



Viruses can silently prime for and trigger central nervous system autoimmune disease

Diethilde J Theil,¹ Ikuo Tsunoda,¹ Fernando Rodriguez,² J Lindsay Whitton,² and Robert S Fujinami¹

¹Department of Neurology, University of Utah School of Medicine, Salt Lake City, Utah, USA; ²Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California, USA

Although many viruses have been isolated from patients with multiple sclerosis (MS), as yet, no one agent has been demonstrated to cause MS. In contrast, epidemiological data indicate that viral infections are associated with exacerbations of MS. Here, we present data showing that virus infections can subclinically prime animals for central nervous system (CNS) autoimmune disease; long after the original infection has been eradicated, a nonspecific challenge/infection can trigger an exacerbation. The priming infectious agent must show molecular mimicry with self-CNS antigens such as glial fibrillary acidic protein (GFAP), myelin associated glycoprotein (MAG) or myelin proteolipid protein (PLP). The subsequent challenge, however, may be nonspecific; complete Freund's adjuvant (CFA), or infection with a recombinant vaccinia virus encoding an irrelevant protein, could trigger CNS disease. In the CNS, we could detect a mononuclear cell infiltration, but no demyelination was found. However, if the pathogenesis of MS is similar to that of this novel animal model for CNS autoimmune disease, our findings could help explain why exacerbations of MS are often associated with a variety of different viral infections. *Journal of NeuroVirology* (2001) 7, 220–227.

Keywords: viruses; autoimmunity; autoimmune diseases; virus diseases; experimental allergic encephalomyelitis; DNA immunization; demyelinating diseases

Introduction

Multiple sclerosis (MS), thought to be an autoimmune disease of the central nervous system (CNS), affects approximately 1/1000 individuals in the United States (Anderson *et al*, 1992). Despite extensive investigation, the etiology and pathogenesis of multiple sclerosis (MS) are still not known. However, many features important for MS have begun to be elucidated. These include: genetics, environmental factors, and infections playing important roles in susceptibility and triggering or exacerbating the disease.

Individuals of Northern European descent have a higher incidence of MS for at least two reasons. First, genetic susceptibility; these people have a higher prevalence of certain histocompatibility anti-

gens (HLA) – HLA3, B7, and especially DR15, Dw2, and –DQw6 (Weinshenker *et al*, 1998). Second, exposure to an unidentified environmental factor at a young age (Martin, 1997; Kalman and Lublin, 1999); migration studies performed by Dean and Kurtzke (Dean and Kurtzke, 1971) and Alter (Alter *et al*, 1971) have shown that individuals living in a high-risk area who migrate to a low-risk area after the age of 15 retain the high risk for developing MS associated with their original geographic location. In contrast, individuals who migrate prior to the age of 15 acquire the risk of the geographical region into which they have moved. These data have been interpreted to indicate that the types of infections in areas of high risk for MS (Kurtzke, 1993) as well as possibly in low-risk regions for MS affect the likelihood of developing disease (Alter *et al*, 1986).

From studies over the past 50 years, no 1 virus or microbe has been identified as a causative agent for MS. However, several reports indicate that viral or bacterial infections can exacerbate MS (Sibley *et al*, 1985; Edwards *et al*, 1998; James, 1988; Andersen *et al*, 1993; Morrison and Nelson, 1994;

Address correspondence to Robert S. Fujinami, Department of Neurology, University of Utah School of Medicine, 30 N 1900 East, RM 3R330, Salt Lake City, UT 84132, USA. E-mail: Robert.Fujinami@hsc.utah.edu

Received 11 December 2000; revised 29 January 2001; accepted 5 March 2001.

Panitch, 1994; Rapp *et al*, 1995; Metz *et al*, 1998). In this study, we provide evidence that certain kinds of infection can prime animals for autoimmune CNS disease, and at a later time infection or other non-specific immunologic stimuli can trigger an attack. We based our model on SJL/J mice, which are highly susceptible to experimental allergic encephalomyelitis (EAE), an animal model for MS (Livingstone *et al*, 1995; Encinas *et al*, 1996; Butterfield *et al*, 1998; Butterfield *et al*, 1999; Charles *et al*, 1999; Teuscher *et al*, 1999). Because the "priming" event for MS takes place before 15 years of age, we primed our mice at 3–4 weeks of age (just prior to puberty); furthermore, because the incidence of MS is higher in women (Smith *et al*, 1999), we used only female mice (Baker *et al*, 1995).

