Regulation of autoimmune encephalomyelitis by toll-like receptors
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ABSTRACT
Experimental autoimmune encephalomyelitis (EAE) is a Th17-mediated autoimmune disease and an animal model for multiple sclerosis (MS). Complete Freund’s adjuvant (CFA) contains pathogen-associated molecular patterns (PAMPs) that bind toll-like receptors (TLRs), and is necessary to induce EAE. Upstream TLR signals modify innate and adaptive immune responses in EAE. In detail, the common TLR adaptor molecule MyD88 is necessary for induction of EAE, and mediates activation of peripheral myeloid dendritic cells (mDCs) and differentiation of autoimmune Th17 cells. The stimulatory TLRs have not yet been identified for Th17 cells. TLR4 down regulates disease severity in EAE and Th17 cell responses, but promotes Th1 cell responses, which may inhibit the differentiation of Th17 cells. Moreover, treatment with a TLR4 ligand tolerizes mice and prevents EAE. TLR9 down regulates disease severity in myelin oligodendrocyte glycoprotein (MOG)-induced EAE, whereas it promotes disease in MOG35–55-induced EAE. Thus MyD88, TLR4 and TLR9 modify the disease process in EAE. Both endogenous and CFA-derived TLR ligands are implicated to modulate the disease process.

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1. Multiple sclerosis and experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disorder of the central nervous system (CNS). It affects young adults with an increasing female predominance and constitutes the most important cause of non-traumatic neurological disability. It is
considered a complex genetic disease and the etiology is likely interplay between genetic and environmental factors. Experimental autoimmune encephalomyelitis (EAE), the animal model for MS, is an invaluable method to study the human disease. Importantly, different EAE models mimic different aspects of MS. Rodents, but also primates, can develop EAE after injection of a myelin protein, e.g., myelin oligodendrocyte glycoprotein (MOG), or homogenized CNS-tissue [1]. Immunodominant peptides, such as MOG35–55 peptide can also be used. In this review, we will mainly discuss two murine EAE models: MOG-protein-induced EAE and MOG35–55 peptide-induced EAE, both in C57BL/6 mice. Although these two EAE models exhibit similar clinical signs, they differ significantly: a) B cells are critical for MOG-induced EAE, but are redundant in MOG35–55-induced EAE [2,3]. This is important, because B cells play a critical role in human MS [4]. b) MOG-induced EAE allows for priming of CD4 and CD8 T cells specific for several MOG peptides, whereas T cell responses in MOG35–55-induced EAE are restricted to one MHC II-binding MOG peptide. c) MOG35–55-induced EAE is a prototype Th17 cell-mediated disease [5], whereas MOG-induced EAE may have an additional encephalitogenic Th1 cell component [1]. d) Only MOG35–55-induced EAE requires more than one injection of the autoantigen.

2. Toll-like receptors and innate immunity

For many years research focused almost entirely on CD4 T cell responses in EAE. Indeed EAE can be induced by injection of CD4 T cells from a mouse with EAE into a healthy syngeneic mouse by adoptive transfer. However, in order to induce EAE, the myelin autoantigens must be emulsified with an adjuvant such as complete Freund's adjuvant (CFA), which contains Mycobacterium tuberculosis, and additional injections of pertussis toxin are required in both the murine MOG- and MOG35–55-induced EAE models discussed herein.

The discovery that certain bacterial molecules can trigger an innate immune response and subsequent adaptive immune responses towards an antigen drew attention to the role of the adjuvant during initiation of CD4 T cell responses in EAE. Bacterial and viral molecules constitute pathogen-associated molecular patterns (PAMPs) that bind toll-like receptors (TLRs). PAMPs can be lipids, lipopeptides, proteins or nucleic acids [6,7]. Initially these exogenous PAMPs were described in the context of infection, but endogenous ligands such as self-molecules from apoptotic cells can also trigger inflammation. In particular, endogenous heat-shock proteins, extracellular molecules from apoptotic cells can also trigger in the context of infection, but endogenous ligands such as self-DNA, self-brinogen, matrix fragments, brinogen, β-defensins and mRNA can ligate human TLRs [8–12]. In a systemic autoimmune disease such as lupus, self DNA in complexes with danger molecules such as HMGB1 stimulates innate immunity and ligates endogenous TLRs [13,14].

TLRs signal through the adaptor protein MyD88 which leads to activation of NF-κB and MAPK and subsequently induced production of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, IL-12 and tumor necrosis factor (TNF). TLR3 and TLR4 are exceptions: TLR3 signals through the MyD88-independent TRIF pathway which leads to transcription of high levels of type 1 interferons (IFNs); TLR4 signals via both the MyD88-dependent pathway and the MyD88-inde-
On the other hand, clinical studies clearly show a detrimental effect of B cells in human MS, and B cell-deficient mice are resistant to MOG-induced EAE, but not to MOG35–55-induced EAE [2,3]. Thus B cells have a detrimental effect in human MS, whereas they appear to have both detrimental and beneficial effects in EAE.

