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Short Communication

In vitro modulation of the multiple sclerosis (MS)-associated retrovirus by cytokines: Implications for MS pathogenesis

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Multiple sclerosis (MS)-associated retrovirus (MSRV) is a component of the human endogenous retrovirus (HERV)-W family, with gliotoxic and superantigenic properties, related to MS clinical progression, and transactivated by viral agents. The authors studied MSRV modulation by cytokines involved *in vivo* in MS course, utilizing peripheral blood mononuclear cells from MSRV-positive and MRSV-negative individuals. Cultured cells from MSRV-negative subjects did not produce virus, whereas spontaneous MSRV release was detected in cultures from MSRV-positive donors; virus release was increased by interleukin (IL)-4 and IL-6 and, to a greater extent, by the detrimental cytokines interferonγ and tumor necrosis factor (TNF)α. Interferonβ, used in MS therapy, inhibits MSRV release. A parallel between the effects of these cytokines on MSRV production *in vitro* and on MS disease *in vivo* is observed, which deserves further elucidations. *Journal of NeuroVirology* (2003) **9,** 637–643.

Keywords: cytokines; endogenous/exogenous retrovirus; MSRV; multiple sclerosis; pathogenesis

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) of unknown etiology and still-debated, immune-mediated pathogenesis, including genetic and environmental (viral) factors (Noseworthy *et al*, 2000). MS patients have peculiar immune responses to viruses, and virus infections are linked to MS relapses (Panitch, 1994). Several viruses have been suggested to be involved in MS etiology over the past few decades (Gonzalez-Scarano and Rima, 1999); in particular, the human herpesvirus (HHV)-6 (Gutierrez *et al*, 2002; see Steiner *et al*, 2001 for a review)and the MS-associated retrovirus (MSRV; Perron *et al*, 1997), a member of the W family of human endogenous retroviruses (HERV; Tristem, 2000), capable of producing infectious virions. We (Dolei *et al*, 2002) recently showed that the extracellular form of MSRV is detectable in the plasma of 100% MS patients and 12% healthy controls from Sardinia, an Italian island at very high risk for MS (Rosati, 2001) and that patients with MSRV-free cerebrospinal fluid (CSF) have a stable MS course, whereas those with MSRV-positive CSF disclose a more severe, treatment-requiring disease (Sotgiu *et al*, 2002).

Cytokines are deeply involved in MS onset and evolution *in vivo*. Aberrant immune responses occur in MS, and it is likely that the spectrum of cytokines produced decisively influences the outcome of the disease (Ozenci *et al*, 2002). Conceptually, an “excess of defense” due to myelin-(auto)reactive CD4+ T cells, along with a cascade of dangerous inflammatory cytokines, is thought to occur within the CNS parenchyma of MS patients (Hohlfeld, 1997; Baranzini *et al*, 2000), leading to oligodendrocyte damage and lesion formation. The cascade of orchestrating cytokines could derive from CNS infiltrating T cells, secondarily recruited inflammatory

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cells, including CD8+ T cells and antigen-presenting cells (APCs). The proinflammatory cytokines that most efficiently recruit additional inflammatory cells and APCs are interferon (IFN)γ and tumor necrosis factor (TNF)α. TNFα itself and other inflammatory products from macrophages contribute to myelin injury and demyelination. Local events, such as the conversion of the dominant Th1 response (characterized by production of interleukin [IL]-2, IFNγ, and TNFα) into a Th2 response with cytokines such IL-10, IL-13, and IL-4, are suggested, though not definitely, to positively affect the outcome of the inflammatory lesion (Hohlfeld, 1997). Elevated levels of IL-6 in peripheral blood and brain of MS patients indicate also dysregulation of IL-6 in this disease (Navikas *et al*, 1996; Baranzini *et al*, 2000). *In vivo*, the systemic administration of IFNγ significantly increases MS exacerbations, with pronounced detrimental effects on cellular immunity and disease activity (Panitch *et al*, 1987); IFNβ, instead, is commonly used as therapeutic agent (Durelli *et al*, 2002).

Despite the fact that MSRV is associated with temporal and clinical progression of MS, little is known about its possible pathogenic role. *In vitro*, a toxic (apoptotic) effect on glial cells (Menard *et al*, 1997) and a superantigenic effect on CD4+ T cells (Perron *et al*, 2000, 2001) have been described. Furthermore, MSRV expression can be transactivated by the human herpes simplex (HSV)-1 (Lafon *et al*, 2002). Data on possible effects of inflammatory cytokines on MSRV are missing. For this purpose, we selected MSRV-positive and MRSV-negative healthy volunteers and evaluated the effects on MSRV release by cultured peripheral blood mononuclear cells (PBMCs) exerted by cytokines supposed to have detrimental, debated, or beneficial effects (TNFα, IFNγ, IL-6, IL-4, and IFNβ; Hohlfeld, 1997; Baranzini *et al*, 2000) on MS immunopathogenesis.

