Journal of NeuroVirology (2000) 6, Suppl 2, S67–S75

www.jneurovirol.com

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?

Hervé Perron*[,1], Jean-Pierre Perin[2], François Rieger[2] and Patrick M Alliel[2]

[1]BioMérieux STELHYS, Chemin de l'Orme, 69280 Marcy l'Etoile, France and [2]Laboratoire 'Neuromodulations interactives et Neuropathologie' INSERM, 17 rue du Fer à Moulin, 75005 Paris, France

Different groups have observed retrovirus particle (RVP) production in cell cultures from patients with multiple sclerosis (MS). This *in vitro* production appeared relatively specific for MS *versus* healthy controls, but was likely to be enhanced or activated by infectious triggers such as Herpesviruses (e.g. HSV, EBV). Independent molecular analysis of retroviral RNA associated with RVP revealed two different genetic families of endogenous retroviral elements (HERV): MSRV/HERV-W and RGH/HERV-H. Interestingly, these sequences were detected by mutually exclusive primers in RT–PCR amplifications. Surprisingly, these two HERV families both contain an ancestral proviral copy inserted in chromosome 7q21-22 region at about 1 kb of distance of each other. Another HERV-W proviral sequence is located within a T-cell alpha-delta receptor (TCR) gene in chromosome 14q11.2 region. Interestingly, these two regions correspond to genetic loci previously identified as potentially associated with 'multigenic' susceptibility to MS and TCR alpha chain genetic determinants have been reported to be statistically associated with MS. A plausible role for infectious agents triggering a co-activation of the chromosome 7q HERV tandem (replicative retrovirus and/or other virus and/or intracellular bacteria) and, eventually, other HERV copies, is discussed. The role of particular HERV polymorphism and the production of pathogenic molecules (gliotoxin and superantigen) possibly associated with retroviral expression are also evoked. An integrative concept of pathogenic 'chain-reaction' in MS involving several step-specific pathogenic 'agents' and 'products' somewhat interacting with particular genetic elements would federate most partial data obtained on MS, including retroviral expression. *Journal of NeuroVirology* (2000) **6,** S67–S75.

Keywords: retrovirus; genetic susceptibility; infection; T-cell receptor; multiple sclerosis

Introduction

Beyond the serological results and animal models which have provided arguments in favour of a potential role for retroviral agents in autoimmune diseases, data concerning the detection of extracellular retrovirus-like particles, their association with reverse transcriptase activity, and their *in vitro* transmissibility have been reported in different human diseases (reviewed in Perron and Seigneurin, 1999). In these autoimmune diseases, virions or retrovirus-like particles seem to be specifically produced by certain cells from infected patients, but not by cells of the same phenotype from healthy controls. The production of these 'retroviral particles' in non-tumoral cells cannot *a priori* be attributed to a physiological and/or ubiquitous phenomenon, since various stimulations (immunological, chemical or viral) of phenotypically identical cells from healthy controls did not induce

*Correspondence: H Perron

such particle production. Such retrovirus-like particles have been reported in several human autoimmune diseases and are now tentatively associated with particular retroviral genomes (reviewed in Perron and Seigneurin, 1999). In multiple sclerosis (MS), retrovirus-like extracellular particles associated with a reverse transcriptase activity were detected by different groups.

Retrovirus in MS cell cultures

Such particles were first described in a primary culture of leptomeningeal cells (LM7) from the cerebrospinal fluid of a patient with MS (Perron *et al*, 1989). The polygenic stimulation by phorbol esters and butyric acid or the superinfection by the herpes simplex virus (HSV-1) increased retrovirus production in the supernatant of the LM7 culture, but induced neither particle production nor specific reverse transcriptase activity in the control cultures (Perron *et al*, 1989). Later, this activity and these retrovirus-like particles were found in series of monocyte cultures from patients with active MS, but not in the culture supernatants from healthy controls, from neurological diseases other than MS and from MS in remission for more than a year (Perron *et al*, 1991).

S. Haahr's team in Denmark made similar observations in cultures of B lymphocytes spontaneously immortalized *in vitro* by Epstein-Barr virus (EBV) (Haahr *et al*, 1991). Another team also established lymphoid cell lines from MS patients, producing retrovirus-like particles associated with a reverse transcriptase activity (Lan *et al*, 1994).

This retrovirus could be endogenous (Rasmussen *et al*, 1993) but, according to experimental results showing no expression in several non-MS cultures (reviewed in Perron and Seigneurin, 1999), the provirus producing extracellular particles was not likely to be present or to be replication-competent in the genome of the controls already tested. A strategy for the molecular characterization of this retroviral expression was therefore primarily based on the RNA genome encapsidated in these particles. Indeed, only particle-associated RNA could permit a preliminary identification, since normal cellular RNA and DNA could contain homologous but not relevant ERV genomes. Using material from cell cultures, purified extracellular particles were produced for a first study by RT–PCR combined with extensive DNase treatments of samples, buffers and enzyme cocktails (Tuke *et al*, 1997). The primers used corresponded to two conserved regions in the retrovirus *pol* gene encompassing a variable region of approximately 100 base pairs. The results of this study have allowed, apart from irrelevant primer concatemers or from fragments shorter than the expected size, the identification of sequences corresponding to the expected *pol* region (Perron *et al*, 1997): a preponderant group (MSRVcpol, representing 85–90% of adequate clones) with about 70% of homology with a human ERV (ERV9) (LaMantia *et al*, 1991), and of a minor group (10–15% of adequate clones) with sequences strongly homologous (over 90%) to ERV9.

