Published in final edited form as:
Curr Top Microbiol Immunol. 2009 ; 336: 155–168. doi:10.1007/978-3-642-00549-7_9.

Toll-Like Receptors in Multiple Sclerosis

Michael K. Racke and
Department of Neurology and Department of Molecular Virology, Immunology & Medical Genetics, The Ohio State University Medical Center, 1654 Upham Drive, 445 Means Hall, Columbus, OH, 43210, USA

Paul D. Drew
Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA DrewPaulD@uams.edu

Abstract

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by inflammation, demyelination, and axonal pathology. The exact causes of MS are unknown, but environmental factors including pathogens are believed to contribute to the development of disease. Toll-like receptors (TLRs) are a family of receptors important in pathogen recognition and host defense. TLRs are expressed by a variety of peripheral immune cells as well as resident cells of the CNS. Studies indicate that TLRs play a significant role in modulating MS, as well as experimental autoimmune encephalomyelitis (EAE), an animal model of MS. This review will discuss the current understanding of the role of TLRs in modulating EAE and MS.

1 Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by demyelination and progressive axonal degeneration. MS is the second leading cause of neurologic disability in young adults following trauma (Anderson et al. 1992). Most patients suffer from the physical, psychological, and financial effects of MS for most of their adult life. In recent years, a variety of therapeutic agents have been developed which slow disease progression (Johnson et al. 1995; Jacobs et al. 1996; Millefiorini et al. 1997). However, there remains no cure for the disease, and more effective therapies are desperately needed. The cause of MS remains unknown; however, an autoimmune process is hypothesized to be involved in disease pathogenesis (Arnason 1983; Martin et al. 1992; McFarland and Martin 2007). Epidemiologic reports and studies examining the disease in identical twins also suggest that both environment and genetics influence the onset and pathogenesis of MS (Sadovnick and Ebers 1993). MS is believed to be principally mediated by $CD4^+$ T cells that are reactive against myelin antigens (Frohman et al. 2006). These cells are activated in the periphery and express adhesion molecules which facilitate interactions with ligands present on vascular endothelial cells, resulting in extravasation across the blood-brain barrier (Compston 2004). Once in the CNS, these myelin-reactive $CD4^+$ T cells contribute to the demyelination and progressive axonal pathology characteristic of MS (Frohman et al. 2005). In addition to $CD4^+$ T cells, $CD8^+$ T cells and B cells are believed to contribute to the pathogenesis associated with MS (Johnson et al. 2007; Nikbin et al. 2007 ; Hauser et al. 2008). In addition to lymphocytes that control adaptive immune responses, dendritic cells and tissue macrophages that regulate innate immune responses also play a

Correspondence to: Paul D. Drew.

role in controlling MS disease pathogenesis. These cells express pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) that recognize pathogen-associated molecular patterns (PAMPs) present on the surface of pathogens. Following ligand binding to TLRs, innate immune cells produce proinflammatory cytokines and can serve as antigen-presenting cells (APCs) to prime naïve T cells to recognize antigens (Takeda and Akira 2005; Hacker et al. 2006). Thus, TLRs play an important role in linking the innate to the adaptive immune response (Bell et al. 2005). Finally, glia including astrocytes and microglia play an important role in protecting the CNS against pathogenic insults. However, when chronically activated, these glia may contribute to the pathogenesis of MS, and this may occur in part through PAMP binding to TLRs present on these cells, which can contribute to the reactivation of myelin-specific autoreactive T cells in the CNS (Sanders and De Keyser 2007; Nair et al. 2008). Interestingly, a variety of resident cells of the CNS express TLRs (Bsibsi et al. 2002). Depending on the TLR evaluated, TLR expression on CNS cells has been demonstrated to contribute to oligodendrocyte and neuron cell death (Lehnardt et al. 2002, 2003) or alternatively to be neuroprotective (Bsibsi et al. 2006). It is also interesting that TLRs are expressed on B cells and T cells and that TLR signaling can directly alter adaptive immune responses (Kabelitz 2007; Lampropoulou et al. 2008).

Several animal models have been used to study MS. In some of these models, the disease is induced by viruses, such as Theiler's virus or Borna disease virus (Miller and Karpus 1994). However, the most common model of MS is termed experimental autoimmune encephalomyelitis (EAE). Active EAE is elicited by immunization with a variety of myelin peptides into organisms including rodents and monkeys. EAE can also be elicited following adoptive transfer of myelin-specific T cells into naïve recipients. Of the EAE models, the most commonly studied are those established in the Lewis rat and in several susceptible mouse strains. Murine models of EAE present several advantages over rat models of EAE (Racke 2001). For example, murine EAE results in a relapsing-remitting disease, similar to the early phase of disease for most MS patients, whereas EAE in the Lewis rat is a monophasic illness. In chronic murine EAE, the pathology observed in the white matter shows much more demyelination than the Lewis rat model, again being more reminiscent of the pathology seen in the CNS of patients with MS. In fact, similarities in pathology between mouse EAE and MS have suggested that autoimmunity plays a role in the development of MS. Finally, with the advent of transgenic and homologous recombination technology, it is increasingly clear that many powerful molecular tools are becoming available to study the immune response in pathologic processes such as murine EAE.