Results

When kept in culture, PBMCs from MSRV-positive donors spontaneously release MSRV. Figure 1**A** reports MSRV detection as bands of a representative RT-PCR. The virus increasingly accumulates in culture medium with time (Figure 1**C**) and upon mitogen stimulation (Figure 1**A**, **B**). Mean values of MSRV release by resting and mitogen-activated PBMCs are reported on the first row of Table 1, and indicate a mean increase of 5.8-fold upon activation, when culture media from equal amounts of cells are analyzed. Mean fold-increase in *individual* donations was 7× in activated versus resting cells; as for cytokine treatments, in untreated cells: TNFα: 10×, IL-6: 14×, IFNβ: 0.26×; in mitogen-activated cells: TNFα: 135×, IL-6: 27×, IL-4: 37×, IFNβ: 0.056×, IFNγ: 71×

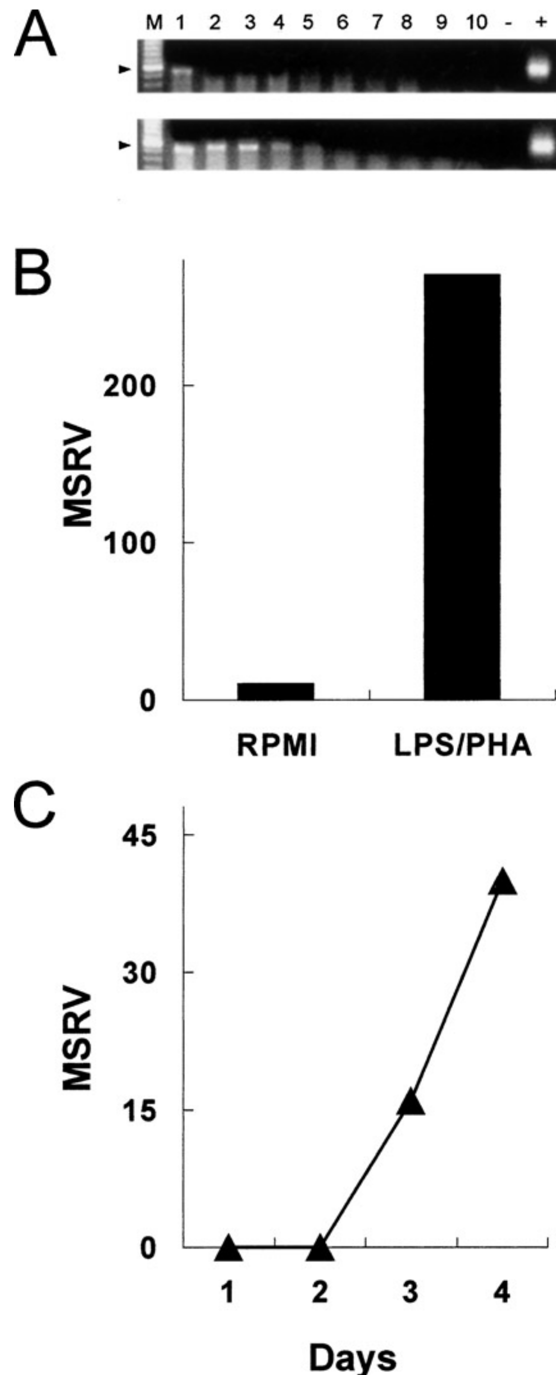

Figure 1 MSRV release with time and mitogen activation by cultured PBMCs from MSRV-positive healthy donors. (**A**) Representative semiquantitative RT-PCR of media from untreated (*upper gel*) and mitogen-activated (*lower gel*) PBMCs after 4 days of culture. Total RNA was extracted, serially diluted (first dilution 1:10, then 1:3 onward), and each dilution was exposed to semiquantitative RT-PCR. M: size markers; 2–10: sample dilutions; −: negative control; +: positive control. The arrow head indicates the 574-bp amplified product. (**B**) Virus accumulation after 4 days of culture expressed as reciprocal of the end point dilutions of the semiquantitative RT-PCR reported in **A**. (**C**) Virus accumulation over time by resting PBMCs, expressed as end point dilutions of semiquantitative RT-PCR of a representative experiment, run in duplicate.

