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Augmented type 1 cytokines and human endogenous retroviruses specific immune responses in patients with acute multiple sclerosis

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***In vitro* antigen- and mitogen-stimulated cytokine production were analysed in multiple sclerosis (MS) patients with either acute (AMS) or stable (SMS) disease and in healthy controls (HC). We also investigated whether immune responses to human endogenous retroviruses (HERV) could be detected in MS and whether these immune responses would be correlated with disease status by analysing cytokine production after stimulation of PBMC with HERV peptides. Results showed that mitogen-stimulated IL-2 and IFN-γ was augmented and IL-10 was decreased in AMS compared to both SMS and healthy controls. Whereas the production of the metabolically active IL-12 (p70 heterodimer), was comparable in SMS, AMS and HC, production of the total IL-12 (p70 heterodimer and the p40 chain) were augmented in SMS compared to both AMS and HC. HERV-peptides IL-2 and IFN-γ production was more frequent and more potent in AMS compared to both SMS patients and HC. HERV-specific type 2 cytokine production was more frequent and potent in SMS compared to AMS and HC. Thus a prevalent type 1 cytokine profile was seen in AMS patients, while IL-10 production predominated in SMS individuals.** *Journal of NeuroVirology* (2000) **6,** S38–S41.

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Introduction

Multiple sclerosis (MS) is a multistep and multifactorial disease. Although the aetiology of MS is still unclear, an immunopathologic mechanism, mainly mediated by the activation of cell mediated immunity (CMI), was suggested to be responsible for the destruction of the myelin sheath (Poser, 1993; Steinman, 1996). Cytokines have been involved in the pathogenesis of MS, thus, increased levels of TNF-α and IFN-γ were detected in MS patients with acute disease (Olsson *et al*, 1990; Navikas *et al*, 1996), and the number of TNF-α and IFN-γ mRNA-expressing lymphocyte was suggested to be different in MS patients in different phases of disease activity (Rieckmann *et al*, 1994; Hohlfeld and Lucas, 1994). Epidemiological and clinical aspects suggest that both genetic and environmental factors could be involved in the aetiology of this condition. In particular current hypotheses indicate that MS could be due to an (auto)-immune process triggered by one or more environmental factors and among these, viruses are suspected of playing an important role (Kurtzke, 1987; Dalgleish, 1987).

Recently it was hypothesised that HERV could be involved in the pathogenesis of MS (Rudge, 1991; Rasmussen *et al*, 1993). HERV are present in the human genome and are estimated to comprise up to 1% of human DNA. Although until a few years ago HERV were not thought to be related to human diseases, the possible involvement of HERV in autoimmune disorders has recently been proposed (Krieg *et al*, 1992; Urnovitz, 1996; Nakagawa and Harrison, 1997).

Our results indicate that a profound and complex immune imbalance is present in MS, including impairment of cytokine production and peculiar immune response to HERV antigens.

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Results

Antigen-stimulated IL-2 production
IL-2 production in response to both FLU (influenza virus vaccine) and ALLO (a pool of irradiated allogeneic PBMCs) as statistically reduced in MS patients compared to controls (FLU: P=0.02; ALLO: P=0.01). Additionally SMS patients produced significantly reduced amounts of IL-2 upon FLU and ALLO stimulation when compared to HC (FLU: P=0.01; ALLO: $P<0.01$). HERV peptides stimulated IL-2 production by PBMC of MS patients more frequently than by PBMC of healthy controls, and by PBMC of AMS patients more frequently than by those of SMS individuals, (Peptide 4.1=33% SMS, 72% AMS, and 4% controls; SMS *versus* AMS $P<0.01$; AMS *versus* HC $P<0.01$). (Peptide P15E=33% SMS, 56% AMS, and 4% controls; AMS *versus* HC $P<0.01$).

