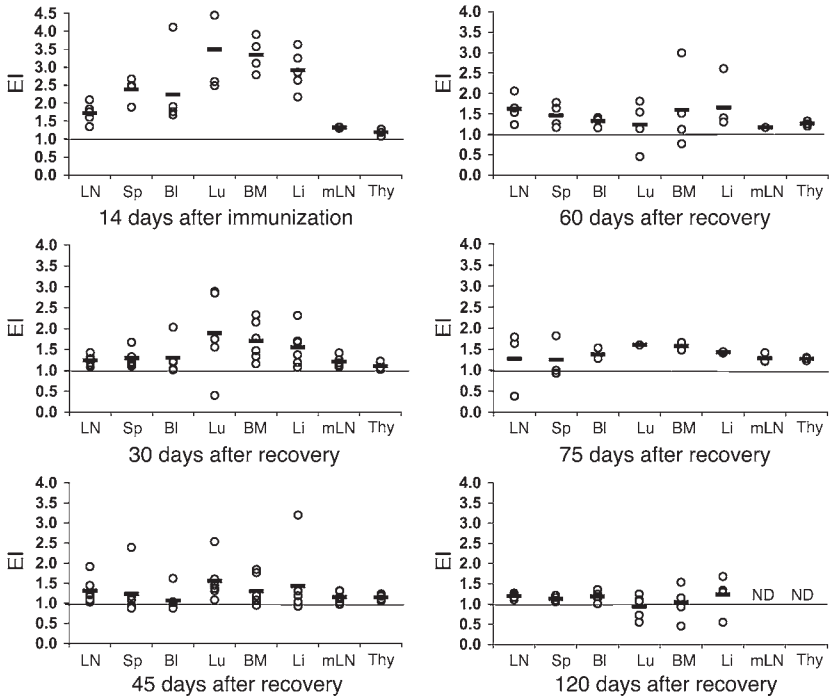

Figure 7
BV8S2/BJ2S7 expansions after recovery. In vitro–stimulated cells from lymph nodes (LN), spleen (Sp), blood (Bl), lung (Lu), bone marrow (BM), liver (Li), mesenteric lymph nodes (mLN), and thymus (Thy) were analyzed immunoscopically at different time points after immunization and recovery. Shown are EIs of the single CDR3 expansion BV8S2/BJ2S7(L9). An EI of 1 represents the particular expansion peak compared with its expansion within the Gaussian distribution. Values above or below this limit indicate expansions or contractions of the peak, respectively. Each symbol represents the EI value from organs of individual mice; mean expression is denoted by horizontal bars. ND, not determined.

the evolution of the immune response (12, 28, 42, 43). The Th1 cells with the greatest dominance, such as BV8S2/BJ2S7(L9) cells, may be the first to die, as a result of a sequence of very effective TCR ligation, costimulation, and expression of death effector molecules such as FasL (29, 30). Indeed, apoptotic elimination of BV8S2 (44–46) and more specifically BV8S2/BJ2S7(L9) (47) MBP-specific T cells has previously been shown to correlate with resolution of EAE (44–47). However, in relapsing-remitting EAE in SJL mice injected with peptide PLP 139–151, either clones of this specificity are maintained in subsequent relapses, while determinant spreading leads to diversification involving other clonotypes (48), or, as shown in some studies, the dominant clonal response disappears and is replaced (49). In MS it has been reported that some clonotypes are maintained over a long period of time in a given patient (50, 51). The results reported here differ in that the dominant, public clonotype was not detected after recovery. Thus, the loss of a pathogenic public clonotype and the apparent absence of its replacement in the remission repertoire suggest an explanation for a "single peak" disease in this mouse model.

