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Journal of Neuroimmunology 186 (2007) 94–103

Journal of Neuroimmunology

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COX-2 inhibitors ameliorate experimental autoimmune encephalomyelitis through modulating IFN-γ and IL-10 production by inhibiting T-bet expression ☆

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Received 8 February 2007; received in revised form 6 March 2007; accepted 7 March 2007

Abstract

The COX-2 inhibitors Rofecoxib (Rof) and Lumiracoxib (Lum) were evaluated in experimental autoimmune encephalomyelitis (EAE), the model of multiple sclerosis (MS). Administration of Rof and Lum significantly reduced the incidence and severity of EAE, which was associated with the inhibition of MOG 35-55 lymphocyte recall response, anti-MOG 35-55 T cell responses, and modulation of cytokines production. In vitro Rof and Lum inhibited primary T cells proliferation and modulated cytokine production. These findings highlight the fact that Rof and Lum likely prevents EAE by modulating Th1/Th2 response, and suggest its utility in the treatment of MS and other autoimmune diseases.

Keywords: Rofecoxib; Lumiracoxib; Experimental autoimmune encephalomyelitis; T cell; Cytokine

1. Introduction

Multiple sclerosis (MS) is the most common central nervous system (CNS) demyelinating disease, affecting approximately 1,000,000 individuals worldwide (Steinman, 2001). Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated autoimmune disease, and serves as an animal model for human MS (Zamvil and Steinman, 1990). Similarities between Experimental autoimmune encephalomyelitis (EAE) and MS are present on many levels. In both diseases, $CD4^+$ and $CD8^+$ T cells can be found in lesions, including evidence of populations of clonally derived T cells, some reactive to myelin proteins. EAE and MS are characterized by damage to the myelin sheath and in both the animal models and in MS there is evidence for axonal degeneration (Steinman and Zamvil, 2005).

In EAE, imbalance in T cell activation contributes to the pathogenesis of autoimmune diseases evidenced by release of pro-inflammatory cytokines, IL-2 and IFN-γ, from Th1 cells (Chitnis and Khoury, 2003; Gor et al., 2003; Sredni-Kenigsbuch, 2002). On the other hand, it is known that anti-inflammatory cytokines such as IL-10 produced by Th2 cells are associated with remissions of the disease (Kennedy et al., 1992). IL-10 is crucial in inflammation with potent anti-inflammatory and immunosuppressive activities

☆ This study was supported in part by grants from the Ministry of Science and Technology of China (2004CB518902) and Shanghai Science and Technology Development Fund (05dz22914).

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doi:10.1016/j.jneuroim.2007.03.012

(Anderson et al., 2004). Some reports have shown that IL-10 is a key regulatory cytokine in EAE, and IL-10-deficient mice are highly susceptible to EAE (Bettelli et al., 1998; Segal et al., 1998; Samoilova et al., 1998). In addition, administration of IL-10 could mitigate the severity of EAE (Nagelkerken et al., 1997; Rott et al., 1994; Slavin et al., 2001).

Previous works described in the literature suggest that T-bet plays a critical role in the progression of EAE as it can promote Th1 development and IFN-γ production (Szabo et al., 2000). In vitro suppression of T-bet during myelin-specific T cell differentiation and in vivo administration of T-bet-specific anti-sense oligonucleotide or siRNA inhibited EAE (Lovett-Racke et al., 2004). T-bet-deficient mice immunized with myelin oligodendrocyte glycoprotein (MOG) are resistant to the development of EAE, with minimal inflammatory infiltrates in the CNS (Bettelli et al., 2004). Recently studies have shown that the treatment of COX-2 inhibitors prevented EAE (Moon et al., 2004; Miyamoto et al., 2006; Muthian et al., 2006), but the underlying mechanisms of action are not fully understood. In this paper, we demonstrate that COX-2 inhibitors could attenuate CNS inflammation and demyelination in EAE by inhibiting lymphocyte proliferation and IL-2/IFN-γ production. This process was accompanied by an increase in IL-10 production by auto-reactive T cells resulted at least in part from a suppression of T-bet expression.

