Published in final edited form as:

J Neuroimmune Pharmacol. 2010 June ; 5(2): 168–175. doi:10.1007/s11481-010-9215-x.

Studies in the Modulation of Experimental Autoimmune Encephalomyelitis

Jane E. Libbey, MS¹, Ikuo Tsunoda, MD, PhD², and Robert S. Fujinami, PhD^{1,*}

¹Department of Pathology, University of Utah School of Medicine, 30 North 1900 East, 3R330 SOM, Salt Lake City, UT 84132

²Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130

Abstract

Experimental autoimmune encephalomyelitis (EAE), an experimental model for multiple sclerosis, can be induced through inoculation with several different central nervous system (CNS) proteins or peptides. Modulation of EAE, resulting in either protection from EAE or enhancement of EAE, can also be accomplished through either vaccination or DNA immunization with molecular mimics of self-CNS proteins. Previously published data on this method of EAE modulation will be reviewed. New data is presented which demonstrates that EAE can also be modulated through the administration of the β -(1,3)-D-glucan, curdlan. Dendritic cells stimulated by curdlan are involved in the differentiation of the interleukin-17 producing subset of CD4⁺ T cells that are recognized effector cells in EAE. Using two different systems to study the effects of curdlan on EAE, it was found that curdlan increased the incidence of EAE and/or the severity of the disease course.

Keywords

Myelin basic protein; myelin proteolipid protein; myelin oligodendrocyte glycoprotein; vaccination; DNA immunization; interleukin-17

Introduction

Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model of multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS) [reviewed in (Tsunoda and Fujinami, 1996)]. The EAE model is used to investigate the possible autoimmune etiology of MS. EAE can be induced in many species of animals, to include the rat, mouse, rabbit, guinea pig and monkey. Factors, such as age, sex and commercial source of the animal, influence an individual animal's susceptibility to the induction of EAE [reviewed in (Tsunoda and Fujinami, 1996)].

Active EAE can be induced through inoculation with spinal cord homogenate or with many different CNS proteins or peptides emulsified in complete Freund's adjuvant (CFA) [reviewed in (Tsunoda and Fujinami, 1996)]. The proteins/peptides commonly used to induce EAE are the proteins that comprise myelin and include myelin basic protein (MBP), myelin proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG)

*Corresponding Author: Robert S. Fujinami, PhD, Department of Pathology, University of Utah School of Medicine, 30 North 1900 East, 3R330 SOM, Salt Lake City, UT 84132, Phone: 801-585-3305, Fax: 801-585-3311, Robert.Fujinami@hsc.utah.edu.

[reviewed in (Tsunoda and Fujinami, 1996)]. PLP is found only in CNS, has a molecular weight of 30 kDa and is highly hydrophobic [reviewed in (Sedzik, 2008)]. PLP is a transmembrane protein that traverses the myelin lipid bilayer four times and as such can act as a proton channel. MBP is a major phosphoprotein of the CNS, has a molecular weight of 18.5 kDa and has a stable coil conformation in solution. A recently proposed function for MBP is in membrane aggregation. MOG is a minor component of the myelin sheath comprising only 0.05-0.1% of the total myelin protein. MOG has a molecular weight of 25 kDa and its function within the CNS remains unknown [reviewed in (Sedzik, 2008)].

Passive EAE can be induced through adoptive transfer of myelin specific T cells into naive animals [reviewed in (Tsunoda and Fujinami, 1996)]. EAE is predominantly mediated by autoreactive CNS specific CD4⁺ T cells; however, myelin specific CD8⁺ T cells also play a role as suppressor/regulatory T cells or effector T cells in EAE pathogenesis. In terms of a regulatory function for CD8⁺ T cells, early studies employing either antibody depletion of CD8⁺ T cells or genetic knockout of the CD8 gene and more recent studies using the B10.PL strain of mice suggest that CD8⁺ T cells may perform a regulatory function in EAE; it has yet to be determined whether the regulatory activity is mediated by myelin specific or nonspecific T cells [reviewed in (Goverman et al, 2005)]. In terms of an effector function for CD8⁺ T cells, Goverman's group has demonstrated that adoptively transferred MBP specific CD8⁺ cytotoxic T lymphocytes (CTL) can mediate autoimmune CNS disease; furthermore, the clinical signs and distribution and type of lesions are distinct from those seen in CD4⁺ T cell-mediated disease and the CD8⁺ CTL-mediated lesions are much more characteristic of MS lesions [(Huseby et al, 2001); reviewed in (Goverman et al, 2005; Ji and Goverman, 2007; Rodriguez, 2007)]. In addition Sun's group has demonstrated that MOG specific CD8⁺ T cells can function as effector cells in EAE and mediate demyelination (Sun et al, 2001). C57BL/6 mice immunized with the MOG₃₅₋₅₅ peptide in CFA generated encephalitogenic CD8⁺ T cells that, upon adoptive transfer, resulted in a more severe disease than direct immunization (Sun et al, 2001). Therefore, both CD4⁺ and CD8⁺ T cells play a role in EAE pathogenesis and most likely MS pathogenesis as both CD4⁺ and CD8⁺ T cells are present in the lesions in both MS and EAE [reviewed in (Johnson et al, 2007)].