It is noteworthy that there was neither reexpansion of BV8S2/BJ2S7(L9) cells nor EAE relapse upon Ac1–9 reimmunization in recovered mice (data not shown). However, the recurrence of EAE in $CD8^+$ T cell–depleted mice (52, 53) indicates that the post-recovery self-reactive repertoire is under regulatory influences in vivo. Indeed, injection of the dominant clone, BV8S2/BJ2S7-DAGGGY, into recovered mice failed to induce EAE (data not shown). Previous work has shown that regulatory TCR-directed $CD4^+$ T cells, in collaboration with TCR-specific $CD8^+$ T cells, are involved in the regulation of EAE in H-2^u mice (3, 10, 53–56). Ongoing immunoscope studies in our laboratory have revealed the expansion of a restricted set of regulatory clonotypes within BV14 that are responsive to a BV8S2 framework 3 antigenic region within the B5 peptide (aa 76–101) (47). These BV14 $CD4^+$ T cells collaborate with $CD8^+$ suppressors that cause apoptosis of the Th1 $CD4^+$ BV8S2 effectors. In this system, the target cells must be activated and specifically bear a BV8S2 TCR; also, Th1 cells have a greater susceptibility than do Th2 cells to the $CD8^+$ suppressor cells. (57). Other regulatory controls via bystander suppression, through signaling by CTLA-4, or by potent mediators such as TGFβ or IL-10 (58–60) may also play a role. The sum total of such regulatory processes can thereby target the activated pathogenic BV8S2/BJ2S7 Th1 clonotype, resulting in its loss from the lymphoid compartment, while other components of the residual Ac1–9–specific repertoire remain.

It is of interest that an extended period seems to be required in order to delete, anergize, or suppress BV8S2/BJ2S7(L9) at peripheral sites; moreover, the mechanisms of tolerance employed might be distinct at different sites. Thus, the positive expression of CTLA-4 on BV8S2/BJ2S7(L9) cells (data not shown) and the cells' lack of IFN-γ production upon cognate stimulation suggest that regulatory activities are playing a role in these sites during recovery. In parallel with our study, public clonotypes have previ-

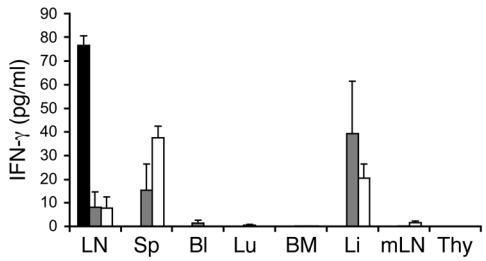

Figure 8
Cells responding to Ac1–9 produce a low level of IFN-γ after recovery. IFN-γ production by cells obtained from different sites at 30 days (gray bars) or 60 days (white bars) after recovery. IFN-γ production by lymph node cells 8 days after immunization is shown as a reference (black bars).