2 Role of Pathogens in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

The cause of MS is unknown. However, clinical evidence suggests an autoimmune response directed against myelin, possibly stimulated by an infectious agent (Martin et al. 1992; Noseworthy 1999). Epidemiologic studies also suggest that MS is triggered by environmental factors, and an infectious agent is the most likely culprit (Kurtzke 1993). Infections can exacerbate the course of MS, but many different viruses and bacteria have been evaluated (Sibley et al. 1985; Compston et al. 1986; Gay et al. 1986; Panitch 1994; Rapp et al. 1995; Sriram et al. 1998; Horwitz and Sarvetnick 1999; Lenz et al. 2001; Du et al. 2002) and no single environmental agent has been definitively linked to MS development. Furthermore, the mechanisms by which pathogens precipitate MS have not been elucidated. One possibility is that antigens from infectious pathogens may activate autoreactive T cells, causing their expansion and leading to clinical disease (Oldstone 1987; Hausmann and Wucherpfennig 1997). However, molecular mimicry—as this process is termed—has found limited experimental support as a cause of autoimmune diseases. Alternatively, MS may be triggered not by a specific pathogen but instead by general

infectious processes. This phenomenon, termed bystander activation, is supported by studies indicating that Theiler's virus can activate APCs and consequently activate autoreactive T cells that do not cross-react with Theiler's virus antigens (Miller et al. 1997; Katz-Levy et al. 1999). Bystander activation has been suggested to be initiated by pathogens that trigger immune processes in the CNS, which is likely mediated by TLRs. Tissue damage then unmasks myelin antigens, resulting in epitope spread and the development of autoimmunity (Vanderlugt and Miller 2002; McMahon et al. 2005; Bailey et al. 2007).

A common clinical occurrence in MS patients is the development of changes in neurological function that appear to be due to MS disease activity but are actually the result of a physiological change such as a urinary tract infection, fever, or electrolyte abnormality. These pseudoexacerbations are usually characterized by a recurrence of old symptoms for short durations, a definable metabolic change, and the disappearance of clinical signs once the metabolic change has been corrected (Tauber et al. 2007). For example, a patient with a prior history of leg weakness can become paraplegic by the fever accompanying a urinary tract infection. Dogma suggests that the reason for the pseudoexacerbation involves elevated temperature producing reversible conduction block in the demyelinated axon. It is this conduction block that produces the recurrence of the old symptoms, in this case paraplegia. If truly due to the above described physiology, most pseudoexcerbations should resolve once the infection is resolved and the fever reduced. However, many MS patients that experience a pseudoexacerbation with a urinary tract infection still require steroids to recover from their symptoms and often never recover completely. These studies suggest that bacterial agents such as *Escherichia coli*, which commonly cause urinary tract infections in many MS patients, may stimulate the innate immune response, which subsequently leads to the activation of autoreactive T cells, thus contributing to the damage that occurs in the CNS during MS exacerbations.

A variety of studies suggest a critical role for pathogens in the development of EAE. Experiments in the 1970s from pioneers in the field of EAE suggested that both mycobacterial components and pertussis toxin were required for disease development following immunization with myelin antigens (Lublin 1982). The McFarlin laboratory demonstrated that EAE could be induced without pertussis toxin using multiple injections of myelin components in complete Freund's adjuvant (CFA) (Brown and McFarlin 1981). More recently, it has become clear that other adjuvants such as CpG DNA, a ligand for TLR9, can also stimulate induction of EAE (Segal et al. 2000; Deng et al. 2003). Interestingly, adjuvants by themselves have limited capacity to induce EAE without concurrent exposure to myelin antigens. An exception occurs with myelin basic protein-specific TCR transgenic mouse, where immunization with CFA alone resulted in the development of EAE (Goverman et al. 1993). In these mice, because of the high frequency of autoreactive T cells, antigen was not required to expand the encephalitogenic T cell pool. Collectively, these studies suggest that pathogens can initiate an encephalitogenic response that contributes to the development of EAE and MS.

3 Toll-Like Receptors: Role in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

3.1 TLRs in MS and EAE: An Overview

Innate immunity represents the first line of defense against pathogens and is principally controlled by phagocytic cells and APCs present in the periphery and in the CNS. Innate immunity is capable of responding rapidly to pathogens, but produces no long-term memory response to a given pathogen. Innate immunity functions through germline-encoded receptors termed PRRs, which include TLRs. Mammalian TLRs were identified based on

sequence homology to the *Drosophila* Toll protein (Brennan and Anderson 2004), and collectively these TLRs recognize PAMPs common to a variety of bacteria, viruses, and fungi. Engagement of TLRs with PAMPs leads to the pathogen-induced release of proinflammatory molecules by cells of the innate immune system.

