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$V_\beta8^+$ T cells protect from demyelinating disease in a viral model of multiple sclerosis

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Abstract

Previous studies illustrated the influence of T cell subsets on susceptibility or resistance to demyelination in the Theiler's murine encephalomyelitis virus (TMEV) model of multiple sclerosis. Genetic segregation analysis showed a correlation with disease phenotype in this model with particular V_β genes. In this study we investigated the contribution of specific V_β TCR to the pathogenesis of virus-induced demyelinating disease. Spectratype analysis of cells infiltrating the CNS early in infection demonstrated an over-representation of $V_\beta8^+$ T cells in mice expressing a susceptible H-2 haplotype. We infected transgenic mice expressing the $V_\beta8.2$ TCR directed against a non-TMEV antigen and found an increase in demyelinating disease in mice of either susceptible or resistant background compared with littermate controls. In addition, depletion studies with an anti-$V_\beta8$-specific antibody in both susceptible (B10.Q) and resistant (C57BL/6) mice resulted in increased demyelination. TCR analysis of VP2-specific cytotoxic T cell clones from mice with a resistant genotype identified only the $V_\beta8.1$ TCR, suggesting that limited T cell diversity is critical to TMEV clearance. Together, these results support a protective role for $V_\beta8^+$ T cells in virus-induced demyelinating disease.

Introduction

Intracerebral infection (i.c.) of mice with Theiler's murine encephalomyelitis virus (TMEV) results in a biphasic disease in susceptible mouse strains. Susceptible mice experience acute encephalitis followed by chronic viral persistence and demyelination, while genetically resistant mice experience only the acute encephalitic phase of the disease and then clear the virus without further pathology (1). Several loci have been identified which influence TMEV-induced demyelination. The D region of the H-2 locus plays a critical role in determining susceptibility or resistance to TMEV-induced demyelination (2–5). Mice on a C57BL background with *f*, *p*, *q*, *r*, *s* or *v* haplotypes are susceptible, while mice with *b*, *d* or *k* haplotypes are resistant (3). Mice with other backgrounds have not been as well studied, although it has been shown that insertion of the D^b (4) or D^d (5) gene into susceptible mice confers resistance to otherwise susceptible strains. Other genes implicated in influencing resistance/susceptibility to demyelination include an area on chromosome 3 near the *Car-2* gene (6), an area on chromosome 6 near the *Tcrb* complex (7), a locus on chromosome 10 near the IFN-γ gene (8), an area on chromosome 18 near the *Mbp* gene (8), and, most recently, a segment of chromosome 14 located between the *Gnrh* and *Glud* genes (9).

A common theme in Theiler's virus immunology is that components of the immune system may be either pathogenic or protective depending on the genetic background of the host. This paradox is illustrated by T cell depletion studies. Depletion of either $CD4^+$ or $CD8^+$ T cells in TMEV-infected mice of susceptible background results in less severe demyelination (10–12). In contrast, depletion of T cell subsets in resistant mice results in induction of demyelination and virus persistence (10,12,13). Therefore, both MHC class I-

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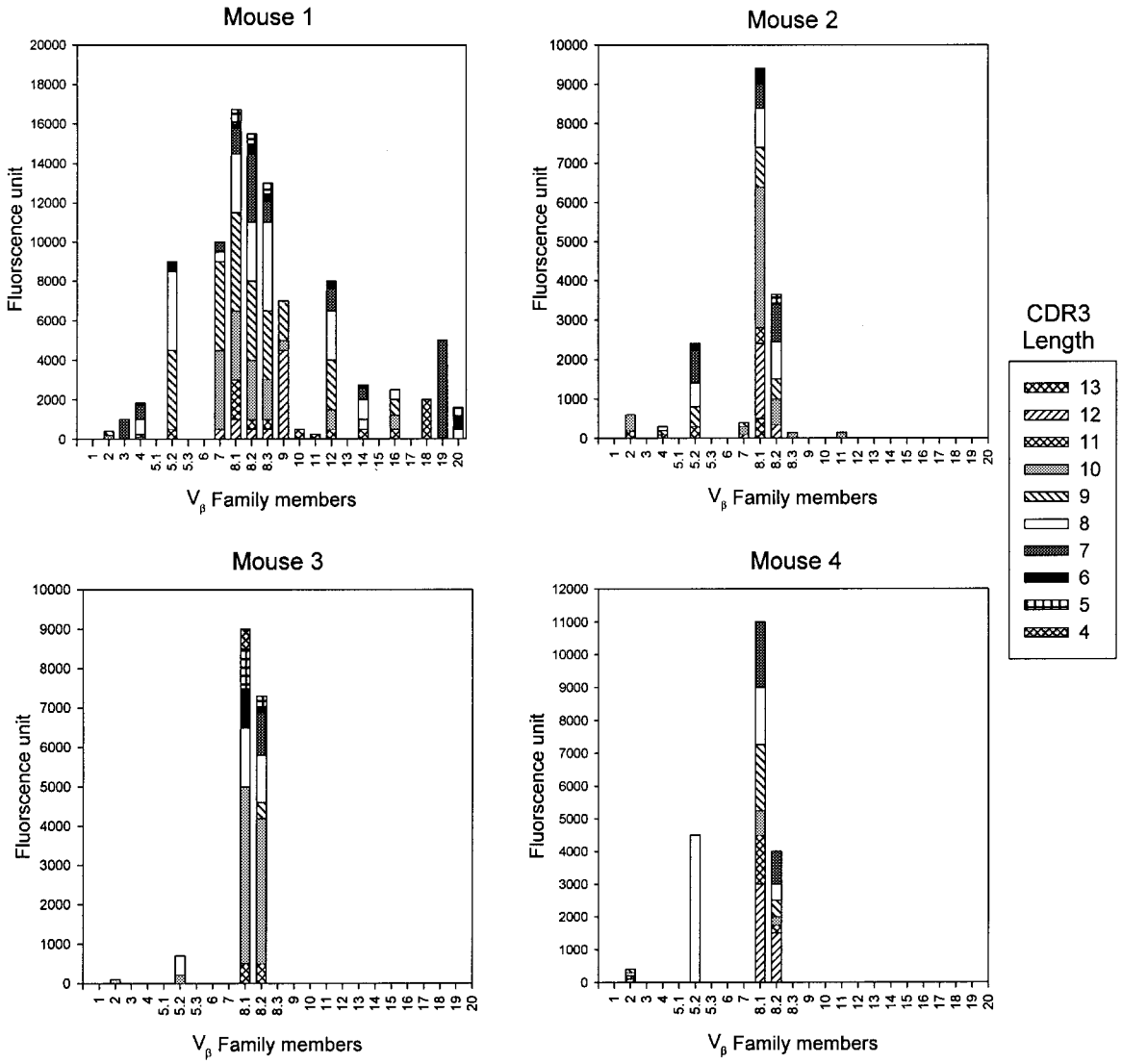