Although, as in many other possible autoimmune diseases, the autoantigens responsible for MS have not been identified, several candidate antigens have been proposed. We selected 3 candidates as potential autoantigens: glial fibrillary acidic protein (GFAP), myelin associated glycoprotein (MAG), and myelin proteolipid protein (PLP) (Yoshimura *et al*, 1985; Sobel *et al*, 1986). Adoptive transfer of T cells specific for GFAP and MAG has been reported to cause CNS disease (Berger *et al*, 1997; Weerth *et al*, 1999), whereas PLP is widely used for induction of EAE in animals (Tsunoda and Fujinami, 1996), and PLP reactive cells have been found in increased numbers in MS patients (Trotter *et al*, 1998; Illés *et al*, 1999). In addition, we previously proposed that molecular mimicry was a viable mechanism for the development of autoimmune CNS disease (Fujinami and Oldstone, 1985). Here, we hypothesized that infection of young females by viruses or microbes having immunologically cross-reactive epitopes could prime for autoimmune disease, and that overt disease would be revealed following a subsequent non-specific immunostimulation. To mirror a viral infection having molecular mimicry, we employed a cDNA encoding PLP fused to ubiquitin (pUPLP); ubiquitin has been demonstrated to target proteins into proteasomes, allowing degradation and presentation mostly through the major histocompatibility complex (MHC) class I pathway, which in turn enhances the induction of CD8⁺ T cells (Rodriguez *et al*, 1998). This scenario would approximate what could occur during virus infection having molecular mimicry, when viral proteins are synthesized within the cytoplasm of the infected cell. As an alternative priming method, we used recombinant vaccinia virus encoding CNS proteins.

Materials and Methods

Mice and induction of disease

Three- to 4-week-old female SJL/J and PL/J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were injected intramuscularly (i.m.)

3 times at 1-week intervals with 100 μ g plasmid, and DNA was dissolved in 1 N saline. On each occasion, 50 μ g was injected into each anterior tibial muscle with a 28-gauge needle. Mice were observed and weighed every second day. One week after the last injection, groups of mice received either (i) PBS emulsified in complete Freund's adjuvant (CFA), (ii) 100 nmol of modified PLP_{139–151} (HSLGKWLGH-PDKF) emulsified in CFA (Barnett *et al*, 1993), (iii) PBS alone subcutaneously (s.c.), at the base of the tail or (iv) 5×10^6 PFU intraperitoneal (i.p.) of VV_{SC11}, a recombinant vaccinia virus encoding β -galactosidase (Barnett *et al*, 1993). Control mice received no DNA injection, and were "challenged" with CFA. Mice were observed and weighed daily.

In other experiments mice were primed with recombinant vaccinia viruses encoding PLP (VV_{PLP}) (Barnett *et al*, 1993), MAG (VV_{MAG}), or GFAP (VV_{GFAP}) [5×10^6 PFU i.p. of recombinant vaccinia virus], and 5 weeks later were challenged with CFA with or without *Bordetella pertussis* (BP). Recombinant viruses were constructed as described in Barnett *et al* (Barnett *et al*, 1993).

Clinical signs of EAE were assessed according to the following criteria: 0=no clinical disease; 1=loss of tail tonicity; 2=mild hind leg paresis; 3=moderate hind leg paralysis; 4=complete paraplegia; and 5=quadriplegia, moribund or death.

Plasmid constructs

The PLP gene was fused to ubiquitin-A76 and subcloned into the *Not* I site of plasmid pCMV, which was derived by excision of the β -galactosidase gene from pCMV β (Clontech, Palo Alto, CA) (Tsunoda *et al*, 1999). The resultant expression vector (pU-PLP) contained the widely expressed immediate-early promoter from cytomegalovirus. A plasmid encoding PLP without ubiquitin (pPLP_{ALL}) (Tsunoda *et al*, 1998) was used as a control. Plasmid DNA was purified from transformed DH5 α *Escherichia coli* with an EndoFree Plasmid Maxi Kit (Qiagen, Chatsworth, CA).

Proliferation assays

The regional lymph nodes and spleens from mice of each group were removed and pooled, and a single cell suspension was prepared. A volume of 0.2 ml containing 2×10^5 cells in RPMI supplemented with 1% glutamine, 1% penicillin/streptomycin, 5×10^{-5} M 2-mercaptoethanol, and 10% fetal bovine serum was added to each well and modified PLP_{139–151}, PLP_{178–191}, or MOG_{92–106} (Amor *et al*, 1994) peptides, which are encephalitogenic in SJL/J mice, were added at 50 μ g/ml. The cells were cultured 72 h, each well was pulsed with 1 μ Ci of tritium thymidine, and cells were cultured another 24 h. Cultures were harvested onto filters using a multiwell cell harvester and counted using standard liquid scintillation techniques. All assays were performed in triplicate.

Histology and immunohistochemistry

Histology and immunohistochemistry were performed as described in Tsunoda *et al* (Tsunoda *et al*, 2000). Slides were coded and read blind.

Results

In humans, infections prior to the age of 15 as well as genetic background are believed to contribute to MS. To investigate whether infections early in life in genetically susceptible individuals could prime for autoimmune disease, we inoculated pUPLP 3 times into young SJL/J mice, which are susceptible to PLP-induced EAE. pUPLP encodes ubiquitin in frame with PLP, leading to enhanced degradation through the proteasome pathway. Because intracellular virus infection leads to presentation of fragments of viral protein by MHC class I molecules (Whitton and Oldstone, 1996), our new model mimics infection with a virus that has molecular mimicry with CNS antigens, but that does not cause cytopathic effects in the host.