With RT–PCR extension approaches on concentrated extracellular particles, the sequence of the complete protease and RT sequences was obtained (Perron *et al*, 1997), contiguous to the first MSRVc*pol* fragment. The virus producing particles containing such retroviral RNA was provisionally called MSRV owing to the origin of its isolation (Multiple Sclerosis associated RetroVirus element). RT–PCR extensions, later permitted progression beyond the 5′ and 3′ ends of this MSRV 'pol' fragment on the RNA encapsidated in such particles and obtaining clones overlapping on the expected regions of a putative retrovirus genome (Komurian *et al*, 1999). Interestingly, the primer binding site (PBS) identified in MSRV RNA 5′ sequences (Komurian *et al*, 1999) best corresponds to a tryptophan (W) t-RNA. Such tryptophan motif had never been reported in PBS from human retroviruses but is the major PBS type in avian oncoviruses.

As illustrated in Figure 1, the phylogenic analysis of the *pol* gene allows linking this gene to the type C oncovirus genes. The phylogenic analysis of the MSRV *env* gene however indicates closer proximity with the type D retroviruses. This phylogenic divergence between retroviral *pol* and *env* genes producing virions with type D morphology has already been described and interpreted as deriving from an ancestral recombination (Sonigo *et al*, 1986). These phylogenic observations are compatible with the morphology of the particles observed in the LM7 cultures (Perron *et al*, 1989) and in monocyte cultures (Perron *et al*, 1991).

At present, no replication-competent MSRV provirus which could encode such particles has been obtained, but such an objective cannot easily be achieved since many ERV copies with strong nucleotide homology with every MSRV regions exist in human DNA (see below).

MSRV-defined endogenous retrovirus family: HERV-W

Indeed, using MSRVprobes obtained from virion-associated RNA, a novel human ERV family has been identified which is different from ERV9 and genetically related to MSRV sequences, as confirmed by phylogenic analyses and particular retroviral features (Blond *et al*, 1999). On the basis of the PBS t-RNA motif generally used for the classification of human endogenous retrovirus families, it was named HERV-W (W=tryptophane). The main characteristics of this HERV-W family which is composed of more or less truncated multicopy elements disseminated in human chromosomes, are summarized in Figure 2. Confirming the genetic organization of the retroviral genome reconstructed from particle-associated RNA (Komurian *et al*,