Fig. 1. Spectratype analysis using V_β-specific primers from RNA of CNS-IL from B10.Q mice at day 7 post-infection with Theiler's virus. A predominance of $V_\beta 8^+$ TCR was identified in these mice. Total RNA was isolated and reverse transcribed using a C_β region-specific primer. Sequential PCR reactions were then performed using V_β-specific primers and a nested C_β primer, followed by primer extension with a 6-carboxyfluorescein-labeled nested C_β primer. Products were run on a polyacrylamide denaturing gel and analyzed as described in Methods.

and II-restricted cells participate in the pathogenesis of disease observed following TMEV infection. β_2-Microglobulin-deficient mice which have a deficiency in both MHC class I and $CD8^+$ T cells develop large demyelinated lesions without significant neurological deficits (14,15). Ab° mice which express reduced levels of MHC class II and $CD4^+$ T cells develop both demyelination and severe clinical disease (16,17). The mice in these studies were of a genetically resistant background, suggesting that both class I and class II participate in resistance to demyelination.

Several studies have tried to dissect the precise contribution of particular TCR genes to susceptibility or resistance to Theiler's virus-induced demyelination. Genetic segregation analysis determined that mice with large deletions in their V_β genes had significantly more demyelination than mice with intact V_β genes (18,19). Genetic analysis using congenic mice with specific deletions in their V_β genes allowed investigators the opportunity to evaluate the relative contributions of specific V_β gene deletions in mice susceptible and resistant to TMEV-induced demyelination. Mice with resistant H-2 genotypes were unaffected by large deletions in their TCR V_β genes, demonstrating that the remaining V_β repertoire was sufficient to protect mice from TMEV-induced damage. Mice of susceptible genotype with large deletions in their TCR V_β genes developed severe demyelination along with virus persistence. Of interest, these mice did not die of overwhelming acute neuronal encephalitis as is seen in *Scid* (20) and $Rag^{-/-}$ mice (21), indicating that the remaining T cell

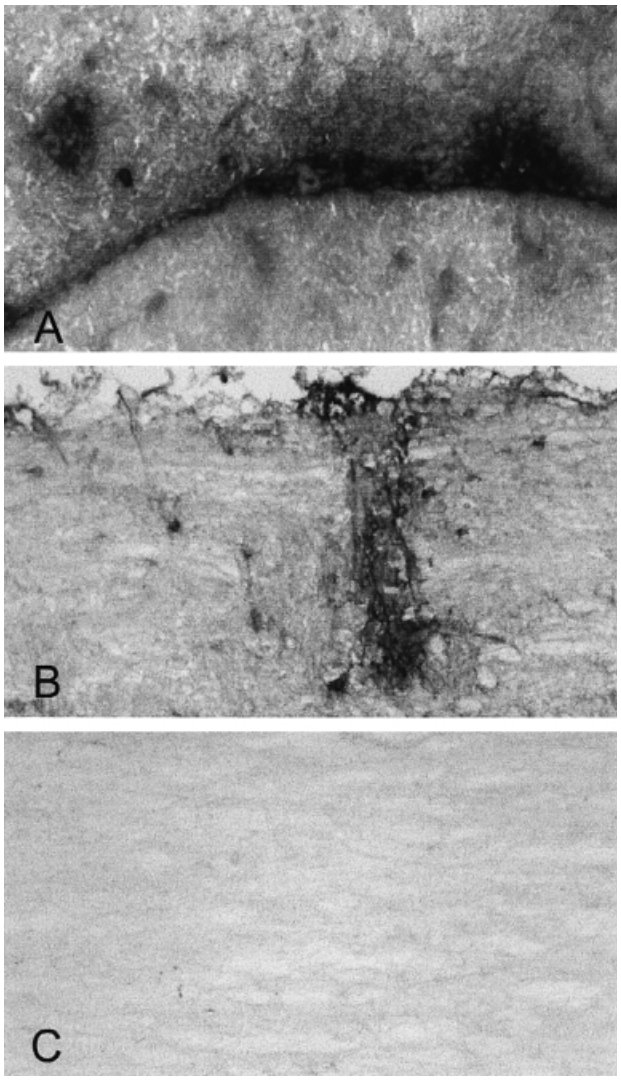

Fig. 2. Expression of $V_\beta 8^+$ cells in the brain (A) and spinal cord (B and C) of B10.Q mice after infection with Theiler's virus. Positively staining cells were observed in the demyelinated lesions of B10.Q mice. Frozen sections were stained using the immunoperoxidase technique with a mAb specific to $V_\beta 8.1$, 8.2 (A and B) or a control antibody (C). The dark reaction product indicates positive staining. No positively staining cells were observed in the CNS of uninfected B10.Q mice.

population in these mice was sufficient to protect from acute disease but nevertheless facilitated progression to chronic demyelinating disease.

