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The multiple sclerosis-associated retrovirus and its HERV-W endogenous family: a biological interface between virology, genetics, and immunology in human physiology and disease

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This mini-review summarizes current knowledge of MSRV (multiple sclerosis–associated retrovirus), founder member of the type W family of human endogenous retroviruses (HERVs), its pathogenic potential and association with diseases. As retrotransposable elements, HERVs behave differently from stable genes, and cannot be studied with ''Mendelian genetics'' concepts only. They also display complex interactions with other HERV families, and with classical viruses. These concepts may contribute to unravelling the etiopathogenesis of complex diseases such as multiple sclerosis, schizophrenia, and other chronic multifactorial diseases. *Journal of NeuroVirology* (2009) **15,** 4–13.

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The discovery of MSRV

The path that led to the discovery of multiple sclerosis–associated retrovirus/type W family of human endogenous retroviruses (MSRV/HERV-W) started in the late 1980s, when leptomeningeal cells were isolated from the cerebrospinal fluid (CSF) of a patient with multiple sclerosis (MS) (Perron *et al.* 1989); these cells, named LM7, harbored an unknown retrovirus, with reverse-transcriptase (RT) activity, that was purified from liters of supernatants (Perron *et al.* 1997a). Amplification by RT–polymerase chain reaction (PCR) (Tuke *et al.* 1997) of virionic RNA, and other approaches, identified a novel retroelement, that was named MSRV (MS-associated retrovirus) (Perron *et al.* 1997b). Complete MSRV sequencing (Komurian-Pradel *et al.* 1999) unraveled a related new family of human endogenous retroviruses (HERVs), which was named HERV-W from its tryptophan t-RNA binding site, identified in MSRV 5' long terminal repeat (LTR) (Blond *et al.* 1999; Komurian-Pradel *et al.* 1999).

HERVs constitute ~8% of our genome and are remnants of ancestral infections by germline-transmitted exogenous retroviruses (reviewed in Dolei, 2006, and Belshaw *et al.* 2007). This retroviral ''passage'' into species DNA is still active and, e.g., presently ongoing in koalas, which are in the midst of a germline invasion by the KoRV koala oncogenic retrovirus (Tarlinton *et al.* 2006). In general, HERVs are highly defective, but complete proviruses have been described (Pavlicek *et al.* 2002). This tremendous amount of genetic material is grouped in at least 31 independently acquired families (Belshaw *et al.* 2005).

The HERV-W family is one of the most studied, after the discovery of its MSRV founder member

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(Perron *et al*. 1989, 1997b). Our haploid genome contains about 70 *gag*, 100 *pro*, and 30 *env* HERV-W–related regions (Voisset *et al*. 2000), but numbers can vary (Mirsattari *et al*. 2001; Zawada *et al*.2003); *in silico* expression data indicate that 22 complete HERV-W subfamilies from chromosomes 1 to 3, 5 to 8, 10 to 12, 15, 19, and X are randomly expressed in various tissues (Kim *et al*. 2008). Presently, this family is active and generates new recombinant copies in cancer cells (Yi *et al*. 2004), retains characteristics of functional retroviral envelopes (An *et al*. 2001; Kim *et al*. 2008), and HERV-W transposition and retrosequence integration have been evidenced in the human genome through interactions with active LINE-1 elements (Costas, 2002; Pavlicek *et al*. 2002). Thus, non-Mendelian genetic rules can apply to HERV-W elements: a key feature to understanding their biology.

None of the known stably inserted HERV-W elements is replication-competent (Blond *et al*. 1999): a study of HERV-W intragenomic spread (Costas, 2002) confirmed that, in the few individuals used for genomewide analysis, the sequenced HERV-W elements lack intact open reading frames (ORFs) in all genes within a single copy. This finding, and the unusual short period of evolutionary time of HERV-Ws ($\sim$5 million years, estimated on average integration age of different subfamilies), suggested that MSRV might be either an exogenous HERV-W, as in animal ERVs (Palmarini *et al*. 1996), or a nonubiquitous replication-competent member, or a partly defective but nonubiquitous copy seldom complemented within the HERV-W family (Perron *et al*. 1997b, 2000; Komurian-Pradel *et al*. 1999; Dolei, 2005; Serra *et al*. 2003). Today, though reasonable arguments in favor of the existence of a replication-competent HERV-W member, which might even be ''exogenous,'' have been provided (Belshaw *et al*. 2005; Costas, 2002; Perron *et al*. 1997b, 1992), the very nature of MSRV remains to be confirmed by future studies (Voisset *et al*. 2008).