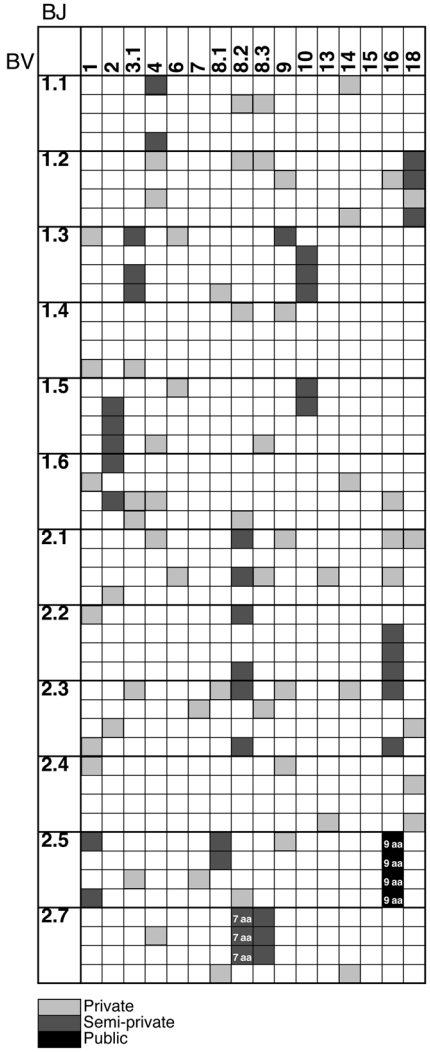

Figure 9
BV/BJ usage of cells responding to Ac1–9 is diverse after recovery. Immunoscopic analyses were performed on stimulated lymph node cells 75 days after recovery as described in the Figure 2 legend. Results are shown for 4 mice and are representative of 2 individual experiments. The 7-aa and 9-aa CDR3 lengths found for BV8S2BJ2S7 and BV16BJ2S5 expansions are denoted. Note that the CDR3 length found for the BV8S2BJ2S7 expansion in this figure is different from the conserved 9-aa sequence found in the previous figures.

ously been found within the repertoire of different MS patients (61–63). Not surprisingly, however, the consensus among researchers is that there is widespread diversity among MS patients. Apparently, clonotypes that are shared among individuals are a minority of the initially available set of myelin-reactive clones in MS. It is nevertheless evident that a predominant driver response would be the most relevant target for therapy. The identification of characteristics of typical driver clonotypes in patients, as proposed in this study, would focus attention on the truly dangerous effectors among a heterogeneous population of autoreactive but nonpathogenic clones. From a therapeutic perspective, focus on a few critical targets promises to more effectively prevent the perpetuation of autoimmunity and should provide greater predictability than some previous approaches (64, 65).

Methods

Mice and induction of EAE. B10.PL/J mice (The Jackson Laboratory) were immunized s.c. at 6–8 weeks of age with 100 μg Ac1–9 (ASQKRPSQR; Macromolecular Resources) and 200 μg *Mycobacterium tuberculosis* H37Ra (Difco) divided among 3 sites on their backs. At the same time and again 2 days later, mice were injected i.p. with 100 ng pertussis toxin (List Biologicals). Disease score was measured as follows: 1, tail paralysis; 2, hind limb weakness; 3, hind limb paralysis; 4, hind and forelimb paralysis; 5, moribund or dead. Transgenic 172.10 mice (kindly provided by J. Goverman, University of Washington Medical Center, Seattle, Washington, USA; ref. 66) were used for generation of T cell clones. This work was approved by the Institutional Animal Care and Use Committee of the Torrey Pines Institute for Molecular Studies.

Adoptive transfer. Cells from spleens and lymph nodes of Ac1–9–immunized mice were cultivated at 6×10^6 cells/ml with 5 μg/ml Ac1–9 and 20 ng/ml IL-12 for 48 hours. The cells were then separated into $CD8^-NK1.1^-$ $BV8.2^+$ and $BV8.2^-CD4^+$ populations by magnetic cell sorting (MACS) using anti-CD8a (Ly-2), anti-CD4 (L3T4), and anti-biotin monoclonal antibodies coupled to MicroBeads (Miltenyi Biotec Inc.) and biotinylated antibodies against NK1.1 (NKR-P1B and NKR-P1C) and BV8.2 (MR5-2) (BD Biosciences). The cells were washed and tested for purity by fluorescence-activated cell sorting before transfer by i.p. injection into naive irradiated (400 rads) mice. A range of cell numbers was tested for each cell population: $0.5–1.5 \times 10^7$ for $BV8S2^+$ cells, $1–5 \times 10^7$ for total $CD4^+$ cells, $1–7 \times 10^7$ for $BV8S2^-$ cells, and $0.5–5 \times 10^7$ for control BV10 cells. Another set of experiments was performed using a T cell clone with the DAGGGY motif in the CDR3 region (range, $0.1–0.5 \times 10^7$ cells injected per mouse) or with $BV8.2^-$ Ac1–9–specific T cell lines (range, $0.5–5 \times 10^7$ cells injected per mouse). The last in vitro stimulation before injection and the adoptive transfer of the clones and cell lines followed the same conditions described above. At the time of transfer and again 2 days later, recipient mice were injected s.c. with 100 ng pertussis toxin (List Biologicals). The mice were monitored daily for clinical signs of EAE.