Ligand binding to TLRs triggers receptor dimerization. TLRs are capable of interacting with a series of adaptor proteins that mediate different signaling pathways. Myeloid differentiation primary response protein 88 (MyD88) is the most widely utilized TLR adaptor protein and mediates signaling through all TLRs except TLR3 (Medzhitov et al. 1998; Muzio et al. 1998; Takeda and Akira 2005). MyD88 interacts with the threonine-serine kinase interleukin (IL)-1 receptor-associated kinase 4 (IRAK4), which upon activation phosphorylates IRAK1 (Cao et al. 1996a; Yamin and Miller 1997; Li et al. 2002). Subsequently, the IRAKs recruit the ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF-6), which polyubiquitinates and activates TAK1 kinase (Cao et al. 1996b). TAK1 kinase activates the IKK complex that triggers the proteolytic degradation of inhibitor κB (I-κB), the inhibitor of nuclear factor κB (NF-κB), which unmasks the nuclear localization signal of NF-κB allowing translocation of this transcription complex from the cytoplasm to the nucleus and activation of a wide variety of NF-κB responsive genes, including genes encoding proinflammatory cytokines and co-stimulatory molecules required for activation of the adaptive immune response. In addition to NF-κB activation, MyD88-dependent signaling results in the activation of transcription factors including AP-1 through a MAPK signaling pathway (Takeda and Akira 2005). Studies involving MyD88-deficiency indicate a critical role of this adaptor protein in response to stimulation through numerous TLRs, including TLR2, TLR4, TLR5, TLR7 and TLR9 (Kawai et al. 1999; Hacker et al. 2000; Schnare et al. 2000; Takeuchi et al. 2000; Hayashi et al. 2001; Hemmi et al. 2002; Takeda et al. 2003).

Ligand binding to TLR3 and TLR4 can stimulate MyD88-independent signaling pathways. These pathways result in the activation of the transcription factor IRF3 and late-phase NF-κB and stimulate the production of genes encoding interferon (IFN)-β and co-stimulatory molecules (Kawai et al. 2001). The adaptor molecule TRIF plays a critical role in signaling through TLR3, while the adaptors TRIF and TRAM are required for MyD88-independent signaling through TLR4 (Fitzgerald et al. 2003b; Oshiumi et al. 2003; Yamamoto et al. 2003; Rowe et al. 2006). Interestingly, the noncanonical I-κB kinases IKKε and TBK1 are required for activation of IRF3 and NF-κB in MyD88-independent signaling pathways (Fitzgerald et al. 2003a).

Adaptive immunity is principally mediated by T and B lymphocytes. These cells are capable of recognizing pathogen-associated antigens in a highly specific manner and provide long-term protection to pathogens. Myelin-reactive T cells are believed to modulate the development of MS and EAE. These myelin-reactive T cells activated in the periphery are capable of moving into the CNS, where they destroy myelin-producing oligodendrocytes and also elicit axonal transaction and neurodegeneration. The role of adaptive immunity in MS has been extensively studied. More recently, the role of the innate immune response in MS has begun to be appreciated. Importantly, it is now recognized that the innate immune response, through the production of proinflammatory cytokines and through antigen presentation, plays a critical role in the activation of myelin-specific autoreactive T cells, thus linking the innate and the adaptive immune system in MS pathogenesis.

Myelin-specific autoreactive $CD4^+$ T cells are believed to contribute to pathogenesis in EAE and MS. $CD4^+$ T cells were originally divided into T helper (Th)1 and Th2 subsets, each exhibiting a distinct effector phenotype and cytokine expression profile (Mosmann and Coffman 1989). Th1 cells produce lymphotoxin and IFN-γ but little IL-4, while Th2 cells

produce IL-4, IL-5, IL-13, and IL-25. Th1 cells are believed to contribute to the development of EAE (Ando et al. 1989), while Th2 cells are believed to suppress EAE (Kennedy et al. 1992; Khoruts et al. 1995; Rocken et al. 1996). More recently, Th17 cells that express IL-17, IL-21, and IL-22 have been identified as a third subset of $CD4^+$ T cells that are important in modulating EAE (Bettelli et al. 2008). Interestingly, cytokines produced by cells of the innate immune system modulate the differentiation and function of $CD4^+$ T cells. This provides a critical link between TLR signaling (innate immunity) and autoreactive T cells (adaptive immunity) that cooperatively modulate the pathogenesis associated with MS and EAE.

3.2 TLRs in MS and EAE: The Current Understanding

Previous studies suggest that TLR signaling through the MyD88-dependent pathway plays a significant role in regulating the development of EAE. MyD88 signaling results in the activation of transcription factors including NF-κB and AP-1, which activate the expression of a variety of genes encoding proinflammatory cytokines and chemokines, as well as molecules important in antigen presentation. Each of the TLRs evaluated except TLR3 are capable of triggering MyD88-dependent signaling. However, TLR4 is capable of triggering MyD88-dependent as well as MyD88-independent signaling. EAE is commonly induced in susceptible mouse and rat strains by immunization with myelin peptides emulsified in CFA. In addition, pertussis toxin is generally administered to animals at the time of immunization, and often a booster of pertussis toxin is administered two days following immunization (Racke 2001). Importantly, *Mycobacterium tuberculosis* present in CFA is believed to activate a variety of TLRs, including TLR1, TLR2, and TLR4 (Hansen et al. 2006). Mice immunized with myelin peptides in the presence of incomplete Freund's adjuvant (IFA) do not develop EAE, but do develop disease when *M. tuberculosis* is added to the adjuvant (Hansen et al. 2006). In addition, it has been appreciated for some time that the TLR9 agonist CpG ODN emulsified in IFA is capable of inducing EAE following immunization with myelin peptides in rodent strains susceptible to EAE (Segal et al. 2000; Deng et al. 2003; Hansen et al. 2006).