MSRV morphology and molecular features

MSRV was repeatedly isolated from cells of MS patients, such as leptomeningeal cells, monocytes, and Epstein-Barr virus (EBV)-transformed B-cell lines (Perron *et al*. 1989, 1997a, 1997b, 1991; Sommerlund *et al*. 1993), and electron microscopy (EM) showed the typical retrovirus-like morphology (Figure 1A). Virionic MSRV RNA was detected in supernatants of peripheral blood mononuclear cells (PBMCs) of MSRV-positive individuals (Serra *et al*. 2003, Nowak *et al*. 2003; Mameli *et al*. 2007), and in serum, plasma, and CSF (Garson *et al*. 1998; Serra *et al*. 2001; Dolei *et al*. 2002; Sotgiu *et al*. 2006a, 2006b Mameli *et al*. 2007, 2008).

Sequencing of overlapping amplicons from extracellular virionic genomes identified a putative 7630-nucleotide-long retroviral organization with RU5, *gag, pol, env,* and U3R regions (Komurian-Pradel *et al*. 1999). These sequences belong to the HERV-W family (Blond *et al*. 1999), classified in the group 2 of the type C–related, class I HERVs (Nelson *et al*. 2003). According to Komurian-Pradel *et al* (1999), the MSRV *gag* gene is 1458 nucleotides (nt) long; the deduced Gag polyprotein has 486 amino acids (aa), with three potential *N*-glycosylation sites. The MSRV *pol* gene encompasses 1187 nt, with a deduced sequence of 398 aa, presenting structural motifs common to several retroviral proteases, reverse transcriptases, and integrases. The MSRV *env* gene is 1629 nt long; the deduced polyprotein has 542 aa, six potential *N*-glycosylation sites, a putative signal peptide (aa 1 to 27), and is divided by a 4-aa cleavage site into a surface moiety (SU, 290 aa, with a putative receptor function) and a transmembrane moiety (TM) of 252 aa (Figure 1B). The TM domain has an extracellular region (including the potentially immunosuppressive domain), an intramembrane domain, and a cytoplasmic tail (Komurian-Pradel *et al*. 1999). The cellular receptors for HERV-W Env are thought to be the ASCT2 and ASCT1 amino acid transporters (Lavillette *et al*. 2002), expressed by both astrocytes and trophoblasts.

At the RNA level, MSRV *env* has $>$94% identity (Mameli *et al*. 2007) with syncytin, the *env* encoded by ERVWE-1, a replication-incompetent HERV-W element located on chromosome 7q21-22 (Blond *et al*. 1999); syncytin is involved in pregnancy (Mi *et al*. 2000) and shares several biological features with MSRV Env protein. Syncytin was renamed syncytin-1, after the discovery of a syncytin-2 gene from the HERV-FRD family (Blaise *et al*. 2003). A major difference between MSRV and syncytin-1 is that only MSRV is found as complete extracellular virus, with RT activity, visualized by EM, and sedimenting at retrovirus buoyant density, whereas syncytin is found only intracellularly or on the plasma membrane (Perron *et al*. 1989, 1997b; Mi *et al*. 2000; Mameli *et al*. 2007). At the protein level, no antibody specific for a unique HERV-W has been identified so far (Perron *et al*. 2005). At the RNA level, based on point mutations of few known RNA sequences, specific primers were proposed to

Figure 1 (**A**) Different electron microscopy pictures of MSRV particles produced in leptomeningeal or macrophage cells (Perron *et al,* 1989, 1993, 1995, 2008). (**B**) Western blot with anti-Env monoclonal antibody on recombinant MSRV-Env protein (encoded by pV14 clone, accession number AF331500.1). *Left,* uncleaved full-length protein. *Right,* partially cleaved protein, with immunodetection of a TM fragment at lower K_d. The protein can autocleave at SU-TM cleavage site, as confirmed by N-terminal sequencing of fragments in QC control production procedures. Thus, under appropriate conditions, the SU or the TM fragment is codetected with full-length protein, depending on the epitope mapping of the used antibody, as in the right lane.

