Regulatory Effects of IFN-β on Production of Osteopontin and IL-17 by CD4⁺ T Cells in MS

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ABSTRACT

Interferon-beta (IFN-beta) currently serves as one of the major treatments for multiple sclerosis (MS). Its anti-inflammatory mechanism has been reported as involving a shift in cytokine balance from Th1 to Th2 in the T cell response against elements of the myelin sheath. In addition to the Th1 and Th2 groups, two other important pro-inflammatory cytokines, IL-17 and osteopontin (OPN), are believed to play important roles in CNS inflammation in the pathogenesis of MS. In this study, we examine the potential effects of IFN-beta on the regulation of OPN and IL-17 in MS patients. We find that IFN-beta used *in vitro* at 0.5-3 ng/ml significantly inhibits the production of OPN in primary T cells derived from peripheral blood mononuclear cells. The inhibition of production of OPN is determined to occur on the CD4+ T cell level. In addition, IFN-beta can be seen to inhibit the production of IL-17 and IL-21 in CD4+ T cells. Further experiments show, in addition to monocytic cytokine mediated suppression of IL-17, that IFN-beta directly acts on CD4+ T cells to regulate OPN and IL-17 expression through type I IFN receptor mediated activation of STAT1 and suppression of STAT3 activity. In an animal system, administration of IFN-beta to EAE mice is shown to ameliorate disease severity. Further, spinal cord infiltration of OPN+ and IL-17+ cells was observed to be decreased in treated EAE mice along with serum levels of OPN and IL-21. Importantly, decreased OPN production by IFN-beta treatment contributes to the reduced migratory activity of T cells. Taken together, the results from both *in vitro* and *in vivo* experiments indicate that IFN-beta treatment can down-regulate OPN and IL-17 production in MS. This study provides new insights into the mechanism of action of IFN-beta in the treatment of MS.

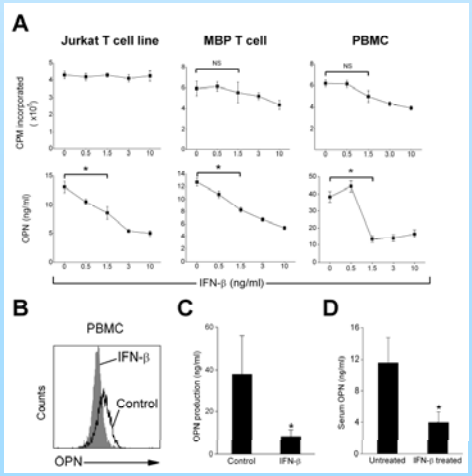
INTRODUCTION

CNS inflammation is considered an important feature in MS pathology and is directly associated with the disease process in MS. Agents that have anti-inflammatory properties have been shown to suppress the disease activity to various degrees. MS is now commonly treated with an immunomodulatory agent, interferon-beta (IFN-beta) which has shown significant treatment efficacy and is thought to involve a number of different mechanisms of action. However, despite extensive clinical experience in the use of IFN-beta, its mechanism of action has not been fully elucidated.

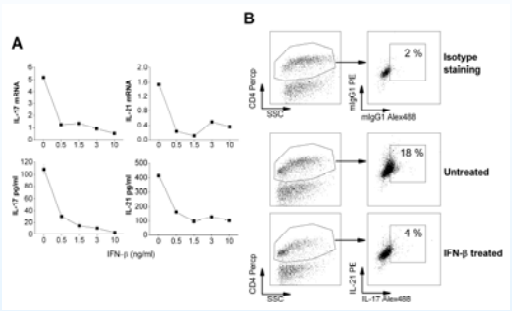
This study was undertaken to evaluate the potential effect and mechanisms of action of IFN-beta on the production of pro-inflammatory or inflammatory cytokines such as OPN and IL-17 in peripheral blood mononuclear cells (PBMC) of patients with MS. In the first part of the study, experiments were designed to directly examine the *in vitro* effect of IFN-beta on the production of OPN and IL-17 in PBMC derived from MS patients and controls. The second part of the study was carried out to evaluate the *in vivo* effectiveness of IFN-beta in suppression of the production of OPN and IL-17 in T cells. To evaluate the *in vivo* effect, EAE mice were treated with IFN-beta. *In vivo* production of IL-17 and OPN were assessed at serum and cellular levels. The findings described here provide new insights into the roles of IFN-beta in the regulation of IL-17 and OPN as an important treatment mechanism.