Isolation of mononuclear cells from nonlymphoid sites. The left ventricle was flushed with 60 ml PBS to obtain blood leukocytes. The spinal cord, lung, liver, bone marrow, and thymus were dissected, homogenized, and centrifuged on a Percoll gradient with 35% and 70% Percoll layers at 24°C for 30 minutes at 500 *g*. Mononuclear cells were collected from the 35%–70% interface, washed, counted, and pelleted for culture or RNA isolation.

Cell culture. Splenocytes and lymph node cells were resuspended in complete RPMI medium and adjusted to 6×10^6 cells/ml; they were then incubated with Ac1–9 peptide for 72 hours. Proliferation was measured by incorporation of 1 μCi [^{3}H]-thymidine (ICN Inc.) during the last 18 hours. Ac1–9–specific T cell clones and lines were generated in complete RPMI medium plus IL-2 without additional cell growth factor for T cell differentiation. T cell lines were established from Ac1–9–immunized mice and were expanded by cycles of resting followed by stimulation with Ac1–9 and irradiated APCs. $CD4^+BV8S2^+$ cells from 172.10 transgenic mice were stimulated with Ac1–9 or concanavalin A and irradiated APCs and seeded in 96-well plates. Positive clones were tested for specificity and then screened by sequencing of the TCR CDR3 motif DAGGGY.

Cytokine capture sorting. IFN-γ– and IL-4–secreting populations were purified using the Mouse IFN-γ Secretion Assay Cell Enrichment and Detection Kit and the IL-4 Secretion Assay Cell Enrichment and Detection Kit (Miltenyi Biotec Inc.) per the manufacturer's instructions.

Isolation of lymphocyte populations with specific cellular markers. Cells were incubated with biotinylated antibodies against CD62L (Miltenyi Biotec Inc.),

mouse FasL.1 (Kay-10), ICAM-1 (3E2), OX40 (OX-86), CD40L (gp39), CD25 (7D4), CD69 (H1.2F3), CD44 (Pgp-1, Ly-24), or CTLA-4 (BD Biosciences), followed by a monoclonal anti-biotin antibody coupled to MicroBeads (Miltenyi Biotec Inc.). Positive populations were sorted using AutoMACS (Miltenyi Biotec Inc.), and samples were studied directly via immunoscope analysis.

TCR repertoire analysis. Repertoire analyses were performed using a protocol modified from Pannetier et al. (18). Analysis was performed on cells from draining lymph nodes (day 8) and spleens (day 14) of naive and immunized mice after restimulation with Ac1–9 or medium alone. Cells infiltrating the spinal cord in each animal were analyzed ex vivo. Total RNA was isolated from cell suspensions of individual samples using RNA isolation kit (QIAGEN) followed by cDNA syntheses using an oligo-dT primer $(dT)_{15}$ (Invitrogen). From each cDNA, PCR reactions were performed using V_β primers (see ref. 18) and a common C_β primer (CACTGATGTTCTGTGTGACA). Using these products as a template, runoff reactions were performed with internal fluorescent primers for the different J_βs (see ref. 18) and analyzed on an ABI 3100 Prism Genetic Analyzer (Applied Biosystems) using GeneScan 2.0 software (Applied Biosystems). The expansion index (EI) was calculated as the percentage of the experimental peak (area of experimental peak divided by the sum of areas of the experimental peaks) divided by the percentage of the control peak (area of control peak divided by the sum of areas of control peaks). Cells from different organs cultured in vitro with Ac1–9 from CFA-injected mice were used as controls. BV8S2/BJ2S7(L9) and BV16S2/BJ2S5(L9) peaks represented approximately 25% and 40%, respectively, of the total area of their respective control peaks. Thus, the maximum EI expected when there were 100% expansions of such peaks would be about 4 and 2.5, respectively.

