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Decreased interferon-α production in response to CpG DNA dysregulates cytokine responses in patients with multiple sclerosis

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Abstract Type I interferons (IFNs), represented by IFN-α and β, activate immune effector cells belonging to the innate and adaptive immune systems. Plasmacytoid dendritic cells (pDCs) produce IFN-α in response to CpG DNA. We aimed to examine the impact of pDC-produced IFN-α on the adaptive immune system in Multiple Sclerosis (MS). Our results demonstrated that CpG DNA-induced IFN-α production was significantly decreased in PBMCs from MS patients. Decreased levels of IL-12 p70, IFN-γ, and IL-17 and increased level of IL-10 were found in CpG DNA-treated PBMCs of healthy subjects unlike in those from MS patients. In samples pre-treated with IFN-α and IFN-β, decreased levels of IL-12 p70, IFN-γ, and IL-17 and increased level of IL-10 were detected in PBMCs from MS patients. These results suggest that CpG DNA-induced decreased IFN-α production causes pro-inflammatory cytokine secretion, and either IFN-α or IFN-β induces anti-inflammatory cytokine secretion in the adaptive immune system in MS.

1. Introduction

MS is an inflammatory demyelinating disease that affects the CNS. Target-specific T cells, which are normally controlled by tolerance mechanisms, are assumed to initiate and perpetuate an autoimmune reaction that leads to severe CNS damage [1]. The mechanisms of initial T-cell activation is

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not known; however, it has been shown that bacterial or viral infections can trigger MS onset and/or relapse via molecular mimicry and induction of Toll-like receptor (TLR) signaling [2]. For established MS patients, the risk of disease exacerbation was found to be increased at the time of or shortly after clinical viral infections [3].

Dendritic cells (DCs) are critically involved in the primary induction of T cell activation as part of the mounting immune response. In humans, there are at least 2 main subsets of peripheral blood DCs: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) [4]. The most striking feature of pDCs distinguishing this subset from other DCs is a vigorous reaction to various pathogens with high secretion of type I interferons (IFNs), which consist of 5 differential subtypes: α, β, ω, ε, κ [5,6]. pDCs, the most important type I IFN-producing cells, are key effector cells in the innate immune system because of their ability to produce large amounts of IFN-α in response to microbial and viral infections [7]. Compared to other peripheral blood mononuclear cells (PBMCs), the pDCs express a high level of TLR9, which recognizes CpG DNA within the early endosomes at the initial phase of viral infection [8]. TLR9 is also expressed at a high level in B cells, and we have previously reported that TLR9-mediated IL-10 production was significantly decreased in the B cells of MS patients [9].

Type I IFNs are inducible cytokines with potent antiviral and anti-proliferative effects. IFN-β, which is one of the type I IFNs, is the first-line treatment for relapsing–remitting MS, and decreases the relapse rate by ~30% with reduction of brain magnetic resonance imaging (MRI) activity [10]. IFN-β is considered to inhibit differentiation from naïve T cells into Th1 and Th17 cells and effector function [11]; however, the mechanisms underlying the action of IFN-β, particularly those via the type I IFN pathway, have not been fully understood.

Considering this background, we aimed to study the impact of the type I IFNs produced by the pDCs on the adaptive immune system of the patients with MS by studying cytokine profiles and their responses upon stimulation with CpG DNA. As a result, we demonstrated that decreased IFN-α production in response to CpG DNA induces pro-inflammatory cytokine secretion in the adaptive immune system of MS patients, and that either IFN-α or IFN-β may induce anti-inflammatory cytokine secretion to correct the development of Th1 and Th17 cells, possibly through the type I IFN pathway.

2. Materials and methods

2.1. MS patients and healthy individuals

We recruited 15 patients with relapsing–remitting MS [age, 36.2 ± 10.5 years (mean ± SD)] and 15 age- and sex-matched healthy subjects (age, 34.8 ± 11.0 years). The diagnosis of MS was established according to Poser's criteria [12], and patients with optico-spinal MS or neuromyelitis optica [13] were excluded. All MS patients were in remission and not treated with disease-modifying drugs, including IFN-β and corticosteroids, and other immunosuppressive drugs for at least 6 months. The study was approved by the ethical committee of Hokkaido University Hospital. All subjects granted written consent forms.

2.2. Measurement of IFN-α secreted from the PBMCs stimulated with CpG DNA

Venous blood was drawn into tubes containing ethylenediamine-tetraacetic acid (EDTA), and peripheral blood mononuclear cells (PBMCs) were freshly separated by standard density centrifugation and Ficoll-Paque (Pharmacia Biotech). The isolated PBMCs were cultured at a density of 2×10^6 cells per well in 24-well, flat-bottomed plates in complete RPMI 1640 medium supplemented with 10% fetal calm serum (HyClone Laboratories, Logan, UT), 100 IU/ml of penicillin, and 100 μg/ml of streptomycin. CpG oligodeoxynucleotide 2216 (5′-ggGGGACGATCGGTCgggggG-3′; HyCult Biotechnology), which is known to selectively activate pDCs, was used optimally at 0.5 or 1.0 μM. The CpG-stimulated cells were then cultured for 24, 48, and 72 h at 37 °C in a humidified atmosphere containing 5% CO_2 before collecting the supernatants. Aliquots of the supernatants were prepared and frozen at −80 °C until batch analysis. IFN-α was assayed using Human IFN-α ELISA kits (PBL Biomedical Laboratories) according to the manufacturer's protocol.

2.3. Measurement of levels of IL-10, IL-12, IL-17, IL-23, and IFN-γ secreted from the PBMCs pre-treated with CpG DNA or type I IFNs

For the detection of IL-10, IL-12, IL-17, IL-23, and IFN-γ from the PBMCs treated with CpG DNA or type I IFNs, we used the protocol described previously by Meyers et al. [14]. As briefly described, freshly isolated PBMCs were cultured at a density of 1×10^5 cells per well in 24-well flat-bottomed plates in complete RPMI 1640 medium supplemented with 10% fetal calf serum, 100 IU/ml of penicillin and100 μg/ml of streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. In these conditions, PBMCs were either pre-treated or not pre-treated with either 1.0 μM of CpG oligodeoxynucleotide 2216 (HyCult Biotechnology), 2000 U/ml of recombinant human IFN-α (PBL Interferon Source), or 2000 U/ml of recombinant human IFN-β1b. To confirm the effects of both IFN-α and IFN-β, 10 μg/ml of B18R (eBioscience), which is a type I IFN neutralizing agent, was added in some conditions [14]. The B18R is a type I IFN receptor encoded by the B18R gene of the western reserve vaccinia virus strain, enabling blockage of both autocrine and paracrine IFN functions [15]. Pre-treated cells were then stimulated with 1 μg/ml of staphylococcal enterotoxin A (SEA) (Toxin Technology, Inc) at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h, and the supernatants were collected. SEA is used for stimulation as it activates a large population of both naïve and effector/memory human T cells in an antigen-presenting cell-dependent manner that allows for the creation of an immune synapse [14]. Aliquots of the supernatants were frozen at −80 °C until batch analysis was performed for measuring cytokines by standard ELISA. IL-10, IL-12 (p70), and IFN-γ were assayed using Human OptEIA ELISA kits (BD Biosciences), and IL-17 and IL-23 were assayed using Human Quantikine ELISA kits (R&D Systems), according to the manufacturer's protocol.