Mitogen-stimulated type 1 and type 2 cytokine production
The production of the type 1 cytokines IL-2 and IFN-γ was significantly increased in MS (IL-2: $P<0.01$; IFN-γ. $P<0.01$) whereas IL-10 production was reduced in MS patients compared to HC ($P<0.01$). Thus, different cytokine profiles were observed in MS patient compared to controls. These changes (increased type 1 cytokine and decreased IL-10 production) were exacerbated in patients suffering from an acute MS episode. Because the metabolically active form of IL-12 is the p70 heterodimer but both the p70 and the metabolically inactive p40 chains can be secreted by cells, we analysed both p70 IL-12 and total IL-12 (p70+p40) production. The results indicate that SAC-stimulated p70 production was comparable in MS and HC and in AMS and SMS patients. In contrast to these results a more complex pattern was obtained when SAC-stimulated production of total IL-12 was examined. Thus, total IL-12 production was: (1) significantly augmented in SMS compared to AMS patients ($P<0.01$); and (2) significantly reduced in AMS compared to HC (P=0.02).

HERV peptides-stimulated cytokine production
Type 1 and type 2 cytokine production by HERV peptides-stimulated PBMC was measured in patients and controls. Peptides 4.1- and 15E-stimulated IL-2 and IFN-γ production was more potent in AMS patients compared to both SMS individuals ($P<0.01$) and controls ($P<0.01$). P23-stimulated PBMC (negative control) did not produce either IL-2 or IFN-γ in any of the groups examined. The type 2 cytokine profile observed was somewhat speculative to that described above. Thus, peptide 4.1-stimulated IL-4 and IL-10 production was more potent in SMS compared to AMS ($P<0.01$ for both cytokines) and to HC (IL-4 $P<0.01$; IL-10 P=0.01). P15E-stimulated IL-4 production was augmented in SMS compared to AMS (P=0.02) and HC (P=0.01). Finally, P15E-stimulated IL-10 production was significantly increased when SMS were compared to AMS (P=0.01) but not when they were compared to HC. Again, neither IL-4 nor IL-10 were produced by PBMC upon stimulation with P23 (negative control). Thus, a predominant HERV peptides-specific type 1 cytokine production is observed in AMS whereas a type 2 cytokine profile dominates in SMS.

HERV-stimulated immune response is modified by disease status
PBMC of two AMS and four SMS patients were retested upon modification of disease expression (AMS → SMS; SMS → AMS). HERV peptide 4.1-specific cytokine production correlated with disease status (similar data were obtained with peptide P15E). Thus the type 1 cytokine profile observed in AMS patients was changed into a type 2 profile once disease went into remission. Similarly, type 2 cytokines were decreased and type 1 cytokines increased when SMS patients underwent an acute episode of disease. Cytokine production was not changed in four other patients (2 AMS; 2 SMS) followed in time and in whom disease status was unmodified.

Discussion

We analysed some immunologic parameters in MS patients with either stable or acute disease to better define the immunopathology underlying this condition.

We observed in MS patients compared to controls that: (1) antigen-stimulated TH function was defective as assessed by IL-2 production, and (2) mitogen-stimulated type 1 cytokine production was increased and type 2 cytokine production was reduced. Mitogen-stimulated IFN-γ and IL-2 production were increased and IL-10 production was decreased in AMS patients compared to SMS patients, suggesting that different cytokine profiles are associated with acute (> type 1 cytokine) or stable (> IL-10) disease. Although SAC-stimulated production of the metabolically -active IL-12 p70 heterodimer was similar in AMS, SMS, and controls, the production of total IL-12 was significantly increased in SMS compared to AMS and significantly reduced in AMS patients compared to controls, suggesting an alteration in the production of the metabolically inactive p40 chains in MS patients. The p40 homodimer subunit of IL-12 has been shown to be antagonists of the biologic effects of IL-12 *in vitro* (Heinzel *et al*, 1997) and was recently demonstrated to elicit a potent immunosuppressive effect on Th1-mediated immune func-

