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Human endogenous retrovirus W in brain lesions: Rationale for targeted therapy in multiple sclerosis

Jack van Horssen [a], Susanne van der Pol [a], Philip Nijland [b], Sandra Amor [b,c], Hervé Perron [d,e,f,*]

[a] Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands
[b] Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
[c] Department of Neuroimmunology Unit, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom
[d] GeNeuro-Innovation, Lyon, France
[e] GeNeuro, Switzerland
[f] University of Lyon, France

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ABSTRACT

Background objective: Attempts to identify a causative agent of Multiple Sclerosis (MS) among environmental viruses have consistently failed suggesting that development of MS is a result from gene-environment interactions. A new pathogenic player within human genes, a human endogenous retrovirus (HERV) was identified from MS cells, named MS-associated retrovirus element (MSRV) and unveiled homologous multicopy HERVs (HERV-W). As independent studies revealed biological features of HERV-W on immune-mediated inflammation and on remyelinating cells, the present study characterized the presence of HERV-W envelope protein (MSRV-Env) at the cellular level, in different MS lesion stages to extend and validate previous studies.
Methods: Immunohistological analysis of HERV-W envelope cellular expression in different lesion stages from a cohort of MS brains versus controls, using well-characterized and highly specific monoclonal antibodies.
Results: HERV-W envelope protein was detected in all MS brains and quite essentially in lesions. Immunohistochemistry showed dominant expression in macrophages and microglia, coinciding with areas of active demyelination, spread over the active lesions, or limited to the rim of active microglia in chronic active lesions or in few surviving astrocytes of inactive plaques. Weak expression was seen in MS normal appearing white matter. In active plaques, few lymphoid cells and astrocytes were also stained. This HERV-W expression was not observed in control brains.
Interpretation: HERV-W was expressed in demyelinated lesions from MS brains, which were all positive for this endogenous pathogenic protein. Pronounced HERV-W immunoreactivity in active MS lesions was intimately associated with areas of active demyelination throughout the successive stages of lesion evolution in MS brains. Based on its pathogenic potential, this HERV-W (MSRV) endogenous toxin thus appears to be a novel therapeutic target in MS. It also has a unique positioning as an early and lifelong expressed pathogenic agonist, acting upstream the pathways in which dysregulated physiological effectors are usually targeted by present therapeutic strategies for MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Histopathological evaluation of MS brain tissue has provided insights into key pathological features of MS, including inflammation, demyelinated lesions in the white and the grey matter, oligodendrocyte loss, defects in remyelination by oligodendrocyte precursor cells (OPC), and axonal and neuronal degeneration (Bien et al., 2012; Chang et al., 2002). To date, a global understanding of etiological factors that could directly and/or indirectly be involved in MS, in its onset, in the lifelong mechanisms of inflammation-driven CNS damage and in the impairment of lesion remyelination, is likely to rely upon a gene-environment interplay (van der Mei et al., 2015;

* Correspondence to: Geneuro, 18, ch des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland.
E-mail address: hp@geneuro.com (H. Perron).

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Hedstrom et al., 2015; Koch et al., 2013).

It therefore appears relevant that the search for pathogenic players in MS led to peculiar elements with both genomic and viral characteristics: Human Endogenous RetroViruses (HERVs), which have repeatedly entered the genome of species through germ-line infections during evolution of species and are now known to represent about 8% of the human genome (Feschotte and Gilbert, 2012).

The idea that a retrovirus could play a role in MS pathogenesis was evoked, but only a classically exogenous and infectious retrovirus such as a Human T-Lymphotropic virus (HTLV) was envisaged (Koprowski et al., 1985). So, when a new human retrovirus was identified in cultures from MS cells (MSRV, for Multiple sclerosis associated RetroVirus) but also unveiled a previously unknown family of homologous and endogenous retroviral sequences (HERV-W) (Blond et al., 1999; Perron et al., 1997; Perron et al., 1991), these findings were initially regarded as anecdotal. Nonetheless, as studies further explored and progressively unveiled unexpected genetic and biological features of these HERVs, it became apparent that HERVs were not merely fossils of past infection and inert relics of ancient genomic invasions, but could display activities with a substantial impact on cellular function, in both health and disease (Feschotte and Gilbert, 2012; Perron and Lang, 2010; Kassiotis, 2014; Sokol et al., 2016; Rolland et al., 2006; Varela et al., 2009). This also highlighted the existence of a new category of pathogens within our genomes behaving as the “enemy within” (Engel and Hiebert, 2010; Volkman and Stetson, 2014). Thus, cumulated results from numerous studies during past decades progressively led to understand how their unique genetic positioning may confer upon them the potential to drive pathogenic cascades leading to MS in response to environmental triggers. HERV-W sequences isolated from MS were shown to produce a bioactive envelope protein (MSRV-Env) engaging pathways leading to final pathognomonic features of Multiple Sclerosis (MS).