Sequencing V_β genes. The cDNA was amplified with a BV8S2/BJ2S7 or BV16/BJ2S5 set of primers and cloned into the pCR2.1 cloning vector from the TOPO TA cloning kit (Invitrogen). Individual colonies were grown in LB medium and plasmid DNA was isolated (QIAGEN), followed by sequencing reactions directly on the plasmid DNA using an M13 (–20) primer and Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems). Reaction products were sequenced for the CDR3 region (ABI 3100 Prism; Applied Biosystems).

Real time PCR. Each V and J standard was established by amplifying samples known to contain T cells using specific V and J primers. A constant amount of cDNA for each dilution of each V, J, or m-cyclophilin (forward, GGCCGATGACGAGCCC; reverse, TGTCTTTGGAACTTTGTCTGCAA) standard (250 nM per primer) was amplified in 25 μl iTaqSYBR Green Supermix with ROX (Bio-Rad). The number of copies of the cDNA target sequence was deduced from a comparison of the measured fluorescence with a standard curve. Each sample was analyzed in triplicate.

Measurement of IFN-γ by ELISA. Cells from different sites were stimulated with Ac1–9. Supernatants were harvested after 72 hours and analyzed by quantitative ELISA. Capture and detection anti-mouse IFN-γ antibodies and recombinant murine cytokine were purchased from BD Biosciences.

Acknowledgments

This work was supported by the National Multiple Sclerosis Society (grant NMSS-RG5643C8T to E.E. Sercarz), the NIH (grants R01 AI042396-0 and R01 AI048077 to E.E. Sercarz), and the Multiple Sclerosis National Research Institute. Peter van den Elzen and Emanual Maverakis initiated these studies. We thank Joan Goverman for providing 172.10 transgenic mice, Janel Suburu and Nazila Daneshjou for technical assistance, and Eric Milner for editorial assistance.

Received for publication February 17, 2006, and accepted in revised form April 30, 2007.

Address correspondence to: Eli E. Sercarz, Division of Immune Regulation, Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, California 92121, USA. Phone: (858) 455-3824; Fax: (858) 455-3715; E-mail: esercarz@tpims.org.

Peter van den Elzen's present address is: Department of Pathology and Laboratory Medicine, University of British Columbia, Children's and Women's Health Centre of BC, Vancouver, British Columbia, Canada.

Emanual Maverakis's present address is: Department of Dermatology, University of California Davis School of Medicine, Sacramento, California, USA.

