

## Review

# Mechanism of experimental autoimmune neuritis in Lewis rats: the dual role of macrophages

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**Summary.** Human peripheral demyelinating diseases, such as Guillain-Barré syndrome (GBS), are characterized by inflammation and demyelination in the peripheral nervous system. Similarities in the pathology between GBS and the animal model of experimental autoimmune neuritis (EAN) indicate that autoimmune responses are involved in both diseases. This article summarizes the general aspects of the EAN model in Lewis rats and discusses the potential role of macrophages in the progression of EAN. A better understanding of macrophages may help to design alternative therapeutic strategies for organ-specific autoimmune diseases, including GBS.

**Key words:** Alternatively activated macrophages, Experimental autoimmune neuritis, Guillain-Barré syndrome, Peripheral nervous system inflammation

### Introduction

Human peripheral neuropathies including Guillain-Barré syndrome (GBS) are organ-specific immune diseases in which cellular and humoral immune components attack the peripheral nervous system (PNS) (Ho and Griffin, 1999). GBS is characterized as an acute neuropathy with inflammatory cell infiltration and demyelination, followed by spontaneous recovery (Griffin et al., 1996; Lu et al., 2000). Because of the presence of demyelinating lesions and the autoimmune nature of GBS, experimental autoimmune neuritis

(EAN) with autoimmune T cell infiltration and demyelination has been suggested as a model for human GBS (Hahn, 1996). Autoimmune T cells and macrophages play an important role in initiating PNS inflammation in both EAN and GBS, possibly through cytokine secretion (Lisak et al., 1997; Zhu et al., 1998) and the activation of various signals, including nuclear factor kappa B (Laura et al., 2006), mitogen-activated protein kinases (Ahn et al., 2004b; Moon et al., 2005; Ahn and Shin, 2006), phospholipase D (Shin et al., 2002), and caveolins (Ahn et al., 2006; Kim et al., 2007). Although autoimmune T cells have been regarded as one of the key cell types in organ-specific autoimmune diseases (Ho and Griffin, 1999; Matsumoto, 2000), macrophages and their secretion of pro-inflammatory mediators play an important role in the progression of PNS pathology. It is believed that macrophages, depending on the phenotype, play either a pro- or anti-inflammatory role or both in EAN (Kiefer et al., 2001). The scenario that macrophages play a pro-inflammatory role originated from the notion that macrophages increase during the peak stage of EAN. In addition, macrophages are known to secrete pro-inflammatory mediators. However, considering that EAN-afflicted rats recover from paralysis spontaneously, macrophages are believed to have an anti-inflammatory function in rat EAN (Kiefer et al., 2001). A population of macrophages, namely alternatively activated M2 phenotype macrophages, are thought to play an immunomodulatory role in EAE (Ahn et al., 2012) and EAN (Zhang et al., 2009b, 2012). Thus, whether macrophages play an immunomodulatory role leading to the spontaneous recovery of paralysis even though they increase in number at the peak stage of EAN must be discussed. Many EAN studies have indicated that macrophages and Schwann cells in the PNS secrete a variety of molecules

including erythropoietin (Ahn et al., 2010b), glial cell line-derived neurotrophic factor (GDNF) (Ahn et al., 2010a), and transforming growth factor-beta 1 (Kiefer et al., 2001), which are strongly associated with modulating PNS inflammation.

For a further understanding of PNS biology in human diseases and animal models, this review will summarize the general features of EAN in Lewis rats as a model of human PNS autoimmune diseases. In addition, the current knowledge on the molecules that are upregulated in EAN lesions will be discussed, with a particular emphasis on pro- and anti-inflammatory mediators in EAN lesions.

### Pathogenesis of EAN in Lewis rats

#### *Neuritogenic antigens*

Synthetic peptide 26, which corresponds to amino acid residues 53-78 of the bovine P2 protein, has been widely used to induce active EAN in Lewis rats (Rostami et al., 1990; Moon et al., 2006). Whole homogenates of the PNS have also been used (Mausberg et al., 2011).