Table 1 Cytokine modulation of MSRV release by cultured PMBCs[a]

	MSRV-positive donors				MSRV-negative donors	
Treatment	*Resting (%)*	P[b]	*Mitogen-activated (%)*	P	*Resting*	*Mitogen-activated*
None	28 ± 22 (100)	—	163 ± 90 (100)	—[c]	<1	<1
TNFα	192 ± 172 (686)	0.0028	12150 ± 8418 (7454)	0.047	nd[d]	<1
IL-6	240 ± 198 (857)	0.01	2025 ± 810 (1242)	0.0015	nd	<1
IL-4	nd		3330 ± 3595 (2042)	0.011	nd	nd
IFNγ	nd		7958 ± 9731 (4882)	0.0011	nd	nd
IFNβ	5 ± 6 (18)	<0.000001	8 ± 3 (5)	0.0015	nd	nd

[a]Cells were kept in culture for 4 days, then media were processed for semiquantitative RT-PCR, to detect extracellular MSRV genomic RNA sequences; data are expressed as mean reciprocal of the end point MSRV-positive dilutions ± standard variations. Standard deviations show some overlapping, due to baseline variations in MSRV production among individuals; however, clear-cut cytokine effects are observed within cells of the same individual (see text for mean increase). From 4 to 11 experiments were evaluated for each experimental conditions. The following cytokine concentrations were used: TNFα (20 pg/ml), IL-6 (500 U/ml), IL-4 (1 ng/ml), IFNγ and IFNβ (1000 IU/ml).
[b]P value obtained from variance analysis of the means.
[c]Variance analysis with respect to untreated cells: $P < .000001$.
[d]nd: not done.

were detected. To be noted that in cultures derived from MSRV-negative individuals, neither spontaneous nor mitogen-induced virus release is detected (Table 1).

The capability of releasing MSRV in culture seems to be a stable feature of cells from MSRV-positive individuals. In fact, one of the healthy volunteers has been withdrawn six different times during more than 30 months; also for experiments to be published elsewhere, evaluation of virus production in culture by resting and mitogen-activated PBMCs from this donor gave mean end point dilutions of 31 ± 30 (range 30–90) and 162 ± 98 (range 90–270), respectively.

MSRV release in culture by PBMCs from virus-positive donors appears to be sensitive to cytokine treatments. Table 1 summarizes overall data obtained with the use of a single concentration of each cytokine. As shown, at the doses used, TNFα, IFNγ, IL-6, and IL-4 increase MSRV release by activated cells, TNFα and IFNγ being the most effective (1.8 and 1.7 log increase, respectively), whereas IFNβ shows dramatically inhibitory effects (1.3 log decrease). Resting cells release smaller amounts of virus, and the effects of cytokines are less pronounced, as expected, but still present, indicating that cell proliferation is not essential for cytokine activity on MSRV. Confidence intervals of Table 1 show some overlapping, due to baseline variations in MSRV production among individuals, however, clear-cut cytokine effects are observed within cells of the same individual.

Figure 2 reports MSRV release in a representative experiment of mitogen-activated PBMC cultures, exposed for 4 days to graded amounts of TNFα, IL-6, IL-4, IFNγ, and IFNβ. Again, at the doses used, TNFα and IFNγ are the most effective stimulatory cytokines, particularly TNFα (Figure 2**A**). Notably, the two interferons exert opposite effects: IFNγ progressively stimulates the MSRV release, up to 1.7 log with respect to the control, whereas the therapeutic agent IFNβ inhibits the virus by ~2 logs.

Discussion

The MSRV novel retrovirus, related to the HERV-W family of endogenous retroviruses, is associated with the production of extracellular, infectious, retroviral particles, both *in vivo* and *in vitro* (Garson *et al*, 1998; Komurian-Pradel *et al*, 1999; Perron *et al*, 2001). MSRV may bear gliotoxic properties (Menard *et al*, 1997) and induce a superantigenic bias in the Vβ T cell response, which could be reproduced with a recombinant MSRV env protein (Perron *et al*, 2001). Additionally, HSV-1 reactivates the expression of MSRV env protein in human neuroblastoma cell lines that contain HERV-W sequences in their genome (Lafon *et al*, 2002), thus supporting the hypothesis that MS pathophysiology can be driven by endogenous retrovirally encoded superantigen with gliotoxic activity, transactivated by a herpesvirus. Consistently, many viral infections are known to precede the clinical relapse in MS patients (Panitch, 1994) and several viruses have been epidemiologically and experimentally linked to MS etiology, particularly the HHV-6 (Gutierrez *et al*, 2002). Therefore, the reactivation of quiescent (endogenous?) viruses by unrelated viruses might be the missing link to explain the role of either viral infection or secondary recruited cytokines in the etiology of autoimmune diseases, such as MS (Rudge, 1991).

