Published in final edited form as:

Vaccine. 2011 April 12; 29(17): 3356–3362. doi:10.1016/j.vaccine.2010.08.103.

Experimental Autoimmune Encephalomyelitis as a Testing Paradigm for Adjuvants and Vaccines

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Abstract

Experimental autoimmune encephalomyelitis (EAE) is an experimental model for multiple sclerosis. EAE can be induced by inoculation with central nervous system (CNS) proteins or peptides emulsified in complete Freund's adjuvant. Protection from EAE, enhancement of EAE or subclinical priming for EAE can occur as a result of either live viral infection or DNA immunization with molecular mimics of CNS proteins or peptides. Here we review the published data describing modulation of EAE through administration of various CNS proteins/peptides introduced via live virus or plasmid DNA and modulation of EAE through choice of adjuvant (immunostimulating agents).

Keywords

Experimental Autoimmune Encephalomyelitis; Vaccination; Adjuvant

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) for which experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model (reviewed in [1]). EAE can be induced in the rat, mouse, rabbit, guinea pig and monkey. EAE can be induced through inoculation with spinal cord homogenate or with many of the CNS proteins or peptides that comprise myelin, emulsified in complete Freund's adjuvant (CFA) (reviewed in [1]). CFA is an oil-in-water emulsion containing killed mycobacteria [2]. Myelin proteins/peptides commonly used to induce EAE include myelin proteolipid protein (PLP), myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) (reviewed in [1]). PLP has a molecular weight of 30 kDa and is found only in the CNS (reviewed in [3]). PLP is a highly hydrophobic transmembrane protein that crosses the lipid bilayer of myelin four times and functions as a proton channel. MBP has a molecular weight of 18.5 kDa and is a major phosphoprotein of the CNS. MBP has a stable coil conformation in solution and may function in membrane aggregation. MOG has a molecular weight of 25 kDa and is only a minor constituent of myelin, contributing only 0.05–0.1% to the total myelin protein. MOG's function within the CNS is as yet unknown (reviewed in [3]).

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Like MS, the clinical course of EAE can be variable depending on the particular myelin protein/peptide used for inoculation and the species and strain of animal inoculated. An individual animal's susceptibility to the induction of EAE is influenced by such factors as age, sex and commercial source of the animal (reviewed in [1]). The two common disease courses that animals experience are an acute monophasic disease course (with or without progression) and a chronic relapsing-remitting disease course (reviewed in [1]). For example and for reference throughout the rest of the text, inoculation of PL/J mice with MBP generally results in an acute monophasic disease without progression [4], inoculation of SJL/J mice with PLP results in a relapsing-remitting disease [5] and inoculation of A.SW and SJL/J mice with MOG₉₂₋₁₀₆ results in chronic progressive disease and relapsing-remitting disease, respectively [6;7]. The acute monophasic disease course may be a more pertinent model for acute disseminated encephalomyelitis, but the relapsing-remitting and chronic progressive disease courses are pertinent models for MS [8]. No matter the disease course, weight loss, ataxia, incontinence and flaccid or spastic hind limb paralysis are common clinical signs of EAE [9]. In general, EAE has been used to model the inflammatory aspects of MS [10]. CNS lesions in both EAE and MS are characterized by inflammation and demyelination; however, MS lesions are commonly found in the brain whereas EAE lesions are predominantly observed in the spinal cord [9]. EAE studies have led to the development of several medications approved for use in MS including glatiramer acetate, mitoxantrone and natalizumab; however, numerous other therapeutics that showed promise in EAE were found to be ineffective or detrimental in MS [10;11]. Therefore, although EAE does not reproduce all aspects of MS pathology, it is still useful and the most widely accepted animal model of MS [11].