The clinical course of EAE can be variable, as is the clinical course of MS. For active EAE, the clinical course depends on both the species of animal inoculated and the particular protein/peptide used for inoculation. For passive EAE, the clinical course again depends on the species of animal inoculated and also on whether CD4⁺ T cells, CD8⁺ T cells or a mixture of both are adoptively transferred. Some animals experience an acute monophasic disease course (progressive) whereas other animals experience a chronic relapsing and remitting disease course [reviewed in (Tsunoda and Fujinami, 1996)]. Clinical signs include weight loss, ataxia, incontinence and flaccid or spastic hind limb paralysis (Fujinami, 2001). Lesions in EAE and early acute lesion in MS are characterized by inflammation and often demyelination; however, the lesions in EAE are predominantly observed in the spinal cord while MS lesions are more common in the brain (Fujinami, 2001).

Modulation of the clinical course of EAE has been accomplished through various means. T helper (Th) 1 cells and Th1-type cytokines [interleukin (IL)-2, tumor necrosis factor (TNF)- α/β , interferon (IFN)- γ] promote EAE, whereas Th2 cells and Th2-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) downregulate the disease [reviewed in (Tsunoda and Fujinami, 1996)]. The clinical course of EAE has also been modulated through the administration of various CNS proteins/peptides. Here we will review the previously published data on modulation of EAE using CNS proteins/peptides and introduce new data on modulation of EAE through the administration of curdlan. Modulation of the clinical course of EAE could be a useful animal model for the disease exacerbations that occur in MS patients in association with viral infections [reviewed in (Libbey and Fujinami, 2009)]. In contrast to

viral infections, no immunization or vaccination has been associated with exacerbations in MS [reviewed in (Fujinami, 2001)]. The development of defined, predictable and reproducible ways to modulate the clinical course of EAE will lead to a greater understanding of the pathogenesis of the disease and to treatments for EAE which may be translated into treatments to prevent either the development of or relapses/exacerbations of MS.

Protection against EAE

One means of modulating EAE is to protect the animal against later development of EAE through vaccination. MBP₁₋₁₁, with the first amino acid acetylated, is the major encephalitogenic peptide from MBP for PL/J mice (Zamvil et al, 1986; Fritz and McFarlin, 1989). PL/J mice infected, via tail scarification [10^6 plaque forming units (pfu) per mouse], with recombinant vaccinia virus (VV) encoding an encephalitogenic peptide of rat MBP (MBP₁₋₂₃) [construction of recombinant VV is as described in (Barnett et al, 1993; Barnett et al, 1996)] do not develop CNS disease and are protected from developing EAE following challenge five weeks later with either whole guinea pig MBP protein (200 µg per mouse) or an acetylated MBP₁₋₂₀ peptide (150 µg per mouse) in CFA (Barnett et al, 1996). Furthermore, the mice were tested for delayed-type hypersensitivity (DTH) reactivity to MBP which is seen in mice that develop EAE. MBP (10 µg) was injected into the right ear of the mice (with PBS as a control in the left ear) 14 days post MBP challenge; it was found that swelling assessed at 24 hours was significantly reduced indicating a decreased DTH response in vaccinated mice (Barnett et al, 1996). The five week recovery time following the vaccination allows the animals to clear VV (McCoy et al, 2006). This protective affect was found to be mouse strain specific (genetic susceptibility) and encephalitogenic determinant specific as well as challenge antigen specific (Barnett et al, 1996). The lack of acetylation of the first amino acid of the MBP peptide as it is synthesized from the recombinant VV could be a factor in the observed protection. In addition, based on the reduced DTH response and other data, the protective affect was hypothesized to be mediated by either anergy or apoptosis of the effector CD4⁺ Th1 cells (Barnett et al, 1996).

Enhancement of EAE

Another way to modulate EAE is to administer substances that enhance the subsequently induced disease. SJL/J mice infected, via tail scarification or intraperitoneal (i.p.) injection (10^6 pfu per mouse), with recombinant VV encoding the complete coding region of rat PLP do not develop CNS disease but are susceptible to development of an enhanced first clinical attack with early onset following challenge five weeks later with various encephalitogenic PLP peptides (PLP₁₃₉₋₁₅₁, modified versions of PLP₁₃₉₋₁₅₁, PLP₁₀₄₋₁₁₇, PLP₁₇₈₋₁₉₁; 150 µg per mouse) in CFA (Barnett et al, 1993; Wang and Fujinami, 1997). Peptides from PLP that are known to be encephalitogenic for SJL mice include PLP₁₀₄₋₁₁₇, PLP₁₃₉₋₁₅₁ and PLP₁₇₈₋₁₉₁ [reviewed in (Sobel et al, 1994; Tuohy, 1994)]. This enhancement affect was found to be challenge antigen specific and was hypothesized to be mediated by a combination of CD4⁺ T cells, CD8⁺ T cells and anti-PLP antibodies (Barnett et al, 1993; Wang and Fujinami, 1997). However, when these mice were followed beyond the enhanced acute phase of disease (2 months post EAE induction), the chronic relapsing-remitting phase of the disease was found to be suppressed as the mice had fewer relapses, decreased CNS pathology (demyelination) and decreased inflammation (meningitis and perivascular cuffing) (Wang et al, 1999). This near permanent remission state was hypothesized to be mediated by decreased T cell reactivity (unresponsiveness or regulated suppression) to the encephalitogenic PLP₁₃₉₋₁₅₁ peptide (Wang et al, 1999).