One week after the last pUPLP injection, the mice were challenged with a nonspecific stimulus (CFA), which has long been used as an adjuvant in many autoimmune disease models such as EAE (Morgan, 1946; Morgan, 1947; Kabat *et al*, 1946; Kabat *et al*, 1947; Freund *et al*, 1947; Kopeloff and Kopeloff, 1947; Morrison, 1947). Ten days after the CFA administration, all animals (5/5) that had been injected with pUPLP showed weight loss, hypomotility and very mild hind limb weakness (Figure 1). Two control groups of mice were followed in parallel.

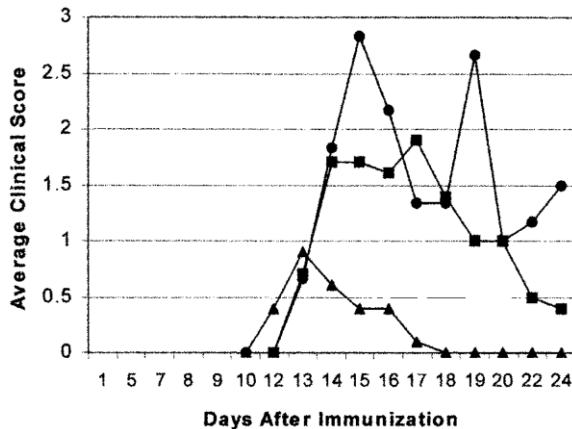


Figure 1 After pUPLP injection, mice were challenged either with CFA (\blacktriangle) or with PLP_{139–151}/CFA (\blacksquare) on day 0. Control EAE was induced in mice with PLP_{139–151}/CFA without pUPLP injection (\bullet). Data are mean clinical scores of 5 mice. Mice were followed for clinical signs of disease according to the following scale: 0 – no clinical disease; 0.5 – loss of tonicity of the distal half of the tail; 1 – complete loss of tail tonicity; 2 – mild hind leg paresis; 3 – moderate hind leg paralysis; and 4 – complete paraplegia. None of the negative control mice that received either pUPLP alone or CFA without pUPLP showed clinical signs.

Table 1 Neuropathology in mice primed with cDNA constructs, followed by CFA challenge^a

Plasmid ^b	CFA	Pathology/total mice ^c
pUPLP	+	4/20
pUPLP	–	0/10
–	+	0/10
pPLP _{ALL}	+	0/10

^aSJL/J mice were primed with cDNA encoding PLP 3 times followed by a challenge of CFA.

^bpUPLP encodes PLP fused with ubiquitin; pPLP_{ALL} encodes PLP alone (Tsunoda *et al*, 1998).

^cNumber of mice that have CNS lesions/total number of mice examined.

One group received PBS instead of the plasmid pUPLP and were sensitized with PLP_{139–151} emulsified in CFA (a stimulus known to induce EAE in SJL/J mice). The other group was injected with pUPLP and then subsequently injected with PLP_{139–151} also emulsified in CFA. As expected, animals in both control groups also developed clinical EAE (Figure 1). Clinical signs did not develop in any mice in 2 negative control groups; the first group had been primed with pUPLP but received no subsequent immunostimulatory challenge; and the second group received CFA alone, without prior priming.

An additional experiment was performed with 4 groups of mice: (i) 20 mice were primed 3 times with pUPLP and challenged with CFA, (ii) 10 mice were primed 3 times with pPLP_{ALL} (PLP construct not encoding ubiquitin) and challenged with CFA, (iii) 10 mice were primed with pUPLP, but no CFA challenge was carried out, and (iv) 10 mice were challenged with CFA, without prior priming (Table 1). A total of 20 mice injected with pUPLP and challenged with CFA were examined for pathologic changes in the CNS. In 4 of these mice, mononuclear cells (MNC) cell infiltrates were found in the leptomeninges (Figure 2a); the lymphocytes present in the perivascular space were identified as being mainly T cells when stained with an anti-CD3 antibody (Figure 2a insert). Although no demyelination was present, occasional areas in the white matter had an edematous appearance. This is similar to the pathology found in acute EAE, which often shows inflammation and edema, but no demyelination. In several models of acute EAE, edema with inflammation, but not demyelination, is believed to cause clinical signs (Levine *et al*, 1966; Simmons *et al*, 1982; Kerlero de Rosbo *et al*, 1985). No lesions or cellular infiltrates were found in mice (30 total) in any of the other 3 groups. The absence of clinical or histological signs in the second group of mice (pPLP_{ALL} priming, CFA challenge) suggests that the initial priming event requires effective MHC class I presentation of the mimicking antigen. MNC from mice injected with pUPLP and further challenged with PLP_{139–151} peptide, when restimulated *in vitro* with the same peptide, had a