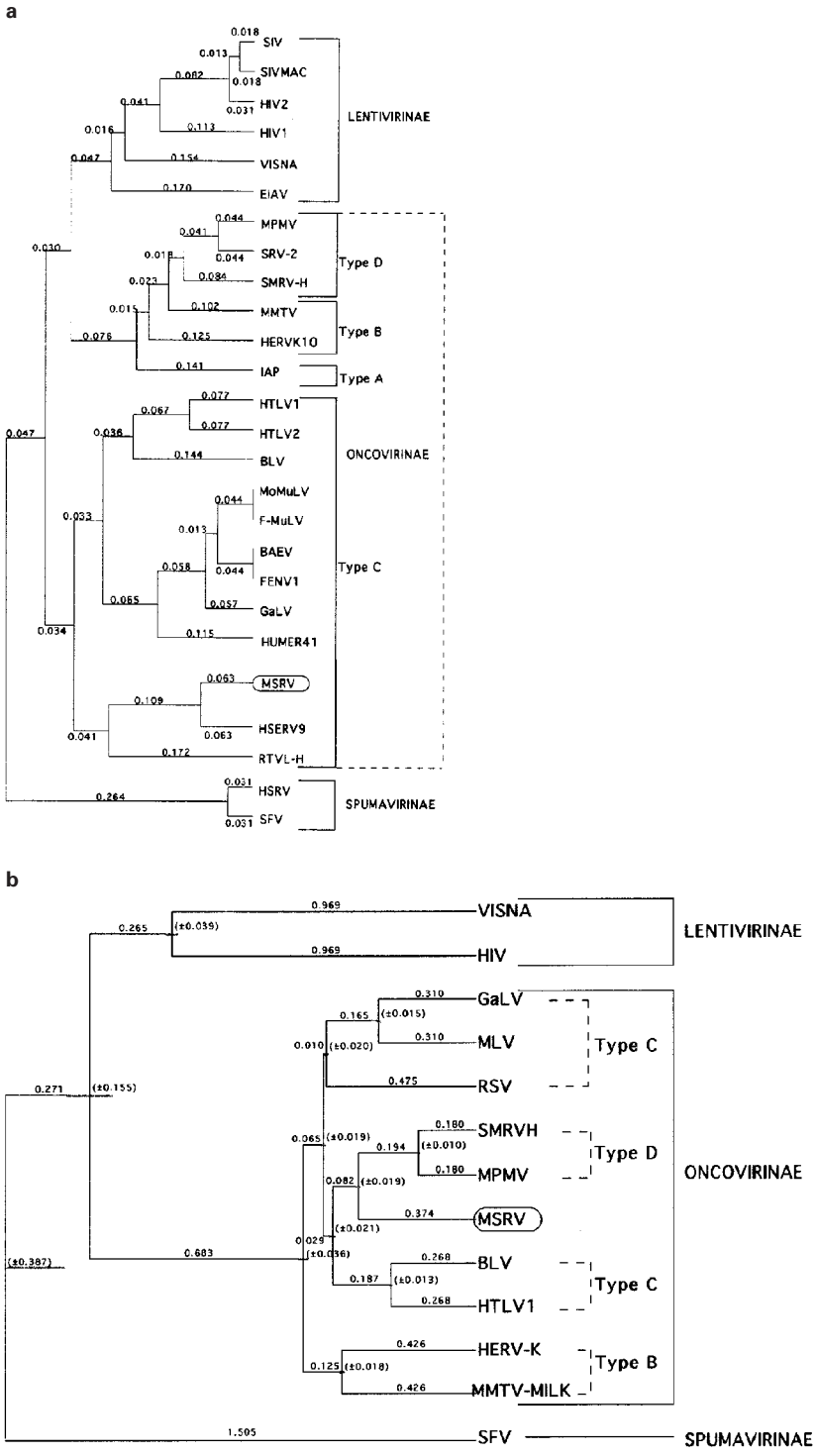

Figure 1 Phylogenic trees. (**A**) *pol* gene. (**B**) *env* gene. The trees have been calculated with GeneWorks® software (U.P.G.M.A. tree) based on the aminoacid sequence encoded by the most conserved region of retroviral *pol* gene (VLPQG....YXDD region) or by the region corresponding to the relatively conserved env region encompassing the immunosuppressive peptide described in oncoviruses. HSRV: Human Spumaretrovirus. EIAV: Equine Infectious Aenemia Virus. BLV: Bovine Leukaemia Virus. HIV1, HIV2: Human Immunodeficiency Viruses type 1 and 2. HTLV1 and HTLV2: Human Leukaemia Viruses type 1 and 2. F-MuLV: Friend-Murine Leukaemia Virus. MoMLV: Moloney-Murine Leukaemia Virus. BAEV: Baboon Endogenous Virus. GaLV: Gibbon Ape Leukaemia Virus. HUMER41: Human Endogenous Retroviral sequence, clone 41. IAP: Intracisternal A-type Particle. MPMV: Mason Pfizer Monkey Virus. HERVK10: Human Endogenous Retrovirus 'K10'. MMTV: Mouse Mammary Tumour Virus. HSERV9 (reference ERV9 clone from cellular RNA): Human sequence of Endogenous Retrovirus 9. MSRV: Multiple Sclerosis associated RetroVirus. SIV: Simian Immunodeficiency Virus. RTVL-H: ReTrovirus-Like sequence 'H'. SFV: Simian Foamy Virus. VISNA: Visna retrovirus. SIV1: Simian Immunodeficiency Virus type 1. SRV-2: Simian Retrovirus type 2. SMRV-H: Squirrel Monkey Retrovirus 'H'.

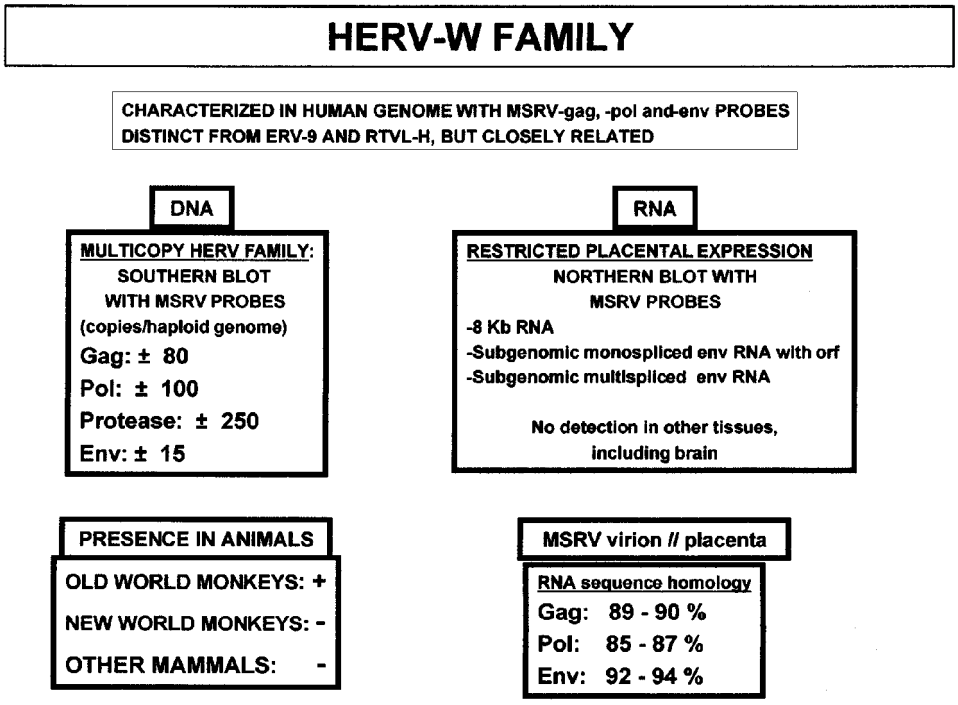

Figure 2 Main genomic characteristics of the HERV-W family.

1999), a complete proviral HERV-W copy (HERV7q) is found in chromosome 7q (Alliel *et al*, 1998) but does not appear to be competent for replication, as well as another one found in chromosome 14q (Alliel *et al*, 1998).