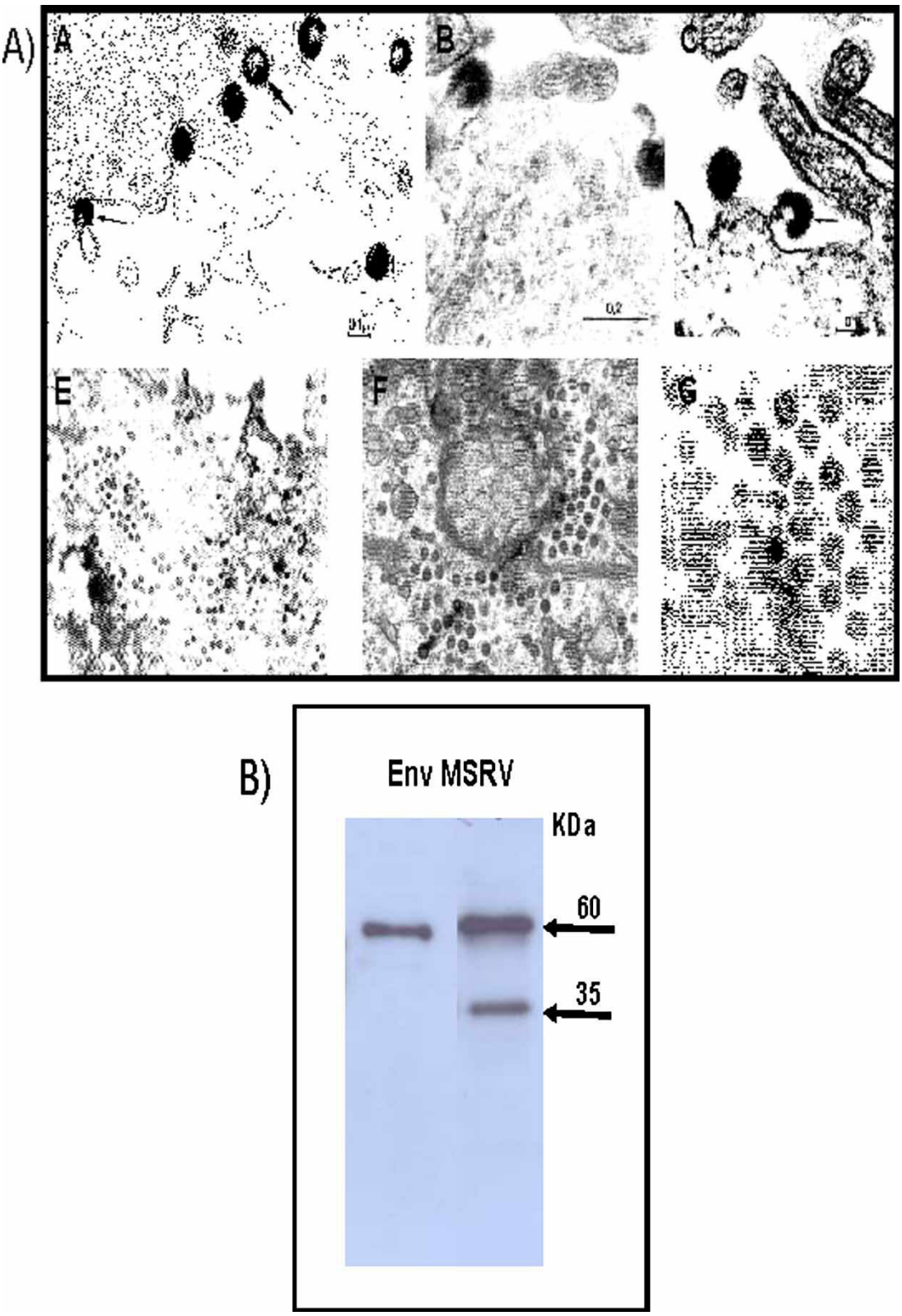

Figure 1 (*Continued*)

discriminate syncytin and MSRV *env* sequences, but MSRV isolates could not be amplified (Antony *et al.* 2007b). Parallel attempts have positively evaluated specific TM regions (Mameli *et al.* 2008a).