Several studies support a role of TLR2 in modulating EAE. TLR2 serves as a ligand for Gram-positive bacteria including *Staphylococcus aureus* and *Streptococcus pneumoniae*. Molecules present in the cell wall of these organisms, such as peptidoglycan (PGN), serve as PAMPs that are capable of activating TLR2. *S. aureus* PGN added to IFA was demonstrated to stimulate the development of EAE in C57BL/6 mice (Visser et al. 2005). Associated in vitro studies indicated that PGN stimulated the maturation of dendritic cells and antigen uptake by these cells. Furthermore, PGN pulsed dendritic cells were demonstrated to stimulate T cell proliferation and drive T cell differentiation toward a Th1 phenotype believed to facilitate development of EAE. These studies suggest that PGN signaling through TLR2 may stimulate dendritic cell maturation, antigen presentation, and production of effector molecules resulting in Th1 cell differentiation and development of EAE. The Laman laboratory also demonstrated that PGN is observed in association with APCs in the CNS of MS patients as well as nonhuman primates (Visser et al. 2006). Furthermore, PGN-laden APCs were increased in the CNS of EAE animals. These studies suggest that PGN and possibly other TLR agonists are capable of accessing the CNS during EAE, which could facilitate the reactivation of myelin-reactive T cells in the target tissue in EAE and MS. More recent studies demonstrated that infection of mice with *S. pneumoniae* was capable of increasing the severity of EAE, although this depended on the timing of infection relative to immunization (Herrmann et al. 2006). Furthermore, these studies demonstrated that the effects of *S. pneumoniae* on EAE were TLR2 dependent, as TLR2-deficient animals did not develop more severe EAE.

TLR4 can trigger both MyD88-dependent and MyD88-independent signaling, which may help to explain the somewhat contradictory findings concerning the effects of TLR4 agonists in modulating EAE. The Kuchroo laboratory utilized mice that express a transgenic TCR (5B6) on a B10.S background that do not develop EAE in spite of exhibiting a high frequency of autoreactive T cells. They demonstrated that these animals did not develop EAE due to the presence of APCs with limited T cell activating capacity (Waldner et al. 2004). These studies further demonstrated that activation of these APCs with TLR4 or TLR9 ligands broke T cell tolerance in these animals, resulting in the development of EAE. Studies by the Kubes laboratory indicated that pertussis toxin, which is commonly co-administered during immunization protocols for EAE, stimulates TLR4 signaling pathways. In addition, pertussis toxin also induced P-selectin expression, increased leukocyte/endothelial cell interactions, and facilitated T cell infiltration into the CNS. Pertussis toxin-mediated signaling and leukocyte extravasation into the CNS were found to be controlled by TLR4, as these effects were not observed in TLR4 knockout mice. The role of TLR4 in pertussis toxin induction of EAE was less clear, however. In general, TLR4 knockout mice were less susceptible to pertussis toxin-induced EAE than wild-type mice. However, this observation varied between individual experiments. In addition, TLR4 knockout mice that developed EAE in response to pertussis toxin developed disability scores that were almost as severe as wild-type animals. These studies suggest that pertussis toxin could be stimulating the TLR4 signaling of both the MyD88-dependent and the MyD88-independent pathways, and/or that pertussis toxin modulation of EAE is only partly dependent on TLR4 (Kerfoot et al. 2004; Racke et al. 2005). Studies in Lewis rats indicated that a combination of the TLR4 agonist LPS and the TLR9 agonist CpG ODN are required for the development of EAE, and that either agent alone added to IFA was capable of eliciting disease. Furthermore, a combination of CpG ODN and the TLR3 agonist poly I:C did not induce EAE (Wolf et al. 2007). These studies support the idea that stimulation of MyD88-independent TLR signaling suppresses EAE.

IRAK-1 plays a critical role in the MyD88-dependent signaling pathway. Interestingly, IRAK-1 knockout mice are resistant to the development of EAE (Deng et al. 2003). T cells derived from IRAK-1 knockout mice have normal TCR signaling but impaired Th1 cell development, suggesting that IRAK-1 is critical for proper T cell priming in the periphery. These conclusions are supported by subsequent studies indicating that although IRAK-1 mice are resistant to disease development using an active immunization protocol, Vα2.3/Vβ8.2 TCR transgenic T cells adoptively transfer disease in IRAK-1 knockout mouse recipients in a manner similar to wild-type recipients. Collectively, these findings suggest that IRAK-1 is critical for priming autoreactive T cells, but IRAK-1 expression is not required in the CNS for disease to occur (Hansen et al. 2006).

MyD88 knockout mice are resistant to the development of active EAE, further supporting a role of MyD88-dependent signaling in disease development (Prinz et al. 2006). Interestingly, T cells derived from MyD88 knockout mice did not respond measurably to their cognate antigen, suggesting that MyD88 knockout mice do not develop active EAE (at least in part) due to inadequate T cell priming in the periphery. However, bone marrow chimera studies indicated that MyD88 expression in the CNS also plays a significant role in controlling the development of EAE. Finally, adoptive transfer of myelin-specific T cells into MyD88 knockout mice resulted in decreased severity of EAE relative to wild-type animals, further supporting an important role for CNS expression of MyD88 in encephalitogenicity during the effector phase of EAE (Prinz et al. 2006).