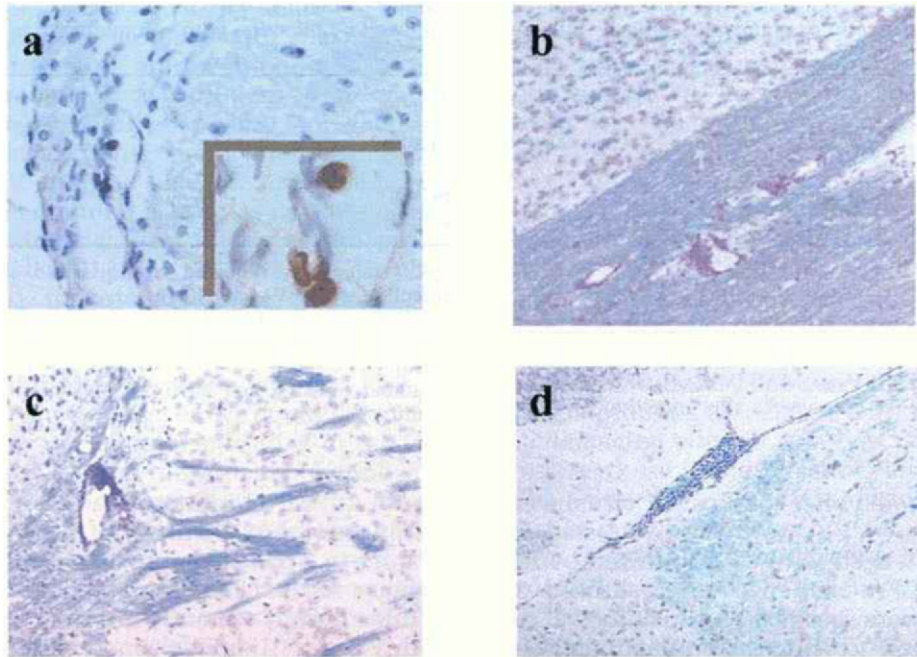


Figure 2 Histopathological changes in the CNS. **a)** Brain section from a mouse primed with pUPLP and challenged with CFA. Mild infiltration of the leptomeninges. CD3⁺ T cells around the vessel (insert). Immunohistochemistry was performed using anti-CD3 antibody. **b)** Spinal cord section from a mouse infected with VV_{MAG} and immunized with CFA. Perivascular cuffing in the white matter of the lumbar spinal cord. **c)** Brain section from a mouse infected with VV_{GFAP} and challenged with CFA. Perivascular cuff in the white matter. **d)** Brain section from a mouse primed with pUPLP and infected with VV_{SC11}. MNC infiltration in the leptomeninges. **b–d)** Luxol fast blue staining that stains myelin.

lower proliferative response compared to the MNC from EAE mice. The proliferative responses of MNC to PLP, from mice vaccinated with pUPLP and challenged with CFA, were higher than the ones seen in the vaccinated group challenged with CFA/PLP. Lymphocytes from mice vaccinated with pUPLP readily

proliferated in response to PLP_{139–151} on days 12 (Figure 3) and 24 post-CFA challenge (not shown).

We next determined whether pUPLP priming could be replaced by priming with a virus having molecular mimicry with self-CNS antigens (Table 2). The 3 recombinant vaccinia viruses used are described in

Lymphoproliferation Assay (SI)

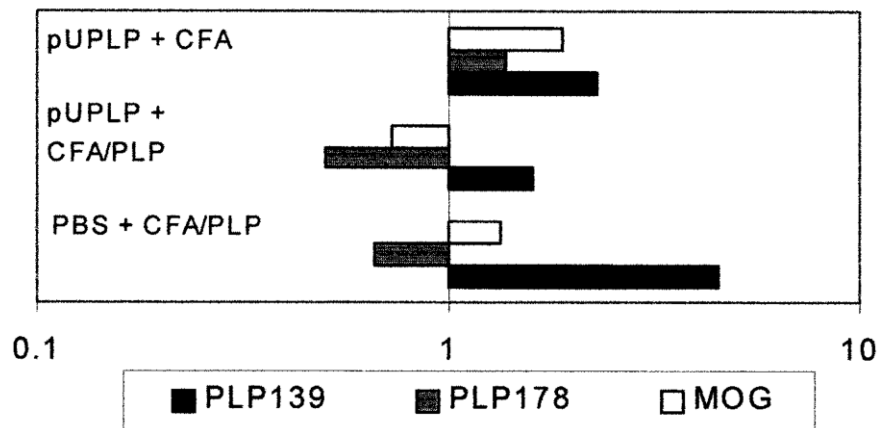


Figure 3 Stimulation indices (SI) of lymphocytes from mice primed with pUPLP and challenged with PLP_{139–151}/CFA or CFA or mice with EAE (PBS + CFA/PLP). Pooled lymphocytes were obtained from 2 mice in each group at day 12 after challenge. Lymphocytes (5×10^5 cells/well) were incubated with 10 nmol/ml of different encephalitogenic peptides (PLP_{139–151}, PLP_{178–191}, and MOG_{92–106}). Lymphocytes from the mice vaccinated with pUPLP and immunized with CFA proliferated to all encephalitogenic peptides.