1. Jingwu, Z., et al. 1992. Myelin basic protein-specific T lymphocytes in multiple sclerosis and controls: precursor frequency, fine specificity, and cytotoxicity. *Ann. Neurol.* **32**:330–338.
2. Olivares-Villagomez, D., Wang, Y., and Lafaille, J.J. 1998. Regulatory CD4(+) T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. *J. Exp. Med.* **188**:1883–1894.
3. Kumar, V., and Sercarz, E.E. 1993. The involvement of T cell receptor peptide-specific regulatory CD4+ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med.* **178**:909–916.
4. Kawakami, N., et al. 2005. Live imaging of effector cell trafficking and autoantigen recognition within the unfolding autoimmune encephalomyelitis lesion. *J. Exp. Med.* **201**:1805–1814.
5. Kawakami, N., et al. 2004. The activation status of neuroantigen-specific T cells in the target organ determines the clinical outcome of autoimmune encephalomyelitis. *J. Exp. Med.* **199**:185–197.
6. Flugel, A., et al. 2001. Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. *Immunity.* **14**:547–560.
7. Sakuma, H., Kohyama, K., Jee, Y., and Matsumoto, Y. 2004. Tracking of V beta 8.2-positive encephalitogenic T cells by complementarity-determining region 3 spectratyping and subsequent Southern blot hybridization in Lewis rats after neuroantigen sensitization. *J. Immunol.* **173**:4516–4522.
8. Ben-Nun, A., Wekerle, H., and Cohen, I.R. 1981. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. *Nature.* **292**:60–61.
9. Lohse, A.W., Mor, F., Karin, N., and Cohen, I.R. 1989. Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells. *Science.* **244**:820–822.
10. Vandenbark, A.A., Hashim, G., and Offner, H. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature.* **341**:541–544.
11. Cibotti, R., et al. 1994. Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in nontransgenic versus HEL transgenic mice. *J. Exp. Med.* **180**:861–872.
12. McHeyzer-Williams, M.G., and Davis, M.M. 1995. Antigen-specific development of primary and memory T cells in vivo. *Science.* **268**:106–111.
13. Cerasoli, D.M., Riley, M.P., Shih, F.F., and Caton, A.J. 1995. Genetic basis for T cell recognition of a major histocompatibility complex class II-restricted neo-self peptide. *J. Exp. Med.* **182**:1327–1336.
14. Lehner, P.J., et al. 1995. Human HLA-A0201-restricted cytotoxic T lymphocyte recognition of influenza A is dominated by T cells bearing the V beta 17 gene segment. *J. Exp. Med.* **181**:79–91.
15. Argaet, V.P., et al. 1994. Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus. *J. Exp. Med.* **180**:2335–2340.
16. Venturi, V., et al. 2006. Sharing of T cell receptors in antigen-specific responses is driven by convergent recombination. *Proc. Natl. Acad. Sci. U. S. A.* **103**:18691–18696.
17. Urban, J.L., et al. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell.* **54**:577–592.
18. Pannetier, C., et al. 1993. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. *Proc. Natl. Acad. Sci. U. S. A.* **90**:4319–4323.
19. Karman, J., Ling, C., Sandor, M., and Fabry, Z. 2004. Initiation of immune responses in brain is promoted by local dendritic cells. *J. Immunol.* **173**:2353–2361.
20. Greter, M., et al. 2005. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat. Med.* **11**:328–334.
21. Bell, R.B., Lindsey, J.W., Sobel, R.A., Hodgkinson, S., and Steinman, L. 1993. Diverse T cell receptor V beta gene usage in the central nervous system in experimental allergic encephalomyelitis. *J. Immunol.*