Pathogenic mechanisms

Several MSRV properties might be pathogenic, such as fusogenicity (Perron *et al.* 1997a), and induction of T-mediated immunopathology; virions induce proinflammatory cytokines and T-lymphocyte responses, with polyclonal T-cell receptor Vβ16 expansion in surface receptor; activation was reproduced by recombinant MSRV Env protein, but not by MSRV Gag, suggesting that MSRV Env triggers proinflammatory, ''superantigen-like,'' abnormal immune responses (Perron *et al.* 2001). Blood cells exposed to MSRV Env SU promptly released proinflammatory cytokines associated with innate immunity, preceding T-lymphocyte activation, through a TLR4/CD14-dependent pathway (Rolland *et al.* 2006). MSRV Env promoted the development of type 1 immune responses upon dendritic cell activation, whose maturation is triggered by MSRV Env SU (Rolland *et al.* 2006). Thus the immunopathogenic cascades associated with chronic inflammatory and/or neurodegenerative diseases may initiate from early innate immunity, towards major dysregulation of T-cell responses, when attracted into a type 1 microenvironment. A pathophysiological role of HERV-W proteins was proposed also in neurons, because detection of MSRV Env in microgliocyte-like cells was associated with MS demyelinated plaques; Gag hyperexpression was seen in demyelinated axons, but was not MS-specific (Perron *et al.* 2005).

A positive feedback loop on MSRV production was observed in PBMCs from MSRV-positive individuals (Serra *et al.* 2003), where virus release was up-regulated by proinflammatory cytokines (but inhibited by interferon β [IFNβ]). These cytokines, in turn, are overproduced in response to MSRV/HERV-W Env by cells from MS patients, and correlate with MS severity (Rolland *et al.* 2005), thus providing a pathogenic amplification loop. Interestingly, cells from MSRV-negative individuals did not produce any MSRV, neither spontaneously nor after treatment with cytokines that enhance MSRV expression in MSRV-positive cells (Serra *et al.* 2003). Therefore, the authors hypothesized that MSRV might be an exogenous member of the HERV-W family (Serra *et al.* 2003).

In vivo, intraperitoneal MSRV injection into humanized severely compromised immunodeficient (SCID) mice caused a T lymphocyte–mediated neuropathology (Firouzi *et al.* 2003), with tumor necrosis factor α (TNFα) overexpression, brain hemorrhages, and death within days. Notably, human T-cell depletion reduced mortality by 95%, thus evidencing an immune-mediated MSRV pathogenicity. Accordingly, proinflammatory properties of experimentally expressed syncytin were observed in the nervous system: death of oligodendrocytes, neuroinflammation, and neurobehavioural deficits of a transgenic mouse (Antony *et al.* 2004, 2007a).

Finally, paralleling MSRV expression in MS macrophages, a gliotoxic factor was observed, causing disorganization of the intermediate filament network, death of astrocytes and oligodendrocytes, and blood-brain barrier breakdown in the rat (Menard *et al.* 1997).

Role of triggering infectious cofactors in HERV-W transactivation

Transactivation might represent the link between HERV-W and other viruses proposed as MS cofactors: brain activation of quiescent HERV-W, e.g., in perivascular macrophages, would initiate Env-associated immunopathogenicity (Ruprecht *et al.* 2006).

In culture, expression of MSRV/HERV-W genes/proteins and HERV-W LTR–directed transcription were activated by herpes simplex virus type 1 and its immediate-early protein, and by influenza virus, but not by rabies virus (Perron *et al.* 1993, Lafon *et al.* 2002; Lee *et al.* 2003; Ruprecht *et al.* 2006; Nellåker *et al.* 2006). Moreover, simultaneous presence of HERV and herpesvirus antigens has synergistic effects on cell-mediated immune responses (Christensen, 2005).

For human herpesvirus-6 (HHV-6), when both MSRV/HERV-W and HHV-6 were monitored within the same brain or blood samples, MSRV/HERV-W was found activated strongly in MS, whereas there were no significant differences between MS patients and controls for HHV-6 presence/replication at both brain and blood level (Mameli *et al.* 2007).

Molecular epidemiology and association with human diseases

When HERV expression is detected, a key problem is to differentiate whether it is cause or effect of the particular disease or physiological condition under study (Dolei, 2006; Voisset *et al.* 2008), but this is true for all viruses.