Evidence suggests that TLR signaling through the MyD88-independent pathway either does not support or suppresses the development of EAE. For example, the TLR3 agonist polyinosinic:polycytidylic acid (poly I:C) does not support the development of active EAE

when immunized with myelin antigens emulsified in IFA (Hansen et al. 2006). Furthermore, treatment of EAE mice with poly I:C suppressed the development of disease (Touil et al. 2006). In these studies, poly I:C treatment resulted in significant production of IFN-β, one of the critical products of the MyD88-independent signaling pathway. Significantly, IFN-β is commonly used in the treatment of MS. Recent studies further support a role of MyD88-independent signaling in suppression of EAE (Guo et al. 2008). In these studies, type I IFN receptor knockout mice developed more severe EAE than wild-type mice. Likewise, TRIF knockout mice lacking this critical adaptor molecule for MyD88-independent signaling also developed more severe disease. The studies further suggested that IFN-β-induced production of IL-27 by cells of the innate immune system played a critical role in suppressing the development of Th17 cells critical to disease development, and that this control was lost in type I IFN receptor and TRIF-deficient animals.

The expression of a variety of TLRs is elevated in the CNS of EAE mice (Zekki et al. 2002; Prinz et al. 2006; Xu and Drew 2007). Agents that specifically modulate TLR signaling pathways may be effective in the treatment of EAE and MS. Recently, we have evaluated the effects of peroxisome proliferator-activated receptors (PPARs), which are members of the nuclear hormone receptor family, on the expression of TLR intermediates by cells of the CNS. We demonstrated that PPAR-α agonists suppress the expression of critical MyD88-dependent signaling intermediates by primary microglia, as well as in the CNS of mice with EAE (Xu et al. 2007). PPAR-γ agonists also suppress the expression of MyD88 signaling intermediates by primary microglia (Xu and Drew 2007). In addition, these PPAR agonists suppressed glial production of IL-12 and IL-23, which are known to play critical roles in the development of Th1 and Th17 cells that stimulate the development of EAE. These studies suggest that PPAR agonists may be effective in the treatment of MS.

4 Summary

Environmental factors including pathogens are believed to stimulate the development of MS. It is clear that TLRs play a significant role in modulating disease. Studies to date suggest that TLRs, which activate MyD88-dependent signaling, contribute to the development of MS, whereas MyD88-independent pathways may mitigate disease severity. TLRs present on cells of the innate immune system are believed to provide critical signals involved in the activation of cells of the adaptive immune system, including autoreactive lymphocytes. However, many questions concerning the role of TLRs in modulating MS remain unanswered. The complex pattern of TLR expression in the periphery and in the CNS, as well as by cells of both the innate and adaptive immune systems, is just beginning to be appreciated. Future studies are needed to understand the detailed mechanisms by which TLRs modulate MS. Importantly, these studies could identify new targets for the treatment of MS.

Abbreviations

APC	Antigen-presenting cell
CFA	Complete Freund’s adjuvant
CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
I-κB	Inhibitor κB
IFN	Interferon
IL	Interleukin

IRAK	Interleukin-1 receptor-associated kinase
MS	Multiple sclerosis
MyD88	Myeloid differentiation primary response protein 88
NF-κB	Nuclear factor κB
PAMP	Pathogen-associated molecular patterns
PGN	Peptidoglycan
PRR	Pattern recognition receptor
Th	T helper
TLR	Toll-like receptor
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain-containing adaptor inducing interferon β

Acknowledgments

This work was supported by grants from the National Institutes of Health and the National Multiple Sclerosis Society.

References

Anderson DW, Ellenberg JH, Leventhal CM, Reingold SC, Rodriguez M, Silberberg DH. Revised estimate of the prevalence of multiple sclerosis in the United States. Ann Neurol. 1992; 31:333–336. [PubMed: 1637140]

Ando DG, Clayton J, Kono D, Urban JL, Sercarz EE. Encephalitogenic T cells in the B10. PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. Cell Immunol. 1989; 124:132–143. [PubMed: 2478300]

Arnason BG. Relevance of experimental allergic encephalomyelitis to multiple sclerosis. Neurol Clin. 1983; 1:765–782. [PubMed: 6209540]

Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4 + T(H)-17 cells in relapsing EAE. Nat Immunol. 2007; 8:172–180. [PubMed: 17206145]

Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Segal DM, Davies DR. The molecular structure of the Toll-like receptor 3 ligand-binding domain. Proc Natl Acad Sci USA. 2005; 102:10976–10980. [PubMed: 16043704]

Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. Nature. 2008; 453:1051–1057. [PubMed: 18563156]

Brennan CA, Anderson KV. Drosophila: the genetics of innate immune recognition and response. Annu Rev Immunol. 2004; 22:457–483. [PubMed: 15032585]