HERV-W physiological expression appears to be restricted to the placenta in which an 8 Kb HERV-W RNA could be detected, by Northern blot analysis using MSRV probes, as well as a spliced RNA corresponding to an HERV-W env orf (Blond *et al*, 1999). No significant expression has been detected in other tissues, including normal brain. RT–PCR techniques which are far more sensitive could nonetheless detect background expression of HERV-W RNA in some tissues with variable results depending on primer sets and PCR cycles. However, the significance of these PCR-detected rare RNA transcripts is difficult to interpret in terms of physiological significance (*c.f.* Figure 3), all the more in the absence of protein expression. In the case of placenta, a physiological expression of an HERV-W env protein during embryonal life may be of great importance in the immunological point of view. For example, this expression could result in an acquired tolerance to this env protein in all individuals – or, e.g., in a subpopulation having HERV-W haplotypes expressed in foetal thymus – but it may also result in an abnormal immune reactivity or in a tolerance breakdown, if an extra-physiological expression occurs in association with a disease process, or during an infection with a genetically homologous retrovirus (*c.f.* Figure 3).

An extra-physiological activation of particular HERV-W elements may therefore be at the origin of most RNA packaged in MS retroviral particles. But, as well-known in animal retroviral families represented by, e.g., murine leukaemia virus (MLV), interactions between replicative strains – of exogenous, endogenous or recombinatorial origin – and endogenous retroviral elements (ERV) also result in significant co-packaging of RNA from co-activated or related ERV copies (*c.f.* Figure 3, Linial and Miller, 1990).

Co-packaging of HERV RNAs in retroviral particles
The presence of defective/co-packaged RNA, possibly resulting from HERVs activation by a common triggering agent, does not *a priori* rule out a possible biological role in the disease, for they may interfere with retroviral expression and/or may be expressed as partial proteins with biological effects. An example of such pathological role played by a defective retroviral RNA is given in murine AIDS (MAIDS): this mouse disease is caused by the association in retroviral particles, of a defective MLV *gag* RNA encoding pathogenic determinants responsible for superantigen-like activity inducing major immune dysfunctions, and of a 'helper' retrovirus genome encoding infectious retroviral particles (Kubo *et al*, 1996).

The significance of a minority of apparently co-packaged ERV9 RNA sequences in our virion isolates (Perron *et al*, 1997) is unclear. As well as the obvious HERV-W defective RNA encapsidation

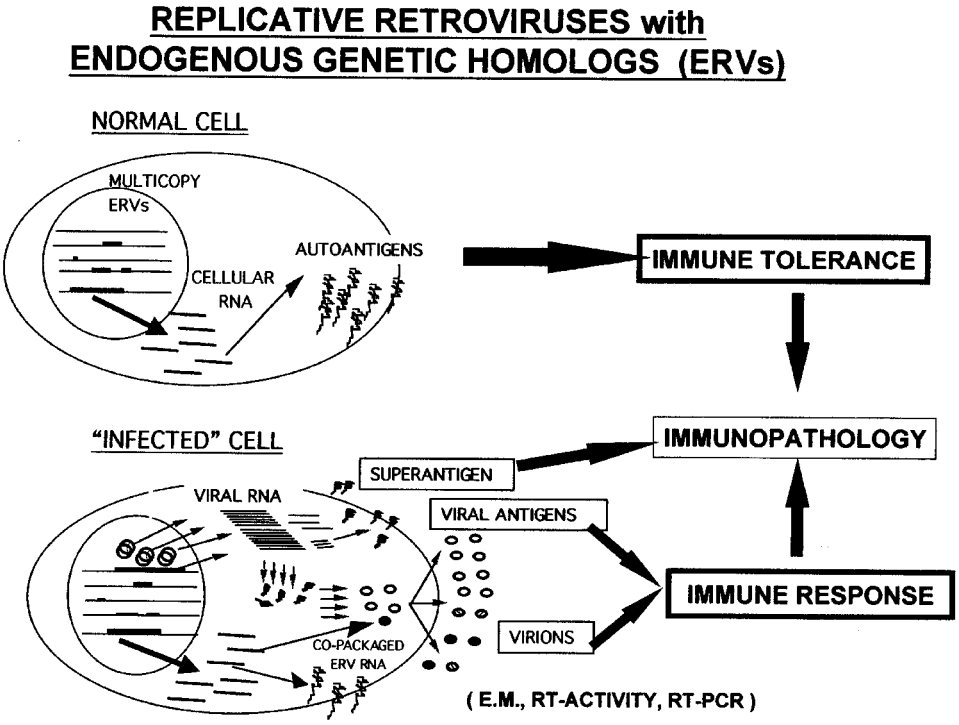

Figure 3 Replicative retroviruses related to multiple homologous endogenous copies: specific features of virion-producing cells. In a healthy cell, multiple copies homologous to a family of ERVs are present in the DNA of the nucleus in a more or less fragmented form; a few of these copies can be transcribed in a 'cellular' RNA form; a few of these RNAs can be translated into a protein which will be tolerated as a 'self' antigen (autoantigen) if its expression is physiological and allows thymic lymphocyte selection. In a cell harbouring an activated replicative strain homologous to an ERV family (e.g., exogenous MMTV, or endogenous polytropic MLV), with a reverse transcription cycle producing extracellular virions, the specific provirus is generally expressed in parallel with homologous ERVs – eventually transactivated by its replication factors targeting similar LTR motifs in the homologous ERVs. The RNAs of the replicative strain allow the synthesis of all the proteins necessary to the formation of virions and the encapsidation of specific 'viral' RNA, but also of closely homologous or even unrelated ERV RNA (Linial and Miller, 1990); they also allow the synthesis of functional enzymes (e.g., reverse transcriptase, protease, integrase). The virions thus released extracellularly are detected by reverse transcriptase activity tests, RT–PCR, even electron microscopy (e.g. negative staining after concentrating particles, or on cross-sections at the surface of productive cells). Non-structural antigens can also be produced in the cell; some of them can be immunogenic, have the properties of a superantigen or be a cytotoxic protein. An immune tolerance to certain ERV-related proteins can elsewhere inhibit the immune response towards corresponding regions of 'infectious' retroviral antigens (total or partial absence of response); conversely, the chronic presentation of 'infectious' antigens in an inflammatory context can cause a breakdown in the tolerance of homologous 'autoantigens' due to ERVs expressed by healthy cells (autoimmunity). The possible presence of a retrovirus superantigen can also set off and/or potentialize the immunopathological phenomena.