By using a VV vector to introduce CNS proteins into the animal, the self-CNS proteins are presented by VV infected cells endogenously through the major histocompatibility complex (MHC) class I pathway (Barnett et al, 1993). This route of presentation allows examination of the CD8⁺ T cell response as well as the CD4⁺ T cell response. Other routes for introduction of CNS proteins into the animal, such as subcutaneous immunization with encephalitogenic peptides in CFA, primarily result in presentation of self-CNS proteins exogenously through the MHC class II pathway (Barnett et al, 1993). This route of presentation only allows for examination of the CD4⁺ T cell response.

Enhancement of EAE can also be achieved by administering substances in ways other than through a viral infection. Intramuscular (i.m.) injection of SJL/J mice (3-4 weeks old; Jackson Laboratory & National Cancer Institute) with plasmid constructs (DNA immunization; 100 µg per mouse; mammalian pCMV expression vector driven by CMV promoter and derived by excision of the β-galactosidase gene from pCMV-β) encoding the whole PLP protein or encephalitogenic epitopes of PLP (PLP₁₃₉₋₁₅₁, PLP₁₇₈₋₁₉₁) did not cause CNS disease in and of itself but did result in an enhanced EAE disease following challenge two weeks later with various encephalitogenic PLP peptides (modified PLP₁₃₉₋₁₅₁, PLP₁₇₈₋₁₉₁; 100 nmol per mouse) in CFA (Tsunoda et al, 1998). These mice experienced more severe disease and frequent relapses when followed for 2-3 months post EAE induction. This enhancement affect was found to be challenge antigen specific and was hypothesized to be mediated by CD4⁺ T cells and anti-PLP antibodies (Tsunoda et al, 1998).

In contrast, another group has demonstrated that DNA immunization could protect the animal against later development of EAE (Ruiz et al, 1999; Garren et al, 2001). In their initial study, i.m. injection of SJL/J mice (6-8 weeks old; Jackson Laboratory) with a plasmid construct (50 µg per mouse; mammalian pTarget expression vector driven by CMV promoter) encoding PLP₁₃₉₋₁₅₁ ameliorated the acute clinical disease that developed following challenge 10 days later with PLP₁₃₉₋₁₅₁ peptide (100 µg per mouse) in CFA (Ruiz et al, 1999). These animals were not followed beyond resolution of the acute phase of the clinical disease. Differences between this study (Ruiz et al, 1999) and the previous study (Tsunoda et al, 1998), that may explain the disparity in the results, include the age and source of the animals used, the DNA vector used, the number of immunizations, the timing of immunizations, the site of immunization, the concentration of immunogen, the timing of the challenge and the length of time the mice were observed following EAE induction. The protective affect was hypothesized to be mediated by anergy of pathogenic T cells (Ruiz et al, 1999).

In a second study, this group injected SJL/J mice (6-8 weeks old; Jackson Laboratory) i.m. with a plasmid construct (100 µg per mouse; pTarget) encoding the same encephalitogenic epitope of PLP as was used above (PLP₁₃₉₋₁₅₁), however this injection was paired with injection of a plasmid construct (100 µg per mouse) encoding the full-length mouse IL-4 protein (Garren et al, 2001). The mice were challenged 7-10 days later with PLP₁₃₉₋₁₅₁ peptide (100 µg per mouse) in CFA. Though there are minor differences in the immunization regimen (age and source of the animals, DNA vector, number of immunizations, timing of immunizations, site of immunization, timing of challenge) between this study demonstrating protection from EAE (Garren et al, 2001) and the previous study demonstrating enhancement of EAE (Tsunoda et al, 1998), the major difference is the presence of IL-4. The protective affect was hypothesized to be mediated by IL-4; secreted IL-4 could act on autoreactive T cells through the IL-4 receptor to activate STAT6 (signal transducer and activator of transcription 6) which in turn results in a shift in the T cells cytokine profile to a Th2-type cytokine profile (more IL-4 and IL-10; less IFN-γ) (Garren et al, 2001).