#### *Induction of active and passive EAN*

Active EAN is induced in susceptible Lewis rats by immunizing PNS myelin or neuritogenic antigens emulsified with complete Freund's adjuvant containing heat-inactivated *Mycobacterium tuberculosis* (H37Ra) (Rostami et al., 1990; Ahn et al., 2010a,b). After immunization, the animals may lose body weight and progressively exhibit tail atony, gait ataxia, and hindlimb paralysis, followed by spontaneous recovery from paralysis (Lee and Shin, 2002; Mausberg et al., 2011). Autoimmune T cells are generated and infiltrate the PNS after immunization of neuritogenic antigens in susceptible animals. EAN lesions are formed, which are associated with the interruption of neural conduction in the PNS. Inflammation in the PNS of susceptible animals is largely matched with behavioral changes, including tail atony, hindlimb paralysis, and recovery. As a result, behavioral scores have been used to evaluate the degree of PNS inflammation.

The inflammatory cell phenotype and tissue reactions have been well evaluated in previous studies on active (Fujioka et al., 2000) and passively induced EAN (Izumo et al., 1985). Briefly, the cell phenotype of EAN lesions is characterized by the infiltration of CD4<sup>+</sup> T cells, macrophages, and other bystander cells (Moon et al., 2006). Focal demyelination is occasionally found in the PNS due to PNS inflammation (Mausberg et al., 2011). After Lewis rats recover from paralysis, the inflammatory response diminishes gradually, and is finally restored to a normal state. EAN is also reproducible through passive transfer of autoimmune T cells in susceptible animals, which is called passive EAN (Izumo et al., 1985; Rostami et al., 1985; Pilartz et

al., 2002). Passive EAN is characterized by an immune response and PNS pathology that largely matches that of active EAN.

The importance of pro-inflammatory cytokines in EAN has been well evaluated in previous reviews (Lisak et al., 1997; Zhu et al., 1997, 1998). Because neuritogenic T cells are the main cell types involved in the induction of EAN, the involvement of pro- and anti-inflammatory cytokines has been further analyzed in rats with EAN following treatment with statins (Li et al., 2011), antibiotics, including doxycycline (Yi et al., 2011) and minocycline (Zhang et al., 2009c), the selective Rho-kinase inhibitor fasudil (Pineda et al., 2011), and the peroxisome proliferator-activated receptor gamma agonist pioglitazone (Ramkalawan et al., 2012). Collectively, the therapeutic or preventive outcome of EAN after drug treatment is caused by the prevalence of anti-inflammatory cytokines in the peripheral immune system, including the spleen and lymph nodes of Lewis rats with EAN (Lisak et al., 1997; Zhu et al., 1997, 1998). Thus, manipulating anti-inflammatory cytokines from peripheral immune organs would be helpful in the design of a therapeutic strategy for EAN and GBS.

### Factors affecting EAN recovery

#### *Apoptosis of inflammatory cells*

Because EAN lesions are associated with the infiltration of T cells and macrophages, it is believed that eliminating these cells as primary targets would result in remission of EAN paralysis. Apoptosis of T cells has been regarded as one of the most important phenomena for the recovery of EAN (Zettl et al., 1994; Ahn et al., 2006; Kim et al., 2007). Although it is unclear which factors induce T cell apoptosis in EAN lesions, it is postulated that autoimmune T cells are primed prior to entry into the PNS or that certain factors, such as nitric oxide (NO) generated in the PNS (Lee and Shin, 2002) and p38 activation (Moon et al., 2005), are strongly associated with the induction of T cell apoptosis. Nevertheless, there are a few lymphocytes in the sciatic nerves of Lewis rats that recover from paralysis, suggesting that the majority of T cells are eliminated, possibly through apoptosis. Although the antigen presentation of T cells to Schwann cells and macrophages may occur in the sciatic nerve during the course of EAN, the reaction, if any, would be minimal, because rats with EAN recover soon after peak paralysis.

