Multiple sclerosis–associated retrovirus and MS prognosis

An observational study

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Abstract—MS-associated retrovirus (MSRV) in the CSF may have gliotoxic properties and could be associated with a more disabling MS. The authors tested this hypothesis in 15 untreated patients with MS: 6 MSRV− and 9 MSRV+ at the time of CSF withdrawal. After a 3-year mean follow-up, MSRV− patients showed a stable MS course, whereas MSRV+ patients had a progressive course ($p = 0.01$).

NEUROLOGY 2002;59:1071–1073

Several intracellular pathogens have been associated with MS in case-control studies but none have been established as relevant to pathogenesis.[1] The MS-associated retrovirus (MSRV), an exogenous member of the HERV-W family, has been found in plasma and CSF of many patients with MS.[2] We estimated MSRV within the CSF of patients with MS in Sardinia, and found it present in 50% of patients at clinical onset,[3] suggesting that it is not the cause of MS.

Because MSRV has gliotoxic effects in vitro,[4] we hypothesized that MSRV+ patients may have more severe demyelination than MSRV− patients. We tested this hypothesis over an average 3-year observational clinical follow-up of untreated patients with active MS.

Patients and methods. *Patients.* We studied 20 consecutive patients with clinical symptoms or signs suggestive of MS and free of immunomodulatory and steroid treatments for at least 3 months before CSF withdrawal. Informed consent was previously obtained. Each patient underwent neurologic examination to identify CNS functional system (FS) involvement and calculate the Kurtzke Expanded Disability Status Scale (EDSS) score.[5] CSF examination for intrathecal immunoglobulin (Ig) production (CSF vs serum Ig to CSF vs serum albumin ratio—Ig index—and oligoclonal bands [OCB]), MRI, and evoked potentials supplemented the evidence for MS diagnosis if clinical criteria were not met. At follow-up, paraclinical tests were negative and MRI was not diagnostic[6] in five such patients, for whom diagnoses of postinfectious encephalomyelitis (2 patients), compressive radiculomyelopathy (1), trigeminal neuralgia (1), and recurrent optic neuritis of unknown origin (1) were made. The remaining 15 patients (women to men ratio: 2.75) had an active course of MS from onset (i.e., they presented neurologic signs and symptoms attributable to demyelination within 2 weeks prior to the study): 5 patients had relapses and 10 were at their onset. Patients at onset were confirmed to have MS according to the Poser criteria[7]: six were diagnosed with clinically probable (CP) MS and five with laboratory-supported definite (LD) MS. The remaining patients with a 1-year history before diagnosis were classified as clinically definite (CD) MS. One patient each with a 2-year clinical history had CPMS and CDMS (table).

Follow-up. After an average interval of 3 years, all 15 patients were individually recontacted. During this period 12 patients had been followed up by different neurologists, who reported that eight patients had had relapses, one had disease progression (see the table), and some had been under immunomodulatory treatments (interferon-β and azathioprine). At the time of last comprehensive clinical re-examination the number of impaired FS and relapses were considered and EDSS score recalculated. Data were compared to those at study entry. Finally, the presence of MSRV was considered and patients stratified accordingly.

MSRV detection. Methods were recently published.[3] Briefly, cell-free CSF samples were precipitated with polyethylene glycol, centrifuged, and treated with bovine Ribonuclease I A (USB Corporation) to avoid cellular RNA contamination; extracted RNA were digested with RNase-free Deoxyribonuclease I to avoid possible DNA contamination and then re-extracted. Coded samples of virionic RNA were used as template for nested reverse transcription PCR (RT-PCR) using primers specific for MSRV-*pol* gene: First PCR amplification with PTpol-A (sense): 5'GGCCAGGCATCAGCCCAAGACTTGA3' and PTpol-F (antisense): 5"TGCAAGCTCATCCCTSRGACCT3. Second PCR amplification with PTpol-B (sense): 5'GACTTGAGCCAGTCCTCATACCT3' and PTpol-E (antisense): 5'CTTTAGGGCCTGGAAAGCCACT3'. Nested PCR amplification was carried out by Taq

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Supported by grants from Regione Autonoma Sardegna, Ministero Università e Ricerca, Istituto Superiore di Sanità, Rome, and Federazione Italiana Sclerosi Multipla (FISM), Genoa, Italy.

Received February 8, 2002. Accepted in final form June 20, 2002.