In this report, we studied the effects exerted on MSRV release *in vitro* by cytokines supposed to have detrimental (TNFα, IFNγ), beneficial (IFNβ), or debated (IL-4, IL-6) effects on MS *in vivo* (Ozenci *et al*, 2002; Hohlfeld, 1997; Hulshof *et al*, 2002), with the aim of finding possible indications on the role of MRSV, if any, in MS pathogenesis. This virus, in fact, has been found in extracellular form in blood from MS patients (Perron *et al*, 1997; Dolei *et al*, 2002), and MSRV-positivity of CSF is a bad prognostic marker (Sotgiu *et al*, 2002). Initially, MS has been postulated to be a Th1 cell–mediated autoimmune disease (Olsson, 1995; Hohlfeld, 1997). However,

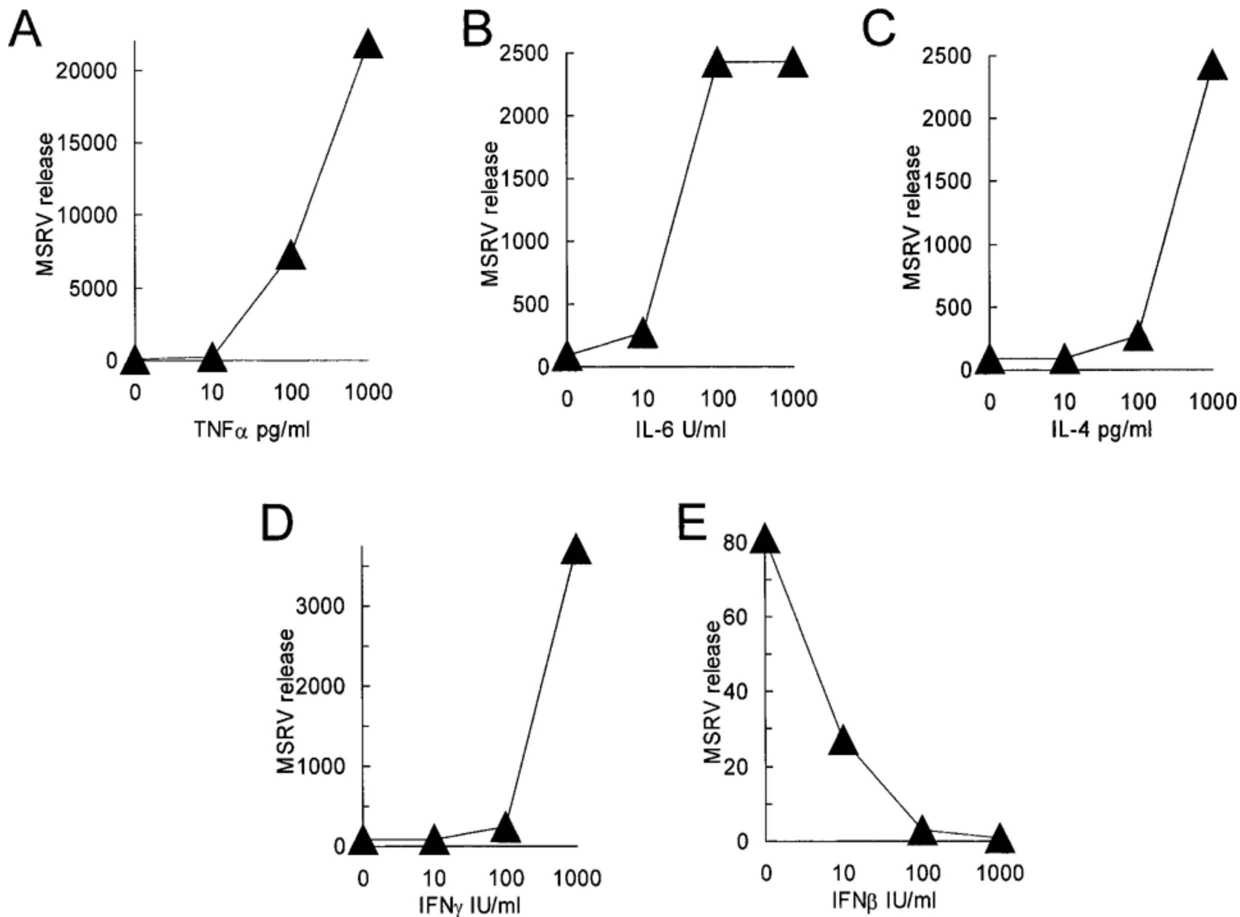

Figure 2 MSRV release in culture by mitogen-activated cells PBMCs from MSRV-positive healthy donors. Cells were exposed for 4 days to graded amounts of TNFα (**A**), IL-6 (**B**), IFNγ (**C**), IL-4 (**D**), and IFNβ (**E**). Data were obtained from one representative experiment with two different donors, and are expressed as reciprocal of the end point MSRV-positive dilution of semiquantitative RT-PCR. For overall variations within experiments, see Table 1.

the dogmatic application of the Th1/Th2 cytokine paradigm to MS is presumably a gross oversimplification (Laman *et al*, 1998) because both Th1 and Th2 cytokines are up-regulated within MS lesions during active disease (Baranzini *et al*, 2000; Ochi *et al*, 2001) and Th2 cells are seen to play important roles in certain stages of lesion formation (Hulshof *et al*, 2002).

