

Molecular mimicry between a viral peptide and a myelin oligodendrocyte glycoprotein peptide induces autoimmune demyelinating disease in mice

Foroozan Mokhtarian^{a,*}, Zhiguang Zhang^a, Yong Shi^a, Efrain Gonzales^a,
Raymond A. Sobel^b

^a Division of Immunology, Department of Medicine, SUNY, Health Science Center and Maimonides Medical Center, 4802 10th Avenue, Brooklyn, NY 11219, USA

^b Department of Pathology, Stanford University School of Medicine and VA Health Care System, Palo Alto, CA, USA

Received 30 June 1998; revised 19 November 1998; accepted 19 November 1998

Abstract

Semliki Forest Virus (SFV) induces an encephalomyelitis followed by demyelination in the brains of C57Bl6/J (B6) mice. To investigate the role of molecular mimicry in the pathogenesis of postviral demyelination, alignment algorithms were used and amino acid homologies between immunogenic epitopes of SFV and myelin autoantigens, myelin basic protein (MBP), myelin proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) were identified. Immunization of B6 mice with SFV proteins induced significant lymphocyte proliferation to SFV E2 peptides and to MOG peptide_{18–32} (which had molecular mimicry with E2_{115–129}), but not to MBP or PLP peptides. Both MOG_{18–32} and E2_{115–129} induced a later-onset chronic EAE-like disease that correlated with the presence of multifocal vacuolation in the CNS white matter. This histopathology was reminiscent of the secondary demyelination seen following SFV infection. Serum antibody responses to the peptides appeared late after immunizations and some samples cross-reacted with other myelin peptides, as well as with the mimicked MOG peptides. These findings suggest that following a CNS viral infection, antibody response to an epitope of virus that exhibits molecular mimicry with a peptide of MOG may contribute to autoimmune mediated injury to CNS myelin. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Autoimmune demyelinating disease; Semliki Forest Virus; Multiple sclerosis

1. Introduction

Semliki Forest Virus (SFV) infection of mice is one of three most useful experimental viral models of multiple sclerosis (MS); the other two being infections with Theiler's and mouse hepatitis virus (Suckling et al., 1978; Fazakerley et al., 1997). These viruses give rise to mononuclear inflammatory demyelinating lesions of the central nervous system (CNS), while no demyelination is observed in the peripheral nervous system. Unlike Theiler's and mouse hepatitis virus, SFV induces an *acute* CNS virus infection

(i.e., without persistence), followed by demyelination after viral clearance. Since SFV is not found in the CNS during demyelination, the mechanisms (i.e., antigens) involved in the late demyelination are not known. Similarly, an argument against viral etiology of MS is that no virus has been consistently isolated from MS patients which can be incriminated as a causative agent of disease. Mice infected with SFV develop a secondary demyelination at 14 to 21 days postinfection. This demyelination, which occurs after the clearance of virus from the brains and spinal cords of mice, also appeared to be T-cell mediated (Fazakerley et al., 1983; Mokhtarian and Swoveland, 1987).

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune demyelinating disease of the CNS which has also been used as an animal model for MS (Mokhtarian et al., 1984; Raine et al., 1984). EAE is induced by T cell responses to certain peptides of myelin proteins including MBP (Beraud et al., 1986; Zamvil et al., 1986), PLP

Abbreviations: SFV: Semliki Forest virus; MOG: myelin oligodendrocyte glycoprotein; PLP: proteolipid protein; MBP: myelin basic protein; EAE: experimental autoimmune encephalomyelitis; CNS: central nervous system

* Corresponding author. Tel.: +1-718-2838432; Fax: +1-718-2836500; E-mail: drfmsuny@idtnet

(Whitham et al., 1991; Sobel et al., 1994; Greer et al., 1996) and recently, MOG (Amor et al., 1994; Kerlero de Rosbo et al., 1995; Mendel et al., 1995). MOG-induced EAE in rats, as in the MBP- and PLP-induced models in mice, was found to be a demyelinating encephalomyelitis, resembling multiple sclerosis (Johns et al., 1995). The encephalitogenic potency of MOG 35–55 in mice is also believed to be comparable to encephalitogenic peptides of MBP and PLP (Amor et al., 1994; Kerlero de Rosbo et al., 1995; Mendel et al., 1995). In B6 mice, EAE can be induced by MOG, using both active immunization and passive transfer techniques (Mendel et al., 1995). This strain, however is relatively resistant to the induction of EAE by MBP or PLP (Tuohy et al., 1988; Endoh et al., 1990; Shaw et al., 1992).

It has been reported that viral infections are able to activate autoreactive T cells in animal models of autoimmune diseases. For example, normal adult hamsters recovered from infection with measles virus, became more susceptible to the induction of EAE than uninfected hamsters (Massanari et al., 1979). Similarly, the JHM strain of mouse hepatitis virus in rats primed them for an autoimmune response to myelin components (Watanabe et al., 1983), and we have shown that infection of B6 mice with SFV triggered susceptibility to EAE induced with MBP (Mokhtarian and Swoveland, 1987). In humans, acute viral infections may similarly activate T and B cells to autoantigens and induce autoimmune responses and diseases such as MS (Johnson et al., 1984; Waksman, 1985), diabetes (Miyazaki et al., 1995) and systemic lupus erythematosus (Mamula et al., 1994).

Molecular mimicry is postulated to be a major mechanism for the activation of autoreactive T cells. Viral peptides with sufficient sequence homology or similarity to self peptides may be able to induce autoimmune responses that lead to disease (Oldstone, 1987). Viruses such as measles, Epstein–Barr, and hepatitis B virus have been shown to activate T cell clones to MBP, as a result of molecular mimicry (Wucherpfenning and Strominger, 1995). Induction of EAE in animals and the activation of MBP-reactive human T cells by viral peptides have provided evidence for a role of molecular mimicry between viral and MBP peptides in the pathogenesis of MS (Fujinami and Oldstone, 1985; Jahnke et al., 1985).

We postulated that the demyelination that occurs following SFV infection of the CNS is mediated through activation of myelin-reactive T cells that recognize mimicked SFV peptides. We have identified homologies between a peptide of a surface protein of SFV (E2) and a MOG peptide, demonstrated cross-recognition by T cells in mice immunized with this SFV peptide, and induced an EAE-like clinical disease and CNS pathology in mice immunized with these peptides. The effector mechanisms appear to involve an antibody response to the mimicked peptide. To our knowledge, this is the first demonstration of an autoimmune CNS disease induced as a consequence

of molecular mimicry between a CNS viral pathogen and a self (myelin) peptide.

2. Materials and Methods

2.1. Alignment of peptide sequences

A Global Alignment Query (<http://genome.eerie.fr/bin/align-guess.cgi>) using three different algorithms or matrices (codaa.mat, Pam250.mat, alprot.mat) was utilized to locate different areas of homology. The major surface glycoprotein of SFV, E2 which has been found to be the target for antibody and T cell responses of mice against SFV (Snijders et al., 1992), was therefore selected for study. The amino acid (aa) sequence of E2 was aligned with those of each of the myelin proteins, MBP, PLP and MOG and was examined for the presence of homologous stretches between the two proteins. Our minimum criterion for homology was the presence of at least three consecutive exact aa matches. Partial homologies, including hydrophobicity, polarity, charge, pK_a , size, and structure of R-group, were also considered in these matched regions. The two matched peptides were then subjected to another computer alignment, described above, to determine the true percent homology between them.