(Komurian *et al*, 1999), this may represent an epiphenomenon without pathological significance or may be meaningful if any abnormal expression of partial HERV products with biological activity ever occurred in this context as discussed above. Indeed, the recent publication by S. Haahr's group in Denmark, of sequence data from four MS B-cell lines isolates (Christensen *et al*, 1998), also suggests co-packaging of RNAs from another cluster of closely-related HERVs having *pol* sequences related to type C oncoviruses. The *gag* and *env* RGH sequences they report in retrovirus particle-associated RNA, belong to the RTVL-H/HERV-H family (Hirose *et al*, 1993). As shown in Figure 1A, MSRV, ERV9 (HSERV9 clone), and RTVL-H *pol* sequences define a common phylogenic 'cluster'.

According to most data obtained in similar cases of retroviral co-packaging (Linial and Miller, 1990), the retroviral genome encoding these particles has the greatest probability to belong to the major family of encapsidated retroviral RNAs; but, conversely a 'helper' retrovirus can be involved in particle production generated by a full-length RNA encapsidated in a minority of 'wild-type' particles only, while distantly related HERV RNAs are packaged in the majority (Linial and Miller, 1990).

As previously described, a non-selective 'pan-retro' RT–PCR approach has amplified retroviral sequence sub-groups in similar proportions (85–90% of 'MSRV-cpol' and 10–15% of ERV-9) from different cultured cell-types and isolates (Perron *et al*, 1997). RGH/RTVL-H *pol* sequences were not encountered among a hundred clones sequenced from our different culture isolates, but we are now aware that the 'Pan-Retro' primers could not amplify this particular HERV family (RTVL-H) due

to major mismatches in the consensus primer regions. In parallel, the PCR approach used by Christensen *et al* (1998), could amplify HERV-H related sequences, but not other families including MSRV/HERV-W and ERV9. Consequently, a subpopulation of co-packaged RGH-like RNAs is likely to have been selectively amplified.

However, fluctuations in the proportions of such co-packaged ERV RNAs may occur during passages in cell cultures and, *in vivo*, during disease evolution. These temporal variations should therefore also be taken into account in further comparative studies.

Tandem HERV-W and RGH proviral copies in chromosome 7q21-22 locus, and HERV-W copy in an alpha/delta T-cell receptor gene on chromosome 14q

Christensen *et al*, have suggested that 'the fact that RGH and MSRV belong to the same subsection of the type C oncovirinae may indicate that we are looking at two features of the same phenomenon' (Christensen *et al*, 1998).

Indeed, this assertion may reveal quite pertinent since: (1) It has recently been outlined that the 7q21-22 region of human chromosome 7 which has already been identified as potentially involved in genetic susceptibility to MS (Charmley *et al*, 1991) contains a complete HERV-W proviral copy named HERV-7q (Alliel *et al*, 1998a) or, more appropriately now, HERV-W/7q. (2) We have now identified an RGH proviral copy (which we name RGH/7q) about 1 kb upstream the HERV-W/7q provirus (*c.f.* Figure 4). Therefore these two HERV 'tandem' copies (RGH/7q and HERV-W/7q) are located within this region of potential genetic susceptibility to MS (Charmley *et al*, 1991). (3) Another HERV-W copy, named HERV-TcR (Alliel *et al*, 1998b) or, more appropriately now, HERV-W/14q, has been found in a region potentially associated with genetic susceptibility to MS in chromosome 14q region (Chataway *et al*, 1998). This copy is present within a gene encoding T-Cell receptor (TCR) alpha/delta chains. (4) Particular TCR alpha chains have elsewhere been reported to be significantly associated with an 'immunogenetic' susceptibility to MS (Sherritt *et al*, 1992). Though discordant findings were reported concerning TCR alpha haplotypes (Hashimoto *et al*, 1992), the TCR gene in chromosome 14q region encodes delta chains as well, which may also display particular association with MS (Nowak *et al*, 1997). More generally, this TCR gene or its chromosomal region remain a possible candidate involved in a multigenic control of susceptibility to MS (Chataway *et al*, 1998; Oksenberg and Hauser, 1997).

These data were obtained by addressing either the determination of chromosomal regions associated with genetic susceptibility to MS – with coincident findings by several groups – either TCR chains haplotypes in MS versus controls, or the identification of retroviral RNA associated with retrovirus-like particles in MS – with two different groups using primers selecting mutually exclusive subpopulations of retroviral sequences.