Subclinical Priming and Subsequent Triggering of EAE

Yet another way to modulate EAE is to subclinically prime the animal with a virus encoding molecular mimics (immunologically cross-reactive epitopes) of self-CNS antigens followed by a nonspecific immunologic stimulus that triggers disease. SJL/J mice infected, via i.p. injection (5×10^6 pfu per mouse), with recombinant VV encoding the entire coding regions of PLP, myelin associated glycoprotein (MAG) or glial fibrillary acidic protein (GFAP) do not develop CNS disease but are susceptible to development of EAE disease following challenge five weeks later with a nonspecific immunostimulant (CFA) (Theil et al, 2001). This priming affect of the viral infection was found to be mouse strain specific (genetic susceptibility). Additional experiments priming SJL/J mice via DNA immunization with a plasmid construct (100 μ g per mouse; i.m. injection) encoding the entire coding region of PLP fused to ubiquitin (to enhance processing through the MHC class I pathway and induction of CD8⁺ T cells) and challenging (one week later) with nonspecific immunostimulants (CFA or a control VV not encoding self-CNS antigens) showed some susceptibility to disease development; however live virus priming resulted in more serious consequences than priming with plasmid DNA (90% of mice developing overt CNS disease compared to 20% of mice developing CNS inflammation, respectively). This priming affect of the DNA immunization was found to be MHC class I specific. Furthermore, the priming agent, either live virus or plasmid DNA, must encode a molecular mimic of a self-CNS antigen, whereas the triggering agent can be nonspecific and need not enter the CNS (Theil et al, 2001). Thus activation of antigen specific cells induced by molecular mimicry can be achieved through nonspecific immunostimulation (bystander activation) (McCoy et al, 2006; Tsunoda et al, 2007) or subtle cross-reactions [(Selin et al, 2004; Kim et al, 2005) reviewed in (Welsh et al, 2006; Selin et al, 2006)].

In an extension of the DNA immunization portion of the above study, SJL/J mice were subclinically primed with the same DNA construct as was used above (PLP fused to ubiquitin), however the trigger in this case was encephalitogenic PLP₁₃₉₋₁₅₁ peptide (100 nmol per mouse) in CFA instead of the nonspecific immunostimulants that were used previously (Theil et al, 2008). Priming and challenging mice in this way resulted in downregulation of the immune response, milder clinical disease, fewer CNS lesions, decreased lymphoproliferative response, decreased IFN- γ production and increased IL-4 production, during the relapse after EAE induction. This decreased immune response was hypothesized to be mediated by activated and expanded CD8⁺ T cells which in turn were regulating the CD4⁺ Th1 encephalitogenic T cells (Theil et al, 2008).

An extension of the live viral subclinical priming portion of the above study was performed in which the nonspecific immunostimulant (CFA) used as challenge was replaced by infection with various live viruses (Tsunoda et al, 2007). SJL/J mice infected, via i.p. injection (10^6 pfu per mouse), with recombinant VV encoding the entire coding region of PLP again did not develop CNS disease. These animals were then challenged five weeks later via i.p. injection with wild-type VV (WR strain; 10^6 pfu per mouse), lymphocytic choriomeningitis virus (LCMV, Armstrong strain; 2.9×10^4 pfu per mouse) or murine cytomegalovirus (MCMV, Smith strain; 2×10^4 pfu per mouse). The outcome after challenge with various live viruses depended on the virus used in the challenge. Mice challenged with wild-type VV or LCMV did not develop EAE whereas mice challenged with MCMV developed CNS inflammation (meningitis and perivascular cuffing) and clinical signs of disease (weight loss and righting reflex disturbances). The ability of MCMV to induce disease in primed mice was hypothesized to be due to the induction of IL-12; IL-12 facilitates the production of IFN- γ by natural killer (NK) cells early after infection and IL-12 and IFN- γ would then activate autoimmune Th1 cells (McCoy et al, 2006; Tsunoda et al, 2007).

Summary of Modulation of EAE using CNS Proteins/Peptides

The consequence of vaccination or immunization with self-CNS proteins (molecular mimics), introduced via replicating organisms (live virus) or plasmid DNA, is variable, unpredictable and likely depends on the antigen, the timing of immunization and the antigen delivery vehicle (Whitton and Fujinami, 1999). If, however, the animal is subclinically primed for the development of disease by either live virus or plasmid DNA, then induction of disease can be accomplished through either molecular mimicry and/or bystander activation by cytokines (i.e. IL-12 and IFN- γ) (McCoy et al, 2006). A summary of the effects of vaccination, DNA immunization and subclinical priming on EAE is given in Table 1.

Modulation of the Disease Course of EAE with Curdlan

Curdlan is a neutral (in charge), essentially linear extracellular polysaccharide (EPS) produced by *Agrobacterium sp.* [reviewed in (McIntosh et al, 2005; Laroche and Michaud, 2007)]. The backbone of the curdlan EPS, a homoglycan, is β -(1,3) linked glucopyranosyl residues (Figure 1). Curdlan molecules may be comprised of as many as 12,000 glucose units, are insoluble in water, alcohols and most organic solvents, but are soluble in dilute bases, dimethylsulphoxide (DMSO) and formic acid. This β -(1,3)-D-glucan, which is tasteless, colorless and odorless, has unique gel-forming properties [reviewed in (McIntosh et al, 2005; Laroche and Michaud, 2007)].

