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Production and functions of IL-17 in microglia

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Abstract

Interleukin (IL)-17-producing helper T cells may play a pivotal role in the pathogenesis of multiple sclerosis. Here, we examined the effects of IL-17 on microglia, which are known to be critically involved in multiple sclerosis. Treatment with IL-17 upregulated the microglial production of IL-6, macrophage inflammatory protein-2, nitric oxide, adhesion molecules, and neurotrophic factors. We also found that IL-17 was produced by microglia in response to IL-23 or IL-1β. Because microglia produce IL-1β and IL-23, these cytokines may act in an autocrine manner to induce IL-17 expression in microglia, and thereby contribute to autoimmune diseases, such as MS, in the central nervous system.

Keywords: Cytokine; IL-17; EAE; MS; Microglia

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder that affects the central nervous system (CNS). Although the etiology of MS is not fully understood, T helper 1 (Th1) cells and the cytokines that they produce are thought to play a role in the development of MS. Recently, interleukin (IL)-17 producing helper T (Th17) cells play important roles in the induction of autoimmune diseases including MS and the corresponding animal model—experimental autoimmune encephalomyelitis (EAE) (Hofstetter et al., 2005, Ishizu et al., 2005, Iwakura and Ishigame, 2006). It has been shown that IL-17 mRNA levels are high in both the cerebrospinal fluid and plaques of MS patients (Matusevicius et al., 1999, Lock et al., 2003). IL-17 is a T cell-derived proinflammatory molecule that stimulates epithelial, endothelial, and fibroblastic cells to produce other inflammatory cytokines and chemokines, including IL-6, macrophage inflammatory protein (MIP)-2, granulocyte-colony stimulating factor (G-CSF), and monocyte chemoattractant protein (MCP)-1 (Aggarwal and Gurney, 2002; Yao et al., 1995; Kennedy et al., 1996; Fossiez et al., 1996; Linden et al., 2000; Cai et al., 1998; Jovanovic et al., 1998; Laan et al., 1999). IL-17 also synergizes with other cytokines such as tumor necrosis factor (TNF)α and IL-1β to further induce chemokine expression (Jovanovic et al., 1998; Chabaud et al., 1998). Although the precise mechanisms that control Th17 cell development have yet to be elucidated, Th17 cells are thought to develop from naïve T helper (Th0) cells via a pathway that is different than the pathways that lead to the development of Th1 and Th2 cells. In the absence of interferon (IFN)γ and IL-4, IL-23 has been shown to maintaining Th17 phenotype in a manner that is not dependent on the transcription factors STAT1, T-bet, STAT4, and STAT6 (Aggarwal et al., 2003; Harrington et al., 2005; Park et al., 2005; Bettelli et al., 2006). Interestingly, a recent study revealed that IL-27 is a critical regulator of IL-17 production. IL-27 receptor-deficient mice were found to generate more IL-17-producing T helper cells and were hypersusceptible to EAE, suggesting that IL-27 negatively regulates the development of Th17 cells (Batten et al., 2006).

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IL-17 levels have been shown to be significantly higher in the cerebrospinal fluid of patients with active optico-spinal MS (Ishizu et al., 2005) and in the CNS of EAE mice (Hofstetter et al., 2005). The effects of IL-17 on CNS cells, however, are unclear. In order to uncover the contribution of IL-17 to inflammatory demyelination in the CNS, we have examined the effects of IL-17 on microglia, which function as antigen-presenting cells and effector cells in the CNS during inflammatory demyelination.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS), human recombinant transforming growth factor (TGF)-β, and mouse recombinant IL-17 and IL-23 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Mouse recombinant IL-1β, TNFα, and IFNγ were purchased from Techne (Minneapolis, MN, USA). Sulfonylamide, *N*-(1-naphthyl)ethylenediamine, and phosphate for Griess reagent (Ignarro et al., 1987) were also purchased from Sigma-Aldrich.

2.2. Cell culture

The protocols for the animal experiments were approved by the Animal Experiment Committee of Nagoya University. All primary cultures were prepared from C57BL/6J mice (Japan SLC, Hamamatsu, Shizuoka, Japan). Microglia were isolated from primary mixed glial cell cultures prepared from newborn mice on day 14 using the "shaking off" method as previously described (Suzumura et al., 1987); the purity of the cultures was almost 100%, as determined by immunostaining with anti-CD11b antibodies. The cultures were maintained in Dulbecco's modified Eagle's minimum essential medium (Sigma-Aldrich) supplemented with 10% fetal calf serum (JRH Biosciences, Lenexa, KS, USA), 5 μg/ml bovine insulin (Sigma), and 0.2% glucose.