2.2. Synthesis of Peptides

Matching peptides were synthesized in slightly longer forms to improve antigenicity. Non-matched peptide, MOG 35–55, was also synthesized as a positive control (Mendel et al., 1995). Peptides were synthesized by an automated peptide synthesizer at the Biochemistry Laboratory of Johns Hopkins University, School of Hygiene and Public Health. Synthetic peptides were analyzed by HPLC, purified, and their compositions were confirmed by aa analysis. Human Fibrinopeptide B (FB) 1–14 and Bovine Fibroblast Growth factor (FGF) 106–120 (Sigma, St. Louis, MO) were used as negative controls.

2.3. SFV antigen

The avirulent A774 strain of SFV with a titer of 3×10^8 plaque-forming units (PFU) per ml on BHK-21 cells was used as stock virus. The virus stock underwent two passages on BHK-21 cells before use in mice. Inactivation of SFV was carried out by UV-irradiation of infectious virus (UV-SFV), as previously described (Mokhtarian and Swoveland, 1987).

2.4. Immunization of mice for lymphoproliferation assay

Female, 8–9 weeks old B6 mice, purchased from the Jackson Laboratory (Bar Harbor, ME), were used in all experiments. For determination of lymphocyte proliferative

Table 1
Peptide alignments of E2 and myelin proteins

Peptides	Sequence ^a	% Identity
A) E2 121–127 and MOG 20–26	AVRACRI : . . . : : AELPCR I	33
B) E2 118–129 and PLP 92–103	TRNAVRACRIQY : . . : . . : : TTGAVRQIFGDY	42
C) E2 137–149 and MBP 56–68	GREKFTIRPHYGK : . . . : . : : : : GKDSHTRTTHYGS	33

^aTwo dots represent complete homology and one dot indicates partial homology as described in Section 2.

The peptides shown in the alignments are sometimes shorter than the actual synthesized peptides.

responses, groups of 10–12 mice were each immunized subcutaneously (s.c.) at four sites with 150 µg of UV-inactivated SFV, E2 115–129 or MOG 18–32, emulsified in an equal volume of Complete Freund's Adjuvant (CFA), containing 30–60 µg of *M. tuberculosis* (Difco Laboratories, Detroit MI), as previously described (Mendel et al., 1995; Kerlero de Rosbo et al., 1995). The immunized animals were boosted with the same injection 10–12 days later. Using this protocol, no mice showed any signs of clinical or histological disease.

2.5. Immunization of mice for induction of EAE

To induce disease, groups of 10–12 mice were each immunized s.c. with 300 µg of E2 115–129, MOG 15–32 or MOG 35–55 (positive control, 12) in CFA supplemented with *M. tuberculosis*, twice on days 0 and 8, as previously described (Mendel et al., 1995). Pertussis toxin from *B. pertussis* was inoculated intraperitoneally with a dose of 400 ng/mouse, immediately and 48 h after the first immunization. Mice receiving pertussis only served as negative controls. Following the encephalitogenic challenge, mice were observed daily for clinical manifestations of EAE and were scored on a scale of 0–5 as follows: 0 = no abnormality, 1 = mild hind limb weakness (some difficulty righting themselves when turned on their back), 2 = moderate hind limb weakness (as in 1), sometimes associated with floppy tail, 3 = weakness of hind limbs accompanied by some forelimb weakness, sometimes more marked on one limb or one side, but not complete paralysis, 4 = hind limb paresis accompanied by mild forelimb weakness, 5 = paralysis of hind limbs, associated with moderate forelimb weakness, and 6 = paraplegia, moribund.

2.6. Lymphocyte proliferation assay

For determination of lymphocyte proliferative responses, 3–4 mice from each group were sacrificed 7 days

following their second immunization with UV-SFV or with peptide, and regional (popliteal and inguinal) draining lymph nodes were collected aseptically. A standard proliferation assay was carried out by seeding $2-3 \times 10^5$ lymph node cells (LNC) in 0.2 ml of RPMI medium in 96-well microtiter plates with UV-SFV and various peptides, each added at concentrations of 50, 25 and 5 µg/ml. The cultures, set up in triplicate, were incubated for 96 h in humidified air plus 5–6% CO₂. For the last 18 h of incubation the cultures were pulsed with 1 µCi of ³H-thymidine (specific activity 20 Ci/nmol). The cultures were harvested on fiberglass filters by standard techniques and incorporation of ³H-thymidine was measured in a liquid scintillation counter. The proliferative responses were expressed as a mean cpm ± SD.

2.7. Antibody determination assay

For determination of antibody responses, mice were either sacrificed and bled at the time of the appearance of clinical disease or at the conclusion of experiment. Blood samples from 2–3 mice of the same group were pooled to obtain sufficient volume of serum. The antibody in the sera of immunized mice was measured by enzyme-linked immunosorbent assay (ELISA). Briefly, flat-bottomed immunolon-2 microtiter plates (Dynatech Laboratories, Alexandria, VA) were coated with 2 µg/ml of UV-irradiated SFV protein or 2 µg/well of each immunization

Table 2
Synthesized peptides used in the study^a

Peptide	Sequence	#AA
<i>SFV peptide</i>		
^b E2 115–129	IQDTRNAVRASRIQY	15
E2 137–151	GREKFTIRPHYGKEI	15
<i>MOG peptide</i>		
^b MOG18–32	DEAELPSRISPGKNA	15
<i>PLP peptide</i>		
PLP 89–104	GFYTTGAVRQIFGDY	15
<i>MBP peptide</i>		
MBP 54–68	GSGKDSHTRTTHYGS	15
<i>Negative Control (non-myelin) peptides</i>		
Human Fibrinopeptide B:		
FB 1–14	EGVNDNEEGFFSAR	14
Bovine Fibroblast Growth factor:		
FGF 106–120	YRSRKYSSWYVAALK	15
<i>Positive Control peptide</i>		
MOG 35–55	MEVGWYRSPFSRVVHLYRNGK	21

^aThe peptides were lengthened to improve antigenicity.

^bThe aa C was substituted with S in synthesis at position 125 in E2 115–129 and at position 24 in MOG 18–32 peptides.

peptide, MOG-35–55, MOG 18–32, E2 115–129 or with the control peptide, FB 1–14, in 0.06 M carbonate buffer, pH 7.6. The plates were then incubated at 4°C for 24 h, washed $\times 3$ with PBS containing 0.05% Tween-20 and blocked with 2% bovine serum albumin (BSA) (Sigma, St. Louis, MO) for 1 h at 37°C. After washing, 100 μ l of 1:100 dilution of sera, in duplicate, were incubated on these solid-phase antigens for 1 h at 37°C, washed again and 100 μ l of peroxidase-conjugated anti-mouse IgG, diluted 1:1000, was added to all wells and incubated for 90 min at 37°C. After the final washes, 200 μ l of *o*-Phenylendiamine (OPD) (Sigma) substrate solution was added to each well. The optical densities (OD) of the wells were read by microtiter plate reader using 495 nm filter (Dynatech Laboratories, Alexandria, VA). Wells containing buffer alone served as background control. Serum sample from pertussis-injected mice was used as normal control. Each sample consisted of pooled sera from 2–3 mice of the same group. The final OD of samples were obtained by

subtracting the OD of negative control from those of samples in the same ELISA. A reactive sample was one which showed a difference of ≥ 0.2 .

2.8. Histopathology

On various days post immunization, mice were sacrificed and their brains and spinal cords were removed. The tissues were processed for paraffin embedment and sections were stained with Luxol fast blue and hematoxylin and eosin. Numbers of inflammatory foci (> 20 perivascular mononuclear cells) were counted in meninges and parenchyma. Each CNS tissue sample was also scored as to the presence or absence of vacuolation/demyelination in the cerebellum, brain stem or spinal cord white matter. Histologic evaluation was performed by an observer blinded to the presence or absence of clinical signs in the animals.