In addition to its gel-forming properties, curdlan has been suggested to be a biological response modifier with the ability to modulate the immune system [reviewed in (McIntosh et al, 2005; Laroche and Michaud, 2007)]. Some of the suggested immunomodulating effects include immunopotentiality, anti-tumorigenicity, anti-infectivity, anti-inflammation, wound repair, radioprotection and anti-coagulation. The bioactivities of β -(1,3)-D-glucans have been shown to be enhanced through modification, such as sulfation, of the polysaccharide. These effects are thought to occur through interactions between the polysaccharide and macrophages or other cells of the immune system via soluble or cell-bound receptors of the innate immune system [reviewed in (McIntosh et al, 2005; Laroche and Michaud, 2007)]. More specifically, it has been reported that curdlan is able to activate antigen presenting cells (macrophages and dendritic cells) that express Dectin-1, a C-type lectin that functions as a receptor for curdlan (Yoshitomi et al, 2005; LeibundGut-Landmann et al, 2007). Dectin-1, stimulated by curdlan, signals either through the spleen tyrosine kinase (Syk) and a caspase-recruitment domain (CARD) adaptor protein, CARD9, or through the p21-activated kinase (Pak) and the Src tyrosine kinase and then through the Raf-1 serine/threonine kinase to activate nuclear factor κ B (NF- κ B) which induces dendritic cell maturation and secretion of the proinflammatory cytokines TNF- α , IL-6 and IL-23 (Yoshitomi et al, 2005; LeibundGut-Landmann et al, 2007; Ruland, 2008; Gringhuis et al, 2009). The activated dendritic cells (efficiently costimulated through CD40-CD40L by T cells), TNF- α , IL-23 and IL-6, together with transforming growth factor (TGF)- β (most likely contributed by dendritic cells or CD25⁺ regulatory T cells), are involved in the differentiation of the IL-17-producing subset of CD4⁺ effector T cells (Th17 cells), and three of these cytokines (TNF- α , IL-6 and TGF- β), as well as the Th17 cells and IL-17 which they produce, are important in EAE pathogenesis (MOG₃₅₋₅₅- and PLP₁₃₉₋₁₅₁-induced in mice; MBP₆₈₋₈₆-induced in rats) (Langrish et al, 2005; Veldhoen et al, 2006; Hofstetter et al, 2007; LeibundGut-Landmann et al, 2007; Iezzi et al, 2009; Xie et al, 2009).

With the emergence of data supporting the role of Th17 cells as effector cells in EAE [reviewed in (Aranami and Yamamura, 2008; Klemann et al, 2009)], we set out to determine whether modulation of these cells, through the administration of curdlan, would result in

modulation of EAE. Since curdlan acts as a Th17 inducer, one would expect exacerbation of EAE upon curdlan administration. However, the effect of curdlan administration may depend on the timing of curdlan administration, the route of curdlan administration, the concentration of the curdlan, the type of EAE inducer used and the type of EAE induced.

Currently we have tested curdlan administration in two different conditions. First, we have tested curdlan administration in MOG₉₂₋₁₀₆-induced EAE in SJL/J mice (unpublished data, Tsunoda-I & Fujinami-RS). Screening of 15-mer peptides, with an 8 amino acid overlap, covering the entire MOG molecule demonstrated that only the MOG₉₂₋₁₀₆ peptide was encephalitogenic in SJL mice (Amor et al, 1994). Sensitization of SJL/J mice with MOG₉₂₋₁₀₆ in CFA (no curdlan) resulted in 1/5 mice (20%) developing relapsing-remitting (RR) EAE with disease onset on day 30 (unpublished data, Tsunoda-I & Fujinami-RS). When the CFA was replaced with curdlan (500 µg per mouse) in incomplete Freund's adjuvant (IFA), 4/5 mice (80%) developed primary progressive (PP) EAE. When curdlan (5 mg per mouse) was administered i.p. to sensitized mice after disease onset (day 53), the one mouse (out of 5; 20%) that had an exacerbation (and would have gone on to develop RR-EAE) lost weight and died 5 days after curdlan administration and two other mice (out of 5; 40%) developed PP-EAE. When curdlan (5 mg per mouse) was administered i.p. one day prior to sensitization of the mice, 3/5 mice developed PP-EAE and 1/5 mice developed secondary progressive (SP) EAE, for a total of 4/5 mice (80%) developing a progressive form of EAE (unpublished data, Tsunoda-I & Fujinami-RS). Therefore administration of curdlan either prior to or at the time of sensitization of SJL/J mice with MOG₉₂₋₁₀₆ resulted in the conversion of a low frequency RR disease to a high frequency progressive disease. Curdlan was also able to convert the disease from an RR course to a progressive course even if given after the onset of disease though at a slightly lower frequency.

Second, we have tested curdlan administration in SJL/J mice subclinically primed with recombinant VV encoding the complete coding region of rat PLP (unpublished data, Tsunoda-I & Fujinami-RS). As described above these subclinically primed mice do not develop CNS disease but were susceptible to the development of disease following administration of a nonspecific immunostimulant (CFA) or infection with MCMV (Theil et al, 2001; Tsunoda et al, 2007). When curdlan (500 µg per mouse) in IFA was administered subcutaneously five weeks after sensitization, all 10 mice (100%) showed mild righting reflex disturbances (clinical signs of disease) and 4/5 mice (80%) showed mild CNS inflammation (meningitis and perivascular cuffing) at 20 days post curdlan challenge (unpublished data, Tsunoda-I & Fujinami-RS). When curdlan (5 mg per mouse) was administered i.p. five weeks after sensitization, only 4/10 mice (40%) showed mild righting reflex disturbances (clinical signs of disease) and there was no obvious neuropathology at 12-14 days post curdlan challenge (unpublished data, Tsunoda-I & Fujinami-RS). Although the curdlan/IFA group received a ten times smaller dose than the curdlan i.p. group, the curdlan/IFA group had a higher frequency of clinical signs and more severe neuropathology. Therefore the route of curdlan administration and the inclusion of IFA may be important factors in disease modulation in this situation.