The possibility that series of totally independent studies could have obtained results coinciding with these human chromosomal loci by chance, now appears quite improbable. Given these striking 'coincidences', the exact role of these HERV-W and RGH endogenous elements in MS genetics and pathogenesis should now be further analysed in the

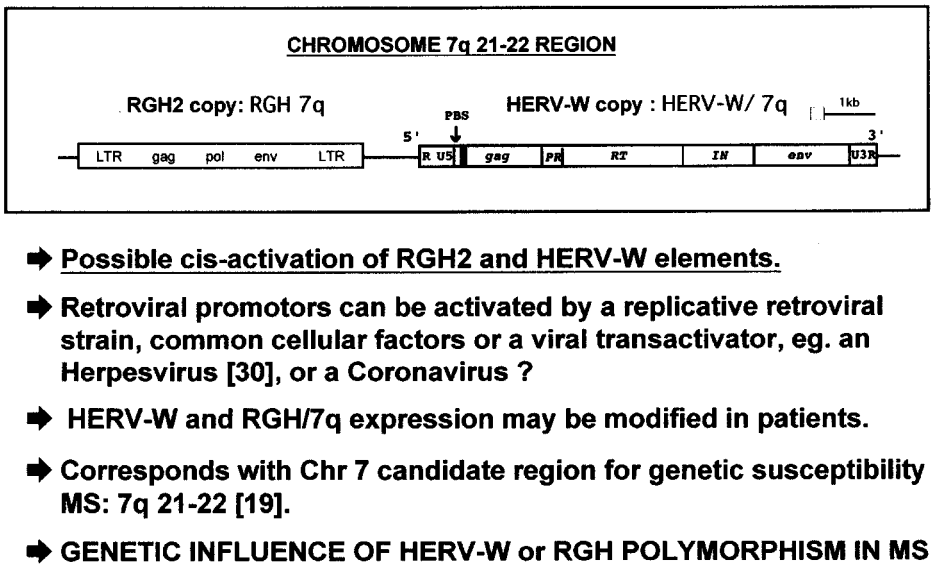

Figure 4 RGH/7q and HERV-W/7q tandem copies on human chromosome 7.

combined lights of retrovirology, immunogenetics and human genomics.

Molecular detection

A previous study had shown that the conserved 'pan-retro' *pol* fragment corresponding to ERV9 and ERV9-like RNA could be amplified in cellular RNA extracted from MS brain tissues (Lefebvre *et al*, 1995). However, Northern blot analysis performed with this small and conserved fragment in rather low stringency conditions, showed ubiquitous and possibly non-specific hybridisation in human multi-tissue RNA preparations. In the cytoplasm of cells obtained from non-MS controls, RNA homologous to MSRV sequences (HERV-W RNA) was also detected using RT–PCR with non-degenerate primers and shown to be expressed in very limited quantity (Perron *et al*, 1999 unpublished data).

However, a study on CSF cell RNA, performed with the pan-retro RT–PCR technique followed by specific MSRV 'ELOSA' hybridisation, showed no detectable MSRV product in several controls with numerous activated lymphocytes in CSF (e.g. viral encephalitis), whereas a positive signal was found in 5 out of 10 MS corresponding to untreated patients (Perron *et al*, 1997). As previously evoked, RT–PCR with MSRV primers is however not specific within cellular or tissue RNA since baseline expression of MSRV-related ERV transcripts can be amplified with MSRV primers tested today in different genes. Its sensitivity is much greater than the 'pan-retro' RT–PCR in which degenerate consensus primers create competitive amplification of any intracellular ERV RNA and, under appropriate conditions, may only allow detection of major retroviral RNA population(s) in a given sample.

Nevertheless, using an RT–PCR technique which selects the MSRV *pol* sequence packaged into extracellular particles, and not cellular or tissue RNA, results suggesting that circulating 'particle-associated' MSRV RNA was detectable in MS sera, preferentially during untreated and active periods, were obtained (Garson *et al*, 1998). These results therefore suggest that, similar to MS cell cultures, an *in vivo* production of extracellular retroviral particles may occur in patients during particular periods. However, optimization of the present complex technical procedures, addressing very low particle numbers and requiring careful hydrolysis of non-packaged RNA and DNA without destruction of particle-associated RNA, are required for further studies and confirmation. Indeed, recent evaluations (Perron *et al*. unpublished data) suggest that, in the present conditions using nested RT–PCR, only statistical detection of low numbers of circulating particles can be achieved. This actually precludes perfect standardisation in the absence of major technical improvements, which obviously would require a non-nested PCR with elevated sensitivity. Alternatively, the 'Pan-retro' technique followed by specific probing, may reveal more adequate as another group obtained convergent data with this technique (Olsson *et al*, 1999), which we had used in our first series with an ELOSA hybridisation technique (Perron *et al*, 1997).

Nonetheless, a better understanding of the retroviral 'system' underlying all these observations would certainly provide means for defining better technical tools and strategies.

'Retroviral system' rather than a 'retrovirus'

Studying the potential role in MS pathogenesis of such retroviral expression therefore requires further confirmation of the retroviral genome(s) contributing to retroviral particle and RT-activity production in MS cells, as well as of the eventual infectious co-factors triggering their expression in MS patients.