tions (rejection of allografts) *in vivo* (Kato *et al*, 1996) and to antagonise directly IL-12-dependent IFN-γ responses in murine endotoxic chock (Heinzel *et al*, 1997). Thus we speculate that the augmented production of total IL-12 observed in SMS patients could include increased quantities of p40 homodimers, allowing for a regulatory mechanism controlling type 1 cytokine production and activation of CMI. To analyse immune responses against HERV in MS patients we selected two previously described peptides: P15E which shares 85% homology with gp21 of HTLV-1 and is known to preferentially suppress mRNA accumulation for type 1 cytokines (Nelson *et al*, 1989; Cianciolo *et al*, 1985; Oostendorp *et al*, 1993; Haraguchi *et al*, 1995), and 4.1, multicopy type C HERV whose transcripts are observed in human tissues (Urnovitz, 1996). Results showed that an immune response to both HERV synthetic peptides is present in MS patients and that this immune response is qualitatively different in patients with acute (type 1 cytokine production and peptide-stimulated proliferation) or stable (type 2 cytokines production) disease and that cytokine profiles are modified by changes in disease expression. The involvement of HERV in pathogenesis could be secondary to their role as superantigens; to molecular mimicry with self antigens; or to both of these mechanisms. In MS an immunopathologic-mediated mechanism is suggested to be responsible for the destruction of the myelin sheath (Poser, 1993; Martin and McFarland, 1995). Thus, because of molecular mimicry with cross-reacting and yet undefined epitopes, T helper cells recognising epitopes of the myelin sheath would secrete type 1 cytokines and activate: (1) myelin-specific CTL with breakage of tolerance and initiation or reactivation of disease (Wucherpfenning and Strominger, 1995; Murray *et al*, 1998); and / or (2) macrophages that would infiltrate the central nervous system and, together with the resident microglia, induce the demyelination process (Gebickehaerter *et al*, 1996; Li *et al*, 1996; Benveniste, 1997; Sriram, 1997). Our data confirm that type 1 cytokines are abnormally produced in the acute phases of the disease.

In conclusion a complex alteration of the immune response is present in MS, including impairment of cytokine production and the detection of immune responses to HERV antigen.

Materials and methods

Patients and controls

Forty-eight patients with multiple sclerosis characterised by clinical and laboratory criteria and followed by the Centro Sclerosi Multipla of the Don Gnocchi Foundation, Milan, Italy, were enrolled in the study. These patients (30 females and 18 males; median age; range=30,1; 21,3 – 40, 4 years) were affected by relapsing-remitting (RR) MS. RR MS had been clinically stable for at least 1 month prior to the study period in 26 patients; these patients were therefore classified as patients with stable MS (SMS). The other 22 RR MS patients presented with clinical re-exacerbation of the disease and were therefore classified as patients with acute MS (AMS). AMS patients were visited and their blood was drawn within 5 days of the onset of the acute episode and always before the onset of steroid-based therapy. Fifty-three healthy donors from the Transfusion Department of the L. Sacco Hospital, Milan, Italy, were utilised as controls. Median age; range (31, 7; 22, 8 – 40, 7 years) and sex (30 females/ 23 males) were comparable between RR MS patients and controls.

Antigen-stimulated IL-2 production

PBMCs were separated on lymphocyte separation medium from blood samples. PBMCs were incubated with: (A) no stimulation (medium background); (B) influenza virus vaccine prepared with a mixture of A/Taiwan, A/Shanghai and B/Victoria, 24 mg/l (final dilution 1 : 1000); (C) a pool of irradiated (50 Gy) allogeneic PBMC (1×10^5 cells per well) from two or more unrelated healthy control volunteers; (D) a synthetic peptide from the P15E the transmembrane envelope protein present in many HERV (also termed CKS-17); (E) HERV 4.1 a multicopy type C HERV termed also HERV-E; or (F) P23 a control non-antigenic peptide from gp160 of HIV-1 (Clerici *et al*, 1992). Pooled human plasma (1 : 20 final) and the anti-IL-2 receptor antibody, humanised monoclonal anti-Tac (Becton-Dickinson; Rutherford, NJ USA) (1 μg/ml) were added to each well 1 h after sensitisation of the PBMC. Culture supernatants were harvested after 7 days, and total IL-2 produced throughout the culture period was determined by testing each supernatant for ability to stimulate the proliferation of an IL-2-dependent mouse continous T lymphocyte line (CTLL).

Cytokine production

PBMCs were either unstimulated, or stimulated with PHA or with the HERV peptides and the non-immunogenic control peptide described above (p23) at 37°C in a moist, 7% CO_2 atmosphere. Supernatants were harvested after 48 h. Production of IL-2, IFN-γ, IL-4, and IL-10 by PBMCs was evaluated with commercially-available ELISA. To evaluate IL-12 production, PBMCs were stimulated with Staphylococcus aureus (SAC) for 48 h. Two commercially available kits measuring either the p70 heterodimer or both the p70 heterodimer, and the p40 chains (total IL-12) were used to measure IL-12 in the supernatants.

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