In addition to EAE induction through direct inoculation (active EAE), EAE can be transferred to naïve animals through adoptive transfer of myelin specific T cells (reviewed in [1]). Although EAE is predominantly mediated by myelin specific CD4⁺ T cells, myelin specific CD8⁺ T cells also play a role in EAE pathogenesis (reviewed in [1]). Both CD4⁺ and CD8⁺ T cells are present in MS and EAE lesions (reviewed in [12]). A regulatory function of CD8⁺ T cells in EAE has been demonstrated using either antibody depletion of CD8⁺ T cells, genetic knockout of the CD8 gene or the B10.PL strain of mice (reviewed in [13]). Whether the regulatory activity is mediated by myelin specific or nonspecific T cells has yet to be determined (reviewed in [13]). An effector function of CD8⁺ T cells in EAE has also been demonstrated. First, adoptively transferred MBP specific CD8⁺ cytotoxic T lymphocytes can mediate autoimmune CNS disease ([14];reviewed in [13;15;16]). The clinical signs, lesion distribution and lesion type are distinct between CD4⁺ T cell-mediated disease and CD8⁺ T cell-mediated disease, and the lesions in CD8⁺ T cell-mediated disease are very representative of MS lesions ([14];reviewed in [13;15;16]). Second, adoptively transferred MOG specific CD8⁺ T cells can mediate autoimmune CNS disease [17]. Adoptive transfer of MOG specific CD8⁺ T cells resulted in a more severe disease and demyelination than direct inoculation with the MOG₃₅₋₅₅ peptide in CFA [17].

Until recently, all EAE models required either direct inoculation (active EAE) or adoptive transfer (passive EAE) for disease induction. Through the use of new transgenic mouse models, the interactions of autoimmune T and B cells and their contributions to the pathogenesis of EAE can be examined in the context of the spontaneous development of disease [18]. A relapsing-remitting disease spontaneously developed in transgenic mice, on a SJL/J background, that expressed a T cell receptor specific for MOG₉₂₋₁₀₆, in the context of I-A^S. This development of disease was dependent on the presence of B cells and MOG. In this model the transgenic autoimmune T cells functioned as helper cells which caused endogenous B cells autoreactive to MOG to expand and to produce autoantibodies pathogenic for the native MOG protein. Importantly, the spontaneous relapsing-remitting

disease in this animal model recapitulates the major features of the most common type of human inflammatory demyelinating disease, relapsing-remitting MS [18].

Modulation of the disease course of EAE has been accomplished through the administration of various types of cells, various types of cytokines and various CNS proteins/peptides. In terms of cells and cytokines, in general, administration of T helper (Th) 1 cells and Th1-type cytokines [interleukin (IL)-2, tumor necrosis factor (TNF)- α/β , interferon (IFN)- γ] promotes EAE, whereas administration of Th2 cells and Th2-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) ameliorates EAE (reviewed in [1]). Although IL-12 (p40p35 heterodimer) plays an important role in Th1 development and can exacerbate EAE, EAE can develop in its absence (p35 deficient mice), suggesting a functional redundancy, possibly through the p40 subunit that occurs in both IL-12 and IL-23 (p40p19 heterodimer) as mice deficient in the p40 subunit are resistant to EAE [19]. IL-23 drives the production of Th17 cells and mice deficient in IL-23 (p19 deficient mice) are resistant to EAE [20]. Th17 cells, the IL-17-producing subset of CD4⁺ effector T cells, have been implicated as effector cells in EAE (reviewed in [21;22]). Th17 cells and IL-17 have been determined to be important in EAE pathogenesis, specifically in MOG₃₅₋₅₅- and PLP₁₃₉₋₁₅₁-induced EAE in mice and in MBP₆₈₋₈₆-induced EAE in rats [20;23-26]. Administration of Th17 cells and IL-17 promotes EAE (reviewed in [22]).

Here we review the published data describing modulation of EAE through administration of various CNS proteins/peptides introduced via live virus or plasmid DNA and modulation of EAE through choice of adjuvant (immunostimulating agents) (Table 1). Administration of CNS proteins/peptides by means of a live viral infection results in endogenous presentation through the major histocompatibility complex (MHC) class I pathway by virally infected cells [27]. Both the CD8⁺ T cell response as well as the CD4⁺ T cell response can be examined following this route of presentation [27]. Active induction of EAE through subcutaneous inoculation with CNS proteins/peptides in CFA primarily results in exogenous presentation through the MHC class II pathway [27]. Only the CD4⁺ T cell response can be examined following this route of presentation. Modulation of the disease course of EAE could be a useful animal model for exacerbations of disease that occur in MS patients in association with viral infections (reviewed in [28]). Immunization or vaccination of MS patients has not been found to be associated with exacerbations (reviewed in [9]).