Summary of Modulation of the Disease Course of EAE with Curdlan

In the two situations tested, MOG₉₂₋₁₀₆-induced EAE in SJL/J mice and SJL/J mice subclinically primed with recombinant VV encoding the complete coding region of rat PLP, curdlan administration appeared to increase the incidence of EAE and/or the severity of the disease course. A summary of the effects of curdlan when used as adjuvant, as a treatment or for the secondary challenge is given in Table 2. These effects likely resulted from curdlan's ability to induce Th17 cells which acted as effector cells. Therefore it appears feasible to modulate EAE by modulating the type of effector cells. Future plans include testing curdlan

administration in other EAE systems. Two of these systems are PLP₁₃₉₋₁₅₁-induced EAE in SJL/J mice and MOG₃₅₋₅₅-induced EAE in C57BL/6 mice. Once modulation with curdlan has been shown to be predictable, then testing of compounds that suppress Th17 cells rather than induce them is in order.

Conclusions

Here we reviewed the previously published data on modulation of EAE using CNS proteins/peptides and introduce new data on modulation of EAE through the administration of curdlan. As modulation of the clinical course of EAE using CNS proteins/peptides is variable, unpredictable and depends on many factors, the usefulness of this means of EAE modulation is minimized. A more direct approach is modulation of EAE through the administration of specific compounds. The administration of curdlan seems to increase the incidence and/or the severity of EAE. This knowledge and an understanding of the mechanism of action of curdlan may lead to the development of defined, predictable and reproducible treatments for the prevention of disease development and/or for the prevention of disease relapses/exacerbations. Once treatments for EAE have been established, these treatments may be translated into treatments for MS. In this way, a predictable means of treating EAE may suggest a predictable means of treating MS.

Acknowledgments

We wish to thank Nikki J. Kirkman, BS, Faris Hasanovic, BS, Daniel J. Doty, and Krystal D. Porter, BS, for excellent technical assistance. We wish to acknowledge Kathleen Borick for the outstanding preparation of the manuscript. This work was supported by funding from NIH 1R01AI058986.

This work was supported by NIH grant 1P01AI058105.

References

- Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu J-M, Baker D. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol* 1994;153:4349–4356. [PubMed: 7525700]
- Aranami T, Yamamura T. Th17 Cells and autoimmune encephalomyelitis (EAE/MS). *Allergol Int* 2008;57:115–120. [PubMed: 18427164]
- Barnett LA, Whitton JL, Wada Y, Fujinami RS. Enhancement of autoimmune disease using recombinant vaccinia virus encoding myelin proteolipid protein. *J Neuroimmunol* 1993;44:15–25. published erratum appears in *J Neuroimmunol* 48:120, 1993. [PubMed: 7684398]
- Barnett LA, Whitton JL, Wang LY, Fujinami RS. Virus encoding an encephalitogenic peptide protects mice from experimental allergic encephalomyelitis. *J Neuroimmunol* 1996;64:163–173. [PubMed: 8632058]
- Fritz RB, McFarlin DE. Encephalitogenic epitopes of myelin basic protein. *Chem Immunol* 1989;46:101–125. [PubMed: 2482036]
- Fujinami RS. Can virus infections trigger autoimmune disease? *J Autoimmun* 2001;16:229–234. [PubMed: 11334487]
- Garren H, Ruiz PJ, Watkins TA, Fontoura P, Nguyen L-VT, Estline ER, Hirschberg DL, Steinman L. Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway. *Immunity* 2001;15:15–22. [PubMed: 11485734]
- Goverman J, Perchellet A, Huseby ES. The role of CD8⁺ T cells in multiple sclerosis and its animal models. *Curr Drug Targets Inflamm Allergy* 2005;4:239–245. [PubMed: 15853746]
- Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Wevers B, Bruijns SCM, Geijtenbeek TBH. Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-κB activation through Raf-1 and Syk. *Nat Immunol* 2009;10:203–213. [PubMed: 19122653]

