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In vitro Th2 deviation of myelin-specific peripheral blood lymphocytes from patients with multiple sclerosis

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Abstract

This study aimed at investigating if selective ex vivo immune deviation of myelin-specific cytokine secretion towards Th2 is possible in blood cells from patients with multiple sclerosis (MS). Interleukin (IL)-4 (Th2) and interferon-γ (Th1) secreting cells were recorded by ELISPOT in 13 MS patients. Deviation was successful in 10 patients. Interleukin-4 alone was most effective in inducing myelin-specific immune deviation in MS patients whereas IL-1 or IL-15 in combination with IL-4 did not improve the results. Further studies and improvements are needed before ex vivo immune deviation can be considered a potential treatment in patients with MS.

Keywords: Enzyme linked immunospot (ELISPOT); Immune deviation; Interferon-γ; Interleukin-4; Multiple sclerosis; Myelin; Peripheral blood mononuclear cells (PBMC)

1. Introduction

After the finding of two different subsets of Th cells in mice (Mosmann et al., 1986), Th1 and Th2, which were later verified to exist in human as well (Salgame et al., 1991), this paradigm has been widely accepted. Human Th1 cells secrete interferon-γ (IFN-γ) and tumor necrosis factor β (TNF-β) (Mosmann and Sad, 1996) and are crucial for the cell mediated immune response to intracellular pathogens including activation of phagocytic cells and cytotoxic reactions (Mosmann and Coffman, 1989). Human Th2 cells on the other hand secrete interleukin-4 (IL-4), IL-5 and IL-9 (Mosmann and Sad, 1996) and promote development of IgE and IgG4 and elimination of extracellular parasites by activation of B-cells, mast cells and basophils (Mosmann and Coffman, 1989).

Multiple Sclerosis (MS) is a chronic inflammatory disease where Th1 like responses from myelin-specific CD4+ T cells, as secretion of pro-inflammatory IFN-γ, are believed to play a major role in the pathogenesis (Hemmer et al., 2002). There are, however, reports contradicting the Th1/Th2 dichotomy in MS where no differences in the Th1 / Th2 ratio were found between MS patients and healthy controls (Franciotta et al., 2000). The myelin-specific T cells that mediate tissue destruction in MS are believed to become activated outside the central nervous system (CNS) in lymphoid tissue (Xiao and Link, 1999) and when they cross the blood brain barrier they will re-encounter their antigen. The restimulated T cells recruit additional T cells and macrophages, induce antibody production and activate macrophages/microglia, which are thought to cooperate in demyelination (Xiao and Link, 1999). The mechanism whereby cells are recruited back to their original tissues is called homing (Tanaka et al., 2004) and is crucial

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for success of transfer of ex vivo immune deviated lymphocytes, where former deteriorating cells return as controllers of inflammation.

Immune deviation is the redirection of the immune response from most often Th1 like responses to Th2 like responses, even though the opposite can also occur (Rocken et al., 1996). It has been shown in several murine studies that cells cultured in IL-4 take on a Th2 like phenotype, and that IL-4 is necessary and sufficient for the redirection (Wang and Mosmann, 2001; Oriss et al., 1997; Abehsira-Amar et al., 1992; Swain et al., 1990). The lack of IL-4 leads to preferential development of Th1 cells (Abehsira-Amar et al., 1992). IL-1 functions as a co-stimulator for the proliferation of Th2, but not Th1 clones, and maximizes the proliferative response of Th2 cells (Wang and Mosmann, 2001; Joseph et al., 1998; Oriss et al., 1997; Chang et al., 1990; Lichtman et al., 1988), but whether IL-1 influences the production of IL-4 is debated (Joseph et al., 1998; Lichtman et al., 1988). The Th2 pattern that results from IL-4 priming is very persistent, and once the polarization has occurred it will be retained in the deviated cells progeny and even in memory cells (Swain, 1993). In vivo generation of Th1 cells retain some flexibility and can be switched into Th2 phenotype (Mocci and Coffman, 1995), where IL-4 induces the phenotypic change. IL-15, in high concentration, can induce proliferation of both naive and memory CD4+ cells and IL-15 together with either IL-12 or IL-4 enhances the proliferation of Th1 and Th2, respectively (Niedbala et al., 2002).

The few reported experiments on human peripheral blood mononuclear cells (PBMC) indicate that IL-4 is important for maintained or increased IL-4 secretion and reduced IFN-γ secretion (Sasama et al., 1998; Gerosa et al., 1996; Murphy et al., 1996). In all these cases strong immunogens were used, not representative of physiological conditions.

In 1995 it was shown that mature T cells, primed towards a Th2 response in vitro and injected into graft receiving patients, could inhibit Th1 like responses and protect against graft-versus-host disease (Krenger et al., 1995). Hence, they concluded that in vitro immune deviated T cells are able to carry out their function in vivo. Ex vivo immune deviation has also been successful in experimental autoimmune encephalomyelitis (EAE) (Racke et al., 1994), the animal model of MS, and in experimental allergic neuritis (EAN) (Ekerfelt et al., 2001), the animal model of Guillain–Barré syndrome. In both cases it was possible to redirect the myelin-specific Th cells ex vivo and re-injection of the deviated Th cells resulted in amelioration of the disease state. This kind of immuno-modulating treatment, which has been suggested to be selective, affecting only disease generating auto-reactive cells T cells, is very attractive since unwanted side effects caused by general immuno-modulation are avoided (Rocken et al., 1996). The use of cell products is a possible future way of treating autoimmune diseases (Ernerudh et al., 2002) but before clinical use a number of issues, including evaluation of protocols for immune deviation, must be clarified.

The aim of this study was to investigate if it is possible to immune deviate lymphocytes from MS patients with the same procedures established to be successful in rodents. Myelin-specific lymphocytes were in vitro deviated in the presence of IL-4, IL-1 and IL-15 and optimal immune deviation conditions were investigated.

2. Materials and methods

2.1. Patients

Thirteen patients with MS, according to McDonald et al. (2001), admitted to the Department of Neurology, University Hospital of Linköping were included in the study. All participants gave informed consent. The mean age of the MS patients was 44 years, ranging from 27 to 67 years, one man and twelve women. Eleven patients had relapsing–remitting MS, one had primary progressive MS and one had secondary progressive MS. Five of the patients received no treatment, seven received IFN-β and one received glatiramer acetate. The characteristics of the MS patients are shown in Table 1. The study was approved by the Local Ethical Committee of the University Hospital, Linköping, Sweden.

2.2. Antigen

The myelin–antigens used to stimulate PBMC from MS patients, peptides from myelin oligodendrocyte glycoprotein (MOG) amino acid sequences 14–39 (ALVGDEVELP-CRISPGKNAYGMELGW) and 63–87 (PEYRGRTELLK-DAIGEGKVTLRIRN), were kindly provided by Erik Wallström, Karolinska Institute, Sweden, and used at a final concentration of 10 μg/ml, which previously was found to

Table 1
Characteristics of the MS patients included in the study

		Males/females	Mean age (range), years	Mean EDSS (range)	Treatment (untreated/IFN-b/GA)
Patients with MS	13	1/12	44 (27–67)	2.7 (0–6.5)	5/7/1
PP	1	0/1	54	6.5	1/0/0
SP	1	0/1	58	6.5	1/0/0
RR	11	1/10	41 (27–67)	2 (0–6)	3/7/1

EDSS: expanded disability status score, GA: glatiramer acetate, IFN-b: interferon-β, MS: multiple sclerosis, SP: secondary progressive, PP: primary progressive, RR: relapsing–remitting.