Modulation of EAE through Viral Infection

Administration of CNS proteins/peptides by means of a live viral infection has been shown to protect the animal against the later development of EAE [29;30]. The major encephalitogenic peptide from MBP for PL/J mice is MBP₁₋₁₁ with the first amino acid acetylated [31;32]. Infection of PL/J mice with a recombinant vaccinia virus (VV) construct that encodes an encephalitogenic peptide of rat MBP (MBP₁₋₂₃) does not in and of itself result in CNS disease and the infected mice are protected from the development of EAE with either whole guinea pig MBP protein or acetylated MBP₁₋₂₀ peptide in CFA, both of which can directly induce EAE [29]. This protection effect, in this case in the form of delayed onset of clinical signs and decreased CNS pathology, was also seen when recombinant VV encoding human MPB was used to infect marmosets (*Callithrix jacchus*) followed by EAE induction with human whole white matter in CFA supplemented with *Bordetella pertussis* cells intravenously, an EAE induction method that results in a relapsing-remitting disease course [30]. Additionally, upon testing the previously recombinant VV infected mice for delayed-type hypersensitivity (DTH) reactivity to MBP, it was found that the DTH response, which normally develops in mice with EAE, was decreased, as indicated by significantly reduced ear swelling after 24 hours [29]. The strain of the mouse (genetic susceptibility), the encephalitogenic determinant synthesized from the

VV construct and the antigen used for inoculation all played a role in the protective affect. Another factor that may have played a role in the observed protection could have been the acetylation missing from the first amino acid of the MBP peptide upon its synthesis from the VV construct. Finally, the protective affect was hypothesized to be mediated by either apoptosis or anergy of the effector CD4⁺ Th1 cells, based on the decreased DTH response and other data [29].

Conversely, administration of CNS proteins/peptides by means of a live viral infection has been shown to enhance the subsequently induced EAE disease. PLP peptides that are encephalitogenic for SJL mice are PLP₁₀₄₋₁₁₇, PLP₁₃₉₋₁₅₁ and PLP₁₇₈₋₁₉₁ (reviewed in [33;34]). Infection of SJL/J mice with a recombinant VV construct encoding the rat PLP protein does not result in CNS disease [27;35]. However, following inoculation with various encephalitogenic PLP peptides (PLP₁₃₉₋₁₅₁, modified versions of PLP₁₃₉₋₁₅₁, PLP₁₀₄₋₁₁₇, PLP₁₇₈₋₁₉₁) in CFA, the mice developed an enhanced first clinical attack with early onset of the acute phase of disease. The antigen used for inoculation played a role in the enhancement affect which may be mediated by a combination of CD4⁺ and CD8⁺ T cells and anti-PLP antibodies [27;35]. In contrast to the enhancement of the acute phase of disease, mice followed into the chronic relapsing-remitting phase of the disease (2 months after inoculation), were found to have fewer relapses, decreased CNS demyelination and decreased inflammation (meningitis and perivascular cuffing) [36]. Decreased T cell reactivity (unresponsiveness or regulated suppression) to the encephalitogenic PLP₁₃₉₋₁₅₁ peptide is thought to mediate suppression of the chronic phase of disease [36].

Administration of CNS proteins/peptides by means of a live viral infection has been shown to subclinically prime animals for the development of disease. Subclinical priming is where the initial exposure to a self-CNS protein or peptide does not, in and of itself, cause disease, however enhanced disease can be induced via subsequent inoculation with CNS proteins/peptides in CFA, as in the enhancement experiment described above. Further, clinical signs of disease can be induced in these subclinically primed animals via other means than inoculation with CNS proteins/peptides in CFA, such as inoculation with a nonspecific immunological stimulus or with an unrelated virus, as described next. SJL/J mice injected with a recombinant VV construct encoding the CNS proteins, PLP, myelin associated glycoprotein (MAG) or glial fibrillary acidic protein (GFAP), did not develop CNS disease. However, these mice were susceptible to the development of EAE following inoculation five weeks later with a nonspecific immunologic stimulus, CFA [37]. The mouse strain (genetic susceptibility) was found to play an important role in the priming affect of the viral infection.

Another study has shown that the nonspecific immunologic stimulus (CFA) used for the inoculation could be replaced with infection with various live viruses [38]. Again, SJL/J mice injected with a recombinant VV construct encoding PLP did not develop CNS disease. Five weeks later, these animals were infected with wild-type VV (WR strain), lymphocytic choriomeningitis virus (LCMV, Armstrong strain) or murine cytomegalovirus (MCMV, Smith strain). The outcome after inoculation depended on the virus used. Mice inoculated with wild-type VV or LCMV did not develop EAE whereas mice inoculated with MCMV developed clinical signs of disease (weight loss and righting reflex disturbances) and CNS inflammation (meningitis and perivascular cuffing). MCMV's ability to induce the production of IL-12 may explain the ability of MCMV to induce disease in these subclinically primed mice [38;39]. IL-12 can facilitate the production of IFN- γ by natural killer (NK) cells early after infection, and IL-12 and IFN- γ together could activate autoimmune Th1 cells [38;39].