The results of the genetic segregation analysis studies support a role for specific TCR genes in resistance/susceptibility to Theiler's virus-induced demyelination (18,22). To further understand the role of particular V_β genes in this model of multiple sclerosis (MS) we used spectratype analysis to identify the TCR V_β genes in the CNS of susceptible B10.Q mice after infection with TMEV and found that $V_\beta 8^+$ T cells were over-represented. We investigated the influence of one of these genes ($V_\beta 8.2$) in genetically susceptible (B10.Q) and resistant (C57BL/6J) mice depleted of $V_\beta 8.2^+$ T cells by

Table 1. Presence of the $V_\beta 8.2$ transgene exacerbates the extent of demyelination in the spinal cord of *H-2*^{b} mice following Theiler's virus infection

Strain	*n*	$V_\beta 8.2$ Tg	Mean pathology score ± SEM (45 days following infection)		
			Gray matter inflammation	Meningeal inflammation	Demyelination
B10.Q	14	–	0.1 ± 0.0	10.5 ± 0.9	15.8 ± 2.4
B10.Q	11	+	0.0 ± 0.0	27.6 ± 3.0[a]	32.8 ± 7.0 [a]
C57BL/6	20	–	0.0 ± 0.0	0.0 ± 5.0	0.2 ± 0.1
C57BL/6	6	+	0.4 ± 0.2	7.8 ± 0.9 [a]	12.5 ± 4.8 [a]

For each mouse, 10–15 spinal cord sections were graded for gray matter inflammation, meningeal inflammation and demyelination. The data are expressed as the percentage of spinal cord quadrants with disease (mean ± SEM). *n* = number of mice. Tg denotes the presence or absence of the $V_\beta 8.2$ transgene.

[a]Differences between demyelination and meningeal inflammation scores were statistically significant between C57BL/6 Tg^- and C57BL/6 Tg^+ ($P < 0.05$) and approached significance between B10.Q Tg^- and B10.Q Tg^+ mice ($P = 0.056$) by the Mann–Whitney rank sum test.

antibody treatment, as well as in mice of the same genotype expressing the $V_\beta 8.2$ transgene. Spectratype analysis of cytotoxic T cell clones from the CNS of resistant *H-2*^{b} mice stimulated *in vitro* identified only $V_\beta 8.1^+$ clones. Our findings support a role for $V_\beta 8^+$ T cells in protection from TMEV-induced demyelination in both B10.Q and C57BL/6J mice.

Methods

Virus

Daniel's strain of Theiler's virus was used for all experiments. The passage history of this virus has been described previously (1).

Mice

C57BL/6J and B10.Q mice were purchased from Jackson Laboratories (Bar Harbor, ME). Transgenic mice used in this study were bred in the Mayo Clinic Immunogenetics Mouse Facility (Rochester, MN). Transgenic C57L (*H-2*^{b}) mice bearing a rearranged $V_\beta 8.2$ TCR cloned from the T cell hybridoma DO-11.10 (ovalbumin specific) were prepared as previously described (23). In some experiments, transgenic mice were backcrossed to B10.Q (*H-2*^{q}) animals. Animals were typed by flow cytometry for haplotype and the presence of the transgene. The antibodies used for this screening were MKDG (*q* specific), AF6/120 (*b* specific) and F23.1 (24).

Flow cytometric analysis

Mice were tail-bled and lymphocytes were isolated over a Ficoll-Hypaque gradient (Pharmacia, Piscataway, NJ). After centrifugation, cells were incubated with antibody on ice, and then washed. The cells were then incubated with a secondary FITC-conjugated $F(ab)'_2$ fragment specific for mouse IgG (Accurate Chemicals, Westbury NY).