150:4085–4092.
22. Ransohoff, R.M., Kivisakk, P., and Kidd, G. 2003. Three or more routes for leukocyte migration into the central nervous system. *Nat. Rev. Immunol.* **3**:569–581.
23. Bousso, P., Levraud, J.P., Kourilsky, P., and Abastado, J.P. 1999. The composition of a primary T cell response is largely determined by the timing of recruitment of individual T cell clones. *J. Exp. Med.* **189**:1591–1600.
24. Hedrick, S.M., et al. 1988. Selection of amino acid sequences in the beta chain of the T cell antigen receptor. *Science.* **239**:1541–1544.
25. Cochet, M., et al. 1992. Molecular detection and in vivo analysis of the specific T cell response to a protein antigen. *Eur. J. Immunol.* **22**:2639–2647.
26. Casanova, J.L., et al. 1992. H-2-restricted cytolytic T lymphocytes specific for HLA display T cell receptors of limited diversity. *J. Exp. Med.* **176**:439–447.
27. Sourdive, D.J., et al. 1998. Conserved T cell receptor repertoire in primary and memory CD8 T cell responses to an acute viral infection. *J. Exp. Med.* **188**:71–82.
28. Fasso, M., et al. 2000. T cell receptor (TCR)-mediated repertoire selection and loss of TCR vbeta diversity during the initiation of a CD4(+) T cell response in vivo. *J. Exp. Med.* **192**:1719–1730.
29. Sabelko-Downes, K.A., Cross, A.H., and Russell, J.H. 1999. Dual role for Fas ligand in the initiation of and recovery from experimental allergic encephalomyelitis. *J. Exp. Med.* **189**:1195–1205.
30. Suvannavejh, G.C., Dal Canto, M.C., Matis, L.A., and Miller, S.D. 2000. Fas-mediated apoptosis in clinical remissions of relapsing experimental autoimmune encephalomyelitis. *J. Clin. Invest.* **105**:223–231.
31. Hovelmeyer, N., et al. 2005. Apoptosis of oligodendrocytes via Fas and TNF-R1 is a key event in the induction of experimental autoimmune encephalomyelitis. *J. Immunol.* **175**:5875–5884.
32. Weinberg, A.D., Wegmann, K.W., Funatake, C., and Whitham, R.H. 1999. Blocking OX-40/OX-40 ligand interaction in vitro and in vivo leads to decreased T cell function and amelioration of experimental allergic encephalomyelitis. *J. Immunol.* **162**:1818–1826.
33. Carboni, S., et al. 2003. CD134 plays a crucial role in the pathogenesis of EAE and is upregulated in the CNS of patients with multiple sclerosis. *J. Neuroimmunol.* **145**:1–11.
34. Grewal, I.S., et al. 1996. Requirement for CD40 ligand in costimulation induction, T cell activation, and experimental allergic encephalomyelitis. *Science.* **273**:1864–1867.
35. Ponomarev, E.D., Shriver, L.P., and Dittel, B.N. 2006. CD40 expression by microglial cells is required for their completion of a two-step activation process during central nervous system autoimmune inflammation. *J. Immunol.* **176**:1402–1410.
36. Archelos, J.J., et al. 1993. Inhibition of experimental autoimmune encephalomyelitis by an antibody to the intercellular adhesion molecule ICAM-1. *Ann. Neurol.* **34**:145–154.
37. Amend, B., et al. 2006. Induction of autoimmunity by expansion of autoreactive CD4+CD62Llow cells in vivo. *J. Immunol.* **177**:4384–4390.
38. Grewal, I.S., et al. 2001. CD62L is required on effector cells for local interactions in the CNS to cause myelin damage in experimental allergic encephalomyelitis. *Immunity.* **14**:291–302.
39. Hurwitz, A.A., Sullivan, T.J., Sobel, R.A., and Allison, J.P. 2002. Cytotoxic T lymphocyte antigen-4 (CTLA-4) limits the expansion of encephalitogenic T cells in experimental autoimmune encephalomyelitis (EAE)-resistant BALB/c mice. *Proc. Natl. Acad. Sci. U. S. A.* **99**:3013–3017.
40. Kuhns, M.S., Epshteyn, V., Sobel, R.A., and Allison, J.P. 2000. Cytotoxic T lymphocyte antigen-4 (CTLA-4) regulates the size, reactivity, and function of a primed pool of CD4+ T cells. *Proc. Natl. Acad. Sci. U. S. A.* **97**:12711–12716.
41. Karandikar, N.J., Vanderlugt, C.L., Walunas, T.L., Miller, S.D., and Bluestone, J.A. 1996. CTLA-4: a negative regulator of autoimmune disease. *J. Exp. Med.* **184**:783–788.
42. McHeyzer-Williams, L.J., Panus, J.F., Mikszta, J.A., and McHeyzer-Williams, M.G. 1999. Evolution of antigen-specific T cell receptors in vivo: preimmune and antigen-driven selection of preferred complementarity-determining region 3 (CDR3) motifs. *J. Exp. Med.* **189**:1823–1838.