Above, the general effects of cytokine administration on EAE were described: Th1 promotes and Th2 ameliorates. This generalization does not always hold true when the cytokines are administered in the context of a recombinant VV infection. Recombinant VVs encoding various cytokines were injected into SJL/J mice at the time of EAE induction, with mouse spinal cord homogenate in CFA supplemented with *B. pertussis* cells intravenously, and again 6 days post inoculation [40]. It was found that IL-6, TNF, IL-1 β , IL-2 and IL-10 all inhibited EAE, IFN- γ had no effect and IL-4 either had no effect or enhanced disease [40].

Modulation of EAE through DNA Immunization

Administration of CNS proteins/peptides by means of DNA immunization has also been shown to enhance the subsequently induced EAE disease. Plasmids were constructed such that expression of the whole PLP protein or encephalitogenic epitopes of PLP (PLP_{139–151}, PLP_{178–191}) was driven by the CMV promoter in mammalian cells [41]. Intramuscular (i.m.) injection of SJL/J mice with these plasmid constructs did not cause CNS disease in and of itself. However, following inoculation two weeks later with various encephalitogenic PLP peptides (modified PLP_{139–151}, PLP_{178–191}) in CFA, the mice developed an enhanced EAE disease. The disease was more severe and the relapses were more frequent in mice followed for 2–3 months after inoculation. The antigen used for inoculation played a role in the enhancement affect which may be mediated by CD4⁺ T cells and anti-PLP antibodies [41].

In contrast, DNA immunization has also been shown to protect the animal against later development of EAE. A plasmid was constructed such that expression of PLP_{139–151} was driven by the CMV promoter in mammalian cells [42;43]. The plasmid used in this construction was the pTarget expression vector [42;43], whereas the plasmid used in the previous construction was derived from the pCMV- β expression vector [41]. An initial study demonstrated that i.m. injection of SJL/J mice with this plasmid construct ameliorated the acute clinical disease that developed following inoculation 10 days later with PLP_{139–151} peptide in CFA [42]. These animals were only followed through the acute phase of the disease. In addition to the different plasmids used and the length of time the mice were followed, other differences, between the previous study where enhancement of disease was seen [41] and this study in which protection was seen [42], include the source and age of the animals, the number, concentration, timing and site of the DNA immunizations and the concentration and timing of the inoculation. Anergy of pathogenic T cells was thought to mediate the protective affect [42].

In an extension of this study, SJL/J mice were injected i.m. with the same plasmid construct as described above (PLP_{139–151} expressed from CMV promoter in pTarget) in conjunction with injection of a plasmid construct encoding the full-length mouse IL-4 protein [43]. Seven-10 days later, the mice were inoculated with PLP_{139–151} peptide in CFA, and the mice were protected. The major difference between this study demonstrating protection from EAE [43] and the previous study demonstrating enhancement of EAE [41] is the presence of IL-4. Secreted IL-4 may act on autoreactive T cells, via the IL-4 receptor, to activate signal transducer and activator of transcription 6 (STAT6) which in turn produces a shift in the cytokine profile of the T cells to a Th2-type cytokine profile (less IFN- γ ; more IL-4 and IL-10), thus mediating protection [43].

Administration of CNS proteins/peptides by means of DNA immunization has also been shown to subclinically prime animals for the development of disease, as shown by the enhancement experiment above, and disease can be induced in these subclinically primed animals via other means than inoculation with CNS proteins/peptides in CFA. SJL/J mice, subclinically primed through i.m. injection of a plasmid construct encoding PLP fused to ubiquitin, which enhances presentation through the MHC class I pathway and induction of

CD8⁺ T cells, showed some susceptibility to disease development following inoculation, one week later, with nonspecific immunologic stimuli (CFA or a control VV not encoding CNS antigens) [37]. However, subclinically priming with live virus, as described in the previous section, resulted in 90% of the mice developing overt CNS disease, whereas subclinically priming with plasmid DNA resulted in only 20% of the mice developing CNS inflammation. Whether the priming agent was live virus or plasmid DNA, the important factor was the presence of a molecular mimic of a CNS antigen. A molecular mimic, in this case, shows similarity or identity to a self-CNS protein or peptide. The inoculating agent, on the other hand, could be nonspecific and did not need to enter the CNS [37]. Thus nonspecific immunostimulation (bystander activation) [38;39] or subtle cross-reactions ([44;45]; reviewed in [46;47]) can activate antigen specific cells induced by molecular mimicry. The priming affect resulting from DNA immunization was MHC class I specific [37].