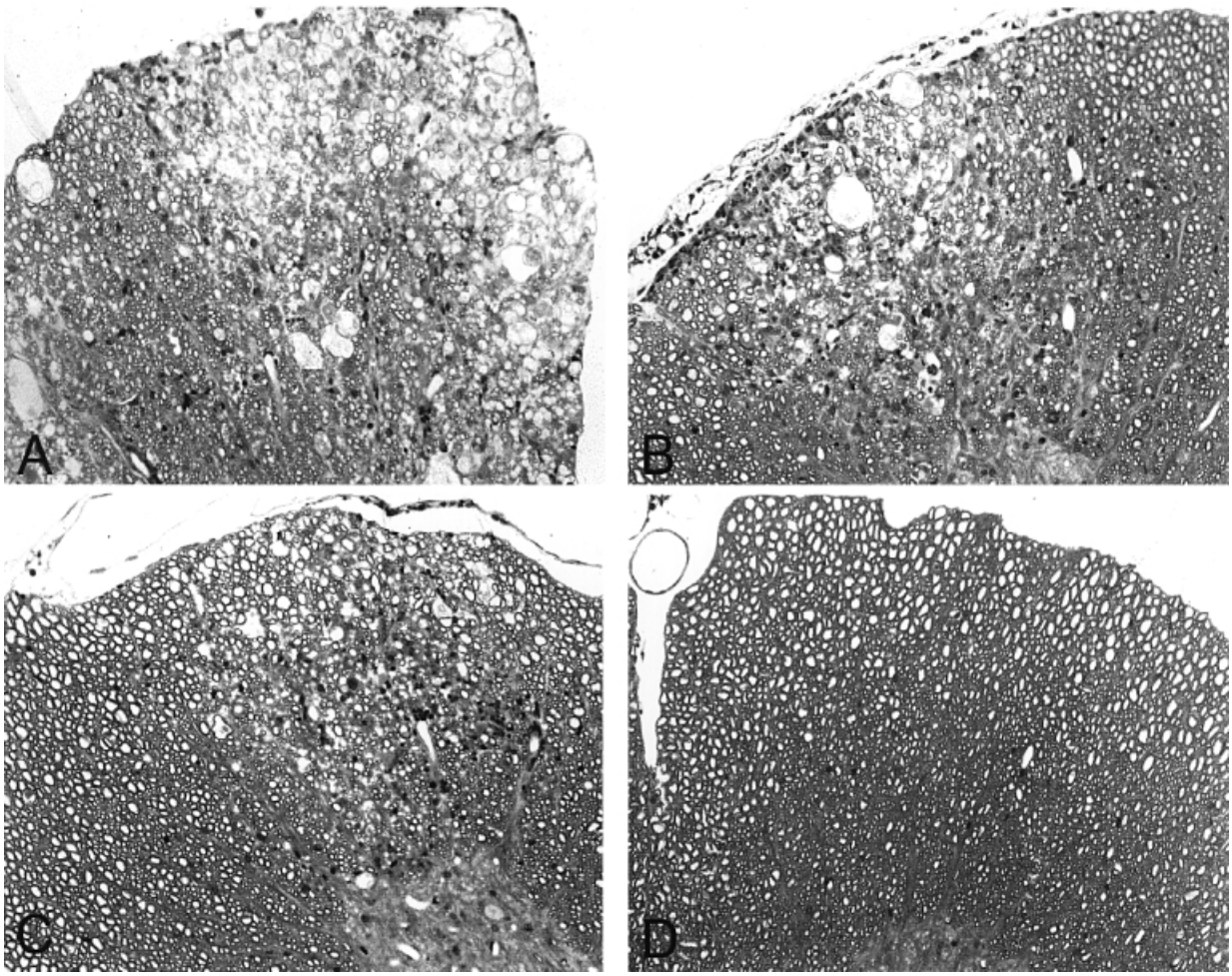

Fig. 3. Demyelination in the spinal cord of $V_\beta 8.2$ transgenic mice at day 45 after infection with Theiler's virus. Note the increased size of the demyelinated lesions in the spinal cords of a B10.Q transgene-positive mouse (A) as compared to the B10.Q transgene-negative littermate (B). Note marked presence of demyelination in $V_\beta 8.2$ transgene-positive C57BL/6 mouse of normally resistant genotype (C) as compared to absence of demyelination in a C57BL/6 transgene-negative mouse (D). Spinal cord sections were embedded in glycol methacrylate plastic and stained with a modified erichrome/cresyl violet stain.

Infection, harvesting and morphology of the CNS

At 4–6 weeks of age, mice were infected i.c. with 2×10^5 p.f.u. of TMEV in a total volume of 10 μl. Forty-five days after infection, mice were perfused with Trump's fixative via intracardiac puncture. The 45 day time-point was chosen because it has been shown previously to distinguish between mice susceptible and resistant to TMEV-induced demyelination (13,25,26). Spinal cord blocks (1 mm) were embedded in glycol methacrylate and stained with a modified erichrome stain with a cresyl violet counterstain (27). Detailed morphologic analysis was performed on 10–15 coronal spinal cord sections from each animal. A total of 2951 spinal cord quadrants was studied. Each quadrant from every third spinal cord block from each animal was scored for the presence or absence of gray matter disease, meningeal inflammation and demyelination without knowledge of genotype or treatment. The score was expressed as a percent of the total number of quadrants with a particular abnormality. A maximum pathologic score of 100 indicated that there was disease in every quadrant of every spinal cord block from a particular mouse (28).

In vivo T cell depletion

One day prior to i.c. infection, mice were i.p. injected with 100 μg of F23.1, an antibody specific for $V_\beta 8$. Mice were tail bled on day 3 post-infection and FACS analysis performed as described above to ensure that depletion was successful. FACS analysis was also performed upon termination of the experiment to confirm the effectiveness of clonal deletion. Control mice were injected with 100 μg of mouse IgG (Sigma, St Louis, MO).

Spectratyping

Spectratyping was performed using the methodology published by Johnston *et al.* (29). Brains and spinal cords were removed at 7 days post-infection. Lymphocytes were isolated over a Percoll (Pharmacia) gradient by centrifugation at 27,000 *g* for 30 min as previously described (30). RNA