Our experiments show that blood cells from MSRV-positive individuals spontaneously release this virus in culture, as observed by RT-PCR detection of virionic RNA sequences, further enhanced upon mitogenic stimulation, which is a known effect in cells chronically infected by exogenous retroviruses. As for endogenous retroviruses, this finding could be expected, because mitogenic activation of pig PBMCs resulted in the activation and release of porcine endogenous retrovirus (Wilson *et al*, 1998), and the human U937 monocytoid cell line, upon treatment with phorbol esters, releases RT activity associated with HERV-H and HERV-K, but not HERV-W, sequences (Johnston *et al*, 2001). On the contrary, the same group (Johnston *et al*, 2001) did not found intracellular HERV-W RNA sequences in normal human monocyte-derived macrophages, and Depil *et al* (2002) did not find significant variation of HERV-K expression in normal human PBMCs after exposure to mitogens. In our study, the capability of PBMCs from MSRV-positive individuals to release MSRV in culture seems to be a stable feature, because it was maintained *in vivo* in our donors for more than 30 months, so far (personal observation). Notably, PBMCs from MSRV-negative individuals do not release MSRV in culture, neither spontaneously nor after treatments. This was in some way a surprise because, if MSRV is an endogenous virus, one would expect that mitogens and/or cytokines would induce it, because the cells do contain the sequence at the DNA level (not shown), as expected (Tristem, 2000). In our opinion, these findings suggest the possibility that MSRV might be an exogenous member of the HERV-W family.

Interestingly, MSRV release by PBMCs in culture is highly sensitive to cytokine treatments. The effect of TNFα, IL-6, IL-4, and IFNγ is stimulatory, and the largest effects are seen with the MS-detrimental TNFα and IFNγ cytokines. On the other hand, the MS-protective IFNβ cytokine exerts a strong inhibitory effect. These findings are seen also in

resting cells, though to a smaller extent (Table 1), thus suggesting that cell proliferation is not essential for cytokine activity on MSRV extracellular release. To our knowledge, this is the first report on the effect of any cytokine on the release of endogenous retroviruses from cells. The cytokine-related modulation of MSRV release, particularly the stimulation by TNFα and IL-6 and the opposite effects of IFNγ and IFNβ, are in line with published data on the effects of inflammatory cytokines on the human retrovirus human immunodeficiency virus (HIV) (Dolei *et al*, 1994, 1998; Pitha, 1994; Fauci, 1996; Conaldi *et al*, 2000; Biolchini *et al*, 2000) and may be connected to MS pathogenic mechanisms as well. Proinflammatory cytokines, such as TNFα and IFNγ that are detrimental in MS (Olsson, 1995; Hohlfeld, 1997), are shown here to stimulate MSRV release in extracellular fluids, whereas IFNβ, beneficial *in vivo* for MS patients (Noseworthy *et al*, 2000; Durelli *et al*, 2002), does block virus release. As for MSRV stimulation by the IL-4 and IL-6 cytokines, this could be in line with published observations that indicate that IL-4 and its receptor actively participate in the formation of chronic MS lesions (Baranzini *et al*, 2000; Ochi *et al*, 2001; Hulshof *et al*, 2002). Increased IL-6 levels in MS patients are said to be implicated in MS pathology (Navikas *et al*, 1996; Baranzini *et al*, 2000), CSF levels of IL-6 being similar between patients with active and stable disease, whereas the difference between stable patients and controls is statistically significant (Miljkovic *et al*, 2002), thus indicating a complex contribute of this cytokine to MS pathogenesis. However, one must be aware of the problems in correlating cytokine expression and effects *in vivo*, due to their short half-lives, paracrine and autocrine modes of action, and complex regulatory feedback loops (Duddy *et al*, 1999). In addition to IFNβ direct antiviral effects, a variety of cross interactions occur among cytokines, and several studies related to IFNβ effects on MS patients showed the interference of IFNβ with IFNγ and IL-12 (Wang *et al*, 2000; Byrnes *et al*, 2002) that is potentially relevant to IFNβ therapeutic mechanisms.

Based on these and other findings discussed above, it can be speculated that during MS active phase of lesion formation, as well as during virus infections, some cytokines, theoretically produced to defense purposes, may activate latent (retro)viruses, which might have pathogenic relevance. In this respect, it should be noted that increased levels of HERV-W and HERV-K RNAs and of TNFα ere found in autopsied brain tissues from MS patients (Johnston *et al*, 2001).

At present, there are only a few indications coming from *in vitro* studies on how MSRV might be pathogenically important in MS, due to its gliotoxic (Menard *et al*, 1997) and superantigenic properties (Perron *et al*, 2001). The latter property by itself suggests a pathogenic loop involving proinflammatory cytokines, regardless whether MSRV expression is a consequence or a cause of a primary inflammatory process. In both cases, given our previous reports on bioclinical correlations between CSF MSRV load and MS progression (Dolei *et al*, 2002; Sotgiu *et al*, 2002), the present study sheds new possible lights on the association of MSRV with MS inflammatory pathogenesis. The reported intriguing parallel between the effects of inflammatory cytokines on MSRV production *in vitro* and on MS disease *in vivo*, particularly with regard to IFNβ, might inspire further studies aimed at MSRV as possible therapy target in MS.