The majority of reports on MSRV and human disease address an association with MS, an inflammatory demyelinating disease of the nervous system, with immune-mediated pathogenesis, involving genetic and environmental (viral) factors (Prat and Antel, 2005). The presence of extracellular MSRV was repeatedly found increased in blood and CSF of MS patients (plasmatic positivity: 53% to 100%) in

cohorts of Caucasians from France, Sardinia, Northern Italy, Poland, Sweden, Spain, and South Africa (Perron *et al.* 1997b; Garson *et al.* 1998; Serra *et al.* 2001; Dolei *et al.* 2002; Nowak *et al.* 2003; deVilliers *et al.* 2006; Arru *et al.* 2008). MSRV load was significantly associated with MS in all ethnic groups studied (Mameli *et al.* 2007; Arru *et al.* 2008). Notably, in the South Africa cohort, MSRV positivity was ~70% in both patients and close relatives (deVilliers *et al.* 2006); in a single family of 19 close relatives with two MS cases, MSRV positivity of healthy relatives was 82% between seniors and adults, but 0% between juniors. This finding could derive from possible spread of an infectious agent between closely related individuals or common inheritance of an unusually 'adulthood-expressive' HERV-W element. This striking parallelism between MSRV positivity, and load, and MS disease (Dolei *et al.* 2002; Sotgiu *et al.* 2002, 2006a; Mameli *et al.* 2007, 2008a; see also Diagnostic and Prognostic Applications) might reflect the spreading of a transmissible agent, which might constitute the environmental risk factor, ''fueling'' MS disease when later triggered by cofactors (Pugliatti *et al.* 2006).

Transcription of HERV-W *pol* and *env* is higher in the brain of MS patients than in controls (Mameli *et al.* 2007; Antony *et al.* 2004, 2006; Johnston *et al.* 2001). By discriminatory real-time assays, these transcripts were either attributed solely to ERVWE1 *env* (Antony *et al.* 2007b), or to both MSRV *env* and ERVWE1 *env* (Mameli *et al.* 2008a).

Patients with optic neuritis (ON), a disease frequently prodromic to MS, were found ~70% MSRV-positive in blood and CSF, compared to ~35% of pathological controls (Sotgiu *et al.* 2006b), whereas in other ON series MSRV viremia was not detected (Petzold *et al.* 2005). In the latter case, it was recently found that certain sample collection and storage condition (in particular tubes and temperature) could be critical for the detection studies (Dolei and Perron, unpublished data).

Anti-HERV-W Env immunostaining was observed in MS brains (in both astrocytes and microglia), but barely present in brains samples from controls with normally appearing brain tissue or affected by non-MS diseases (Mameli *et al.* 2007; Antony *et al.* 2007a). A multicentric study evaluated different monoclonal antibodies against HERV-W Gag and Env on MS and control brains (Perron *et al.* 2005); MS-specific expression patterns of HERV-W proteins were found in MS lesions, involving mainly endothelial and microglial cells; a physiological expression of HERV-W Gag was detected in neurons from normal and non-MS brains, with up-regulation in demyelinated axons of MS or progressive multifocal leukoencephalopathy.

Humoral and cellular HERV-W–specific immune responses were restricted to isolated epitopes in MS patients (Jolivet-Reynaud *et al.* 1999; Clerici *et al.* 1999; Trabattoni *et al.* 2000), mostly identified in CSF (Jolivet-Reynaud *et al.* 1999). Immunofluorescence and immunospot assays detected an anti-HERV-W response in only 1 of 50 individuals tested, demonstrating a generalized immunotolerance against HERV-W antigens (Ruprecht *et al.* 2008). Therefore HERV-W antigens may be recognized as ''self-antigens,'' thus bypassing many immune defenses, at variance with HERV-K proteins, whose expression in human tumors induces antigen-specific immune response (Wang-Johanning *et al.* 2008), that can be related to reduced survival probability (Hahn *et al.* 2008).

Proinflammatory cytokines induced by MSRV Env SU protein were evaluated in PBMCs from MS patients and controls, finding overproduction in patients that correlated with MS severity (Rolland *et al.* 2005). As previously quoted, proinflammatory cytokines stimulated MSRV release by PBMCs from carrier individuals (Serra *et al.* 2003): this argues again for a pathogenic feedback loop, as often encountered in virus biology. Cells from MS patients in acute phase have a prevalent type 1 cytokine profile, whereas stable individuals have a type 2 cytokine profile (Clerici *et al.* 1999; Trabattoni *et al.* 2000).