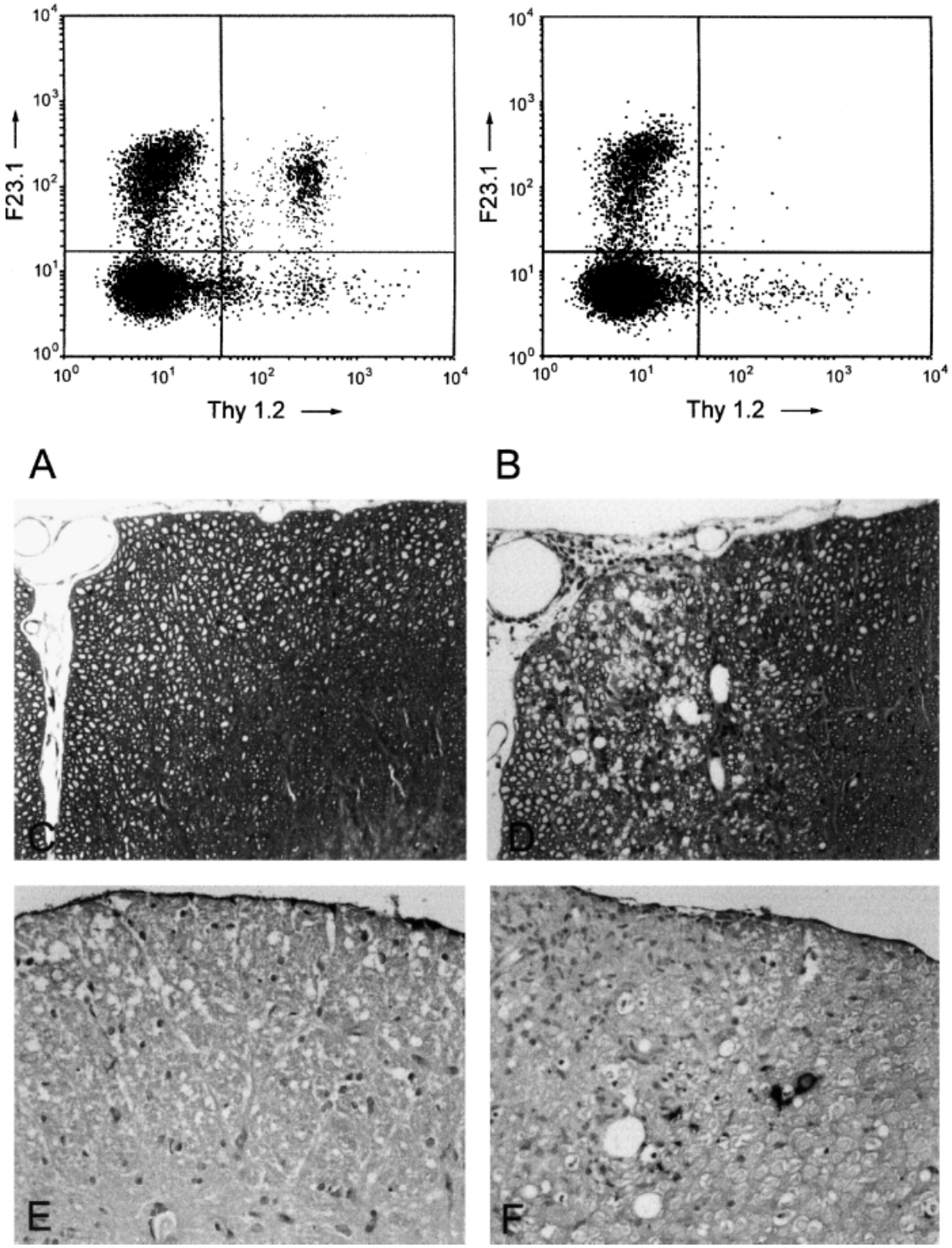

Fig. 4. Flow cytometric analysis of peripheral blood lymphocytes of C57BL/6 mice following *in vivo* treatment with F23.1 mAb. Mice were i.p. injected with either a control IgG (A) or F23.1 (B). Double staining was performed as indicated in Methods using antibodies to Thy-1.2 (*x*-axis) and F23.1 (*y*-axis). At day 45 after infection with Theiler's virus, spinal cords were removed for morphometric analysis. Normal resistant C57BL/6 mice treated with the control IgG had no inflammation or demyelination (C). C57BL/6 mice depleted of $V_\beta 8^+$ T cells had prominent levels of demyelination and meningeal inflammation (D). Spinal cords sections were embedded in glycol methacrylate plastic and stained with a modified erichrome/cresyl violet stain. No virus antigen was detected in transgene-negative littermate controls (E). TMEV antigen was detected in the spinal cord of $V_\beta 8.2$ transgenic mice on a C57BL/6 background (F). TMEV antigen staining was performed on paraffin-embedded sections with a rabbit polyclonal antisera using the ABC immunoperoxidase technique.

Table 2. V_β 8^+ T cells protect *H-2^b* mice from spinal cord demyelination following Theiler's virus infection

Strain	*n*	Treatment	Mean pathology score ± SEM (45 days following infection)		
			Gray matter inflammation	Meningeal inflammation	Demyelination
B10.Q	3	F23.1	2.7 ± 0.4	26.9 ± 12.3	33.6 ± 15.0
B10.Q	4	MsIgG	0.4 ± 0.8	30.0 ± 18.4	14.8 ± 12.2
C57BL/6	5	F23.1	2.0 ± 1.3	33.1 ± 5.5[a]	15.8 ± 3.6[a]
C57BL/6	5	MsIgG	0.9 ± 1.0	7.7 ± 5.3	1.8 ± 0.8

For each mouse, 10–15 spinal cord sections were graded for gray matter inflammation, meningeal inflammation and demyelination. The data are expressed as the percentage of spinal cord quadrants with disease (mean ± SEM). *n* = number of mice. Mice were treated with either F23.1 mAb to deplete of $V_\beta 8$ T cells or an isotype control (mouse IgG).

[a]Differences between demyelination or meningeal inflammation scores were statistically significant between C57BL/6 F23.1-treated mice and the isotype controls ($P < 0.05$).

was extracted from the CNS-infiltrating lymphocytes (CNS-IL) using the Total RNA extraction kit (5′-3′, Boulder, CO) as per the manufacturer's protocol. Twenty-four V_β-specific RT-PCR were performed using 10 ng total RNA per reaction, a C_β region-specific primer and previously published V_β region-specific primers (29). Following further amplification with a second nested C_β primer, the amplicons underwent single-stranded primer extension using a third nested C_β region primer end-labeled with 6-carboxyfluorescein. The resulting products were run on a 4.75% polyacrylamide/6 M urea denaturing gel. Product length was determined using the Prism Genescan software package (version 2.0.1; Applied Biosystems, Foster City, CA). Data analysis was performed using the Genotyper 1.1 software package (ABI).

Immunoperoxidase staining

Sections (10 μm thick) were cut and fixed in chilled acetone. Sections were stained with a mouse-anti-mouse $V_\beta 8.1$, 8.2-specific mAb (clone MR5-2; PharMingen, San Diego, CA). In some cases, paraffin-embedded sections of spinal cords were deparaffinized in xylene and rehydrated through an