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Table *Demographic and clinical features of the two patient groups at study entry and at follow-up*

Patient no./sex/ age (duration), y	Study entry			Interval, y	Follow-up		
	Poser	OCB (index)	EDSS (FS)		No. relapse (rate)	Rx	EDSS (FS)
MSRV−							
1/F/35 (0)	LSD	− (+)	2.5 (2)	2.5	1 (0.4)	—	2 (2)
2/F/25 (0)	CP	− (−)	2 (1)	3	0 (0)	—	1 (1)
3/F/30 (2)	CP	− (+)	1 (2)	3.5	1 (0.28)	—	1 (1)
4/M/36 (0)	LSD	+ (+)	1.5 (1)	3.5	0 (0)	—	1.5 (1)
5/F/28 (0)	CP	− (−)	2 (1)	3	1 (0.33)	—	2 (1)
6/M/27 (0)	CP	ND	3.5 (1)	2.5	0 (0)	—	2 (1)
Mean	30.2 (age)		2.1 (1.3)	3.0	0.5 (0.2)		1.6 (1.2)
SD	4.4		0.9 (0.5)	0.4	0.5 (0.2)		0.5 (0.4)
MSRV+							
7/F/22 (2)	CD	+ (+)	2 (1)	3	1 (0.33)	AZA	3 (3)
8/F/25 (0)	CP	− (−)	2 (1)	2.5	0 (0)	—	1 (1)
9/M/26 (1)	CD	− (+)	1 (1)	3.5	1 (0.28)	—	2 (2)
10/F/36 (1)	CD	+ (+)	3.5 (1)	3	SP (—)	AZA	6 (3)
11/F/27 (1)	CD	+ (+)	1 (2)	3.2	3 (0.93)	IFNβ	4 (3)
12/M/28 (0)	CP	− (−)	1.5 (1)	3	0 (0)	—	1.5 (2)
13/F/17 (0)	LSD	+ (+)	2.5 (1)	3	3 (1)	IFNβ	4 (3)
14/F/26 (0)	LSD	+ (+)	3 (1)	2.8	0 (0)	AZA	3 (1)
15/F/42 (0)	LSD	+ (+)	3 (2)	3.5	2 (0.57)	IFNβ	4 (3)
Mean	27.7 (age)		2.2 (1.2)	3.1	1.3 (0.4)		3.2 (2.4)
SD	7.4		0.9 (0.4)	0.3	1.3 (0.4)		1.5 (0.9)
p Value					0.11 (0.52)	0.028	0.01 (0.01)

MRI findings were compatible with MS diagnosis in all patients.

MSRV− = CSF-negative patients; MSRV+ = CSF-positive patients; duration (0) = MS onset; Poser = diagnostic criteria for MS[7] (CP = clinically probable; LSD = laboratory supported definite; CD = clinically definite); OCB = CSF oligoclonal band; index = immunoglobulin (Ig) index: CSF vs serum Ig to CSF vs serum albumin ratio; EDSS = Kurtzke Expanded Disability Status Scale score[13]; FS = number of impaired CNS functional systems; Rate = mean annual relapse rate (no. relapses/years of follow-up); Rx = treatment (AZA = azathioprine 2 mg/Kg; IFNβ = interferon-beta [1a or 1b]); *p* values = Fisher exact test (FET) or analysis of variance (two-tailed FET).

DNA polymerase (DYNAzyme) and created a 435-bp long product, identified after agarose gel electrophoresis and ethidium bromide staining under ultraviolet light. Controls included PCR of RNA not exposed to RT with betaglobin gene or MSRV-specific primers, PCR of cDNA samples without template (negative control), and samples of human cell DNA (positive control). MSRV was repeatedly searched for from the same CSF sample. The specificity of the amplified products was confirmed by dideoxy sequencing and analysis in six patients by using the BLASTN program, NIH (Bethesda, MD).

Data analysis. Statistical analysis was carried out by means of Epi Info 2000 Database. The two-tailed Fisher exact test (FET) or analysis of variance and the Maentel–Haenszel χ^2 (stratified cell-count cohort studies) were used as significance tests.

Results. *Study entry.* Nine patients with MS were MSRV+ and six were MSRV−. Mean age (27.7 vs 30.2), number of impaired FS (mostly monosymptomatic), EDSS score (2.2 vs 2.1), and MRI findings (see the table)[6] did not significantly differ in the two groups. A nonsignificant lower rate of CSF immunologic abnormalities was evident in the MSRV− group: 6 of 9 (67%) MSRV+ vs 1 of 5 (20%) MSRV− patients had OCB; 7 of 9 (78%) MSRV+ vs 4 of 5 (80%) MSRV− patients had positive IgG index (not done in one MSRV− patient).