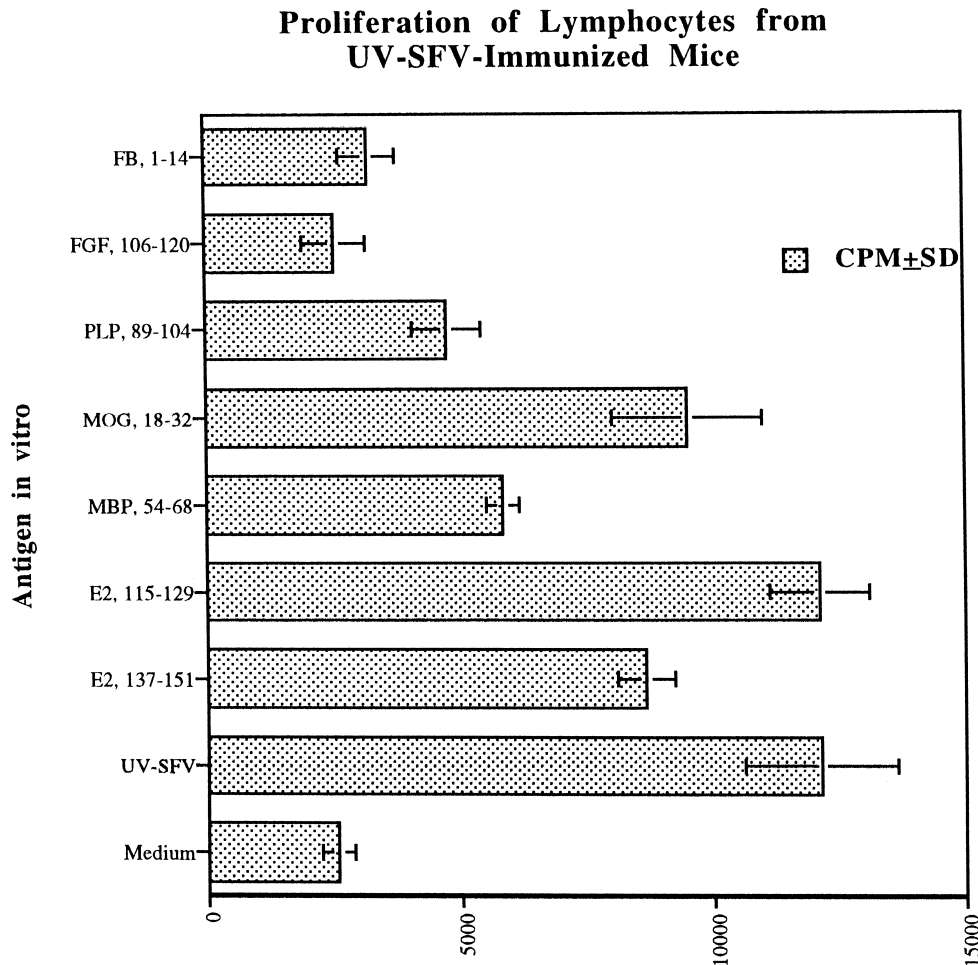


Fig. 1. Analysis of epitopes recognized by lymphocytes of mice immunized with UV-SFV. Mice were immunized twice (150 μ g antigen/mouse in CFA) with 10–12 days interval. Antigen and peptides were added with the concentration of 25 μ g/ml. Proliferative responses of lymphocytes are shown as counts per minute (CPM) \pm standard deviation (SD) of tritiated thymidine uptake. The data shown are an average from three different experiments each consisting of 3 mice. Mean background CPM was 2564 \pm 322. CPM for SFV was 12136 \pm 1516, for E2 137–151; 8670 \pm 566, for E2 115–129; 12116 \pm 987, for MBP 54–68; 4844 \pm 325, for MOG 18–32; 9506 \pm 1487, for PLP 89–104; 4751 \pm 678, for FGF 106–12; 2543 \pm 623, and for FB 1–14; 3210 \pm 554.

3. Results

3.1. Homologies between epitopes of SFV and proteins of myelin

The two major peptides of SFV, which are both parts of its helper T cell epitope, E2, 137–151 and E2 115–129 (Snijders et al., 1992), were the primary targets for homology studies. The initial alignments of aa sequences of SFV, E2 with those of MBP, PLP and MOG, indicated that there were few exact matches of 3 consecutive aa combined with some partial homologies in the same areas, with no gaps or breakage in the alignment (data not shown). These areas were aligned again, in the short forms, using the same program, and aa homologies were verified (Table 1). Significant homologies were found between the aa sequences of E2 121–127 and MOG 20–26, and between E2 118–129 and PLP 92–103 (Table 1). Another significant homology was between E2 137–149 and MBP 56–68. Based on the sequence homology between E2 peptides and those of MOG and PLP and MBP and to ensure antigenicity, the two SFV E2 peptides and their matching regions were synthesized in slightly longer versions as shown in Table 2.

3.2. Lymphocyte proliferation responses of SFV-immunized mice

In initial studies, immunization of B6 mice with UV-SFV produced highly significant proliferative responses to

SFV proteins and to the two major surface peptides of SFV: E2 115–129, E2 137–151 (Fig. 1). Lymphocytes of UV-SFV-immunized mice proliferated more vigorously to MOG 18–32, which had no interruptions in the homology area (Table 1), than to other myelin and control peptides ($p < 0.001$, in each case) (Fig. 1). Although some reactivity with other myelin peptides, PLP peptide 89–104 and MBP peptide 54–68, was seen, these responses were not as strong ($p > 0.05$) as those with MOG 18–32. MOG 18–32 did not stimulate lymphocytes from normal B6 mice, *in vitro* (data not shown). Responses to unrelated peptides, Fibroblast growth factor 106–120 (FGF) and Fibrinopeptide B 1–14 (FB) were minimal (Fig. 1).

3.3. Lymphocyte proliferation of peptide-immunized mice

The results described above indicated that molecular mimicry between E2 115–129 and MOG 18–32 may have been the reason for the observed cross reactivity of the lymphocytes of UV-SFV-immunized mice with MOG 18–32.

Immunization of mice with E2 115–129 led to a proliferative response of lymphocytes to this peptide, and again to a strong cross-reactive response to MOG 18–32 (Fig. 2). The cross-reactive response to MOG 18–32 was stronger than to PLP 89–104, and to MBP 54–68.

Lymphocytes from mice immunized with MOG 18–32 produced a strong proliferative response to this peptide but