Methods

Eight healthy volunteers, previously shown to be MSRV-positive ($N = 6$) or MSRV-negative ($N = 2$), informedly consented to blood withdrawal. Donors had no history of MS in their families, did not show symptoms or signs of disease, nor had been exposed to immunomodulatory treatments by at least 3 months before blood withdrawals. Mean age was 36.4 ± 10.3. From two to four withdrawals occurred for each donor, during a period of 24 months.

PBMCs were prepared as described (Dolei *et al*, 1998) and cultured (10^6 cells/ml) for 4 days as such (resting PBMCs) or under mitogen activation with lipopolysaccharide (LPS) (10 pg/ml; Sigma Aldrich srl, Milan) plus phytohemaglutinin (PHA) (1 μg/ml, Boheringer Mannheim Gmbh), with/without the following human recombinant cytokines: TNFα (Amersham International plc, Amersham), IL-6 (Amersham), IL-4 (Boheringer), IFNγ (Amersham), and IFNβ (Sigma), in RPMI 1640 medium (Flow Lab, Irvine, CA), supplemented with 10% fetal calf serum (Gibco Laboratories, Paisley) and antibiotics. Cell viability was assessed by trypan blue exclusion, and more than 95% of the cells excluded trypan blue in each experiment.

Published procedures were followed for sample collection and preparation of extracellular MSRV genomic RNA (Dolei *et al*, 2002; Perron *et al*, 1997; Garson *et al*, 1998). Culture medium from equal amounts of cells were filtered through 0.45-nm filter membranes (Millex, Millipore, Bedford, MA), in order to eliminate cells and cell debris. The medium was then precipitated with PEG_{8000} polyethylene glycol (10% *w/v*) for 24 h, centrifuged at $5000 \times g$ for 20 min, and treated with bovine ribonuclease IA (USB, Cleveland, OH; 8 units/μl for 1 h at 25°C), to avoid contamination by cellular RNAs containing endogenous retroviral sequences. RNAs were added of glycogen carrier, then extracted by the guanidine thiocyanate method, and subsequently digested by RNase-free deoxyribonuclease I (Amersham Life Science, Buckinghamshire, UK; 100 units/μl for 1 h at 37°C), to avoid possible DNA contamination. Samples were diluted in RNase-free, diethylpyrocarbonate-inactivated water by using RNase-free tips. RNAs were then extracted again. Only extracellular, encapsidated RNAs were

therefore available for molecular studies, according also to literature (Perron *et al*, 1997; Garson *et al*, 1998; Dolei *et al*, 2002).

Samples of virionic RNAs were used as template for semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR) using MSRV *pol*–specific primers, essentially as described (Dolei *et al*, 2002; Perron *et al*, 1997; Biolchini *et al*, 2000). Briefly, 2-μg aliquots of RNAs were serially diluted and each dilution underwent reverse transcription into DNA, by using oligo-dT as primer and M-MLV RT (GIBCO BRL Life Technologies, Frederick, MD) as published (Biolchini *et al*, 2000; Dolei *et al*, 2002), followed by PCR amplification of DNA products, in a Hybaid Thermal Cycler (Omnigene, Ashford, Middlesex, UK) utilizing primers specific for the MSRV *pol* gene (Garson *et al*, 1998): PTpol-A (sense): 5′GGCCAGGCATCAGCCCAAGACTTGA3′ and PTpol-F (antisense): 5′TGCAAGCTCATCCCTSR GACCT3′. PCR amplification was carried out by 2 units/50 μl of Taq DNA polymerase (DYNAzyme, Riihitontuntie, Finland) and 25 pmol of each primer pair: initial step 94°C/5 min; 40 cycles of 94°C/30 s, 54°C/1 min, 72°C/1.5 min; and final extension step 72°C/5 min. This created an amplification product 574 bp long, which was identified using 0.7% agarose gel electrophoresis and ethidium bromide staining, under ultraviolet (UV) light, and then photographed. A representative gel image is shown in Figure. 1**A**. Controls included PCR of RNAs not exposed to RT with primers specific for the β-globin gene (primer pair: PC04/GH20; Synthetic Genetics, San Diego, CA) or with MSRV-specific primers (to ensure the absence of contaminating cellular DNA sequences and of endogenous retroviral DNA sequences, respectively), PCR of cDNA samples without template (negative control), and samples of human cell DNA (positive control). Cellular RNA from PBMCs of MSRV-negative individuals was also included. Absence of cellular RNAs was also tested when the RNase batch was changed, by using the PC04/GH20 primer pair (Synthetic Genetic) specific for the β-actin gene (Dolei *et al*, 1998). Data were expressed as reciprocal of each end point MSRV-positive dilution in semiquantitative RT-PCR. Prevention measures against cross-contamination were employed (Kwok and Higuchi, 1989); in particular, sample processing and PCR amplification were carried out in separate laboratories, with different equipment.