#### *Regulatory T cells*

The involvement of regulatory T cells has been suggested as a factor for the amelioration of EAN through the secretion of anti-inflammatory mediators (Lisak et al., 1997; Zhang et al., 2009a). This hypothesis is further supported by EAN therapeutic experiments (Zhang et al., 2009b; Li et al., 2011). Although the proportion of regulatory T cells in EAN lesions is not

high (Zhang et al., 2009a), their importance in the modulation of immune activation has been raised in the control of immune alterations (Peterson, 2012), particularly through the upregulation of anti-inflammatory cytokines.

#### *Apolipoprotein E in EAN*

The involvement of apolipoprotein E in EAN has been a focus in apolipoprotein E deficient mice, in which apolipoprotein E not only inhibits the onset of EAN, but also enhances the termination of immune responses in the PNS through modulation of T cell, macrophage, and Schwann cell functions (Zhang et al., 2010a, 2011a). In either case, amelioration of EAN is associated with the suppression of immune activation caused by autoimmune T cells and bystander macrophages and the activation and/or switching of anti-inflammatory actions in immune cells and Schwann cells in the PNS.

#### **Dual role of macrophages**

##### *Pro- and anti-inflammatory functions*

The dual role of macrophages has been clearly discussed in a previous review (Kiefer et al., 2001; Shin et al., 2012). It is believed that macrophages can be functionally polarized into pro-inflammatory (M1) and alternatively activated M2 phenotypes (Martinez et al., 2008; Cassetta et al., 2011; Sica and Mantovani, 2012).

In concert with autoimmune T cells in EAN, macrophages play a role in antigen presentation and also contribute to initiating PNS inflammation by secreting pro-inflammatory mediators, including tumor necrosis factor (TNF)- $\alpha$  (Kiefer et al., 2001), and the generation of NO through inducible nitric oxide (iNOS) (Lee and Shin, 2002; De La Hoz et al., 2010). There is general agreement that macrophages, namely the classically activated M1 phenotype, play an important role when EAN is initiated. The importance of macrophages has been further supported, because elimination of macrophages in rats immunized with neurotogenic antigen ameliorates the paralysis (Jung et al., 1993), suggesting that macrophages are strongly involved in the initiation of EAN, possibly through the stripping of myelin, as well as the secretion of pro-inflammatory mediators, including TNF- $\alpha$ . Although the involvement of macrophages via TNF- $\alpha$  secretion is well confirmed in the EAN induction stage of Lewis rats (Kiefer et al., 2001; De La Hoz et al., 2010), the roles of TNF- $\alpha$  and iNOS remain controversial. This is because the clinical severity and pathological lesions in the PNS of tumor necrosis factor receptor 1 (TNFR1) (p55) deficient (TNFR1 $^{-/-}$ ) mice are more severe than those of wild-type EAN mice (Lu et al., 2007). Similarly, experimental autoimmune encephalomyelitis (EAE), a variant form of an organ-specific central nervous system (CNS) autoimmune disease, is accelerated in iNOS deficient (knockout) mice, coinciding with greatly increased

numbers of Ag-specific Th1 cells in the peripheral immune system as well as in the CNS (Dalton and Wittmer, 2005). Thus, the role of either TNF- $\alpha$  or iNOS, or both, varies depending on the stage of autoimmune inflammation in animal models.