In particular, immunological effects of MSRV particles revealed to induce superantigen-like activation of T-lymphocytes (Perron et al., 2001), itself conditioned to an upstream activation of innate immunity causing pro-inflammatory effects through the Toll-like receptor 4 (TLR4) pathway (Rolland et al., 2006). These effects were shown to be specifically due to the HERV-W encoded envelope protein (MSRV-Env) (Rolland et al., 2006; Perron et al., 2001), which displays original and different biological effects compared to another known HERV-W full-length envelope protein, now named Syncytin (Duperray et al., 2015). MSRV-Env was also shown to promote macrophage and dendritic cells differentiation, anti-myelin oligodendrocyte glycoprotein autoimmunity and experimental autoimmune encephalomyelitis (EAE) in C57/BL/6 mice, an animal model of MS, (Perron et al., 2013). Parallel studies on glial cells had showed gliotoxicity induced by MSRV particles with reverse-transcriptase activity (Menard et al., 1997), but its molecular origin and mechanisms of action long remained unknown. However, it was recently discovered that transiently expressed TLR4 in early differentiating oligodendrocyte precursor cells (OPC) exposed them to an interaction with MSRV-Env causing a defect of myelin production through a differentiation blockade of OPC (Kremer et al., 2013). These observations indicated potential therapeutic application when a specific monoclonal antibody was shown to neutralize the immune pathogenic activity of MSRV-Env *in vitro* and *in vivo* (Rolland et al., 2006; Curtin et al., 2015) and, also, to neutralize its neuroglial pathogenic effects by reversing the inhibition of myelin protein production by OPC (Kremer et al., 2014).

This appeared relevant when considering that other studies confirmed the enhanced d expression of HERV-W/MSRV elements in patients with MS and the *ex-vivo* and *post-mortem* detection of its envelope protein (Dolei et al., 2002; Garson et al., 1998; Mameli et al., 2007; Sotgiu et al., 2010).

Importantly, the HERV-W envelope was detected in MS pathognomonic lesions, i.e. within brain demyelinated lesions, using monoclonal antibodies (mAbs) raised against the MSRV-Env protein encoded by virion RNA isolated from MS cell cultures (Perron et al., 2001; Komurian-Pradel et al., 1999). Successive studies have repeatedly and independently confirmed the presence of HERV-W envelope (here named MSRV-Env) within MS lesions with various specific mAbs, as detailed in Table 1. Such results obtained after examination of brain regions and lesions from a total of 55 MS cases from 6 different centers and countries have consolidated the evidence for the presence of HERV-W Env protein expression in MS.

These observations reported its detection in astroglial or macrophage-like cells within MS lesions and in endothelial cells within Marburg's type hyperacute lesions. However, which immune or glial cells expressed this protein in the CNS and where, during the development of the lesions and according to their different stages of activity until the end of MS patient's life (when autopsy material is collected), still remained open questions. Further determining when such an expression arose and localized according to MS lesion development required a systematic analysis of MS brains, from first perivascular inflammatory infiltrates to late burnt out plaques.

To address these questions, the present study performed a systematic analysis of HERV-W envelope protein (MSRV-Env) distribution and cellular localization in different stages of MS lesion development, using a large cohort of well-characterized MS samples with one of the 3 highly specific monoclonal antibodies that were previously used to examine its expression in sections of MS lesions (Perron et al., 2012).

Here we show that MSRV-Env protein is abundantly present in MS brain lesions and is intimately associated with active areas of demyelination where macrophages and microglia represent the predominant cell type expressing MSRV Env. Only moderate expression was observed in reactive astrocytes throughout active and chronic active lesion areas, while rarely in inactive lesion areas. Expression in perivascular macrophages and in neighboring endothelial cells appeared quite essentially in early demyelinated areas.

2. Materials and methods

2.1. Autopsy material

Brain samples from 20 patients with clinically diagnosed and neuropathologically confirmed MS were obtained at rapid autopsy and immediately fixed in buffered formalin (in collaboration with The Netherlands Brain Bank, Amsterdam; Dr. I. Huitinga, coordinator). The Netherlands Brain Bank received permission to perform autopsies for the use of tissue and for access to medical records for research purposes from the ethics committee of the VU Medical Center (Amsterdam, The Netherlands). Six cases without neurological disease were selected as controls. Tissue samples from control cases were taken from the subcortical white matter or corpus callosum, regions where most MS lesions were encountered and therefore analyzed. MS tissue samples were selected on the basis of postmortem MRI and lesions were classified according to validated histopathological criteria as previously published (De Groot et al., 2001). MRI-guided white matter lesions were thus obtained from different locations in every case. Hence, cases with different type of lesion stages was included in the present MS cohort. However, different types of lesions were also obtained from individual brains as lesional activity can still be detected at autopsy in most cases (van Horssen et al., 2012).