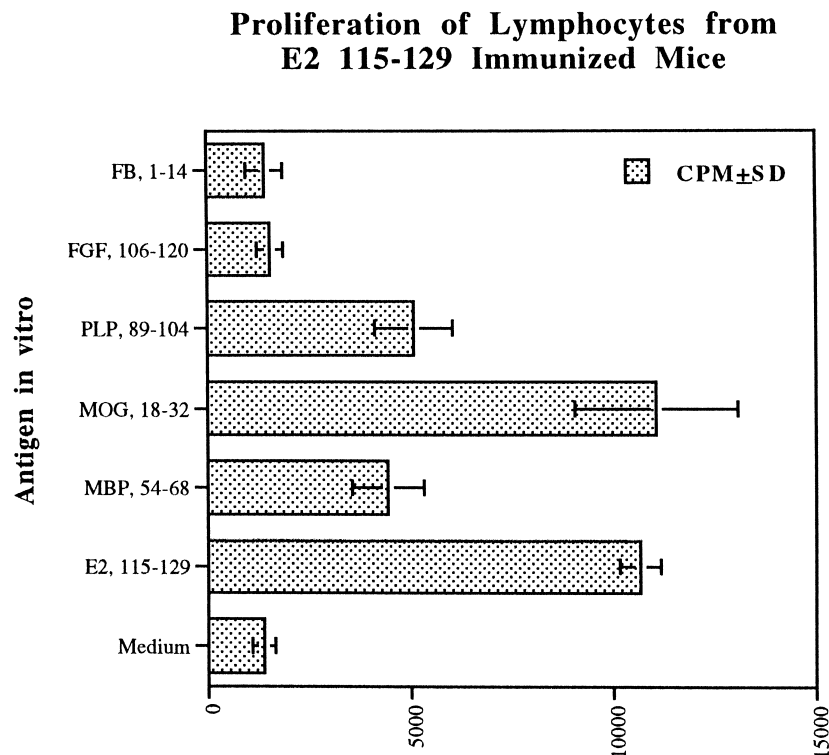


Fig. 2. Analysis of epitopes recognized by lymphocytes of mice immunized with E2 115–129. See legend for Fig. 1. Mean background CPM was 1370 ± 282 . CPM for E2 115–129 was 10667 ± 510 , for MOG 18–32: 11068 ± 2015 , for PLP 89–104; 5080 ± 963 , for MBP 54–68; 4434 ± 886 , for FGF 106–12; 1543 ± 323 and FB 1–14; 1416 ± 454 .

did not exhibit cross-reactive responses with other peptides tested, including E2 115–129 (data not shown).

3.4. Induction of acute and chronic EAE-like diseases by peptides

Since MOG 35–55 is the only MOG peptide, reported to induce EAE in B6 mice (Mendel et al., 1995), this peptide was used as positive control. MOG 35–55 induced EAE in 2 of 6 mice on day 13 after the first immunization. The affected mice showed mild to moderate hind limb weakness and one had floppy tail with average clinical score (ACS) of 1.5 (Fig. 3). Three more mice developed moderate hind limb weakness, on days 14 and 15 post-immunization (pi), while the first two gradually progressed to weakness of both hind and fore limbs (ACS = 2). By day 16 after immunization, 5 of 6 (83%) MOG 35–55-immunized mice were affected with signs of EAE (ACS = 2.5) (Fig. 3). The MOG-35–55 experiment was terminated on day 16 and mice were sacrificed for histological and antibody assays. One mouse remained unaffected during this observation period.

Initially, immunization with MOG 18–32 induced clinical EAE in four out of seven (57%) mice. Two mice developed mild to moderate hind limb weakness, on day 13 and another two on day 14 pi, with ACS = 1.5. Three of these mice progressed only to moderate hind limb weakness while one of the first two developed weakness of both hind and forelimbs, by day 16 after immunization (ACS = 2.3) (Fig. 3). The MOG-18–32 experiment was also terminated on day 16 and mice were sacrificed for histological and antibody assays.

Immunization with E2 115–129 did not result in clinical disease during the first 16 days of observation, but 2 of 6 mice developed a mild to moderate hind limb weakness with difficulty to roll over (ACS = 1.5), at 20 days pi, progressing to moderate hind limb weakness on day 28 pi. Another two mice exhibited moderate hind limb weakness on day 28 pi (ACS = 2). A total of 4 of 6 (66%) of E2 115–129-immunized mice were affected with signs of EAE-like disease.

In a second experiment mice were observed for a longer period of time. MOG 35–55 induced an acute EAE followed by a chronic phase, as previously reported (Mendel

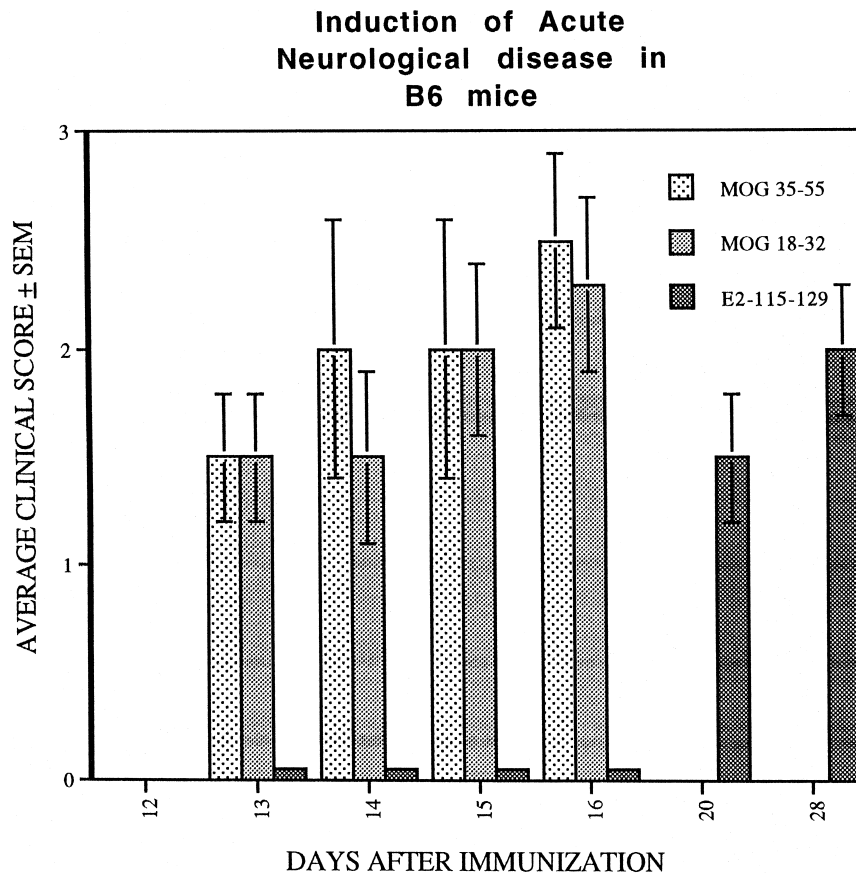


Fig. 3. Development of acute clinical EAE in 6 MOG 35–55, 8 MOG 18–32 and 7 E2 115–129 immunized C57Bl6 mice. Mice were inoculated for disease development with 300 μ g/mouse of peptide, as described in Section 2, and were followed daily for clinical signs of disease. The mean daily clinical score \pm SEM is shown.

et al., 1995). In MOG 18–32-immunized mice, the first signs of EAE appeared on day 13 pi, increased with time and peaked on days 24–34 post immunization (Fig. 4).

Immunization with E2 115–129 did not induce EAE-like disease until day 20, increased with time and the signs were still increasing on day 40 pi, when the experiment was terminated (Fig. 4).

Mice immunized with peptides without pertussis or with CFA and pertussis alone did not show clinical disease.

In summary, MOG 35–55 and MOG 18–32 induced EAE with onset beginning on days 10–12 pi and progressing to a chronic phase and the E2 115–129 peptide induced a milder chronic EAE-like disease, beginning on day 20 pi.

3.5. Antibody responses

Samples (pooled sera) from peptide immunized/pertussis-injected and pertussis (only)-injected B6 mice were examined for antibody (ab) production by ELISA. Samples from MOG 35–55-immunized mice showed high reactivity with this peptide at 2–3 weeks and 5–6 weeks pi (Table 3). Samples from MOG 18–32-immunized mice, however, did not react with the specific peptide (MOG

18–32) until 5–6 weeks pi, at which time 1 of 3 samples reacted with MOG 18–32 and cross-reacted with E2 115–129. Three of 4 samples from MOG 18–32-immunized mice showed high reactivity with specific peptide by 5–6 weeks pi. One of two samples from E2 115–129-immunized mice had developed a low reactivity with E2 115–129 and cross reaction with MOG 18–32, at 3 weeks pi (Table 3). Three of four samples from E2 115–129-immunized mice reacted specifically with this peptide at 4–5 weeks pi (Table 3). In short, MOG 35–55-immunized mice developed higher levels of specific antibody, at an earlier time point, than MOG 18–32-immunized mice. E2 115–129-immunized mice also developed antibody similar to those in MOG 18–32-immunized mice, but the antibody was detected at an earlier time point.