Table 2 Neuropathology in mice infected with recombinant vaccinia virus encoding myelin or nonmyelin antigens followed by CFA challenge

Virus	Challenge	Mouse Strain	Pathology/Mice	
			Exp. I	Exp. II
VV _{PLP}	CFA	SJL/J	5/5	5/5
VV _{MAG}	CFA	SJL/J	5/5	2/5
VV _{GFAP}	CFA	SJL/J	5/5	4/5
VV _{SC11}	CFA	SJL/J	0/9	0/5
VV _{PLP}	CFA	PL/J		0/4
VV _{GFAP}	CFA	PL/J		0/5

SJL/J mice or PL/J mice were infected with recombinant vaccinia virus encoding either PLP (VV_{PLP}), MAG (VV_{MAG}), GFAP (VV_{GFAP}), or β -galactosidase (VV_{SC11}). Five weeks after infection, CFA was injected with or without *BP*.

Materials and Methods. Mice (5 mice per virus) were infected with VV_{PLP}, VV_{MAG}, or VV_{GFAP}, rested for 5 weeks, then challenged with CFA followed by *BP*. At 1 month post-CFA challenge, mice showed no clinical signs and were sacrificed for histological examination. The majority of mice had inflammatory lesions in the brain or spinal cord (Table 2). As shown in Figures 2b and 2c, MNC infiltrates were evident within the white matter and meninges. When these regions were stained with an anti-CD3 antibody, most of the inflammatory infiltrating cells were identified as T cells. As a negative control, 9 mice were primed with VV_{SC11}, a recombinant vaccinia virus that encodes β -galactosidase but no CNS proteins. β -galactosidase has no known molecular mimicry with PLP or CNS antigens. Following the CFA challenge, no inflammatory changes were seen in the CNS of VV_{SC11} infected mice. Thus, this second experiment showed the encephalitogenicity of VV_{PLP}, VV_{MAG}, and VV_{GFAP} (Table 2).

To demonstrate the genetic influence on the priming event, we infected PL/J mice with two highly encephalitogenic recombinant vaccinia viruses, VV_{PLP} and VV_{GFAP}, and later challenged them with CFA; none of 10 PL/J mice primed with recombinant viruses showed either clinical signs or pathological changes in the CNS. Therefore, genetic background seems to play an important role in this disease model, as in EAE and MS. These data indicate that infections having molecular mimicry can subclinically prime animals for autoimmune CNS disease and at a later time a nonspecific infection or challenge could initiate overt disease.

To determine whether a nonspecific virus infection of primed animals would produce the same outcome as CFA challenge, the following experiment was performed. Five mice were primed 3 times with pUPLP, rested for 1 week, then infected with VV_{SC11}. Mice were followed for 2 weeks and then sacrificed. No mice showed clinical signs during the 2-week observation period. Brain and spinal cords were removed and examined for pathologic changes. As shown in

Table 3 Neuropathology in mice primed with cDNA constructs, followed by infection with recombinant vaccinia virus encoding β -galactosidase^a

Plasmid ^b	Challenge	Pathology/total mice ^c
pUPLP	VV _{SC11}	1/5
—	VV _{SC11}	0/5
pCMV β	VV _{SC11}	0/5
pPLP _{ALL}	VV _{SC11}	0/5

^aSJL/J mice were injected with plasmid 3 times, followed by infection with VV_{SC11}.

^bpUPLP encodes PLP fused with ubiquitin; pCMV β encodes β -galactosidase, and pPLP_{ALL} encodes PLP alone (Tsunoda *et al*, 1998).

^cNumber of mice that have CNS lesions/total number of mice examined.

Figure 2d, MNC infiltrates were evident within the meninges of the CNS of one of these mice. None of the 15 mice in 3 control groups showed any pathological changes in the CNS (Table 3). These control groups (5 mice/group) were: (i) unprimed mice challenged with VV_{SC11}, (ii) mice primed with pCMV β followed by VV_{SC11} challenge, and (iii) mice primed with pPLP_{ALL} and challenged with VV_{SC11}. Therefore, in mice primed with pUPLP, subsequent challenge with either VV_{SC11} (Table 3) or CFA (Table 1) induced CNS inflammation in 20% of mice. However, because non-specific (CFA) challenge induced autoimmune CNS disease in approximately 90% of mice which had been primed with vaccinia viruses encoding self-CNS antigens (Table 2), the consequences of molecular mimicry appear to be more serious following live virus priming than after priming with plasmid DNA.