43. Savage, P.A., Boniface, J.J., and Davis, M.M. 1999. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity.* **10**:485–492.
44. Pender, M.P., McCombe, P.A., Yoong, G., and Nguyen, K.B. 1992. Apoptosis of alpha beta T lymphocytes in the nervous system in experimental autoimmune encephalomyelitis: its possible implications for recovery and acquired tolerance. *J. Autoimmun.* **5**:401–410.
45. McCombe, P.A., Nickson, I., Tabi, Z., and Pender, M.P. 1996. Apoptosis of V beta 8.2+ T lymphocytes in the spinal cord during recovery from experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein. *J. Neurol. Sci.* **139**:1–6.
46. Tabi, Z., McCombe, P.A., and Pender, M.P. 1994. Apoptotic elimination of V beta 8.2+ cells from the central nervous system during recovery from experimental autoimmune encephalomyelitis induced by the passive transfer of V beta 8.2+ encephalitogenic T cells. *Eur. J. Immunol.* **24**:2609–2617.
47. Madakamutil, L.T., Maricic, I., Sercarz, E., and Kumar, V. 2003. Regulatory T cells control autoimmunity in vivo by inducing apoptotic depletion of activated pathogenic lymphocytes. *J. Immunol.* **170**:2985–2992.
48. Fritz, R.B., Wang, X., and Zhao, M.L. 2000. Alterations in the spinal cord T cell repertoire during relapsing experimental autoimmune encephalomyelitis. *J. Immunol.* **164**:6662–6668.
49. Vanderlugt, C.L., et al. 2000. Pathologic role and temporal appearance of newly emerging autoepitopes in relapsing experimental autoimmune encephalomyelitis. *J. Immunol.* **164**:670–678.
50. Goebels, N., et al. 2000. Repertoire dynamics of autoreactive T cells in multiple sclerosis patients and healthy subjects: epitope spreading versus clonal persistence. *Brain.* **123**:508–518.
51. Wilson, D.B., et al. 1997. Results of a phase I clinical trial of a T-cell receptor peptide vaccine in patients with multiple sclerosis. I. Analysis of T-cell receptor utilization in CSF cell populations. *J. Neuroimmunol.* **76**:15–28.
52. Jiang, H., Zhang, S.I., and Pernis, B. 1992. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science.* **256**:1213–1215.
53. Jiang, H., et al. 2003. Regulatory CD8+ T cells fine-tune the myelin basic protein-reactive T cell receptor V beta repertoire during experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U. S. A.* **100**:8378–8383.
54. Lider, O., Reshef, T., Beraud, E., Ben-Nun, A., and Cohen, I.R. 1988. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science.* **239**:181–183.
55. van den Elzen, P., et al. 2004. Limited clonality in autoimmunity: drivers and regulators. *Autoimmun. Rev.* **3**:524–529.
56. Kumar, V., and Sercarz, E. 2001. An integrative model of regulation centered on recognition of TCR peptide/MHC complexes. *Immunol. Rev.* **182**:113–121.
57. Jiang, H., Braunstein, N.S., Yu, B., Winchester, R., and Chess, L. 2001. CD8+ T cells control the TH phenotype of MBP-reactive CD4+ T cells in EAE mice. *Proc. Natl. Acad. Sci. U. S. A.* **98**:6301–6306.
58. Tang, Q., and Bluestone, J.A. 2006. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol. Rev.* **212**:217–237.
59. Roncarolo, M.G., et al. 2006. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* **212**:28–50.
60. Fehervari, Z., and Sakaguchi, S. 2004. CD4+ Tregs and immune control. *J. Clin. Invest.* **114**:1209–1217. doi:10.1172/JCI200423395.
61. Lozeron, P., Chabas, D., Duprey, B., Lyon-Caen, O., and Liblau, R. 1998. T cell receptor V beta 5 and V beta 17 clonal diversity in cerebrospinal fluid and peripheral blood lymphocytes of multiple sclerosis patients. *Mult. Scler.* **4**:154–161.
62. Hong, J., et al. 1999. A common TCR V-D-J sequence in V beta 13.1 T cells recognizing an immunodominant peptide of myelin basic protein in multiple sclerosis. *J. Immunol.* **163**:3530–3538.
63. Monteiro, J., Hingorani, R., Pergolizzi, R., Apatoff, B., and Gregersen, P.K. 1995. Clonal dominance of CD8+ T-cell in multiple sclerosis. *Ann. N. Y. Acad. Sci.* **756**:310–312.
64. Bielekova, B., et al. 2000. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med.* **6**:1167–1175.
65. Kappos, L., et al. 2000. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. *Nat. Med.* **6**:1176–1182.
66. Goverman, J., et al. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell.* **72**:551–560.