In experiments using the protocol to maximize lymphocyte proliferation responses rather than disease induction, immunization of mice with E2 115–129 and MOG 18–32, induced a low titer of specific antibody to E2 115–129, but did not produce detectable levels of antibody to MOG 18–32 (data not shown).

Due to cross reactive antibody responses seen in some samples obtained from E2 115–129- and MOG 18–32-immunized mice, as described above, we decided to investi-

Induction of Acute and Chronic Neurological disease in B6 mice

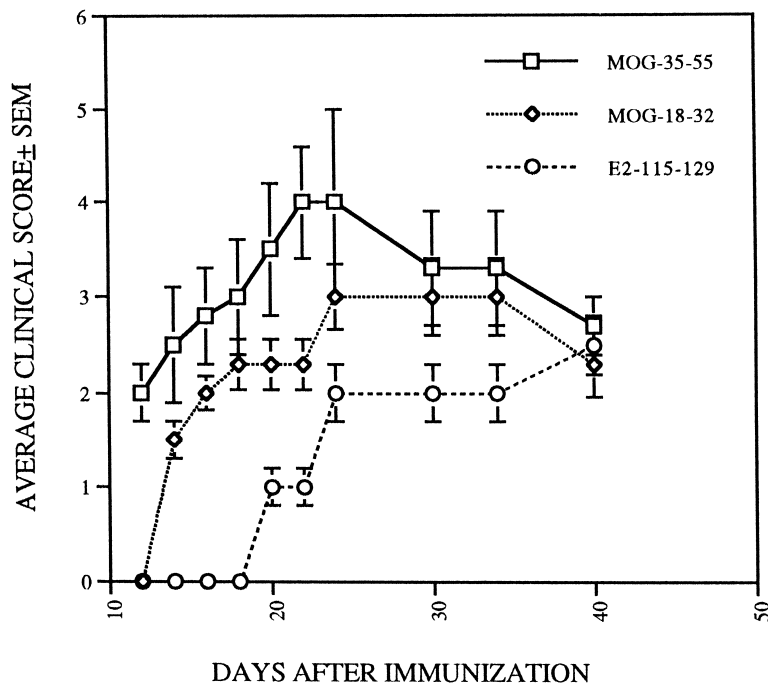


Fig. 4. Development of chronic EAE in 7 MOG 18–32 and 8 E2-115–129 immunized C57Bl6 mice. Mice were inoculated for disease development with 300 μ g/mouse of peptide, as described in Section 2, and were followed daily for clinical signs of disease. The mean daily clinical scores \pm SEM are shown.

Table 3
Antibody responses in peptide-immunized mice

Immunizing peptide	Antibody positive * /total, at weeks post immunization		
	2–3 weeks	4–5 weeks	5–6 weeks
MOG 35–55 ^A	1/3	ND	4/4
MOG 18–32 ^B	0/3	1/3 * *	3/4
E2 115–129 ^C	1/2 * *	2/3	3/4

*The numbers indicated are those of samples (each from 2–3 mice) that reacted with the immunizing peptide.

* *The positive sample in these groups reacted with both MOG 18–32 and E2 115–129.

^AThe OD of one of three samples at 2–3 weeks post immunization (pi) was 1.6; at 5–6 weeks (pi), the average OD of samples was 1.15.

^BThe OD of the sample at 4–5 weeks pi was 0.55 for MOG and 0.56 for E2 peptide; at 5–6 weeks pi, the average OD for 3 positive samples was 1.01.

^CThe OD of the sample at 2–3 weeks pi was 0.3 for E2 and 0.2 for MOG peptide; at 4–5 weeks pi, the average OD of the 2 positive samples was 0.75; at 5–6 weeks pi, the average OD for three positive samples was 0.9.

gate the phenomenon of epitope spreading in the antibody response. Therefore, more samples obtained from mice at the time of clinical disease were tested for reactivity with several peptides of SFV and myelin. Serum samples from MOG 18–32-immunized mice (day 18 pi) showed minimal antibody response to this peptide and to the other mimicked and non mimicked peptides (Fig. 5), despite the clinical signs of EAE at this time point. One of two samples from E2 115–129-immunized mice (day 21 pi) reacted not only with the specific peptide, but also with SFV proteins, and a few of other mimicked and non-mimicked peptides. The strongest cross reaction was with non mimicked peptide, MBP 64–75. The sera also reacted with PLP 89–104, MOG 10–32 and MOG 35–55 (Fig. 5). To further investigate the role of epitope spreading in

antibody response to SFV, serum samples from both SFV-infected and UV-SFV-immunized mice were similarly assayed and found to be reactive with all the above peptides, but non reactive with negative control peptides, FB (1–14) and FGF (106–120) (data not shown).

3.6. Histology

3.6.1. E2-115–129-immunized mice

CNS tissues of nine E2 115–129-immunized mice sacrificed on days 24–35 were examined by histology. Six of these mice had shown clinical signs with average clinical score (ACS) = 2 ± 0.3 (Table 4). Only one of six mice had a small number of mononuclear cell inflammatory infiltrates in the CNS (Fig. 6A, Table 4), but all six with clinical signs had foci of white matter vacuolation in the brain stem and spinal cord (Fig. 6B). This white matter vacuolation was not associated with glial cell proliferation, although an increase in immunoreactivity for glial fibrillary acidic protein was detected in some of these samples (not shown). Bielschowsky silver impregnation preparations on selected samples showed preservation of axons in the areas of vacuolation (not shown).

3.6.2. MOG 18–32-immunized mice

CNS tissues of seven MOG 18–32-immunized mice were examined by histology. Four of seven of the immunized mice showed clinical signs with ACS = 2.3 ± 0.3 (Table 4). Two of these mice which were killed on day 16 pi showed inflammatory cell infiltration: one mouse had very marked meningitis with minimal parenchymal infiltrates (Fig. 6C, Table 4). One mouse, that had shown severe clinical disease and died on day 18 pi, had moderate inflammation but marked vacuolation in the brain stem (Fig. 6D). Two mice in this group sacrificed at later time points had no CNS inflammation, but both showed white matter vacuolation (Table 4).

3.6.3. MOG 35–55-immunized and other control mice

All MOG 35–55-immunized mice showed clinical signs with ACS = 4 ± 0.6 , and all but one had marked meningeal

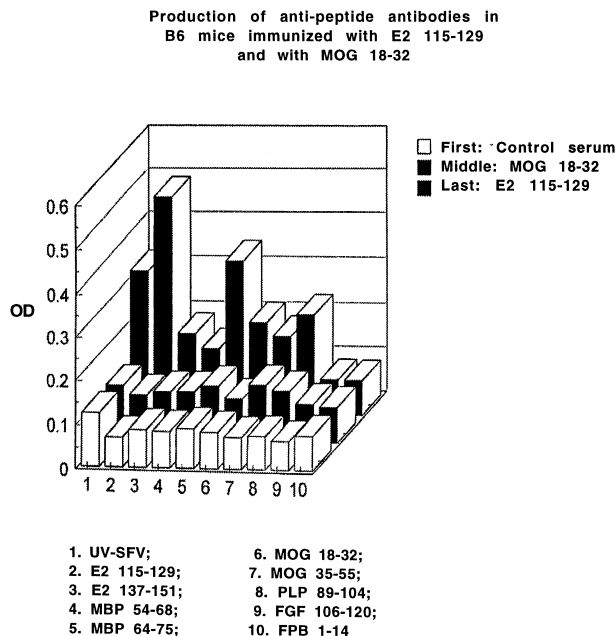


Fig. 5. Production of anti-peptide antibodies after immunization of C57Bl6 mice with SFV E2 115–129 and with MOG 18–32. See legend of Fig. 3 for immunization protocol of mice from which the sera were obtained. Microtiter plates were coated with 2 μ g of each listed peptide and ELISA were performed as described in Section 2.