Brown AM, McFarlin DE. Relapsing experimental allergic encephalomyelitis in the SJL/J mouse. Lab Invest. 1981; 45:278–284. [PubMed: 6792424]

Bsibsi M, Persoon-Deen C, Verwer RW, Meeuwsen S, Ravid R, Van Noort JM. Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. Glia. 2006; 53:688–695. [PubMed: 16482523]

Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol. 2002; 61:1013–1021. [PubMed: 12430718]

Cao Z, Henzel WJ, Gao X. IRAK: a kinase associated with the interleukin-1 receptor. Science. 1996a; 271:1128–1131. [PubMed: 8599092]

Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. TRAF6 is a signal transducer for interleukin-1. Nature. 1996b; 383:443–446. [PubMed: 8837778]

Compston A. The pathogenesis and basis for treatment in multiple sclerosis. Clin Neurol Neurosurg. 2004; 106:246–248. [PubMed: 15177777]

Compston DA, Vakarelis BN, Paul E, McDonald WI, Batchelor JR, Mims CA. Viral infection in patients with multiple sclerosis and HLA-DR matched controls. Brain. 1986; 109(Pt 2):325–344. [PubMed: 3456817]

Deng C, Radu C, Diab A, Tsen MF, Hussain R, Cowdery JS, Racke MK, Thomas JA. IL-1 receptor-associated kinase 1 regulates susceptibility to organ-specific autoimmunity. J Immunol. 2003; 170:2833–2842. [PubMed: 12626533]

Du C, Yao SY, Ljunggren-Rose A, Sriram S. *Chlamydia pneumoniae* infection of the central nervous system worsens experimental allergic encephalitis. J Exp Med. 2002; 196:1639–1644. [PubMed: 12486106]

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol. 2003a; 4:491–496. [PubMed: 12692549]

Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J Exp Med. 2003b; 198:1043–1055. [PubMed: 14517278]

Frohman EM, Filippi M, Stuve O, Waxman SG, Corboy J, Phillips JT, Lucchinetti C, Wilken J, Karandikar N, Hemmer B, Monson N, De Keyser J, Hartung H, Steinman L, Oksenberg JR, Cree BA, Hauser S, Racke MK. Characterizing the mechanisms of progression in multiple sclerosis: evidence and new hypotheses for future directions. Arch Neurol. 2005; 62:1345–1356. [PubMed: 16157741]

Frohman EM, Racke MK, Raine CS. Multiple sclerosis: the plaque and its pathogenesis. N Engl J Med. 2006; 354:942–955. [PubMed: 16510748]

Gay D, Dick G, Upton G. Multiple sclerosis associated with sinusitis: case-controlled study in general practice. Lancet. 1986; 1:815–819. [PubMed: 2870312]

Goverman J, Woods A, Larson L, Weiner LP, Hood L, Zaller DM. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. Cell. 1993; 72:551–560. [PubMed: 7679952]

Guo B, Chang EY, Cheng G. The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. J Clin Invest. 2008; 118:1680–1690. [PubMed: 18382764]

Hacker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, Kamps MP, Raz E, Wagner H, Hacker G, Mann M, Karin M. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature. 2006; 439:204–207. [PubMed: 16306937]

Hacker H, Vabulas RM, Takeuchi O, Hoshino K, Akira S, Wagner H. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. J Exp Med. 2000; 192:595–600. [PubMed: 10952730]

Hansen BS, Hussain RZ, Lovett-Racke AE, Thomas JA, Racke MK. Multiple toll-like receptor agonists act as potent adjuvants in the induction of autoimmunity. J Neuroimmunol. 2006; 172:94–103. [PubMed: 16360885]

Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould A, Smith CH. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med. 2008; 358:676–688. [PubMed: 18272891]

Hausmann S, Wucherpfennig KW. Activation of autoreactive T cells by peptides from human pathogens. Curr Opin Immunol. 1997; 9:831–838. [PubMed: 9492986]

Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001; 410:1099–1103. [PubMed: 11323673]

Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002; 3:196–200. [PubMed: 11812998]

Herrmann I, Kellert M, Schmidt H, Mildner A, Hanisch UK, Bruck W, Prinz M, Nau R. *Streptococcus pneumoniae* Infection aggravates experimental autoimmune encephalomyelitis via Toll-like receptor 2. Infect Immun. 2006; 74:4841–4848. [PubMed: 16861672]

Horwitz MS, Sarvetnick N. Viruses, host responses, and autoimmunity. Immunol Rev. 1999; 169:241–253. [PubMed: 10450521]

Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, Fischer JS, Goodkin DE, Granger CV, Simon JH, Alam JJ, Bartoszak DM, Bourdette DN, Braiman J, Brownscheidle CM, Coats ME, Cohan SL, Dougherty DS, Kinkel RP, Mass MK, Munschauer FE 3rd, Priore RL, Pullicino PM, Scherokman BJ, Whitham RH, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Ann Neurol. 1996; 39:285–294. [PubMed: 8602746]

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]

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Schiffer RB. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. Neurology. 1995; 45:1268–1276. [PubMed: 7617181]

Kabelitz D. Expression and function of Toll-like receptors in T lymphocytes. Curr Opin Immunol. 2007; 19:39–45. [PubMed: 17129718]