Discussion

Our study indicates that, following an initial early priming event by an antigen that can mimic self-epitopes, subsequent immunostimulation (for example, during a nonspecific virus infection) could trigger inflammatory changes within the CNS, or overt disease. Importantly, whereas the original event must prime for an autoimmune response against a self-antigen, the subsequent virus does not need to have molecular mimicry with the target self-CNS protein, nor need it directly infect the CNS. This model mirrors the epidemiologic data supporting MS as being a disease relating to environment, genetics, and age.

Our data are compatible with those of others (reviewed in Goverman, 1999) where transgenic mice were made only expressing the T-cell receptor (TCR) specific for an encephalitogenic peptide of myelin basic protein. When raised under normal conditions, these mice had a high incidence of EAE, but under specific pathogen-free conditions, the mice had a much lower incidence of spontaneous EAE.

In our model, we found that sensitization of young genetically susceptible animals with plasmids encoding self-CNS proteins followed by a nonspecific immunologic challenge was sufficient to induce inflammatory changes within the CNS. Our data suggest that the first encounter with microbes can set the stage for autoimmune disease. In addition, the MHC class I presentation pathway is important. Previously, we reported significant lymphoproliferative responses against PLP_{139–151} following CFA challenge of pPLP_{ALL} inoculated mice (Tsunoda *et al*, 1998). In contrast, control mice that were injected with pCMV β with or without CFA challenge showed no lymphoproliferative response against PLP. In pPLP_{ALL}-primed mice, however, we could detect neither clinical signs nor histologic lesions in the CNS (Tsunoda *et al*, 1998), and these data are confirmed in the present report. In contrast, mice primed with pUPLP, which enhances MHC class I presentation, show clinical signs after CFA challenge, suggesting that ubiquitination or processing and presentation through MHC class I is an important contribution, and perhaps a necessary component.

In our study, we could prime for autoimmune disease not only with a cDNA or a virus encoding the highly encephalitogenic myelin antigen, PLP, but also with the less encephalitogenic myelin antigen, MAG, and a nonmyelin antigen, GFAP. This is the first report that demonstrates MAG and GFAP as potential CNS antigens in mice. Previously, Weerth *et al* demonstrated that a mild inflammatory disease could be induced in Lewis rats after adoptive transfer of MAG specific T cells. However, sensitization with MAG peptides failed to induce acute EAE (Weerth *et al*, 1999). Berger *et al* showed that adoptive transfer of GFAP-specific T cell lines could cause CNS inflammation in Lewis rats, while ovalbumin or purified protein derivative (PPD) specific T cells could not (Berger *et al*, 1997). Our results are in accord with these previous reports; CNS inflammation was induced with recombinant vaccinia virus encoding CNS antigens, VV_{PLP}, VV_{MAG}, and VV_{GFAP}, but not

with VV_{SC11}, a vaccinia virus encoding a non-CNS protein, β -galactosidase.

We propose that a virus having molecular mimicry with a self-CNS antigen may be involved in some of the early priming events occurring in young genetically susceptible MS individuals. The first agent need not cause obvious autoimmunity or disease; instead, these are provoked by a subsequent non-specific challenge. Potentially deleterious priming events were induced not only by recombinant vaccinia viruses encoding proteins found in oligodendrocytes, but also by a virus encoding the astrocytic protein GFAP. Therefore, autoimmunity induced by molecular mimicry can cause harm not only when it affects proteins associated with myelin; it may also be damaging when it involves neighboring proteins such as those found in astrocytes. In addition, using VV_{PLP} and VV_{GFAP}, we could induce CNS inflammation in most SJL/J mice, but not in PL/J mice. Therefore, genetic background seems to play an important role in the pathogenesis of this model similar to that in "classical" EAE and MS.

Our data would explain why no single virus has been demonstrated to have a cause-and-effect relationship with MS. We would also predict that there is a window of susceptibility where animals can be primed and rendered susceptible to subsequent challenge. Outside this window, individuals would be refractory to disease induction. This could help explain why MS is seen mostly in individuals between the age of 20 and 40 and then the incidence decreases.

Acknowledgements

The authors would like to thank Jane E Libbey, MS, Loren S Jack, Jenny Zhang, and Stephanie Harkins for excellent technical assistance. We are grateful to Ms Kathleen Borick for her help in preparation of the manuscript. This work was supported by NIH AI42525 (RSF) and by NIH AI37186 (JLW).

References

- Alter M, Okihiro M, Rowley W, Morris T (1971). Multiple sclerosis among Orientals and Caucasians in Hawaii. *Neurology* **21**: 122–130.
- Alter M, Zhang ZX, Davanipour Z, Sobel E, Zibulewski J, Schwartz G, Friday G (1986). Multiple sclerosis and childhood infections. *Neurology* **36**: 1386–1389.
- Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu J-M, Baker D (1994). Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol* **153**: 4349–4356.
- Andersen O, Lygner P-E, Bergström T, Andersson M, Vahlne A (1993). Viral infections trigger multiple sclerosis relapses: a prospective seroepidemiological study. *J Neurol* **240**: 417–422.
- Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH (1992). Revised estimate of the prevalence of multiple sclerosis in the United States. *Ann Neurol* **31**: 333–336.
- Baker D, Rosenwasser OA, O'Neill JK, Turk JL (1995). Genetic analysis of experimental allergic encephalomyelitis in mice. *J Immunol* **155**: 4046–4051.
- Barnett LA, Whitton JL, Wada Y, Fujinami RS (1993). Enhancement of autoimmune disease using recombinant vaccinia virus encoding myelin proteolipid protein [published erratum appears in *J Neuroimmunol* 1993;**48**:120]. *J Neuroimmunol* **44**: 15–25.