From the present data, particle-associated MSRV RNA detection in MS may reflect: (1) a human endogenous retrovirus (HERV-W) haplotype particular to MS DNA, encoding viral particles and triggered by an heterologous viral transactivator (e.g. herpesvirus as suggested by Perron *et al*, 1993) and/or by other infectious co-factors (e.g. Coronaviruses or Chlamydiae?); (2) an exogenous proviral DNA, member of a genetically homologous HERV-W family, interfering with particular endogenous copies (such as the RGH+HERV-W/7q tandem, and the HERV-W/14q) at the origin of defective RNA co-encapsidated in extracellular particles; (3) a stimulation and rather selective co-encapsidation of particular HERV copies (from the HERV-W/ERV9/RGH genetic cluster), by a 'helper' retrovirus possibly related to one of these families; (4) a multi-complementation of partially defective HERV-W, ERV9 and/or RGH copies transactivated by infectious co-factors, with possible direct interactions between RGH7q and HER-W/7q RNAs.

However, in each case, a common transcriptional pattern of RGH/7q and HERV-W/7q could be transactivated, thus resulting in mixed RNA co-packaging in particles, whatever the virion-encoding genome(s) would reveal to be.

Epiphenomenon or role in MS pathogenicity?

Ever since this retroviral particle and RT-activity production has been reported in MS cell cultures, we considered that the expression of a latent or endogenous retrovirus might only be an epiphenomenon in the disease process but, if this was not the case, that this expression should be associated with the production of 'neuro- and/or immuno-pathogenic' molecules (Rasmussen *et al*, 1993).

Given the possible contribution of a superantigen in immune-mediated inflammation and in autoimmunity (Rudge, 1991), the existence of a superantigen associated with this retroviral production is one important question to be addressed.

Concerning potential cytotoxic activities targeting CNS cells, a more advanced study has now

evidenced the production of a gliotoxic activity co-expressed with retroviral activity in MS macrophage cultures and co-purifying with a protein fraction with an apparent molecular weight of 17 kDa on 12% SDS–PAGE gels (Ménard *et al*, 1997). This 'gliotoxin' causes apoptotic death of macroglial cells (astrocytes and oligodendrocytes) *in vitro* and its activity appears to be resistant to proteases in physiological conditions as well to heating at 70°C for 30 min but is inactivated at 100°C (Ménard *et al*, 1998a). This characteristic gliotoxic activity was further detected in patients' CSF and urine and apparently correlated disease activity (Malcus-Vocanson *et al*, 1998; Ménard *et al*, 1998b).

The induction and/or the co-expression of such neuro- and immuno-pathogenic molecules may therefore provide a direct or indirect link with the disease, which may reveal easier to evaluate than a possible 'chain-reaction' in which a complex retroviral system appears to be involved.

Conclusion

Any component of such a pathogenic 'chain-reaction' involving infectious triggering agents, activated endogenous/genetic elements and induced pathogenic molecules may provide useful biological markers for diagnosis, prognosis and/or therapeutic monitoring, or may even constitute a key target for novel and complementary therapeutic approaches. In parallel, the challenge for a fundamental research strategy might be to identify all key pieces of a puzzle helping reconstituting the correct picture.

This review and the numerous but partial data reported here show that new questions, possibly pertinent but very complex, have been addressed with recently developed techniques. They have obviously not been answered clearly and definitely. Nonetheless, all the converging data already gathered now provide tools which can be used to further explore such important questions as to evaluate novel concepts.

References

Alliel PM, Perin JP, Pierig R, Nussbaum JL, Menard A, Rieger F (1998a). Endogenous retroviruses and multiple sclerosis. II. HERV-7q. *C R Acad Sci* III **321:** 857–863.

Alliel PM, Perin JP, Pierig R, Rieger F (1998b). An endogenous retrovirus with nucleic acid sequences similar to those of the multiple sclerosis associated retrovirus at the human T-cell receptor alpha, delta gene locus. *Cell Mol Biol* (Noisy-le-grand) **44:** 927–931.

Blond JL, Beseme F, Duret L, Bouton O, Bedin F, Perron H, Mandrand B, Mallet F. (1999). Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. *J Virol* **73:** 1175–1185.

Charmley P, Beall SS, Concannon P, Hood L, Gatti RA (1991). Further localization of a multiple sclerosis susceptibility gene on chromosome 7q using a new T cell receptor beta-chain DNA polymorphism. *J Neuroimmunol* **32:** 231–240.

Chataway J, Feakes R, Coraddu F, Gray J, Deans J, Fraser M, Robertson N, Broadley S, Jones H, Clayton D, Goodfellow P, Sawcer S, Compston A (1998). The genetics of multiple sclerosis: principles, background and updated results of the United Kingdom systematic genome screen. *Brain* **121:** 1869–1887.

Christensen T, Dissing-Sørensen P, Riemann H, Hansen HJ, Møller-Larsen A (1998). Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis. *Lancet* **352:** 1033.

Garson JA, Tuke PW, Giraud P, Paranhos-Baccala G and Perron H (1998) Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. *Lancet* **199:** 351–33.

Haahr S, Sommerlund M, Moller-Larsen A, Nielsen R, Hansen HJ (1991). Just another dubious virus in cells from a patient with multiple sclerosis? *Lancet* **337:** 863–864.

Hashimoto LL, Mak TW, Ebers GC (1992). T cell receptor alpha chain polymorphisms in multiple sclerosis. *J Neuroimmunol* **40:** 41–48.

Hirose Y, Takamarsu M, Harrada F (1993). Presence of env genes in members of the RTVL-H family of human endogenous retrovirus-like lements. *Virology* **192:** 52–61.

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.