Circulating virionic MSRV/HERV-W RNA was detected by RT-PCR in a small percentage of healthy donors (mean 8.9% ± 6.2%; from the studies of Garson *et al.* 1998; Serra *et al.* 2001; Dolei *et al.* 2002; Gaudin *et al*; 2000; Karlsson *et al.* 2004; Arru *et al.* 2008). The MSRV viremia occurs also in non-MS neurological patients, but the frequency of MSRV detection (Dolei *et al.* 2002; Nowak *et al.* 2003; Karlsson *et al.* 2004; Sotgiu *et al.* 2006b), and viral load (Mameli *et al.* 2007), are significantly lower than in MS; when these patients were stratified with respect to the disease, MSRV presence was higher in non-MS patients with inflammatory diseases, with significant differences from both MS patients and healthy donors (Dolei et al. 2002).

Schizophrenia and MS share several features, such as epidemiology and multifactorial pathogenesis (Karlsson *et al.* 2001; Dolei, 2006). Virionic MSRV/HERV-W RNA was detected in the CSF of 29% subjects with recent-onset schizophrenia and 5% subjects with chronic schizophrenia, but not in controls (Karlsson *et al.* 2001), and in the plasma of 17% individuals with recent-onset schizophrenia (Karlsson *et al.* 2004). Because the ASCT2 and ASCT1 amino acid transporters are presumed HERV-W receptors, HERV-W might impair neuronal glutamine uptake, interfering with glutamatergic transmission (Karlsson *et al.* 2004). PBMCs of recent-onset schizophrenia patients had elevated levels of HERV-W *gag* transcripts from the PTD015 putative gene on chromosome 11q13.5 (Yao *et al.* 2008), in contrast to diminished HERV-W Gag detection in cortical neurons of brains from similar patients (Weis *et al.* 2007a,b). Because HERV-W Gag expression appears physiological in brain neurons

(Perron *et al.* 2005), this diminution could derive from neuronal depletion in certain schizophrenia forms (Karlsgodt *et al.* 2007). In monozygotic discordant twins, the affected individual may present *de novo* HERV-W integrations (Deb-Rinker *et al.* 1999, 2002). One study on brain from patients with schizophrenia and bipolar disorders and controls did not find significant difference in HERV-W *env* transcription (Frank *et al.* 2005), contrary to a previous study with different primers (Yolken *et al.* 2000), but here again methodological aspects (choice of primers, PCR conditions, patient's material processing, and accuracy of selected control groups) should be debated. Moreover, HERV-W Env and Gag were significantly detected in sera of schizophrenic patients with subacute systemic inflammation (Perron *et al.* 2008).

Virionic MSRV/HERV-W RNA was detected in synovial fluids of a small proportion of rheumatoid arthritis patients, without differences from controls (Gaudin *et al.* 2000). In psoriasis, another T lymphocyte–mediated multifactorial disorder, retrovirus-like particles were detected in skin lesions, urine, and stimulated lymphocytes (Dalen *et al.* 1983). Psoriatic lesions express the HERV-W, -K and -E families, and a new variant sequence of the HERV-W family was detected in 67% lesional skin samples, 9% normal, and 21% pathological controls (Moles *et al.* 2005), but role of HERVs in the disease is unknown.

Diagnostic and prognostic applications

In MS, a demanding task is to predict individual clinical course and therapy outcome; so far, no suitable (bio)marker has been established, although reliable clinical predictors are crucial for identifying appropriate candidates for early or aggressive therapies, or for therapeutic monitoring (Bergamaschi 2007; Mameli *et al.* 2008). A direct parallelism was found between MSRV positivity and load (in blood, CSF, and brain samples) and MS temporal and clinical stages, as well as active/remission phases (Dolei *et al.* 2002); at MS onset, 50% of CSFs were MSRV positive, and positivity increased with progression. Importantly, MSRV presence in CSF at MS onset was related to poor prognosis; starting from otherwise comparable conditions, after 3 and 6 years mean EDSS (expanded disability status scale), annual relapse rate, therapy requirement, and progression to secondary-progressive MS were significantly higher (Figure 2) in patients with detectable MSRV CSF load at onset (Sotgiu *et al.* 2002, 2006a).