Table 4
Clinical and histologic findings in immunized mice^a

Peptide immunization	Incidence of clinical disease	Average severity \pm SEM	Incidence of CNS inflammation	Total inflammation foci mean \pm SE ^b	Incidence of white matter vacuolation ^c
E2 115–129	6/9	2.0 \pm 0.3	1/6	2	6/9
MOG 18–32	4/7	2.3 \pm 0.3	2/7	10 \pm 9	4/7
MOG 35–55	6/6	4.0 \pm 0.6	5/6	84 \pm 39	4/6
CFA	0/3	0	0/3	0	0/3

^aCombined data from experiments 1 and 2 for mice that were examined for histopathology. Mice were sacrificed from day 16 to 35 pi.

^bMeningeal and parenchymal foci in brain and spinal cord sections were counted by single observer blinded to the clinical status of the mice.

^cTissue samples were scored for the presence or absences of white matter vacuolation. In MOG 35-immunized mice, vacuolation was associated with inflammatory infiltrates whereas in E2- and MOG 18-immunized mice vacuolation was present despite the lack of inflammation.

and parenchymal mononuclear cell infiltrates with associated white matter vacuolation (demyelination) typical of acute EAE (Fig. 6E and Table 4).

The white matter vacuolation seen in E2 115–129, MOG 18–32 and MOG-35–55 immunized mice, resembled the secondary demyelination seen in the cerebellar

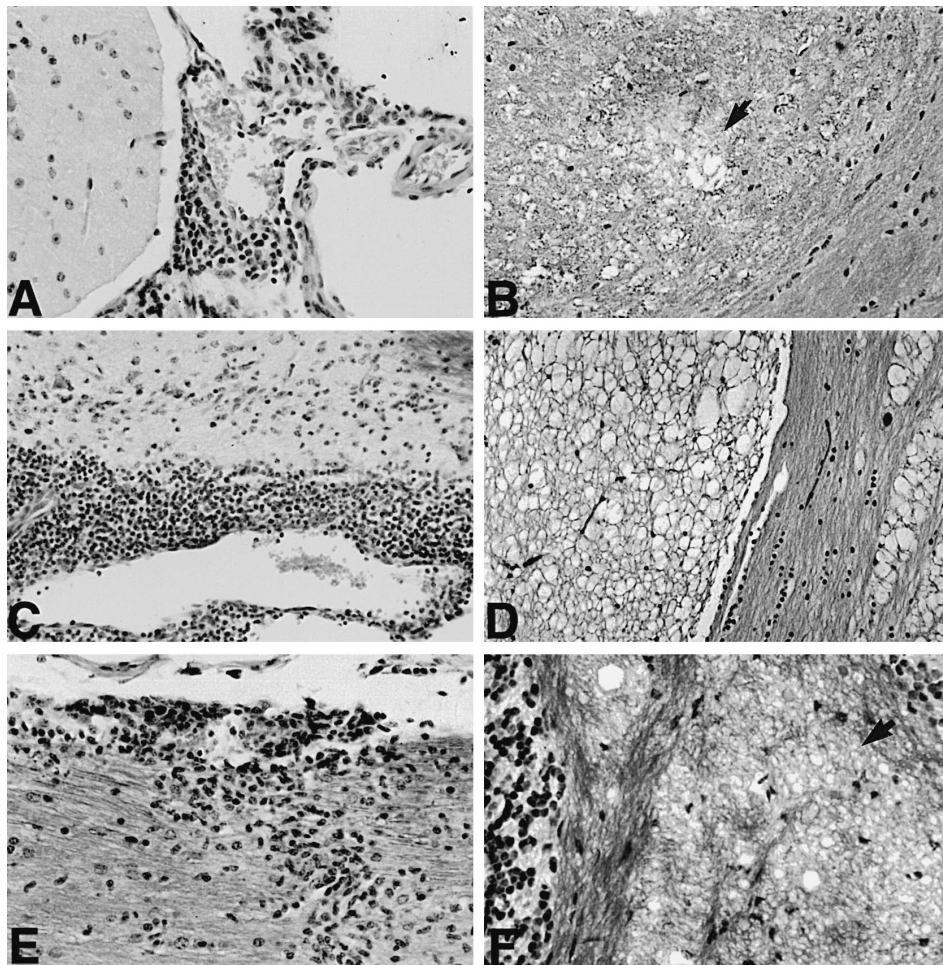


Fig. 6. All are stained with Luxol fast blue-hematoxylin and eosin. A. A mononuclear cell inflammatory focus in the leptomeninges adjacent to the cerebellum (left side of the field) in an E2 peptide-immunized mouse that showed clinical signs, 80 \times . B. Vacuolation in the brain stem white matter (arrow) of an E2 115–129-immunized mouse that showed mild clinical signs, 80 \times . C. Marked mononuclear cell leptomeningeal infiltrate adjacent to the cerebrum (upper portion of the field) of a MOG 18–32 immunized mouse that had EAE, and was sacrificed on day 18 post-immunization, 60 \times . D. Marked focal vacuolation (left and far right sides of field) in brain stem white matter of a MOG 18–32-immunized mouse that showed severe clinical disease and died on day 18 pi. Intact myelin is seen in the center right of the field 80 \times . E. Meningeal and parenchymal mononuclear cell infiltration with associated perivascular demyelination in the spinal cord white matter of a MOG 35-immunized mouse with typical signs of EAE, 80 \times . F. White matter vacuolation (arrow) in the cerebellum of a SFV-infected B6 mouse sacrificed on day 21. No inflammation or macrophages are seen in this area, 100 \times .

white matter of B6 mice, following the clearance of SFV on days 15–21 postinfection (Fig. 6F).

CFA-immunized mice showed no clinical signs at any time point and had no CNS inflammation or vacuolation of white matter.

Mice immunized with UV-SFV and with peptides, but without pertussis, to generate T cell proliferative responses did not show any histological evidence of EAE when examined at 4–5 weeks after immunization.

4. Discussion

SFV infection induces viral encephalomyelitis in mice. The virus enters the CNS and multiplies in the brains and spinal cords on days 3–7 following peripheral inoculation with the virus. SFV infection of B6 mice is cleared from the brains and spinal cords by day 10 postinfection (Mokhtarian et al., 1996), and is followed by a secondary demyelination, predominantly affecting cerebellar white matter, at 14 to 21 days postinfection (Fazakerley et al., 1983, 1997; Mokhtarian and Swoveland, 1987). We found that the T cell responses to the viral peptides occur simultaneously with responses to mimicked myelin peptides (Mokhtarian et al., 1994, data not shown), and the demyelination also appeared to be T-cell mediated (Fazakerley et al., 1983; Mokhtarian and Swoveland, 1987). The present findings, however, suggest that an antibody response to an epitope of SFV that exhibits molecular mimicry with a peptide of MOG, contributes to the demyelinating lesions induced following SFV infection. It should be emphasized, however, that these autoimmune mediated lesions are probably initiated and preceded by a T cell mediated inflammatory response at the time of peak clinical disease and viral clearance (Mokhtarian et al., 1996).