In contrast, when SJL/J mice were subclinically primed with the PLP-ubiquitin fusion construct and then inoculated with PLP₁₃₉₋₁₅₁ peptide in CFA, in place of the nonspecific immunologic stimuli, the immune response was downregulated [48]. During the relapse after inoculation, the mice had a milder clinical disease, fewer CNS lesions, a decreased lymphoproliferative response, decreased IFN- γ production and increased IL-4 production. Activated and expanded CD8⁺ T cells, which regulate CD4⁺ Th1 encephalitogenic T cells, are thought to mediate this decreased immune response [48].

Modulation of EAE through Choice of Adjuvant

Modulation of the clinical course of EAE has been accomplished by altering the adjuvant. Administration of MOG₉₂₋₁₀₆ peptide in CFA subcutaneously at the base of the tail to A.SW mice resulted in a primary progressive (PP) disease course, in which disease continuously progresses from the onset [6;7]. However, supplementing the MOG₉₂₋₁₀₆/CFA injection into A.SW mice with *B. pertussis* cells intravenously resulted in a secondary progressive (SP) disease course, in which progressive disease follows a relapsing-remitting course. In the case of A.SW mice, the choice of adjuvant, CFA alone or CFA plus *B. pertussis*, which is an inducer of a mixed Th1/Th17-type immune response in mice [49;50], played a very important role in determining whether the animals would experience a PP or SP disease course, and supplementation with *B. pertussis* appeared to be protective. In contrast, administration of MOG₉₂₋₁₀₆ peptide in CFA to SJL/J mice resulted in a relapsing remitting disease course, whether *B. pertussis* was present or not [6;7]. Whereas clinical, histological and immunological differences between A.SW and SJL/J mice injected with MOG₉₂₋₁₀₆/CFA with or without *B. pertussis* could clearly be based on strain differences, when comparing A.SW mice injected with MOG₉₂₋₁₀₆/CFA with *B. pertussis* to A.SW mice injected with MOG₉₂₋₁₀₆/CFA without *B. pertussis*, differences included the presence (without *B. pertussis*) or absence (with *B. pertussis*) of MOG specific antibodies, of the immunoglobulin (Ig) G subtype, in the serum [6] and significantly lower spleen weights in those mice without *B. pertussis* supplementation [7].

Supplementation with plasmid DNA encoding CpG motifs has also been demonstrated to modulate the clinical course of EAE. Unmethylated CpG dinucleotides present in bacterial DNA are also an inducer of a Th1-type immune response [51]. Unmethylated CpG islands elicit an adjuvant effect through their ability to function as ligands for Toll-like receptors (TLRs), membrane bound pattern recognition receptors expressed on leukocytes [51]. Administration of the pCMV plasmid, derived from the pCMV- β vector by excision of the β -galactosidase gene, which contains 20 CpG motifs, i.m. into SJL/J mice, followed two weeks later by inoculation with modified PLP₁₃₉₋₁₅₁ or PLP₁₇₈₋₁₉₁ peptide in CFA resulted in an enhanced disease [52]. The clinical signs of disease were more severe and there was an increase in demyelinating lesions and an enhancement in the production of Th1-promoting

cytokines (IFN- γ and IL-12). The antigen used for inoculation played a role in the enhancement effect [52]. In contrast, administration of pCMV plasmid three weeks prior to inoculation with MOG_{92–106} peptide in CFA in A.SW mice resulted in protection from disease [6]. Instead of developing PP-EAE, half of the DNA-injected mice showed no clinical signs of disease and only one mouse died of SP-EAE [6].