- Hofstetter HH, Toyka KV, Tary-Lehmann M, Lehmann PV. Kinetics and organ distribution of IL-17-producing CD4 cells in proteolipid protein 139-151 peptide-induced experimental autoimmune encephalomyelitis of SJL mice. *J Immunol* 2007;178:1372–1378. [PubMed: 17237384]
- Huseby ES, Liggitt D, Brabb T, Schnabel B, Öhlén C, Goverman J. A pathogenic role for myelin-specific CD8⁺ T cells in a model for multiple sclerosis. *J Exp Med* 2001;194:669–676. [PubMed: 11535634]
- Iezzi G, Sonderegger I, Ampenberger F, Schmitz N, Marsland BJ, Kopf M. CD40-CD40L cross-talk integrates strong antigenic signals and microbial stimuli to induce development of IL-17-producing CD4⁺ T cells. *Proc Natl Acad Sci USA* 2009;106:876–881. [PubMed: 19136631]
- Ji Q, Goverman J. Experimental autoimmune encephalomyelitis mediated by CD8⁺ T cells. *Ann N Y Acad Sci* 2007;1103:157–166. [PubMed: 17376824]
- Johnson AJ, Suidan GL, McDole J, Pirko I. The CD8 T cell in multiple sclerosis: Suppressor cell or mediator of neuropathology? *Int Rev Neurobiol* 2007;79:73–97. [PubMed: 17531838]
- Kim S-K, Cornberg M, Wang XZ, Chen HD, Selin LK, Welsh RM. Private specificities of CD8 T cell responses control patterns of heterologous immunity. *J Exp Med* 2005;201:523–533. [PubMed: 15710651]
- Klemann C, Je Raveney B, Oki S, Yamamura T. Retinoid signals and Th17-mediated pathology. *Jpn J Clin Immunol* 2009;32:20–28.
- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005;201:233–240. [PubMed: 15657292]
- Laroche C, Michaud P. New developments and prospective applications for β (1,3) glucans. *Recent Pat Biotechnol* 2007;1:59–73. [PubMed: 19075833]
- LeibundGut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV, Schweighoffer E, Tybulewicz V, Brown GD, Ruland J, Reis e Sousa C. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007;8:630–638. [PubMed: 17450144]
- Libbey JE, Fujinami RS. Potential Triggers of MS. *Results Probl Cell Differ*. 2009 epub ahead of print (1-8-09).
- McCoy L, Tsunoda I, Fujinami RS. Multiple sclerosis and virus induced immune responses: Autoimmunity can be primed by molecular mimicry and augmented by bystander activation. *Autoimmunity* 2006;39:9–19. [PubMed: 16455578]
- McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1 $\rightarrow$ 3)- β -D-glucans. *Appl Microbiol Biotechnol* 2005;68:163–173. [PubMed: 15818477]
- Rodriguez M. Effectors of demyelination and remyelination in the CNS: implications for multiple sclerosis. *Brain Pathol* 2007;17:219–229. [PubMed: 17388953]
- Ruiz PJ, Garren H, Ruiz IU, Hirschberg DL, Nguyen L-VT, Karpuj MV, Cooper MT, Mitchell DJ, Fathman CG, Steinman L. Suppressive immunization with DNA encoding a self-peptide prevents autoimmune disease: modulation of T cell costimulation. *J Immunol* 1999;162:3336–3341. [PubMed: 10092787]
- Ruland J. CARD9 signaling in the innate immune response. *Ann N Y Acad Sci* 2008;1143:35–44. [PubMed: 19076343]
- Sedzik J. Myelin sheaths and autoimmune response induced by myelin proteins and alphaviruses. I. Physicochemical background. *Curr Med Chem* 2008;15:1899–1910. [PubMed: 18691047]
- Selin LK, Brehm MA, Naumov YN, Cornberg M, Kim S-K, Clute SC, Welsh RM. Memory of mice and men: CD8⁺ T-cell cross-reactivity and heterologous immunity. *Immunol Rev* 2006;211:164–181. [PubMed: 16824126]
- Selin LK, Cornberg M, Brehm MA, Kim SK, Calcagno C, Ghersi D, Puzone R, Celada F, Welsh RM. CD8 memory T cells: cross-reactivity and heterologous immunity. *Semin Immunol* 2004;16:335–347. [PubMed: 15528078]
- Sobel RA, Greer JM, Kuchroo VK. Minireview: Autoimmune responses to myelin proteolipid protein. *Neurochem Res* 1994;19:915–921. [PubMed: 7528354]

- Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS. Myelin antigen-specific CD8⁺ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 2001;166:7579–7587. [PubMed: 11390514]
- Theil DJ, Libbey JE, Rodriguez F, Whitton JL, Tsunoda I, Derfuss TJ, Fujinami RS. Targeting myelin proteolipid protein to the MHC class I pathway by ubiquitination modulates the course of experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2008;204:92–100. [PubMed: 18706703]
- Theil DJ, Tsunoda I, Rodriguez F, Whitton JL, Fujinami RS. Viruses can silently prime for and trigger central nervous system autoimmune disease. *J NeuroVirol* 2001;7:220–227. [PubMed: 11517396]
- Tsunoda I, Fujinami RS. Two models for multiple sclerosis: Experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *J Neuropathol Exp Neurol* 1996;55:673–686. [PubMed: 8642393]
- Tsunoda I, Kuang L-Q, Tolley ND, Whitton JL, Fujinami RS. Enhancement of experimental allergic encephalomyelitis (EAE) by DNA immunization with myelin proteolipid protein (PLP) plasmid DNA. *J Neuropathol Exp Neurol* 1998;57:758–767. [PubMed: 9720491]
- Tsunoda I, Libbey JE, Fujinami RS. Sequential polymicrobial infections lead to CNS inflammatory disease: Possible involvement of bystander activation in heterologous immunity. *J Neuroimmunol* 2007;188:22–33. [PubMed: 17604850]
- Tuohy VK. Peptide determinants of myelin proteolipid protein (PLP) in autoimmune demyelinating disease: a review. *Neurochem Res* 1994;19:935–944. [PubMed: 7528355]
- Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals mediated by transforming growth factor- β initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol* 2006;7:1151–1156. [PubMed: 16998492]
- Wang L-Y, Fujinami RS. Enhancement of EAE and induction of autoantibodies to T-cell epitopes in mice infected with a recombinant vaccinia virus encoding myelin proteolipid protein. *J Neuroimmunol* 1997;75:75–83. [PubMed: 9143240]
- Wang L-Y, Theil DJ, Whitton JL, Fujinami RS. Infection with a recombinant vaccinia virus encoding myelin proteolipid protein causes suppression of chronic relapsing-remitting experimental allergic encephalomyelitis. *J Neuroimmunol* 1999;96:148–157. [PubMed: 10337913]
- Welsh RM, Kim SK, Cornberg M, Clute SC, Selin LK, Naumov YN. The privacy of T cell memory to viruses. *Curr Top Microbiol Immunol* 2006;311:117–153. [PubMed: 17048707]
- Whitton JL, Fujinami RS. Viruses as triggers of autoimmunity: facts and fantasies. *Curr Opin Microbiol* 1999;2:392–397. [PubMed: 10458987]
- Xie L, Li X-K, Funeshima-Fuji N, Kimura H, Matsumoto Y, Isaka Y, Takahara S. Amelioration of experimental autoimmune encephalomyelitis by curcumin treatment through inhibition of IL-17 production. *Int Immunopharmacol* 2009;9:575–581. [PubMed: 19539560]
- Yoshitomi H, Sakaguchi N, Kobayashi K, Brown GD, Tagami T, Sakihama T, Hirota K, Tanaka S, Nomura T, Miki I, Gordon S, Akira S, Nakamura T, Sakaguchi S. A role for fungal β -glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J Exp Med* 2005;201:949–960. [PubMed: 15781585]
- Zamvil SS, Mitchell DJ, Moore AC, Kitamura K, Steinman L, Rothbard JB. T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature* 1986;324:258–260. [PubMed: 2431317]

Figure 1.
Structure of curdlan.

Table 1

Summary of modulation of EAE using CNS proteins/peptides

Mouse strain	Mode of administration	Protein/peptide	Secondary challenge	Effect on EAE
PL/J	Viral Infection	MBP ₁₋₂₃	Whole MBP Acetylated MBP ₁₋₂₀	Protection
SJL/J		PLP	Vaccinia virus LCMV	
			MCMV	Develop EAE
		PLP MAG GFAP	CFA	90% of mice have overt CNS disease
		PLP	PLP ₁₃₉₋₁₅₁ Modified PLP ₁₃₉₋₁₅₁ PLP ₁₀₄₋₁₁₇ PLP ₁₇₈₋₁₉₁	Enhanced acute phase and suppressed chronic phase
	DNA Immunization	PLP ₁₃₉₋₁₅₁ PLP ₁₃₉₋₁₅₁ + IL-4 Ubiquitin-PLP	PLP ₁₃₉₋₁₅₁	Protection
			CFA Vaccinia virus	Suppressed chronic phase 20% of mice have CNS inflammation
		PLP PLP ₁₃₉₋₁₅₁ PLP ₁₇₈₋₁₉₁	Modified PLP ₁₃₉₋₁₅₁ or PLP ₁₇₈₋₁₉₁	Enhancement

CFA complete Freund's adjuvant, CNS central nervous system, EAE experimental autoimmune encephalomyelitis, GFAP glial fibrillary acidic protein, IL-4 interleukin-4, LCMV lymphocytic choriomeningitis virus, MAG myelin associated glycoprotein, MBP myelin basic protein, MCMV murine cytomegalovirus, PLP myelin proteolipid protein

Table 2

Effects of Curdlan as Adjuvant and Treatment

Mouse Strain	EAE Induction	Adjuvant	Treatment	Disease & Day of Onset	Days of Death
SJL/J	MOG ₃₂₋₁₀₆	CFA	None	1/5 RR @ day 30	–
		IFA + Curdlan	None	4/5 PP	24, 24, 34, 30
		CFA	Curdlan i.p. day 53	2/5 PP	64, 103
		CFA	Curdlan i.p. day -1	1/5 (weight loss only) (would be RR if lived) 3/5 PP	58 21, 32, 34

CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis; IFA, incomplete Freund's adjuvant; i.p., intraperitoneal injection; MOG, myelin oligodendrocyte glycoprotein; PP, primary progressive; RR, relapsing-remitting; SP, secondary progressive.

Table 3

Effects of Curdlan as Secondary Challenge.

Mouse Strain	Mode of Administration	Protein	Secondary Challenge	Effect on Mice
SJL/J	Viral Infection	PLP	CFA	Develop EAE
			MCMV	
			IFA + Curdlan subcutaneously	10/10 mild righting reflex disturbance 4/5 mild CNS inflammation
			Curdlan i.p.	4/10 mild righting reflex disturbance

CFA, complete Freund's adjuvant; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; IFA, incomplete Freund's adjuvant; i.p., intraperitoneal injection; MCMV, murine cytomegalovirus; PLP, myelin proteolipid protein;