Katz-Levy Y, Neville KL, Girvin AM, Vanderlugt CL, Pope JG, Tan LJ, Miller SD. Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. J Clin Invest. 1999; 104:599–610. [PubMed: 10487774]

Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity. 1999; 11:115–122. [PubMed: 10435584]

Kawai T, Takeuchi O, Fujita T, Inoue J, Muhlradt PF, Sato S, Hoshino K, Akira S. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol. 2001; 167:5887–5894. [PubMed: 11698465]

Kennedy MK, Torrance DS, Picha KS, Mohler KM. 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. 1992; 149:2496–2505. [PubMed: 1527389]

Kerfoot SM, Long EM, Hickey MJ, Andonegui G, Lapointe BM, Zanardo RC, Bonder C, James WG, Robbins SM, Kubes P. TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. J Immunol. 2004; 173:7070–7077. [PubMed: 15557205]

Khoruts A, Miller SD, Jenkins MK. Neuroantigen-specific Th2 cells are inefficient suppressors of experimental autoimmune encephalomyelitis induced by effector Th1 cells. J Immunol. 1995; 155:5011–5017. [PubMed: 7594508]

Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev. 1993; 6:382–427. [PubMed: 8269393]

Lampropoulou V, Hoehlig K, Roch T, Neves P, Calderon Gomez E, Sweenie CH, Hao Y, Freitas AA, Steinhoff U, Anderton SM, Fillatreau S. TLR-activated B cells suppress T cell-mediated autoimmunity. J Immunol. 2008; 180:4763–4773. [PubMed: 18354200]

Lehnardt S, Lachance C, Patrizi S, Lefebvre S, Follett PL, Jensen FE, Rosenberg PA, Volpe JJ, Vartanian T. The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci. 2002; 22:2478–2486. [PubMed: 11923412]

Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci USA. 2003; 100:8514–8519. [PubMed: 12824464]

Lenz DC, Lu L, Conant SB, Wolf NA, Gerard HC, Whittum-Hudson JA, Hudson AP, Swanborg RH. A *Chlamydia pneumoniae*-specific peptide induces experimental autoimmune encephalomyelitis in rats. J Immunol. 2001; 167:1803–1808. [PubMed: 11466406]

Li S, Strelow A, Fontana EJ, Wesche H. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc Natl Acad Sci USA. 2002; 99:5567–5572. [PubMed: 11960013]

Lublin FD. Delayed, relapsing experimental allergic encephalomyelitis in mice. Role of adjuvants and pertussis vaccine. J Neurol Sci. 1982; 57:105–110. [PubMed: 7153781]

Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. Annu Rev Immunol. 1992; 10:153–187. [PubMed: 1375472]

McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007; 8:913–919. [PubMed: 17712344]

McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med. 2005; 11:335–339. [PubMed: 15735651]

Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell. 1998; 2:253–258. [PubMed: 9734363]

Millefiorini E, Gasperini C, Pozzilli C, D'Andrea F, Bastianello S, Trojano M, Morino S, Morra VB, Bozzao A, Calo A, Bernini ML, Gambi D, Prencipe M. Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome. J Neurol. 1997; 244:153–159. [PubMed: 9050955]

Miller SD, Karpus WJ. The immunopathogenesis and regulation of T-cell-mediated demyelinating diseases. Immunol Today. 1994; 15:356–361. [PubMed: 7916948]

Miller SD, Vanderlugt CL, Begolka WS, Pao W, Yauch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat Med. 1997; 3:1133–1136. [PubMed: 9334726]

Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989; 7:145–173. [PubMed: 2523712]

Muzio M, Natoli G, Saccani S, Levrero M, Mantovani A. The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). J Exp Med. 1998; 187:2097–2101. [PubMed: 9625770]

Nair A, Frederick TJ, Miller SD. Astrocytes in multiple sclerosis: a product of their environment. Cell Mol Life Sci. 2008; 65:2702–2720. [PubMed: 18516496]

Nikbin B, Bonab MM, Khosravi F, Talebian F. Role of B cells in pathogenesis of multiple sclerosis. Int Rev Neurobiol. 2007; 79:13–42. [PubMed: 17531836]

Noseworthy JH. Progress in determining the causes and treatment of multiple sclerosis. Nature. 1999; 399:A40–A47. [PubMed: 10392579]

Oldstone MB. Molecular mimicry and autoimmune disease. Cell. 1987; 50:819–820. [PubMed: 3621346]

Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol. 2003; 4:161–167. [PubMed: 12539043]

Panitch HS. Influence of infection on exacerbations of multiple sclerosis. Ann Neurol. 1994; 36 Suppl:S25–S28. [PubMed: 8017885]

Prinz M, Garbe F, Schmidt H, Mildner A, Gutcher I, Wolter K, Piesche M, Schroers R, Weiss E, Kirschning CJ, Rochford CD, Bruck W, Becher B. Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. J Clin Invest. 2006; 116:456–464. [PubMed: 16440059]

Racke MK. Experimental autoimmune encephalomyelitis (EAE). Chap 9. Curr Protoc Neurosci. 2001 Unit 9.7.