RESULTS

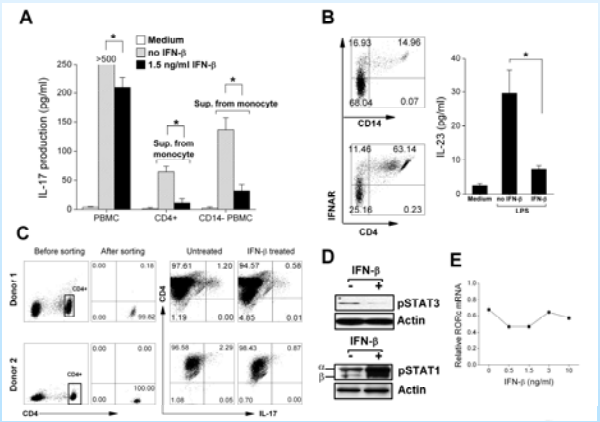
In vitro effects of IFN-beta on the expression of OPN in a leukemic T cell line, primary T cells and peripheral blood mononuclear cells

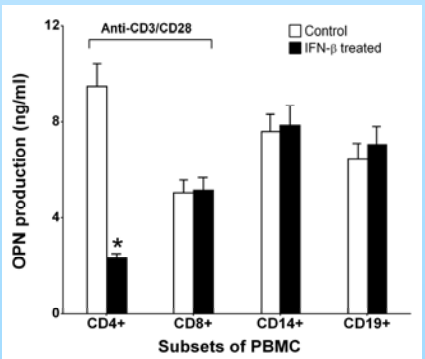


In vitro effect of IFN-beta on Th17 cells

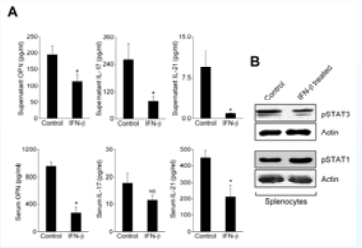


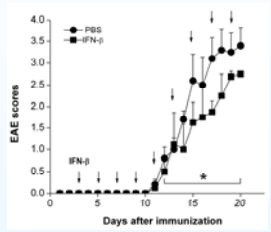
IFN-beta depresses IL-17 secretion by CD4+ T cells in the absence of monocytes and Effect of IFN-beta on inhibition of IL-17 is mediated by STAT3 in CD4+ T cells

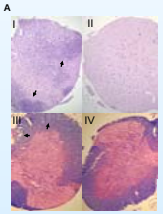


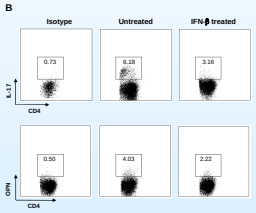


Inhibition of IL-17, IL-21 and OPN production in vivo in EAE mice after subcutaneous injection of IFN-beta

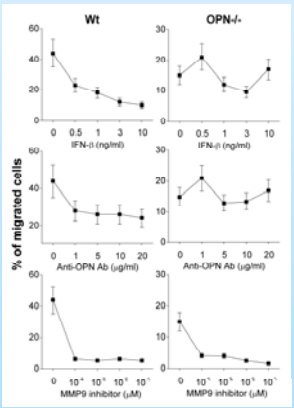








IFN-beta inhibits RANTES-induced T cell migration in part through reduced production of OPN



CONCLUSIONS

IFN-β regulates the production of the pro-inflammatory cytokine osteopontin and IL-17 in CD4⁺ T cells *in vitro* experiments.

IFN-β inhibits the activity of Th17 cells directly through the activation of STAT-1 and suppression of STAT-3 as well as indirectly through the simulation of monocytes.

In IFN-β treated MS patients, there are significant decreases of the production of osteopontin and IL-17 in peripheral T cells.

In the brain and spinal cord of EAE mice treated with mIFN-β *in vivo*, the production of IL-17 and osteopontin are markedly decreased.

The migratory activity of inflammatory T cells is impaired when the expression of osteopontin is blocked.

The down-regulations of osteopontin and IL-17 in CD4⁺ T cells are important mechanisms of IFN-β in the treatment for MS.