SFV infection is the first example in which an *acute* CNS virus infection (i.e., without persistence) triggers an autoimmune mediated CNS injury about 2 weeks after the clearance of virus from the CNS. The cross-reactive T cell responses observed in this study suggested a possible molecular mimicry, i.e., at the level of I-A binding (Babbitt et al., 1986; Hill et al., 1991; Madden et al., 1993; Gautam et al., 1994), between viral and self peptides. These cross-reactive responses were greatest to E2 115–129 and to MOG 18 to 32, a pair of mimicked peptides with the longest continuous stretch of combined complete and partial homologies. These findings indicate that in order for molecular mimicry between two peptides to result in cross proliferation, a minimum of three exact aa homologies combined with partial homologies in the same region, in an uninterrupted manner, was needed.

Immunization of B6 mice with a surface peptide of SFV, E2-115–129, led to an autoimmune lymphocyte proliferative response to its mimicked peptide of MOG,

18–32. Immunization of mice with either of these two peptides induced a similar EAE-like disease with white matter vacuolation. Furthermore, the white matter vacuolation seen in E2 115–129 and MOG 18–32-immunized mice, resembled the secondary demyelination seen in the cerebellar white matter of B6 mice, following the clearance of SFV on days 15–21 postinfection (Fig. 6F). We use the term ‘white matter vacuolation’ rather than ‘secondary demyelination’ because it more accurately describes the histologic appearance of the CNS lesions. Based on clinical and histological features, similar autoimmune responses appear to be the mechanism responsible for the white matter vacuolation (secondary demyelination) seen in the CNS of B6 mice after immunization with E2 115–129 and MOG 18–32, and 15–21 days following SFV-infection.

The significance of the white matter vacuolation, in patients and animal models, and the mechanisms by which it occurs are not completely understood. The majority of E2 115–129-immunized mice that showed clinical disease had white matter vacuolation whereas only one of six E2 115–129-immunized mouse with clinical signs showed CNS inflammation. Similarly, the majority of MOG 18–32 immunized mice which showed clinical signs and were sampled for histology had white matter vacuolation yet only two of seven had CNS inflammation. Despite the low incidence and extent of inflammation in the E2 115–129 and MOG 18–32 immunized mice, clinical disease and white matter vacuolation (as illustrated in Fig. 5) were evident in the majority of these mice. More extensive inflammation with associated vacuolation and clinical disease was present in MOG 35–55 immunized mice, used as positive controls. Neither clinical disease, inflammation or white matter vacuolation were present in CFA-immunized mice, used as negative controls. These findings suggest an association between vacuolation and clinical disease because there were no other histologic abnormalities in these mice that correlate with the presence of clinical signs. Since the majority of E2 115–129 and MOG 18–32 immunized mice were tested after they showed signs of clinical disease, i.e., late, it is possible that inflammation of the CNS of these mice may have resolved by that time.

We speculate that a small, perhaps transient, cellular immune response in the CNS of these peptide-immunized mice might have led to sufficient breakdown of the blood-brain barrier to have permitted the anti-peptide antibodies induced by E2- or MOG peptide immunization to leak into the CNS and induce tissue injury. The support for this interpretation is the fact that the antibody responses to all three peptides were not detected in the sera until 4–5 weeks after immunization, probably after the antibody producing cells migrated from the CNS into the periphery. The role of antibody was especially evident in E2 115–129 immunized mice, in which the later onset of clinical disease coincided with the antibody production (which was earlier than the other two peptide groups). Furthermore,

epitope spreading to other peptides occurred in the E2 115–129 immunized mice. The sera of E2 115–129 immunized mice also cross reacted with MBP 64–75, which has some mimicry with E2 137–151. The role of this epitope spreading is under further investigation. Previous studies have shown that injection of anti-MOG antibodies at the time when blood-brain barrier is breached induces extensive CNS demyelination in Lewis rats (Schluesener et al., 1987; Linington et al., 1988; Piddlesden et al., 1993). We have also found that SFV-infection of B6 mice induced anti MOG antibody with a high titer (data not shown). In recombinant MOG-tolerized marmosets, a pattern of white matter vacuolation similar to the pattern we observed in SFV-infected and peptide-immunized mice, was attributed to pathogenetic autoantibodies (Genain et al., 1996). This is in contrast to the usual TH1 cytokine production seen in acute viral encephalomyelitis (Mokhtarian et al., 1994; Mokhtarian et al., 1996) and EAE (Selmaj and Raine, 1988; Kennedy et al., 1992).

In this report, we have shown that autoimmunity may develop when peptides of a microorganism (such as a virus) have only a short sequence homology with host self peptides. Although there may not be a clear and direct relationship between the induction of clinical disease and the extent of conventional (EAE-like) inflammatory pathology in the CNS of MOG 18–32 and E2 115–129-immunized mice, the use of these peptides and disease and pathology induced by them, permits dissection of both the immune response components and the pathology. Although not all disease manifestations are necessarily attributed to molecular mimicry mechanisms, individual mimicked responses may, nevertheless, make significant contributions both to clinical disease expression and to tissue injury. One important aspect of this model is that the mimicked viral and MOG peptides appear to be the epitope for the pathogenic antibody production. In other words, while T cells reactive with a certain epitope on E2 result in inflammation and clearance of SFV, antibody to E2 115–129 epitope appears to mediate injury to the white matter. Our previous studies and those of others have shown that E2 115–129 is part of a T cell epitope in both BALB/c (H-2^d) and B6 (H-2^b) mice. Furthermore, portions of the SFV T cell epitopes for the induction of antibody are within E2 115–141 and E2 127–151 and a DTH-inducing epitope was found to be within the region of E2 137–151 in BALB/c mice (Snijders et al., 1992).

This study provides further support for the involvement of environmental pathogens in the induction of autoimmune diseases, when an optimal number of autoreactive T and B cells are present in the host. It also shows that whereas T cells are necessary to recruit lymphocytes into the CNS, white matter injury may be mediated by autoantibodies that are cross-reactive with an epitope of a CNS virus.

This work was supported by Maimonides Research and Development Foundation and by NIH grant NS26773.

Acknowledgements

We like to thank Dr. Dj. Shirazian for critically reviewing and Agatha Manganaro for typing the manuscript.