IL-17 is a pro-inflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines, and cell adhesion molecules. (Komiyama et al., 2006). Recent reports illustrate that IL-17 is crucially involved in the cytokine network as an effector cytokine in EAE. Strong MOG-specific IL-17 production could be detected both in the spleen representing the immune periphery with the induction phase and the CNS as the target organ of the autoimmune T-cell response in acute EAE. Neutralization of IL-17 with a monoclonal antibody also ameliorated the disease course. (Hofstetter et al., 2005). The development of EAE was significantly suppressed in IL-17(−/−) mice, and adoptive transfer of IL-17(−/−) $CD4^+$ T cells inefficiently induced EAE in recipient mice (Komiyama et al., 2006).

In the present study, we immunized C57BL/6 mice with MOG 35-55 to induce to EAE, a T-cell-mediated experiment model of chronic and non-relapsing EAE, and examined the effects of Rofecoxib (Rof) and Lumiracoxib (Lum) on EAE. Our results demonstrate that Rof and Lum significantly reduced the incidence and severity of the disease, which was associated with direct inhibition of T cell proliferation, modulating Th1/Th2 response and suppressing IL-17 expression.

2. Materials and methods

2.1. Reagents

Rofecoxib (Rof) and Lumiracoxib (Lum) were provided by the National Center for Drug Screening. Peptide MOG 35-55 (MEVGWYRSPFSRVVHLYRNGK) was synthesized by Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China). The sequence was confirmed by amino acid analysis and mass spectroscopy, and the purity was greater than 95%. Complete Freund's adjuvant CFA and *Mycobacterium tuberculosis* H37Ra were purchased from Difco (Detroit, MI, USA). *Bordetella pertussis* toxin (PTX) and 3, 3′, 5, 5′-tetramethylbenzidine were supplied by Sigma-Aldrich. (St. Louis, MO, USA). RPMI 1640 was bought from GIBCO/Life Technologies Inc. (Gaithersburg, MD, USA), and fetal calf serum (FCS) obtained from Hyclone Laboratories (Logan, Utah, USA). [^{3}H] thymidine was provided by Shanghai Institute of Applied Physics, Chinese Academy of Science (Shanghai, PR China). The ELISA kits for IL-2, INF-γ and IL-10 were procured from PharMingen (San Diego, CA, USA).

2.2. Induction, treatment, and clinical evaluation of EAE

Female C57BL/6 mice, aged between 6 and 8 weeks, were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences. The animals were housed in a specific pathogen-free environment. Experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica. Murine active EAE model was produced as described previously (Fu et al., in press). Briefly, C57BL/6 mice were immunized on day 0 by s.c. injection with 100 μl of an emulsion of MOG 35-55 peptide in CFA. Each mouse additionally received PTX via i.p. injection on day 0 and day 3 post-immunization (p.i.). Rof or Lum (50 mg/kg) was dissolved in PBS containing 0.5% sodium CM-cellulose and daily administered orally following the immunization and continued throughout the study ($n=10$ mice). To examine the therapeutic efficacy, Rof or Lum (50 mg/kg) was administered from days 14 to 30 p.i. The dose of Rof or Lum was chosen on the basis of preliminary experiments. Control mice received orally an equal volume of PBS containing 0.5% CM-cellulose ($n=10$).

2.3. Histopathological analysis

To assess the degree of CNS inflammation and demyelination, mice in vehicle, Rof or Lum treated groups were anaesthetized by i.p. injection of sodium pentobarbital (30 mg/kg) on day 21 (at the peak of the disease) and perfused with 20 ml cold PBS. Spinal cords were dissected and fixed in 10% formalin. Five-micrometer thick transverse sections were taken from cervical, upper thoracic, lower thoracic, and lumbar regions of the spinal cord (four sections per mouse). The sections were stained with H&E to examine inflammation. Signs of inflammation in the anterior, posterior, and two lateral columns (four quadrants) of the section were scored under microscope by the standard described previously (Fu et al., in press).

2.4. Splenocyte response assay

The in vivo and in vitro effects of COX-2 inhibitors on neural antigen specific T cell proliferation were measured by [^{3}H] thymidine incorporation assay. Spleens ($n=10$) were removed from MOG-immunized mice treated with vehicle or COX-2 inhibitors on day 14 p.i. Splenocyte (5×10^5 cells/well) suspensions were prepared from various treatment groups as