- Berger T, Weerth S, Kojima K, Linington C, Wekerle H, Lassmann H (1997). Experimental autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. *Lab Invest* **76**: 355–364.
- Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Sudweeks J, Rose J, Teuscher C (1999). Genetic analysis of disease subtypes and sexual dimorphisms in mouse experimental allergic encephalomyelitis (EAE): Relapsing/remitting and monophasic remitting/nonrelapsing EAE are immunogenetically distinct. *J Immunol* **162**: 3096–3102.
- Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C (1998). New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. *J Immunol* **161**: 1860–1867.
- Charles PC, Weber KS, Cipriani B, Brosnan CF (1999). Cytokine, chemokine and chemokine receptor mRNA expression in different strains of normal mice: implications for establishment of a Th1/Th2 bias. *J Neuroimmunol* **100**: 64–73.
- Dean G, Kurtzke JF (1971). On the risk of multiple sclerosis according to age at immigration to South Africa. *Br Med J* **3**: 725–729.
- Edwards S, Zvartau M, Clarke H, Irving W, Blumhardt LD (1998). Clinical relapses and disease activity on magnetic resonance imaging associated with viral upper respiratory tract infections in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **64**: 736–741.
- Encinas JA, Lees MB, Sobel RA, Symonowicz C, Greer JM, Shovlin CL, Weiner HL, Seidman CE, Seidman JG, Kuchroo VK (1996). Genetic analysis of susceptibility to experimental autoimmune encephalomyelitis in a cross between SJL/J and B10.S mice. *J Immunol* **157**: 2186–2192.
- Freund J, Stern ER, Pisani TM (1947). Isoallergic encephalomyelitis and radiculitis in guinea pigs after one injection of brain and mycobacteria in water-in-oil emulsion. *J Immunol* **57**: 179–193.
- Fujinami RS, Oldstone MBA (1985). Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* **230**: 1043–1045.
- Goverman J (1999). Tolerance and autoimmunity in TCR transgenic mice specific for myelin basic protein. *Immunol Rev* **169**: 147–159.
- Illés Z, Kondo T, Yokoyama K, Ohashi T, Tabira T, Yamamura T (1999). Identification of autoimmune T cells among in vivo expanded CD25⁺ T cells in multiple sclerosis. *J Immunol* **162**: 1811–1817.
- James WH (1988). Further evidence in support of the hypothesis that one cause of multiple sclerosis is childhood infection. *Neuroepidemiology* **7**: 130–133.
- Kabat EA, Wolf A, Bezer AE (1946). Rapid production of acute disseminated encephalomyelitis in Rhesus monkeys by infection of brain tissue with adjuvants. *Science* **104**: 362–363.
- Kabat EA, Wolf A, Bezer AE (1947). The rapid production of acute disseminated encephalomyelitis in Rhesus monkeys by infection of heterologous and homologous brain tissue with adjuvants. *J Exp Med* **85**: 117–128.
- Kalman B, Lublin FD (1999). The genetics of multiple sclerosis. A review. *Biomed Pharmacother* **53**: 358–370.
- Kerlero de Rosbo N, Bernard CCA, Simmons RD, Carnegie PR (1985). Concomitant detection of changes in myelin basic protein and permeability of blood–spinal cord barrier in acute experimental autoimmune encephalomyelitis by electroimmunoblotting. *J Neuroimmunol* **9**: 349–361.
- Kopeloff LM, Kopeloff N (1947). Neurologic manifestations in laboratory animals produced by organ (adjuvant) emulsions. *J Immunol* **57**: 229–237.
- Kurtzke JF (1993). Epidemiologic evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* **6**: 382–427.
- Levine S, Simon J, Wenk EJ (1966). Edema of the spinal cord in experimental allergic encephalomyelitis. *Proc Soc Exp Biol Med* **123**: 539–541.
- Livingstone KD, Sudweeks JD, Blankenhorn EP, Hickey WF, Teuscher C (1995). Susceptibility to actively-induced murine experimental allergic encephalomyelitis is not linked to genes of the T cell receptor or CD3 complexes. *Autoimmunity* **21**: 195–201.
- Martin R (1997). Genetics of multiple sclerosis—how could disease-associated HLA- types contribute to pathogenesis? *J Neural Transm Suppl* **49**: 177–194.
- Metz LM, McGuinness SD, Harris C (1998). Urinary tract infections may trigger relapse in multiple sclerosis. *Axone* **19**: 67–70.
- Morgan IM (1946). Allergic encephalomyelitis in monkeys in response to injection of normal monkey cord. *J Bacteriology* **51**: 614.
- Morgan IM (1947). Allergic encephalomyelitis in monkeys in response to injection of normal monkey nervous tissue. *J Exp Med* **85**: 131–140.
- Morrison LA (1947). Disseminated encephalomyelitis experimentally produced by the use of homologous antigen. *Arch Neurol & Psychiat* **58**: 391–416.
- Morrison W, Nelson J (1994). Environmental triggers in multiple sclerosis. Fact or fallacy? *Axone* **16**: 23–26.
- Panitch HS (1994). Influence of infection on exacerbations of multiple sclerosis. *Ann Neurol* **36 Suppl**: S25–S28.
- Rapp NS, Gilroy J, Lerner AM (1995). Role of bacterial infection in exacerbation of multiple sclerosis. *Am J Phys Med Rehabil* **74**: 415–418.
- Rodriguez F, An LL, Harkins S, Zhang J, Yokoyama M, Widera G, Fuller JT, Kincaid C, Campbell IL, Whitton JL (1998). DNA immunization with minigenes: low frequency of memory cytotoxic T lymphocytes and inefficient antiviral protection are rectified by ubiquitination. *J Virol* **72**: 5174–5181.
- Sibley WA, Bamford CR, Clark K (1985). Clinical viral infections and multiple sclerosis. *Lancet* **1**: 1313–1315.
- Simmons RD, Bernard CCA, Singer G, Carnegie PR (1982). Experimental autoimmune encephalomyelitis. An anatomically-based explanation of clinical progression in rodents. *J Neuroimmunol* **3**: 307–318.
- Smith ME, Eller NL, McFarland HF, Racke MK, Raine CS (1999). Age dependence of clinical and pathological manifestations of autoimmune demyelination. Implications for multiple sclerosis. *Am J Pathol* **155**: 1147–1161.
- Sobel RA, van der Veen RC, Lees MB (1986). The immunopathology of chronic experimental allergic encephalomyelitis induced in rabbits with bovine proteolipid protein. *J Immunol* **136**: 157–163.
- Teuscher C, Butterfield RJ, Ma RZ, Zachary JF, Doerge RW, Blankenhorn EP (1999). Sequence polymorphisms