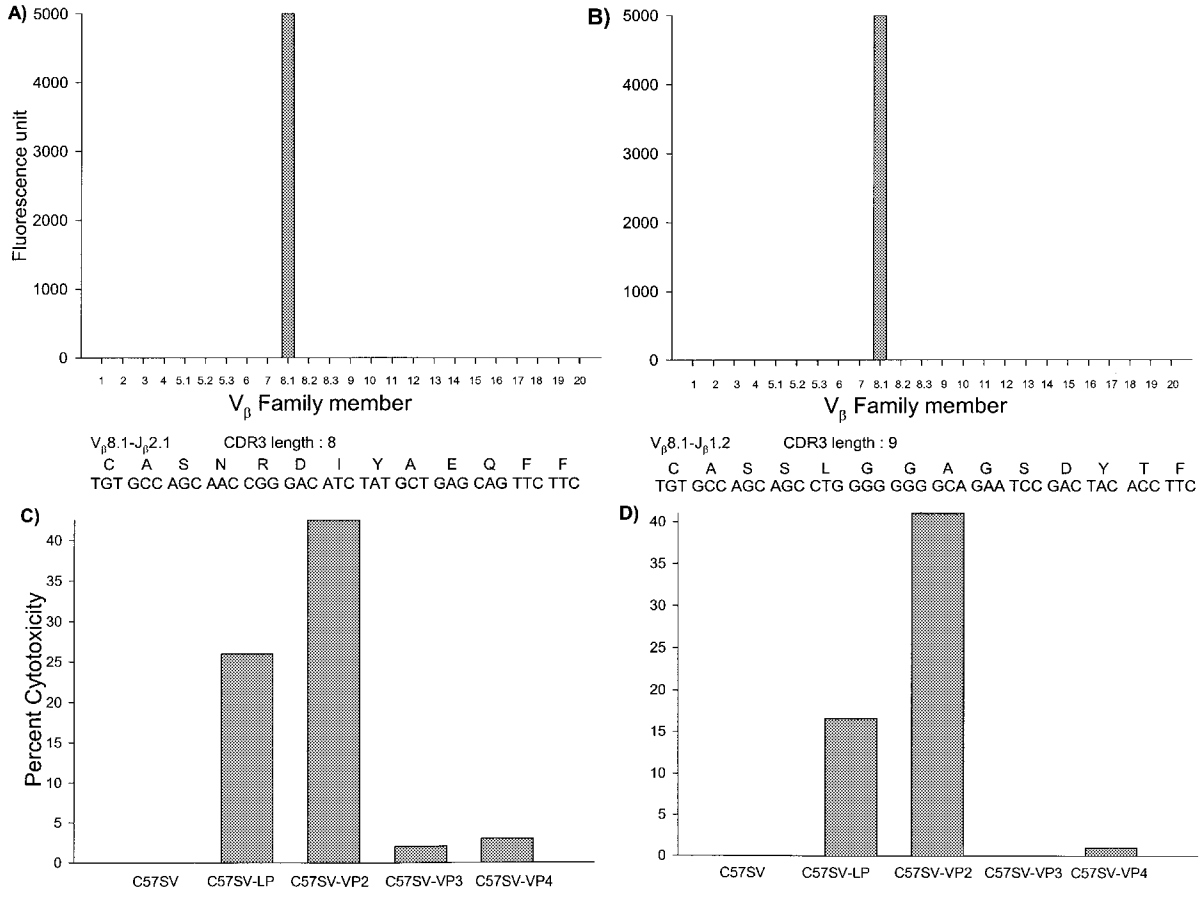

Fig. 5. Spectratype analysis of two VP2-stimulated CTL clones from H-2^b mice. Only $V_\beta 8.1^+$ TCR were identified (A and B). Total RNA was isolated and reverse transcribed using a C_β region-specific primer. Sequential PCR reactions were then performed using V_β-specific primers and a nested C_β primer, followed by primer extension with a 6-carboxyfluorescein-labeled nested C_β primer. Products were run on a polyacrylamide denaturing gel and analyzed as described in Methods. Cytotoxicity assays using the clones in (A) and (B) as effector cells demonstrate that these clones are VP2-specific as they only recognize and kill C57SV target cells which are transfected and express the immunodominant peptide contained in the VP2 capsid protein (C and D respectively). No CTL epitopes have been mapped to the VP3 or VP4 capsid proteins. LP transfectants express the entire left-hand side of the TMEV genome which contains VP2, VP3 and VP4. No cytotoxicity was detected in the untransfected C57SV cells.

ethanol series (100, 95, 90, 75% and PBS) prior to staining with a polyclonal rabbit anti-TMEV sera. The avidin–biotin immunoperoxidase technique was used (Vector, Burlingame, CA), as described previously (31). Slides were developed with Hanker Yates reagent (Polysciences, Warrington, PA), using hydrogen peroxide as the substrate. Slides were lightly counterstained with Mayer's hematoxylin.

Cytotoxic T lymphocyte (CTL) assay

C57SV cells transfected with the genes encoding the capsid proteins VP2, VP3 or VP4 were used as targets as previously described (30). In addition, cells expressing all three capsid proteins (LP) were used. Untransfected C57SV cells were used as a negative control. On the day of assay, target cells were labeled with 200 μCi of ^{51}Cr-labeled sodium chromate (Amersham Life Sciences, Arlington Heights, IL), washed with RPMI and resuspended to 2×10^4/ml in RPMI with 5% FCS. The target cell suspensions (100 μl) were placed in 96-well round-bottom microtiter plates (Nalgene Nunc International, Naperville, IL). CNS-IL from Theiler's virus-infected mice (day 7 post-infection) were used as effector cells in this assay. The CNS-IL were resuspended to 2×10^6/ml in RPMI with 5% FCS and 2-fold serial dilutions were made to provide E:T ratios of 100:1 to 6.25:1. The effector cell suspensions (100 μl) were added in triplicate to the targets, resulting in final E:T ratios of 100:1, 50:1, 25:1, 12.5:1 and 6.25:1. Six wells of targets also received medium alone or 10% Triton X-100 (Sigma) to determine spontaneous release and maximum release of chromium from targets respectively. Plates were incubated for 5 h at 37°C in 5% CO_2. Supernatants were harvested with Skatron supernatant collection system (Skatron, Sterling, VA) and assayed in a γ-counter (Gamma 5500; Beckman Instruments, Irvine, CA) for the amount of radioactivity. Mean values were calculated from triplicate wells and results were expressed as percent specific lysis according to the formula [(experimental c.p.m. – spontaneous c.p.m.)/(maximum c.p.m. – spontaneous c.p.m.)]×100%.

Statistics

Data was analyzed using either the Student's *t*-test for normally distributed data or the Mann–Whitney rank-sum test for data that were not normally distributed.

Results

Spectratype analysis of CNS-IL from B10.Q mice demonstrates an over-representation of $V_\beta 8.1$ and $V_\beta 8.2$ TCR genes at day 7 post-infection with TMEV

Genetic segregation analysis has suggested that mice with large deletions in the V_β complex have increased levels of demyelination compared to mice with intact V_β genes (18,22). Previous work from our laboratory attempted to identify specific V_β genes involved in TMEV-induced pathology using RT-PCR analysis of the CNS-IL. While no specific TCR V_β genes were determined to be prominent, a caveat to the study was the relatively non-quantitative nature of the assay (32). More recently the technique of spectratyping was developed to more precisely identify V_β gene transcripts present in defined anatomical sites. This technique involves cDNA synthesis with a C_β gene primer, PCR amplification with specific V_β gene and C_β gene primers, followed by electrophoresis and subsequent identification. Spectratyping has been utilized successfully in identifying V_β gene usage in tumors (33,34), graft rejection (29,35) and TMEV (36). In this study, susceptible B10.Q mice were i.c. infected with Theiler's virus (Daniel's strain). At 7 days post-infection mice were sacrificed and CNS-IL were isolated (30). Total RNA was extracted from the CNS-IL, followed by cDNA synthesis and PCR amplification of V_β genes using specific primers. Spectratype analysis revealed restricted diversity in the number of V_β genes expressed by the CNS-IL of the B10.Q mice and a bias toward $V_\beta 8$ gene usage in three of four of the mice examined with preferential expression of the $V_\beta 8.1$ and $V_\beta 8.2$ genes (Fig. 1). There was reduced V_β gene diversity in the remaining mouse, but the restriction towards $V_\beta 8$ gene usage was not as striking as in the other mice. To determine whether $V_\beta 8^+$ T cells infiltrate the lesions of mice susceptible to TMEV-induced demyelinating disease, immunohistochemical staining was performed on spinal cord sections of mice showing virus persistence. Lesions were examined at day 45 post-infection, a time when well-formed infiltrated lesions are apparent in the white matter of the spinal cord. Abundant $V_\beta 8.1$, 2^+ cells were observed in the brains (Fig. 2A) and spinal cord lesions (Fig. 2B) of TMEV-infected B10.Q mice. No $V_\beta 8.1$, 2^+ cells were found in the CNS of non-infected B10.Q mice.