In contrast to the modulation of EAE seen upon supplementation with plasmid DNA encoding CpG motifs, it has been reported that CpG motifs can not replace CFA in the direct induction of EAE [53]. Administration of PLP_{139–151} peptide in CFA to SJL/J mice resulted in induction of active disease (clinically and histologically), DTH and high levels of IL-17. Replacement of CFA with the CpG oligodeoxynucleotide, CpG 1826, emulsified in incomplete Freund's adjuvant (mineral oil alone) abrogated all of these responses, thus demonstrating a difference in encephalitogenicity between CFA and CpG motifs [53].

Mechanisms of modulation

The mechanisms of modulation of EAE, by administration of various CNS proteins/peptides introduced via live virus or plasmid DNA and through choice of adjuvant, have not been fully elucidated in all cases. However, by comparing and contrasting the immunological basis of the modulation (if examined) in the examples presented (Table 1) and the effect on disease (protection/suppression or occurrence/enhancement), some generalizations can be made. Protection from or suppression of EAE is characterized by a reduced DTH response [29;36;53], a reduced antigen specific lymphoproliferative response [30;36;42;48], a decrease in Th1-type cytokines (IL-2, IFN- γ) [42;43;48], an increase in Th2-type cytokines (IL-4, IL-10) [36;43;48] and a decrease in IL-17 [53]. Occurrence/enhancement of EAE is characterized by an increased antigen specific lymphoproliferative response [41], an increase in Th1-type cytokines (IFN- γ) [52], an increase in antigen specific antibodies [6;35], a shift from predominantly the IgG1 isotype to the IgG2a and/or 2b isotypes [35;52] and an increase in IL-12 [38]. Therefore, protection, which can be seen as tolerance/anergy, can occur through apoptosis, regulated suppression by CD8⁺ T cells or a lack of activation of CD4⁺ Th1 and Th17 effector T cells accompanied by an increase in Th2 cells and cytokines. Enhancement can occur via activation of Th1 effector cells and proinflammatory cytokine production and through the presence of pathogenic antibodies. The problem with applying this knowledge to the prevention/treatment of EAE is that there is no way to predict a priori which combination of vaccination/EAE induction techniques will yield which results.

Conclusion

Here we have reviewed the published data describing modulation of EAE through administration of various CNS proteins/peptides introduced via live virus or plasmid. The consequence of introducing molecular mimics of CNS proteins/peptides, via live virus (replicating organisms) or plasmid DNA, is variable, and likely depends on the antigen, the timing of antigen administration and the vehicle used for antigen delivery [54]. Subclinical priming through live virus or plasmid DNA administration allows disease to be induced through either molecular mimicry and/or bystander activation by cytokines (i.e. IL-12 and IFN- γ) ([39];reviewed in [55]).

EAE can also be modulated through the choice of adjuvant. Adjuvants are agents that stimulate the immune system [51]. This immune stimulation can occur through various, different pathways. Alum, short for any aluminum salt to include aluminum hydroxide and aluminum phosphate, has been the most common adjuvant used in human and veterinary vaccines since its use as an adjuvant was discovered in 1926 (reviewed in [56;57]).

Aluminum hydroxide is a more potent adjuvant than aluminum phosphate (reviewed in [56]). Alum functions as an adjuvant by provoking the secretion of uric acid which in turn activates inflammatory dendritic cells [51]. Alum, which does not contain any TLR ligands, can induce a Th2-type immune response [51]. In most cases, CFA is a more potent adjuvant than alum (reviewed in [56]). CFA, which contains microbial TLR ligands (for TLRs 2, 4 and 9), is an inducer of a Th1-type immune response [53]. Although it contains TLR ligands, CFA can elicit a robust antibody response to T-cell-dependent antigens in TLR-deficient mice [58]. The muramyl dipeptide component of CFA is thought to be the ligand recognized by nucleotide-binding oligomerization domain-2, thus accounting for the TLR-independent induction of an immune response by CFA (reviewed in [59]). *B. pertussis* and CpG motifs also induce a Th1-type immune response [53]. Unmethylated CpG oligodeoxynucleotides and CpG motifs present on plasmid DNA are recognized as TLR ligands by TLR9 on B cells and plasmacytoid dendritic cells in humans and on monocytes, macrophages and myeloid dendritic cells in mice [53;60]. CpG motifs present on plasmid DNA, but not CpG oligodeoxynucleotides, can elicit an immune response in TLR9-deficient mice (reviewed in [59]). It is the double-stranded nature of the plasmid DNA that is thought to mediate this response through an as yet undetermined mechanism (reviewed in [59]). Newer adjuvants are under development with the goal of producing highly purified, fully defined, standardized vaccines with greater potency and lower toxicity (reviewed in [61]). Subunit vaccines, containing highly purified recombinant proteins plus synthetic analogues as adjuvant, are the next step, beyond whole-cell vaccines, towards this goal (reviewed in [61]). The first adjuvant to be approved for use in humans (Europe) since alum is MF59 (reviewed in [57;61]). MF59 is a fully defined oil-in-water microemulsion adjuvant that is generally more potent than alum (reviewed in [57;61]). In addition, highly refined mineral oil-based adjuvants (Montanide ISA 51) and metabolizable oil-based adjuvants (Montanide ISA 720) are promising adjuvants for future vaccines [62].