The involvement of M2 phenotype macrophages has been associated with the therapeutic effects of a plant-derived phenyl aziridine precursor (Zhang et al., 2009b) and a potent histone deacetylase inhibitor in rat EAN (Zhang et al., 2010b). Attenuated EAN is associated with an altered balance of M1/M2 macrophages in TNF- $\alpha$ -deficient mice (Zhang et al., 2012). Furthermore, Tim3 $^{+}$  macrophages, regarded as the M2 phenotype, appear in the later stages of rat EAN (Zhang et al., 2011b). In a variant CNS autoimmune disease model including EAE in Lewis rats, the involvement of alternatively activated macrophages by arginase-1 expression has been postulated to contribute to EAE remission (Mikita et al., 2011; Ahn et al., 2012). Taken together, it is highly possible that classical macrophages, namely the M1 phenotype (Kiefer et al., 2001; Jung et al., 1993), may play a role in initiating EAN lesions. Alternatively, M2 phenotype macrophages (Kiefer et al., 2001; Zhang et al., 2011b) in concert with regulatory T cells (Zhang et al., 2009a) at the peak and recovery stages of EAN may counteract pro-inflammatory mediators from Th1 cells and M1 macrophages and contribute to the remission of EAN lesions.

#### **Macrophage mediators in EAN**

In addition to the involvement of autoimmune T cells in the course of EAN, macrophages in concert with T cells are crucial for the amplification and effector phases. Specifically, they damage the myelin sheath by phagocytic attack and release inflammatory mediators, such as toxic radicals and arachidonic acid metabolites, in GBS and EAN (Hartung and Toyka, 1990; Kiefer et al., 2001; Shin et al., 2003; De La Hoz et al., 2010). It is believed that M1 phenotype macrophages, which secrete pro-inflammatory mediators, are involved in the induction stage of EAN. M2 phenotype macrophages have also been found in EAN lesions and may secrete many beneficial mediators including GDNF (Ahn et al., 2010a), erythropoietin (Ahn et al., 2010b), netrin-1 (Moon et al., 2006), heat-shock protein 70 (Zhang et al., 2009d), and osteopontin (Ahn et al., 2004a) in EAN lesions.

##### *Osteopontin*

Osteopontin functions in cell adhesion, chemotaxis, prevention of apoptosis, inhibition of iNOS, and cellular signaling by binding with integrin and CD44 receptors (Xie et al., 2001; Rittling, 2011; Shin, 2012). It has been suggested that osteopontin secreted by macrophages during the early stage of EAN may facilitate inflammatory cell migration as seen in EAE (Wang and Denhardt, 2008; Braitch and Constantinescu, 2010; Shin,

2012). In addition to the pro-inflammatory role of osteopontin in various types of inflammation (Morimoto et al., 2010; Rittling, 2011; Uede, 2011), osteopontin may function as an anti-inflammatory mediator in macrophages *in vitro* by suppressing iNOS (Gao et al., 2007; Rittling, 2011). In addition, it may play a role in CNS remyelination and neuroprotection (Braithc and Constantinescu, 2010; Wu et al., 2011), as well as in PNS injury (Jander et al., 2002). The involvement of osteopontin in the survival and migration of cancer cells (Wai and Kuo, 2004; Wai et al., 2006) is different from that of organ-specific autoimmune nervous system diseases. It is postulated that osteopontin, via inhibition of NO generation in Schwann cells and macrophages, may play a role in modulating PNS inflammation in rat EAN. The neuroimmunomodulatory roles of osteopontin in M2 phenotype macrophages remain to be elucidated in rat autoimmune PNS disease models.

### Conclusion and prospective

This review summarizes the general features of rat EAN and updates the recent data on macrophages in rat EAN following a comprehensive review (Kiefer et al., 2001). Considering the spontaneous recovery of EAN paralysis in Lewis rats, this model would be useful for studying autoimmune reactions in the PNS and for examining alternatively activated macrophages that are involved in the remission of PNS inflammation. A precise understanding of the various molecules expressed by macrophages in EAN-recovered rats will aid in the establishment of new therapeutic strategies for human PNS autoimmune diseases. The dual role of macrophages in the PNS and CNS remains to be clarified in relation to nervous system homeostasis.

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Accepted January 18, 2013