A $V_\beta 8.2$ transgene exacerbates the extent of demyelination in the spinal cords of Theiler's virus-infected H-2^b mice

Previous studies utilizing mice bearing the $V_\beta 8.2$ TCR transgene directed against ovalbumin have demonstrated an altered pattern of susceptibility to various pathological conditions, including collagen-induced arthritis (37), experimental autoimmune thyroiditis (38) and graft versus host disease (39). Prior data have demonstrated that >92% of the $CD8^+$ T cells and 77% of $CD4^+$ T cells express the $V_\beta 8.2$ TCR in these transgenic mice, while only 14 and 9% of the $CD8^+$ and $CD4^+$ T cells express $V_\beta 8.2$ in non-transgenic mice (37). To determine the effect of the $V_\beta 8.2$ TCR in our model system, $V_\beta 8.2$ transgenic mice on a background resistant to TMEV (*H-*2^b) were infected i.c. with Theiler's virus. In addition, $V_\beta 8.2$ transgene-positive mice were also backcrossed to B10.Q mice (background susceptible to demyelination) and screened by flow cytometry for both *H-*2^q and the transgene. Mice homozygous for both *q* and the transgene were subsequently infected with TMEV. At 45 days post-infection, spinal cords were removed, embedded in glycol methacrylate, and examined for the presence or absence of gray matter disease, meningeal inflammation and demyelination. Transgene-positive B10.Q mice had increased levels of demyelination compared with the B10.Q transgene-negative littermate controls (Fig. 3A and B). The increased demyelination levels approached significance ($P = 0.056$; Table 1). In mice on a background resistant to demyelination, expression of the $V_\beta 8.2$ TCR transgene resulted in statistically significant increased levels of demyelination compared to the non-transgenic control mice ($P < 0.003$; Table 1; Fig. 3C and D respectively). The extent of demyelination in transgene

positive C57BL/6 was similar to the extent of demyelination in transgene negative normally susceptible B10.Q mice.

Depletion of $V_\beta 8^+$ T cells enhances TMEV-induced demyelination in mice of resistant genotype

In experimental autoimmune encephalomyelitis (EAE), the autoimmune model of MS, $V_\beta 8.2^+$ T cells have been demonstrated to be encephalitogenic. Furthermore, EAE cannot be induced in rats depleted of $V_\beta 8.2^+$ T cells (40). To determine whether $V_\beta 8^+$ T cells participate in protection or pathogenesis in TMEV infection, mice susceptible (B10.Q) or resistant (C57BL/6J) to TMEV-induced demyelination were i.p. injected with either the F23.1 antibody (specific for $V_\beta 8^+$ T cells) or a control IgG. One day later mice were i.c. injected with TMEV. At day 3 post-infection, depletion was confirmed by flow cytometric analysis of peripheral blood lymphocytes. Depletion was considered successful if $<1\%$ of the T cells were positive when stained with the F23.1 antibody (Fig. 4A and B). Flow cytometry of the peripheral blood lymphocytes was also performed at the end of the experiment to ensure the effectiveness of depletion. Only those mice with $>99\%$ of the T cells negative for $V_\beta 8$ were involved in the analysis. Depletion of $F23.1^+$ cells resulted in an increased, but not statistically significant level of demyelination in susceptible B10.Q mice at day 45 post-infection (Table 2). However, depletion of $V_\beta 8^+$ T cells from resistant C57BL/6 mice resulted in statistically significant increased levels of demyelination (Table 2 and Fig. 4D) compared with control-treated mice (Fig. 4C). All of the mice expressing a resistant haplotype converted to the demyelinating phenotype following depletion of $V_\beta 8^+$ T cells. The extent of demyelination in resistant mice depleted of $V_\beta 8.2$ was similar to that observed in normal susceptible B10.Q mice. There was a 15-fold increase of demyelinating pathology in *H-2^b* mice depleted of $V_\beta 8.2^+$ T cells as compared to non-depleted infected mice. In addition, TMEV antigens were detected in the white matter of infected C57BL/6 mice depleted of $V_\beta 8^+$ T cells (Fig. 4F), while no TMEV antigens were detected in control-treated infected C57BL/6 mice (Fig. 4E). Together, these data indicate that $V_\beta 8^+$ T cells play an integral role in protecting mice on a resistant background from virus-induced demyelination. Because the extent of gray matter disease was not affected by anti-$V_\beta 8$ antibody treatment, and because the mice did not succumb to encephalitis and death during the acute disease, this indicates that cells other than $V_\beta 8^+$ T cells contribute to protection from acute encephalitis.

TMEV-specific CTL cell clones are $V_\beta 8.1^+$

Previous data from our laboratory and others have demonstrated that cytotoxic T cell responses to TMEV are critical to resistance to demyelination. In addition, *H-2^b* CTL are directed against an immunodominant $VP2_{121-130}$ peptide in the context of H-2D^b (41,42). This is the only CTL epitope that has been identified in *H-2^b* mice following TMEV infection. One hypothesis regarding the critical role of $V_\beta 8^+$ T cells in *H-2^b* mice is that these T cells recognize the $VP2_{121-130}$ peptide. To test this hypothesis, CNS-IL were isolated from C57BL/6 mice at day 7 post-infection and re-stimulated *in vitro* with VP2-transfected fibroblasts. Spectratype analysis of two individual VP2-specific CTL clones revealed exclusive $V_\beta 8.1$ usage (Fig. 5A–D). These data would suggest that the $V_\beta 8.1$ TCR is critical to the development of the protective CTL response observed in *H-2^b* mice.