Based on their different modes of action, different adjuvants can modulate EAE in different ways. Examination of some of the newly developed adjuvants for their ability to modulate EAE may be in order. Knowledge of the various TLR-dependent and TLR-independent modes of action of different adjuvants 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 have been established for EAE, these treatments may be translated into treatments for MS.

Acknowledgments

We wish to acknowledge Kathleen Borick for the outstanding preparation of the manuscript.

This work was supported by NIH grant 1P01AI058105.

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Table 1

EAE modulation.

Modulation method	Vaccine	EAE induction method			Effect
		Immunogen	Animal	Adjuvant	
Viral Infection	VV-MBP ₁₋₂₃	Whole MBP or Acetylated MBP ₁₋₂₀	PL/J mouse	CFA + BP	Protection from acute monophasic disease without progression (PL/J mouse) or relapsing-remitting disease (marmosets)
	VV-MBP	Whole human white matter	Marmosets		
	VV-PLP	PLP ₁₃₉₋₁₅₁	SJL/J mouse		Enhanced acute phase and suppressed chronic relapsing-remitting phase
	VV-MAG	Modified PLP ₁₃₉₋₁₅₁			
		PLP ₁₀₄₋₁₁₇			
	VV-GFAP	PLP ₁₇₈₋₁₉₁			
		VV LCMV		None	Protection from relapsing-remitting disease
		MCMV			Developed clinical signs of disease
		None		CFA + BP	Overt CNS disease in 90% of mice
	VV-IL-6	Mouse spinal cord homogenate			Inhibited EAE
	VV-TNF				
	VV-IL-1 β				
	VV-IL-2				
	VV-IL-10				
	VV-IFN- γ				No effect
	VV-IL-4				No effect or enhanced disease
DNA Immunization	pTargeT-PLP ₁₃₉₋₁₅₁	PLP ₁₃₉₋₁₅₁		CFA $\pm$ BP	Ameliorated acute disease
	pTargeT-PLP ₁₃₉₋₁₅₁ + pTargeT-IL-4				Suppressed chronic relapsing-remitting phase
	pCMV-Ubiquitin- PLP	None VV		CFA	CNS inflammation in 20% of mice
	pCMV-PLP	Modified PLP ₁₃₉₋₁₅₁ or PLP ₁₇₈₋₁₉₁		None	Enhanced relapsing-remitting disease
	pCMV-PLP ₁₃₉₋₁₅₁		A.SW mouse	CFA	Protected from chronic progressive disease
	pCMV-PLP ₁₇₈₋₁₉₁	MOG ₉₂₋₁₀₆			
Adjuvant	pCMV (CpG)				Primary progressive disease
	None			CFA + BP	Secondary progressive disease

Modulation method	Vaccine	EAE induction method			Effect
		Immunogen	Animal	Adjuvant	
			SJL/J mouse	CFA	Relapsing-remitting disease
				CFA + BP	
		PLP ₁₃₉₋₁₅₁		CpG 1826 + IFA	Protected from relapsing-remitting disease

BP *Bordetella pertussis*, CFA complete Freund's adjuvant, CNS central nervous system, EAE experimental autoimmune encephalomyelitis, GFAP glial fibrillary acidic protein, IFA incomplete Freund's adjuvant, IFN interferon, IL interleukin, LCMV lymphocytic choriomeningitis virus, MAG myelin associated glycoprotein, MBP myelin basic protein, MCMV murine cytomegalovirus, MOG myelin oligodendrocyte glycoprotein, PLP myelin proteolipid protein, TNF tumor necrosis factor, VV vaccinia virus