Follow-up. At the time of study entry, two MSRV− patients at clinical onset already met criteria for definite MS.[7] Two of the remaining four met criteria for definite MS after the second attack (mean interval 1.6 years). Two patients have presented a rather stable course, still falling in the CPMS category. Of the nine MSRV+ patients, seven met criteria for definite MS at study entry. Of the remaining two patients with CPMS, one had clinical relapse after 8 months (CDMS), and one was categorized as having CDMS 1 year later[7] based on impairment in a formerly normal FS.

As for the MSRV− group, number of impaired FS and EDSS score were not significantly different between the first and second evaluation. Conversely, in MSRV+ patients a more disabling course was observed, with a higher number of impaired FS (2.4 vs 1.2; $p = 0.01$) and mean EDSS score (3.2 vs 1.6; $p = 0.01$; see the table and the

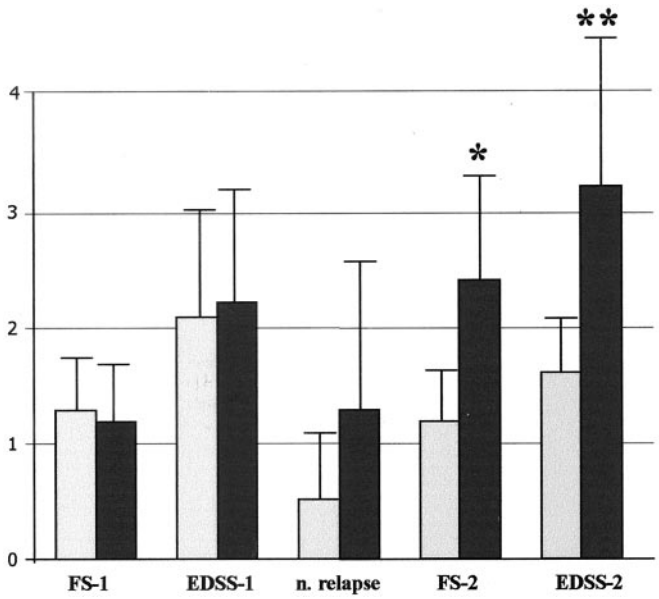

*Figure. Comparison of clinical evolution of the two MS groups (light gray bars = MS-associated retrovirus [MSRV]− and dark gray bars = MSRV+). After an average 3-year period, clinical re-examination disclosed significant differences between the two groups. In MSRV+ patients, a disabling course was evident, in terms of the number of functional systems (FS) impaired as well as a significantly higher mean Expanded Disability Status Scale (EDSS) score. Average number of relapses per patient was also higher in the MSRV+ group, although not significantly. FS-1 = number of impaired FS at study entry; FS-2 = at follow-up. EDSS-1 = EDSS score at entry; EDSS-2 = at follow-up. On the Y axis, numbers indicate the number of FS, the EDSS score, or the relapse number. Significant differences are indicated (*p = 0.015; **p = 0.01).*

figure). The average number of relapses (1.3 vs 0.5) and the mean annual relapse rate (0.4 vs 0.2) were also higher in the MSRV+ group (see the figure), although not significantly. No MSRV− patients required immunoregulatory drugs, whereas six MSRV+ patients underwent treatment with interferon-β or azathioprine (FET $p = 0.028$). One MSRV+ patient developed a secondary progressive course.

Discussion. Evidence on MS natural history derived from life-table analyses of large MS populations refers to a broad range of MS forms, including malignant, benign, or even asymptomatic. The age at onset, the nature of the first symptoms, the remittent or progressive form at onset, the time lag between the first two relapses, and the time interval to EDSS grade 4 appear to be predictors of MS course in the long run.[8,9] Also, a more precise analysis of axonal loss, gliosis, and demyelination, even in the normal-appearing white matter, by means of new MRI techniques may have effective clinical applications.[10] Prognostic predictive factors are being analyzed in the attempt to identify patients with an aggressive course who may benefit from early treatments, with respect to their high costs.

Our study results suggest a new prognostic factor for MS. The presence of MSRV in the CSF of patients with MS correlates to disability progression on clinical examination. On the contrary, MSRV− patients present a rather stable and treatment-free MS course. Our evidence appears to be supported by data from in vitro studies on MSRV pathogenetic relevance in MS.[4] However, if MSRV only represents a pathogenetic epiphenomenon, it might still be considered a prognostic marker in MS. A larger patient sample size, an extension of the follow-up period, and a correlation with the rate of conversion from isolated syndromes to CDMS based on MRI criteria[6] are needed to better corroborate these hypotheses.

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