A recent study (Mameli *et al.* 2008) found that plasmatic MSRV load of naive patients with active MS was directly related to MS duration; longitudinal evaluation of patients during 1 year of IFNβ therapy showed that MS progression index (EDSS/MS years) was reduced for the majority of patients, whose viremia became rapidly undetectable. Notably, one patient had atypically high viremia at enrolment and, after initial virus inhibition and clinical benefit, had MSRV reemergence, accompanied by strong progression with therapy failure. The authors concluded that evaluation of plasmatic MSRV could be considered the first prognostic marker for the individual patient to monitor disease progression and therapy outcome (Mameli *et al.* 2008).

This possibility is reinforced by studies of patients with optical neuritis (Sotgiu *et al.* 2006b): conversion of ON patients to full-blown MS was evaluated during ~20 months, with respect to MSRV positivity (blood and CSF), finding that 33.3% MSRV-positive and 0% MSRV-negative patients developed MS ($P = .03$).

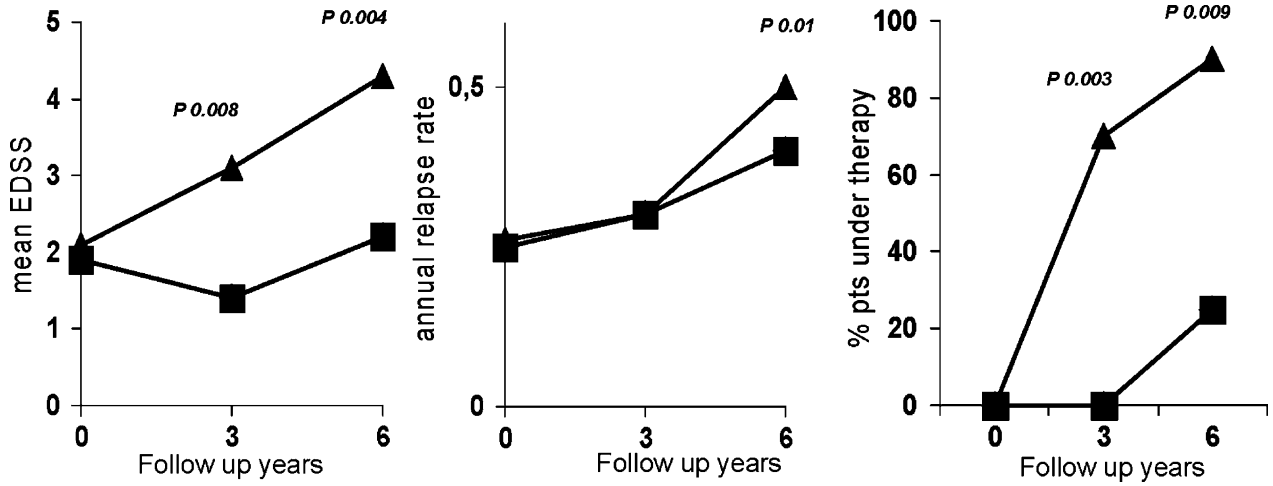

Figure 2 Association of the presence of MSRV virions in the CSF at MS onset with disability accumulation (*left*), higher rate of clinical relapse (*middle*), and therapy requirement (*right*). Eighteen MS patients were subjected to a 3- and 6-year follow-up based on the presence (*triangles*) or absence (*squares*) of extracellular MSRV in the CSF at study entry (Sotgiu *et al*, 2002, 2006a).

Concluding remarks and perspectives

At present, it is unclear whether the detection of MSRV/HERV-W/syncytin expression simply represents an epiphenomenon (e.g., the abnormal expression of endogenous HERV-W or an unrelated coinfection) or whether it might play some part in pathogenesis (Dolei, 2006; Perron and Seigneurin, 1999). In a domain where many questions still remain unanswered, there is experimental evidence linking the presence and regulation of MSRV (the first HERV-W element detected and purified as retroviral particles carrying RT and the corresponding HERV-W RNA, in LTR, *gag*, *pol*, and *env* regions) with MS features. Indeed, we are only beginning to understand a yet poorly explored domain in human biology, that of endogenous retroviruses. Classical exogenous viruses, bacteria, and parasites have been widely studied during the past century, thus elucidating many causes of diseases, among which ''infectious'' diseases predominate. At the dawn of the present century, we seem to have approached yet unraveled complex biological entities, which are pointing to new concepts in virology, genetics, and human physiopathology. This gives hope of a novel avenue for elucidating the multiparametric causes and intricate pathogenic mechanisms of complex diseases, such as MS and schizophrenia.

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