Kubo Y, Kakimi K, Higo K, Kobayashi H, Ono T, Iwama Y, Kuribayashi K, Hiai H, Adachi A, Ishimoto A (1996). Possible origin of murine AIDS (MAIDS) virus: conversion of an endogenous retroviral p12gag sequence to a MAIDS-inducing sequence by frameshift mutations. *J Virol* **70:** 6405–6409.

LaMantia G, Maglione D, Pengue G, DiCristofano A, Simeone A, Lanfrancone L, Lania L (1991). Identification and characterization of novel human endogenous retroviral sequences preferentially expressed in undifferentiated embryonal carcinoma cells. *Nucleic Acids Res* **19:** 1513–1520.

Lan XY, Zeng Y, Zhang D, Hong ML, Wang DX, Zhang YL, Feng ZJ, Tang MH, Feng BZ (1994). Establishment of a human malignant T lymphoma cell line carrying retrovirus-like particles with RT activity. *Biomed Environ Sci* **7:** 1–12.

Lefebvre S, Hubert B, Tekaia F, Brahic M, Bureau JF (1995). Isolation from human brain of six previously unreported cDNAs related to the reverse transcriptase of human endogenous retroviruses. *AIDS Res Hum Retroviruses* **11:** 231–237.

Linial ML, Miller AD (1990). Retroviral RNA packaging: sequence requirements and implications. In: *Retroviruses strategies of replication, Current Topics in Microbiology and Immunology* **157**. Swanstrom R, Vogt PK. (eds). Springer-Verlag: Berlin, pp 125–152.

Malcus-Vocanson C, Giraud P, Broussolle E, Perron H, Mandrand B, Chazot G (1998). A urinary marker for multiple sclerosis. *Lancet* **351:** 1330.

Ménard A, Amouri R, Dobransky T, Charriaut-Marlangue C, Pierig R, Benjelloun N, Cifuentes-Diaz C, Gandhour S, Belliveau J, Gascan H, Lyon-Caen O, Perron H, Rieger F (1998a). A Gliotoxic Factor in Multiple Sclerosis. *J Neurol Sci* **154:** 209–221.

Ménard A, Amouri R, Michel M, Marcel F, Brouillet A, Belliveaud J, Geny C, Deforges F, Malcus-Vocanson C, Dobransky T, Armstrong M, Lyon-Caen O, Mandrand B, 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.

Ménard A, Pierig R, Pelletier J, Bensa P, Belliveau J, Mandrand B, Perron H, Rieger F (1998b). Detection of a gliotoxic activity in the cerebrospinal fluid from multiple sclerosis patients. *Neurosci Lett* **245:** 49–52.

Nowak JS, Michalowska-Wender G, Januszkiewicz D, Wender M (1997). Limited junctional diversity of V delta 5-J delta 1 rearrangement in multiple sclerosis patients. *Mol Chem Neuropathol* **30:** 95–100.

Oksenberg JR, Hauser SL (1997). New insights into the immunogenetics of multiple sclerosis. *Curr Opin Neurol* **10:** 181–185.

Olsson P, Ryberg B, Awad R, Ammoun S, Yin H, Hjalmarsson S, Blomberg J (1999). Retroviral RNA related to ERV9/MSRV in a human serum: a new sequence variant. *AIDS Res Hum Retroviruses* **15:** 591–593.

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 and The Collaborative Research Group on Multiple Sclerosis (1997). Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. *Proc Natl Acad Sci USA* **94:** 7583–7588.

Perron H, Geny C, Laurent A, Mouriquand C, Pellat J, Perret J, Seigneurin JM (1989). Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. *Research in Virology* **140:** 551–561.

Perron H, Gratacap, Lalande B, Laurent A, Geny C, Mallaret M, Schuller E, Stoebner P, Seigneurin JM (1991). Isolation of retrovirus from patients with multiple sclerosis. *Lancet* **337:** 862–863.

Perron H, Suh M, Lalande B, Gratacap B, Laurent A, Stoebner P, Seigneurin JM (1993). Herpes Simplex Virus ICP0 and ICP4 immediate-early proteins strongly enhance expression of a retrovirus harboured by a leptomeningeal cell-line from multiple sclerosis. *J Gen Virol* **74:** 65–72.

Perron H, Seigneurin JM (1999). Retroviral sequences associated with extracellular particles in autoimmune diseases: epiphenomenon or possible role in aetiopathogenesis? *Microbes and Infection* **1:** 309–322.

Rasmussen HB, Perron H, Clausen J (1993). Do endogenous retroviruses and related genetic elements contribute to the development of inflammatory and degenerative nervous system diseases? *Acta Neurologica* **88:** 190–198.

Rudge P (1991). Does a retrovirally encoded superantigen cause multiple sclerosis? *Neurol Neurosurg Psychiatry* **54:** 853–855.

Sherritt MA, Oksenberg J, de Rosbo NK, Bernard CC (1992). Influence of HLA-DR2, HLA-DPw4, and T cell receptor alpha chain genes on the susceptibility to multiple sclerosis. *Int Immunol* **4:** 177–181.

Sonigo P, Barker C, Hunter E, Wain-Hobson S (1986). Nucleotide sequence of Mason-Pfizer monkey virus: an immunosuppressive D-type retrovirus. *Cell* **45:** 375–385.

Tuke PW, Perron H, Bedin F, Beseme F, Garson JA (1997). Development of a pan-retrovirus detection system for multiple sclerosis studies. *Acta Neurol Scand* (Suppl): **169:** 16–21.