Discussion

The concept that a particular V_β gene may be associated with susceptibility or resistance to disease has been well documented (37,39,43,44) The correlation between $V_\beta 8$ family members and altered susceptibility to disease has been documented in autoimmune disease [i.e. EAE (40,45)and influenza (44)]. This has particular relevance to potential therapeutic strategies for human disease, since depletion of a particular V_β T cell population may hold promise for clinical improvement.

Despite the generation of a diverse T cell response in the CNS of TMEV-infected mice, predominant expression of $V_\beta 8.1^+$ and $V_\beta 8.2^+$ T cells was detected using spectratyping in susceptible B10.Q mice during the acute phase of the disease. This finding is significant, as previous studies in the autoimmune model of MS (EAE) have demonstrated that $V_\beta 8^+$ T cells are the predominant T cell population responsible for disease induction. *In vivo* depletion of $V_\beta 8.2^+$ T cells prevents EAE in otherwise susceptible animals (40). In addition, $V_\beta 8.2^+$ T cells have been shown to be pathogenic in experimental autoimmune uveitis (47), experimental autoimmune neuritis (48) and experimental autoimmune thyroiditis (38). Therefore, it is a reasonable hypothesis that $V_\beta 8^+$ T cell populations may contribute to the pathogenesis of virus-induced demyelination in susceptible strains of mice. In resistant mice TCR analysis of VP2-specific cytotoxic T cell clones identified only a single TCR, $V_\beta 8.1$, suggesting that limited T cell diversity is important for TMEV clearance.

To analyze the influence of a skewed TCR repertoire in the TMEV model, we infected mice expressing the $V_\beta 8.2$ TCR transgene with Theiler's virus. Because of the skewed T cell repertoire, it was postulated that mice of a resistant background would not clear early virus infection and would develop persistence. It was unknown however was whether the mice would succumb to acute neuronal infection as observed in *Scid* mice (49) or whether they would survive the acute disease to develop demyelination. Both *H-2^b* and *H-2^q* transgene-positive mice experienced demyelination following infection with TMEV, but only the *H-2^b* $V_\beta 8.2$ TCR transgenic mice experienced significantly more demyelination than their non-transgenic littermate controls. One interpretation of these results is that $V_\beta 8.2^+$ T cells, if not regulated by other T cell populations, may participate in the generation of TMEV-induced demyelination if virus is allowed to persist in the CNS. However, because only 77% of the $CD4^+$ cells express the transgene, it is more likely that demyelination is the result of an insufficient number of T cells directed against viral peptide of a non-$V_\beta 8.2$ specificity. This situation would be analogous to experiments from our lab demonstrating that adoptive transfer of an insufficient number of splenocytes into a *Scid* mouse (on a background resistant to demyelination) results in demyelination and virus persistence instead of protection from disease (20). An additional interpretation of these results is that epitope spreading is enhanced in the $V_\beta 8.2$ transgene-positive mice. Studies by Miller *et al*.

have demonstrated that by day 52 post-infection TMEV-infected mice develop a vigorous immune response against $PLP_{139–151}$ that is partially responsible for the chronic demyelination observed in this model (53). Autoreactive T cells to this epitope have been demonstrated in these $V_\beta 8.2^+$ mice in the EAE model of demyelination (54).

To better study the influence of this TCR on disease progression in the TMEV model of demyelinating disease, we performed *in vivo* depletion studies in strains susceptible (B10.Q) and resistant (C57BL/6) to Theiler's virus-induced demyelination. The mice depleted of $V_\beta 8^+$ T cells did not succumb to early encephalitis observed in *Scid* (49), $Rag^{-/-}$ (21) or MHC class I/class II double knockout mice (50). Therefore, the remaining T cell, B cell, NK cell and macrophage populations are sufficient to protect against the neuronal disease. Normally resistant mice experienced significant levels of demyelination following depletion of $V_\beta 8^+$ T cells. The levels of disease present in the mice on a background resistant to TMEV-induced demyelination following depletion were similar to the levels seen in undepleted susceptible strains of mice. These data suggest that in the TMEV model of MS $V_\beta 8^+$ T cells are important for ameliorating demyelinating disease, as opposed to being pathogenic as has been proposed for EAE (40).

The results reported here contrast with studies by Mussette *et al*. using TMEV-infected SJL/J mice (36). In their study, no selective expansion of V_β populations was observed, despite similar technical approaches. These disparate results could be related to subtle differences in the virus inoculum, the time-points after infection examined or the method of RT-PCR. In the study by Mussette, later times (days 21 and 45) after infection were examined in SJL/J mice (36) in contrast to the 7 day time-point in our experiments. It may be that there is an increase in non-specific lymphocytes infiltrating demyelinated lesions at later times after infection, such that the predominance of $V_\beta 8$ is not apparent. However, the most likely explanation for the differences observed between the two studies are the differences in the backgrounds of the mice. Almost all that is known regarding the immunogenetics of TMEV-induced disease is based on the C57BL background which was used for our experiments (3,15–17,25,51,52). Minimal studies have been performed on the genetics of demyelinating disease in SJL/J mice.

In the *in vivo* depletion studies presented here, data suggests that $V_\beta 8^+$ T cells are important in protecting resistant strains of mice from demyelination. In contrast $V_\beta 8.2^+$ cells may be critical for induction of autoimmune-mediated demyelination. These results illustrate the potentially complex interactions that must be considered when evaluating the potential of TCR-based strategies for the treatment of human disease when the inciting antigen is unknown.

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Abbreviations

EAE	experimental autoimmune encephalomyelitis
CNS-IL	CNS-infiltrating lymphocytes
CTL	cytotoxic T lymphocyte
i.c.	intracerebral infection
MS	multiple sclerosis
TMEV	Theiler's murine encephalomyelitis virus;

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