Racke MK, Hu W, Lovett-Racke AE. PTX cruiser: driving autoimmunity via TLR4. Trends Immunol. 2005; 26:289–291. [PubMed: 15922942]

Rapp NS, Gilroy J, Lerner AM. Role of bacterial infection in exacerbation of multiple sclerosis. Am J Phys Med Rehabil. 1995; 74:415–418. [PubMed: 8534384]

Rocken M, Racke M, Shevach EM. IL-4-induced immune deviation as antigen-specific therapy for inflammatory autoimmune disease. Immunol Today. 1996; 17:225–231. [PubMed: 8991384]

Rowe DC, McGettrick AF, Latz E, Monks BG, Gay NJ, Yamamoto M, Akira S, O'Neill LA, Fitzgerald KA, Golenbock DT. The myristoylation of TRIF-related adaptor molecule is essential

for Toll-like receptor 4 signal transduction. Proc Natl Acad Sci USA. 2006; 103:6299–6304. [PubMed: 16603631]

Sadovnick AD, Ebers GC. Epidemiology of multiple sclerosis: a critical overview. Can J Neurol Sci. 1993; 20:17–29. [PubMed: 8467424]

Sanders P, De Keyser J. Janus faces of microglia in multiple sclerosis. Brain Res Rev. 2007; 54:274–285. [PubMed: 17383006]

Schnare M, Holt AC, Takeda K, Akira S, Medzhitov R. Recognition of CpG DNA is mediated by signaling pathways dependent on the adaptor protein MyD88. Curr Biol. 2000; 10:1139–1142. [PubMed: 10996797]

Segal BM, Chang JT, Shevach EM. CpG oligonucleotides are potent adjuvants for the activation of autoreactive encephalitogenic T cells in vivo. J Immunol. 2000; 164:5683–5688. [PubMed: 10820244]

Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet. 1985; 1:1313–1315. [PubMed: 2860501]

Sriram S, Mitchell W, Stratton C. Multiple sclerosis associated with *Chlamydia pneumoniae* infection of the CNS. Neurology. 1998; 50:571–572. [PubMed: 9484408]

Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol. 2005; 17:1–14. [PubMed: 15585605]

Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003; 21:335–376. [PubMed: 12524386]

Takeuchi O, Kaufmann A, Grote K, Kawai T, Hoshino K, Morr M, Muhlradt PF, Akira S. Cutting edge: preferentially the *R*-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. J Immunol. 2000; 164:554–557. [PubMed: 10623793]

Tauber SC, Nau R, Gerber J. Systemic infections in multiple sclerosis and experimental autoimmune encephalomyelitis. Arch Physiol Biochem. 2007; 113:124–130. [PubMed: 17922308]

Touil T, Fitzgerald D, Zhang GX, Rostami A, Gran B. Cutting edge: TLR3 stimulation suppresses experimental autoimmune encephalomyelitis by inducing endogenous IFN-beta. J Immunol. 2006; 177:7505–7509. [PubMed: 17114417]

Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol. 2002; 2:85–95. [PubMed: 11910899]

Visser L, Jan de Heer H, Boven LA, van Riel D, van Meurs M, Melief MJ, Zahringer U, van Strijp J, Lambrecht BN, Nieuwenhuis EE, Laman JD. Proinflammatory bacterial peptidogly-can as a cofactor for the development of central nervous system autoimmune disease. J Immunol. 2005; 174:808–816. [PubMed: 15634902]

Visser L, Melief MJ, van Riel D, van Meurs M, Sick EA, Inamura S, Bajramovic JJ, Amor S, Hintzen RQ, Boven LA, t Hart BA, Laman JD. Phagocytes containing a disease-promoting Toll-like receptor/Nod ligand are present in the brain during demyelinating disease in primates. Am J Pathol. 2006; 169:1671–1685. [PubMed: 17071591]

Waldner H, Collins M, Kuchroo VK. Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. J Clin Invest. 2004; 113:990–997. [PubMed: 15057305]

Wolf NA, Amouzegar TK, Swanborg RH. Synergistic interaction between Toll-like receptor agonists is required for induction of experimental autoimmune encephalomyelitis in Lewis rats. J Neuroimmunol. 2007; 185:115–122. [PubMed: 17341432]

Xu J, Drew PD. Peroxisome proliferator-activated receptor-gamma agonists suppress the production of IL-12 family cytokines by activated glia. J Immunol. 2007; 178:1904–1913. [PubMed: 17237441]

Xu J, Racke MK, Drew PD. Peroxisome proliferator-activated receptor-alpha agonist feno-fibrate regulates IL-12 family cytokine expression in the CNS: relevance to multiple sclerosis. J Neurochem. 2007; 103:1801–1810. [PubMed: 17727629]

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003; 301:640–643. [PubMed: 12855817]

Yamin TT, Miller DK. The interleukin-1 receptor-associated kinase is degraded by proteasomes following its phosphorylation. J Biol Chem. 1997; 272:21540–21547. [PubMed: 9261174]

Zekki H, Feinstein DL, Rivest S. The clinical course of experimental autoimmune encephalomyelitis is associated with a profound and sustained transcriptional activation of the genes encoding toll-like receptor 2 and CD14 in the mouse CNS. Brain Pathol. 2002; 12:308–319. [PubMed: 12146799]