References

- Amor, S., Groome, N., Linington, C., Morris, M.M., Dornmair, K., Gardinier, M.V., 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.
- Babbitt, B.P., Matsueda, G., Haber, E., Unanue, E.R., Allen, P.M., 1986. Antigenic competition at the level of peptide-Ia binding. *Proc. Natl. Acad. Sci. U.S.A.* 83, 4509–4513.
- Beraud, E., Reshef, T., Vandenbark, A.A., Offner, H., Fritz, R., Chou, C.-H., Bernard, D., Cohen, I.R., 1986. Experimental autoimmune encephalomyelitis mediated by T lymphocyte lines: genotype of antigen-presenting cells influences immunodominant epitope of basic protein. *J. Immunol.* 136, 511–517.
- Endoh, M., Kunishita, T., Nihei, J., Nishizawa, M., Tabira, M., 1990. Susceptibility to proteolipid apoprotein and its encephalitogenic determinants in mice. *Int. Arch. Allerg. Appl. Immunol.* 92, 433–438.
- Fazakerley, J.K., Amor, S., Nash, A.A. (1997). *Animal Model Systems of MS*. In: Russel, W.C. (Ed.), *Molecular biology of multiple sclerosis*, Wiley, pp. 255–273.
- Fazakerley, J.K., Amor, S., Webb, H.E., 1983. Reconstitution of Semliki Forest virus infected mice, induces immune mediated pathological changes in the CNS. *Clin. Exp. Immunol.* 52, 115–120.
- Fujinami, R.S., Oldstone, M.B.A., 1985. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 230, 1043–1045.
- Gautam, A.M., Lock, C.B., Smilek, D.E., Pearson, C.I., Steinman, L., McDevitt, H.O., 1994. Minimum structural requirements for peptide presentation major histocompatibility complex class II molecules: implications in induction of autoimmunity. *Proc. Natl. Acad. Sci. U.S.A.* 91, 767–771.
- Genain, C.P., Abel, K., Belmar, N., Vilinger, F., Rosenberg, D.P., Linington, C., Raine, C.S., Huser, S.L., 1996. Late complications of immune deviation therapy in a nonhuman primate. *Science* 274, 2054–2057.
- Greer, J.M., Sobel, R.A., Sette, A., Southwood, S., Lees, M.B., Kuchroo, V.K., 1996. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. *J. Immunol.* 156, 371–379.
- Hill, M.C., Hayball, J.D., Allison, A.A., Rothbard, J.B., 1991. Conformational and structural characteristics of peptides binding to HLA-DR molecules. *J. Immunol.* 147, 189–197.
- Jahnke, U., Fischer, E.H., Alvord, E.C. Jr., 1985. Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. *Science* 229, 282–284.
- Johns, T.G., Kerlero de Rosbo, N., Menon, K.K., Abo, S., Gonzales, M.F., Bernard, C.C.A., 1995. Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J. Immunol.* 154, 5536–5541.
- Johnson, R.T., Griffin, D.E., Hirsch, R.L., Wolinsky, J.S., Roedenbeck, S., De Soriano, I.L., Vaisberg, A., 1984. Measles encephalomyelitis—Clinical and immunologic studies. *N. Engl. J. Med.* 310, 137–141.
- Kennedy, M.K., Torrance, D.S., Picha, K.S., Mohler, K.M., 1992. Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. *J. Immunol.* 149, 2496–2505.
- Kerlero de Rosbo, N., Mendel, I., Ben-Nun, A., 1995. Chronic relapsing experimental autoimmune encephalomyelitis with a delayed onset and an atypical clinical course, induced in PL/J mice by myelin oligodendro-

- drocyte glycoprotein (MOG)-derived peptide: preliminary analysis of MOG T cell epitopes. *Eur. J. Immunol.* 25, 985–993.
- Linington, C., Bradle, M., Lassmann, H., Brunner, C., Vass, K., 1988. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin oligodendrocyte glycoprotein. *Am. J. Pathol.* 130, 443–454.
- Madden, D.R., Garboczi, D.N., Wiley, D.C., 1993. The antigenic identity of peptide-MHC complexes: a comparison of the conformation of five viral peptides presented by HLA-A2. *Cell* 75, 693–708.
- Mamula, M.J., Fatenejad, S., Craft, J., 1994. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J. Immunol.* 152, 1453–1461.
- Massanari, R.M., Paterson, P.Y., Lipton, H.L., 1979. Potentiation of experimental allergic encephalomyelitis in hamsters with persistent encephalitis due to measles virus. *J. Infect. Dis.* 139, 297–303.
- Mendel, I., Kerlero de Rosbo, N., Ben-Nun, A., 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V β expression of encephalitogenic T cells. *Eur. J. Immunol.* 25, 1951–1959.
- Miyazaki, I., Cheung, R.K., Gaedigk, R., Hui, M.F., Van ter Meulen, J., Rajotte, R.V., Dosch, H.M., 1995. T cell activation and anergy to islet cell antigen in type I diabetes. *J. Immunol.* 154, 1461–1469.
- Mokhtarian, F., Swoveland, P., 1987. Predisposition to EAE induction in resistant mice by prior infection with Semliki Forest virus. *J. Immunol.* 138, 3264–3268.
- Mokhtarian, F., McFarlin, D.E., Raine, C.S., 1984. Adoptive transfer of myelin basic protein sensitized T cells produces chronic relapsing demyelinating disease in mice. *Nature* 309, 356–358.
- Mokhtarian, F., Shi, Y., Zhu, P.-F., Grob, D., 1994. Immune responses, and autoimmune outcome, during virus infection of the central nervous system. *Cell. Immunol.* 157, 195–210.
- Mokhtarian, F., Wesselingh, S.L., Choi, S., Maeda, A., Griffin, D.E., Sobel, R.A., Grob, D., 1996. Production and role of cytokines in the CNS of mice with acute viral encephalomyelitis. *J. Neuroimmunol.* 66, 11–22.
- Oldstone, M.B.A., 1987. Molecular mimicry: immunologic cross-reactivity between dissimilar protein (microbial and self) that share common epitopes can lead to autoimmunity. *Cell* 50, 819–820.
- Piddlesden, S.J., Lassmann, H., Zimprich, F., Morgan, B.P., Linington, C., 1993. The demyelinating potential of antibodies to MOG is related to their ability to fix complement. *Am. J. Pathol.* 143, 555–564.
- Raine, C.S., Mokhtarian, F., McFarlin, D.E., 1984. Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse. *Neuropathologic Analysis Lab. Invest.* 51, 534–546.
- Schluessener, H.J., Sobel, R.A., Linington, C., Weiner, H.L., 1987. A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J. Immunol.* 139, 4016–4021.
- Selmaj, K.W., Raine, C.S., 1988. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. *Ann. Neurol.* 23, 339–346.
- Shaw, M.K., Ho Kim, C., Lisak, R.P., Tse, H.Y., 1992. A combination of adoptive transfer and antigenic challenge induces consistent murine experimental autoimmune encephalomyelitis in C57Bl6 mice and other reputed resistant strains. *J. Neuroimmunol.* 39, 139–150.
- Snijders, A., Benissa-Trouw, B.J., Visser-Vernooi, H.J. et al., 1992. A delayed-type hypersensitivity-inducing T cell epitope of Semliki forest virus mediates effective T helper activity for antibody production. *Immunology* 77, 322–329.
- Sobel, R.A., Greer, J.M., Kuchroo, V.K., 1994. Minireview: autoimmune responses to myelin proteolipid protein. *Neurochem. Res.* 19, 915–921.
- Suckling, A.J., Pathak, S., Jagelman, S., Webb, H.E., 1978. Virus-associated demyelination: a model using avirulent Semliki Forest virus infection of mice. *J. Neurol. Sci.* 39, 147–154.
- Tuohy, V.K., Sobel, R.A., Lees, B., 1988. Myelin proteolipid protein-induced experimental allergic encephalomyelitis: variations of disease expression in different strains of mice. *J. Immunol.* 140, 1868–1873.
- Waksman, B., 1985. Mechanisms in multiple sclerosis. *Nature* 318, 104–105.
- Watanabe, R., Wege, H., TerMeulen, V., 1983. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature* 305, 150–153.
- Whitham, R.H., Jones, R.E., Hashim, G.A., Hoy, C.M., Wang, R.Y., Vandenbark, A.A., Offner, H., 1991. Location of a new encephalitogenic epitope (residues 43 to 64) in proteolipid protein that induces relapsing experimental autoimmune encephalomyelitis in PL/J and (SJL \times PL) F_1 mice. *J. Immunol.* 147, 3803–3808.
- Wucherpfenning, K.W., Strominger, J.L., 1995. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 80, 695–705.
- Zamvil, S.S., Mitchell, D.J., Moore, C.A., Kitamura, K., Steinman, L., Rothbard, J.B., 1986. T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature* 324, 258–260.