References

Baranzini SE, Elfstrom C, Chang SY, Butunoi C, Murray R, Higuchi R, Oksenberg JR (2000). Transcriptional analysis of multiple sclerosis brain lesions reveals a complex pattern of cytokine expression. *J Immunol* **165:** 6576–6582.

Biolchini A, Curreli S, Ziccheddu M, Serra C, Dolei A (2000). Opposite effects of interferonγ on CCR5 and CXCR4 expression and on entry of M- and T-tropic HIV in epithelial cells. *AIDS* **14:** 7–9.

Byrnes AA, McArthur JC, Karp CL (2002). Interferon-beta therapy for multiple sclerosis induces reciprocal changes in interleukin-12 and interleukin-10 production. *Ann Neurol* **51:** 165–174.

Conaldi PG, Bottelli A, Wade-Evans A, Biancone L, Baj A, Cantaluppi V, Serra C, Dolei A, Toniolo A, Camussi G (2000). HIV-1 persistent infection and cytokine induction in mesangial cells: a potential mechanism for HIV-associated glomerulosclerosis. *AIDS* **14:** 2045–2047.

Depil S, Roche C, Dussart P, Prin L (2002). Expression of a human endogenous retrovirus, HERV-K, in the blood cells of leukemia patients. no significant variation of HERV-K expression in normal PBMCs after exposure to different factors (PHA, gamma irradiation, 5-azacytidine) that potentially modulate HERV expression. *Leukemia* **16:** 254–259.

Dolei A, Arca MV, Tilocca F, Pietravalle M, Alemanno L, Toniolo A, Ameglio F (1994). Mutual interactions of HIV-1 and cytokines in adherent cells during acute infection. *Arch Virol* **134:** 157–168.

Dolei A, Biolchini A, Serra C, Curreli S, Gomes E, Dianzani F (1998). Increased replication of T-cell-tropic HIV strains and CXC-chemokine receptor-4 induction in T cells treated with macrophage inflammatory protein (MIP)-1α, MIP-1β and RANTES β-chemokines. *AIDS* **12:** 183–190.

Dolei A, Serra C, Mameli G, Pugliatti M, Sechi S, Cirotto MC, Rosati G, Sotgiu S (2002). Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients. *Neurology* **58:** 471–473.

Duddy ME, Armstrong MA, Crockard AD, Hawkins SA (1999). Changes in plasma cytokines induced by interferon-β1a treatment in patients with multiple sclerosis. *J Neuroimmunol* **101:** 98–109.

Durelli L, Verdun E, Barbero P, Bergui M, Versino E, Ghezzi A, Montanari E, Zaffaroni M (2002). Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year prospective randomised multicentre study (INCOMIN). *Lancet* **359:** 1453–1460.

Fauci AS (1996). Host factors and the pathogenesis of HIV-induced disease. *Nature* **384:** 529–534.

Garson J, Tuke PW, Giraud P, Paranhos-Baccala G, Perron H (1998). Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. *Lancet* **351:** 33.

Gonzalez-Scarano F, Rima B (1999). Infectious aetiology in multiple sclerosis: the debate continues. *Trends Microbiol* **7:** 475–477.

Gutierrez J, Vergara MJ, Guerrero M, Fernandez O, Piedrola G, Morales P, Maroto MC (2002). Multiple sclerosis and human herpesvirus 6. *Infection* **30:** 145–149.

Hohlfeld R (1997). Biotechnological agents for the immunotherapy of multiple sclerosis. Principles, problems and perspectives. *Brain* **120:** 865–916.

Hulshof S, Montagne L, De Groot CJ, Van Der Valk P (2002). Cellular localization and expression patterns of interleukin-10, interleukin-4, and their