Astrocyte-enriched cultures were prepared as described previously (Kuno et al., 2006). Briefly, the mixed glial cell cultures were trypsinized after the microglia were collected, and replated in Petri dishes. After this procedure was repeated three times, the cultures that had undergone four passages were used as the astrocyte-enriched cultures. The purity of the cultures were more than 80% as determined by immunostaining with anti-glial fibrillary acidic protein (GFAP). Peritoneal macrophages were collected from mice intraperitoneally injected with thioglycolate 48 h prior to collection. T cell-rich lymphocytes were separated from mouse spleens. Neuronal cultures were prepared from mice at embryonic day 17 as described previously (Takeuchi et al., 2005). Briefly, cortices were dissected and freed of meninges. Cortical fragments were dissociated into single cells using dissociation solution, and they were resuspended in Nerve-Cell Culture Medium (serum-free conditioned medium from 48-h rat astrocyte confluent cultures based on Dulbecco's modified Eagle's minimum essential medium/F-12 with N2 supplement, Sumitomo Bakelite, Akita, Japan). The purity of the cultures was more than 95% as determined by NeuN-specific immunostaining.

2.3. Expression of IL-17 receptors

The mRNA expression of the IL-17 receptor was examined using reverse transcription-polymerase chain reactions (RT-PCRs). Microglia, astrocytes, or neurons were cultured for 3 days before total cellular RNA was extracted using an RNase Mini Kit (Qiagen). cDNA encoding the IL-17 receptor was examined by RT-PCR analysis using SuperScript II (Invitrogen), AmpliTaq DNA polymerase (Applied Biosystems), and the specific primers shown in Table 1. Amplification within the linear range using 5 μl of each cDNA sample was achieved following 30 cycles in a DNA thermal cycler under conditions that were optimized for each set of primers.

The protein level of IL-17 receptor expression was examined using Western blot analysis. Samples (20 μg/well) were electrophoresed on 7.5% SDS-polyacrylamide gels (Invitrogen) according to the Laemmli method (Laemmli and Favre, 1973). After electrophoresis, proteins were transferred from the gels to nitrocellulose membranes (Amersham Bioscience, Buckinghamshire, UK) using standard procedures (Towbin et al., 1979). Nonspecific binding was blocked with 5% nonfat dry milk in TBST buffer (5 mM Tris–HCl, pH 7.6, 136 mM NaCl, 0.05% Tween 20) for 1 h. Blots were incubated for 12 h at 4 °C with rat anti-mouse IL-17 receptor antibody (R&D Systems) (1:1000 dilution). Blots were washed four times in TBST: the first time for 20 min and 10 min each time thereafter. We then incubated the washed blots for 1 h at room temperature with a

Table 1
Primer sequences used for RT-PCR analysis

GAPDH sense, 5′-ACTCACGGGAAATTCAACG
GAPDH antisense, 5′-CCCTGTTGCTGTAGCCGTA
IL-17R sense, 5′-CTAAACTGCACGGTCAAGAAT
IL-17R antisense, 5′-ATGAACCAGTACACCCAC
TNFα sense 5′-ATGAGCACAGAAAGCATGATCCGC
TNFα antisense 5′-CCAAAGTAGACCTGCCCGGACTC
IL-1β sense, 5′-ATGGCAACTGTTCCTGAACTCAACT
IL-1β antisense, 5′-CAGGACAGGTATAGATTCTTTCCTTT
IL-6 sense, 5′-ATGAAGTTCCTCTCTGCAAGAGACT
IL-6 antisense, 5′-CACTAGGTTTGCCGAGTAGGATCTC
MIP-2 sense, 5′-CCGGCTCCTCAGTGCTG
MIP-2 antisense, 5′-GGTCAGTTAGCCTTGCCTTT
IL-17 sense, 5′-CAGGACGCGCAAAACATGA
IL-17 antisense, 5′-GCAACAGCATCAGAGAGACACAGAT
iNOS sense, 5′-CCCTTCCGAAGTTTCTGGCAGCAGC
iNOS antisense, 5′-GGCTGTCAGAGCCTCGTGGCTTTGG
NGF sense, 5′-CATAGCGTAATGTCCATGTTGTTCT
NGF antisense, 5′-CTTCTCATCTGTTGTCAACGC
BDNF sense, 5′-AGCCTCCTCTGCTCTTTCTG
BDNF antisense, 5′-TTGTCTATGCCCCTGCAGCC
GDNF sense, 5′-ATTTTATTCAAGGCCACCATTA
GDNF antisense, 5′-GATACATCCACACCGTTTTAGC
MHC class II antigen sense, 5′-AAGAAGGAGACTGTCTGGATGC
MHC class II antigen antisense, 5′-TGAATGATGAAGATGGTGCCC
ICAM-1 sense, 5′-TTCACACTGAATGCCAGCTC
ICAM-1 antisense, 5′-GTCTGCTGAGACCCCTCTTG
VCAM-1 sense, 5′-ATTTTCTGGGGCAGGAAGTT
VCAM-1 antisense, 5′-ACGTCAGAACAACCGAATCC