- in the chemokines Scya1 (TCA-3), Scya2 (monocyte chemoattractant protein (MCP)-1), and Scya12 (MCP-5) are candidates for eae7, a locus controlling susceptibility to monophasic remitting/nonrelapsing experimental allergic encephalomyelitis. *J Immunol* **163**: 2262–2266.
- Trotter JL, Pelfrey CM, Trotter AL, Selvidge JA, Gushleff KC, Mohanakumar T, McFarland HF (1998). T cell recognition of myelin proteolipid protein and myelin proteolipid protein peptides in the peripheral blood of multiple sclerosis and control subjects. *J Neuroimmunol* **84**: 172–178.
- Tsunoda I, Fujinami RS (1996). Two models for multiple sclerosis: Experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *J Neuropathol Exp Neurol* **55**: 673–686.
- Tsunoda I, Kuang L-Q, Tolley ND, Whitton JL, Fujinami RS (1998). Enhancement of experimental allergic encephalomyelitis (EAE) by DNA immunization with myelin proteolipid protein (PLP) plasmid DNA. *J Neuropathol Exp Neurol* **57**: 758–767.
- Tsunoda I, Kuang L-Q, Theil DJ, Fujinami RS (2000). Antibody association with a novel model for primary progressive multiple sclerosis: Induction of relapsing-remitting and progressive forms of EAE in *H2^S* mouse strains. *Brain Pathol* **10**: 402–418.
- Tsunoda I, Tolley ND, Theil DJ, Whitton JL, Kobayashi H, Fujinami RS (1999). Exacerbation of viral and autoimmune animal models for multiple sclerosis by bacterial DNA. *Brain Pathol* **9**: 481–493.
- Weerth S, Berger T, Lassmann H, Linington C (1999). Encephalitogenic and neuritogenic T cell responses to the myelin-associated glycoprotein (MAG) in the Lewis rat. *J Neuroimmunol* **95**: 157–164.
- Weinshenker BG, Santrach P, Bissonet AS, McDonnell SK, Schaid D, Moore SB, Rodriguez M (1998). Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. *Neurology* **51**: 742–747.
- Whitton JL, Oldstone MBA (1996). Immune response to viruses. In: *Fields Virology*. Fields BN, Knipe DM, Howley PM (eds). Third edition Lippincott-Raven: Philadelphia, pp 345–374.
- Yoshimura T, Kunishita T, Sakai K, Endoh M, Namikawa T, Tabira T (1985). Chronic experimental allergic encephalomyelitis in guinea pigs induced by proteolipid protein. *J Neurol Sci* **69**: 47–58.