receptors in multiple sclerosis lesions. *Glia* **38:** 24–35.
Johnston JB, Silva C, Holden J, Warren KG, Clark AW, Power C (2001). Monocyte activation and differentiation augment human endogenous retrovirus expression: implications for inflammatory brain diseases. *Ann Neurol* **50:** 434–442.
Komurian-Pradel F, Paranhos-Baccala G, Bedin F, Ounanian-Paraz A, Sodoyer M, Ott C, Rajoharison A, Garcia E, Mallet F, Mandrand B, Perron H (1999). Molecular cloning and characterization of MSRV-related sequences associated with retrovirus-like particles. *Virology* **260:** 1–9.
Kwok S, Higuchi R (1989). Avoiding false positives with PCR. *Nature* **339:** 237–238.
Lafon M, Jouvin-Marche E, Marche PN, Perron H (2002). Human viral superantigens: to be or not to be transactivated? *Trends Immunol* **23:** 238–239.
Laman JD, Thompson EJ, Kappos L (1998). Balancing the Th1/Th2 concept in multiple sclerosis. *Immunol Today* **19:** 489–490.
Menard A, Amouri R, Michel M, Marcel F, Brouillet A, Belliveau J, Geny C, Deforges L, Malcus-Vocanson C, Armstrong M, Lyon-Caen O, Mandrand B, Dobransky T, Rieger F, Perron H (1997). Gliotoxicity, reverse transcriptase activity and retroviral RNA in monocyte/macrophage culture supernatants from patients with multiple sclerosis. *FEBS Lett* **413:** 477–485.
Miljkovic DJ, Drulovic J, Trajkovic V, Mesaros S, Dujmovic I, Maksimovic D, Samardzic T, Stojsavljevic N, Levic Z, Mostarica Stojkovic M (2002). Nitric oxide metabolites and interleukin-6 in cerebrospinal fluid from multiple sclerosis patients. *Eur J Neurol* **9:** 413–418.
Navikas V, Matusevicius D, Soderström M, Frederikson S, Kivisakk P, Ljungdahl A, Hojeberg B, Link H (1996). Increased interleukin-6 mRNA expression in blood and cerebrospinal fluid mononuclear cells in multiple sclerosis. *J Neuroimmunol* **64:** 63–69.
Noseworthy JH, Lucchinetti C, Rodriguez M, Weinhenker BG (2000). Multiple sclerosis. *New Engl J Med* **343:** 938–962.
Ochi H, Wu XM, Osoegawa M, Horiuchi I, Minohara M, Murai H, Ohyagi Y, Furuya H, Kira J (2001). Tc1/Tc2 and Th1/Th2 balance in Asian and Western types of multiple sclerosis (MS), HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and hyper-IgEaemic myelitis. *J Neuroimmunol* **119:** 297–305.
Olsson T (1995). Critical influences of the cytokine orchestration on the outcome of myelin antigen-specific T-cell autoimmunity in experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunol Rev* **144:** 245–268.
Ozenci V, Kouwenhoven M, Link H (2002). Cytokines in multiple sclerosis: methodological aspects and pathogenic implications. *Mult Scler* **8:** 396–404.
Panitch HS (1994). Influence of infection on exacerbations of multiple sclerosis. *Ann Neurol* **36:** S25–S28.
Panitch HS, Hirsch RL, Schindler J, Johnson KP (1987). Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology* **37:** 1097–1029.
Perron H, Garson JA, Bedin F, Beseme F, Paranhos-Baccala G, Komurian-Pradel F, Mallet F, Tuke PW, Voisset C, Blond JL, Lalande B, Seigneurin JM, Mandrand B (1997). Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. *Proc Natl Acad Sci U S A* **94:** 7583–7588.
Perron H, Jouvin-Marche E, Michel M, Ounanian-Paraz A, Camelo S, Dumon A, Jolivet-Reynaud C, Marcel F, Souillet Y, Borel E, Gebuhrer L, Santoro L, Marcel S, Seigneurin JM, Marche PN, Lafon M (2001). Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal Vbeta16 T-lymphocyte activation. *Virology* **287:** 321–332.
Perron H, Perin JP, Rieger F, Alliel PM (2000). Particle-associated retroviral RNA and tandem RGH/HERV-W copies on human chromosome 7q: possible components of a 'chain-reaction' triggered by infectious agents in multiple sclerosis? *J NeuroVirol* **6 (Suppl 2):** S67–S75.
Pitha PM (1994). Multiple effects of interferon on the replication of human immunodeficiency virus type 1. *Antiviral Res* **24:** 205–219.
Rosati G (2001). The prevalence of multiple sclerosis in the world: an update. *Neurol Sci* **22:** 117–140.
Rudge P (1991). Does a retrovirally encoded superantigen cause multiple sclerosis? *J Neurol Neurosurg. Psychiatry* **54:** 853–855.
Sotgiu S, Serra C, Mameli G, Pugliatti M, Rosati G, Arru G, Dolei A (2002). Multiple sclerosis (MS)-associated retrovirus and MS prognosis: an observational study. *Neurology* **59:** 1071–1073.
Steiner I, Nisipianu P, Wirguin I (2001). Infection and the etiology and pathogenesis of multiple sclerosis. *Curr Neurol Neurosci Rep* **1:** 271–276.
Tristem M (2000). Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. *J Virol* **74:** 3715–3730.
Wang X, Chen M, Wandinger KP, Williams G, Dhib-Jalbut S (2000). IFN-beta-1b inhibits IL-12 production in peripheral blood mononuclear cells in an IL-10-dependent mechanism: relevance to IFN-beta-1b therapeutic effects in multiple sclerosis. *J Immunol* **165:** 548–557.
Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, Burd P (1998). Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. *J Virol* **72:** 3082–3087.