3.4. Modulation of EAE by TLR9

Mycobacteria are rich in unmethylated CpG DNA that binds to TLR9. The role for TLR9 in EAE has therefore been examined. Marta et al. show that TLR9−/− mice exhibits exacerbated symptoms of MOG-induced EAE (Fig. 1) [18]. Pro-inflammatory cytokine expression by splenic mDCs and T cells is similar in TLR9−/− and wild-type mice, although MOG-stimulated TLR9−/− splenocytes express more IL-6. This suggests that other cells than mDC or T cells express more IL-6 in TLR9−/− mice [18].

In sharp contrast, Prinz et al. show that TLR9−/− mice exhibit ameliorated symptoms of MOG35–55-induced EAE [19]. The decreased EAE susceptibility in TLR9−/− mice implicates an endogenous TLR9-dependent signal, because CNS inflammation is reduced very late (day 30) in the disease course [19]. It is unlikely that CFA-derived TLR9 ligands influence the disease course at this late time point. In another study, clinical signs of MOG35–55-induced EAE in mice with TLR9−/− B cells were similar to mice with TLR9+/+ B cells [24].

There are several putative explanations for the conflicting results. A critical difference is that the mice were immunized once with MOG in the study by Marta et al., whereas the immunization with MOG35–55 was given twice in the study by Prinz et al. The boost may have obscured the down regulatory role of TLR9 observed after MOG immunization. Furthermore, MOG protein induces pathogenic B cell responses and demyelinating autoantibodies similar to human MS. Interestingly, a similar dual role of TLR9 occurs in models for experimental lupus [12]. Kim et al. describe several possible mechanisms: (a) an anti-inflammatory net effect for TLR9 signaling despite its role in pathogenic antibody production; (b) induction of cross-tolerance upon repeated stimulation of one TLR family and (c) the role of TLR9 in the induction of tolerance [25].

3.5. TLR function in neuroinflammation

TLRs are expressed on cells of the CNS and can influence local CNS immune responses. There is a marked increase in expression of TLRs in MS brain lesions and cerebro-spinal fluid mononuclear cells as well as in EAE brain lesions [26,27]. Human microglia, but also murine microglia, expresses costimulatory molecules and MHC and has a role as antigen-presenting cell to reactivate infiltrating T cells. Microglia mounts specific activation programs suited to different TLR signals and links innate and adaptive immune responses where CD4 T cells get activated and/or proliferate. Signaling through TLR3, but not through TLR2 or TLR4, elicits IFN-α production whereas all three TLR mediate up-regulation of co-stimulatory molecules. Furthermore, TLR3 signals cause

![Fig. 1. Schematic illustration of a proposed mechanism for induction of EAE. Signaling via MyD88 is essential for initiation of EAE and mediates mDC IL-6 and IL-23 production and T cell IL-23R production. MOG-specific Th17 cells are primed, differentiated and maintained in the presence of mDC IL-6 and IL-23. The regulatory role of TLR4 or TLR9 on IL-17 production may act via downregulation of DC activation, and/or via dampened priming of encephalitogenic Th17 cells responses. Lines with bars represent inhibition.](https://example.com/fig1.png)
Th1 polarization with increased IFN-γ secretion concomitant with increased CD4 T cell death [28]. TLR signals, therefore, are potent modulators of microglial activation programs.

LPS-induced tolerance to cerebral ischemia was demonstrated using low dose systemic administration of LPS to render rats tolerant to ischemic brain damage induced by middle cerebral artery occlusion [29]. Upon TLR4 ligation, LPS-tolerant cells fail to recruit MyD88 to TLR4 and to activate IRAK-1 and NF-κB thereby leading to a block in TNF expression, in contrast to what occurs in naïve cells [30].

Moreover, Glezner I et al. injected Tween 20 into cerebral tissue of C3H/HeJ mice and observed that co-injection of LPS reverted the neurotoxic effect of the detergent, except in mice lacking TLR4 function [31]. Thus innate immunity and TLR4 signaling in microglia can protect neurons in the presence of toxic agents and trigger transcription factor activation of genes involved in oligodendrocyte progenitor cell recruitment and myelin repair.

4. Conclusions

MyD88 is required for the development of EAE, but the stimulatory TLRs that recognize CFA-derived PAMPs to differentiate and activate Th17 cells have not yet been identified. Proinflammatory cytokine expression by mDCs and subsequent Th17 cell responses depend on MyD88 signaling (Fig. 1). TLR4 and TLR9 have down regulatory roles and subsequent Th17 cell responses depend on MyD88 in MOG-induced EAE, but TLR9 has the opposite role in the innate immune system. Science 2002;296:298–300.


