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Research Report

Diminished cytokine and chemokine expression in the central nervous system of GMF-deficient mice with experimental autoimmune encephalomyelitis

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ABSTRACT

Pro-inflammatory cytokines/chemokines are implemented in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model with clinical and pathological similarities to multiple sclerosis. We have previously shown that over-expression of glia maturation factor (GMF) in glial cells cause excessive production and secretion of pro-inflammatory cytokines/chemokines sufficient to destroy the myelin-forming oligodendroglial cell *in vitro*. In this present investigation, we evaluate the expression of pro-inflammatory mediators in the central nervous system (CNS) of GMF+/+ (wild type) mice and GMF–/– (GMF-knockout) mice at the peak of EAE induced by immunization with MOG 35–55 peptide. GMF+/+ (Wt) mice developed severe EAE with a maximal mean clinical score of 3.6 ± 0.5 by day 16 post-immunization, whereas GMF-KO mice showed significantly delayed EAE with an average onset on day 26 pi with reduced mean clinical score of 1.3 ± 0.3. Three of fifteen Wt mice as compared to none of GMF-KO mice died of EAE. Encephalitogenic cells from Wt mice transferred to recipient GMF-KO mice caused very mild and with low incidence of EAE. We determined the differences in the expression of cytokines, IFN-γ, TNF-α, IL-1 β, IL-6, IL-4, IL-10, and chemokines, MIP-1, MIP-2, IP-10, MCP-1, GM-CSF mRNA by quantitative real-time RT–PCR in brain and spinal cord. Our results demonstrate significantly low levels of pro-inflammatory cytokines/chemokines in the CNS of GMF-KO mice and increased expression in Wt mice with EAE. Our data suggest that GMF play a critical role in CNS inflammation.

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1. Introduction

Multiple sclerosis (MS) is a chronic, relapsing–remitting inflammatory demyelinating disease of central nervous system. The pathogenesis of the disease is characterized by the activation and infiltration of mononuclear cells, predominantly antigen-specific CD4+ and CD8+ T cells and B cells, in the central nervous system, reactivation by resident antigen-presenting microglial cells, secretion of proinflammatory cytokines/chemokines along with generation of other inflammatory mediators, such as complement, highly reactive free radicals (ROS, RNS), resulting in the demyelination of axons (Genain and Hauser, 2001; Iglesias et al., 2001; Noseworthy et al., 2000). Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS produced in laboratory animals by immunization with myelin-derived antigens. The disease is induced in susceptible mice, rat, or nonhuman primates by immunization with myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) or by the transfer of autoreactive CD4+ T cells (Linington et al., 1993; Tuohy, 1994). The disease EAE progresses by infiltration of autoreactive, myelin-specific T cells and activated macrophages into the CNS. These events start a series of inflammatory reactions in addition to direct toxicity of infiltrating cells and antibody-mediated toxicity. Cytokines and chemokines play an important role in the initiation and maintenance of the inflammatory environment in EAE. Precise mechanisms of action of these inflammatory mediators remain unclear.

Recent studies on glia maturation factor (GMF), a brain-specific protein, isolated, sequenced and cloned in our laboratory (Kaplan et al., 1991; Lim et al., 1989, 1990), have given new insights into its role in immunomodulatory and proinflammatory functions in CNS (Lim and Zaheer, 1996; Lim et al., 2000; Zaheer et al., 2002, 2004, in press, 2007). We have established that overexpression of GMF in astrocytes leads to immune activation of microglia, through secretion of granulocyte–macrophage–colony stimulating factor (GM-CSF), and resulting in production of several pro-inflammatory mediators that destroy oligodendrocytes, the myelin-forming cells in CNS. We hypothesize that intracellular GMF is involved in the pathogenesis of inflammatory diseases of the central nervous system such as MS and EAE. More recently, we have shown that GMF–/– mice (GMF-KO) were resistant to EAE induced by active immunization with MOG 35–55 antigen, whereas GMF +/+ (Wt) mice developed the disease characterized by significantly increased inflammation and demyelination in the central nervous system (Zaheer et al., 2007). In the present study, to analyze the inflammatory and immune mechanisms underlying the beneficiary effects of GMF deficiency in mice, we compared the development of EAE in wild type (Wt) and GMF-KO mice in both active and adoptive transfer models. To test our hypothesis that GMF exerts its effects by modulating the cytokine/chemokine profile in the CNS microenvironment, we studied the expression profile of cytokines, chemokines and inducible nitric oxide synthase (iNOS) during the progression of EAE in brain and spinal cord of GMF-KO and Wt mice.

2. Results

2.1. *GMF-KO mice are relatively resistant to MOG-induced EAE in active and passive transfer models*

GMF-dependent expression of several pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, GM-CSF and IP-10, are responsible for the destruction of myelin-forming oligodendrocytes in the central nervous system (Zaheer et al., in press). In the present study we investigated the involvement of GMF in both, the induction and the effector phases of EAE. For this purpose, we immunized GMF-deficient (GMF-KO) and control wild type (Wt) mice (8- to 10-week-old female) with MOG 35–55 peptide, and followed EAE development. Results in Fig. 1A show that all fifteen Wt mice developed EAE within 16 days with a maximal mean clinical score of 3.6±0.5. In the GMF-KO group only four mice out of sixteen developed EAE with a maximal mean clinical score of 1.3±0.3 in 26 days. There was

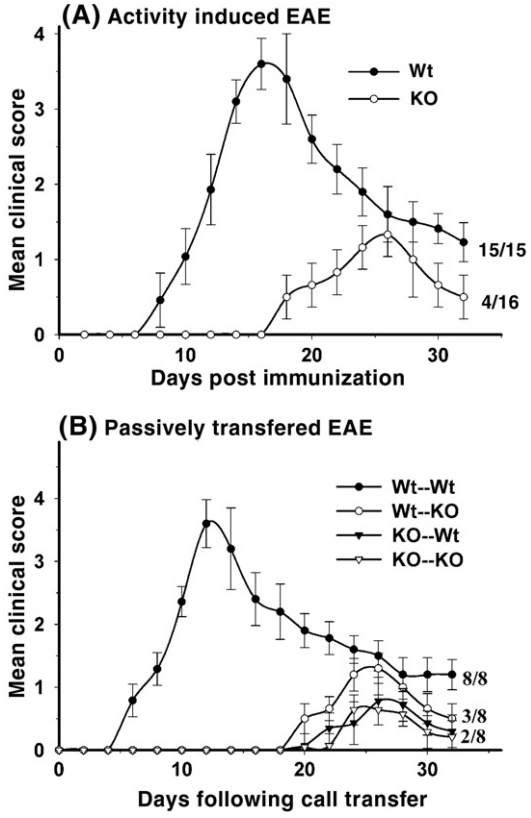

Fig. 1 – (A) Reduced severity, decreased incidence, and delay in onset of EAE in GMF-KO mice. GMF-KO (KO) and wild type (Wt) mice were immunized with MOG 35–55 peptide in CFA as described in Experimental procedures. Data are presented as mean EAE score and error bars represent ± SEM (n = 16 for GMF-KO, and n = 15 for Wt mice). These results represent data from three independent experiments. (B) Clinical score of EAE in GMF-KO and Wt recipient mice that received MOG 35–55-reactive T cells generated from KO or Wt mice. Data are presented as the EAE score (mean ± S.E.M.) in each group of eight recipient mice. Data shown represent two independent experiments.