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Research Paper

Human Endogenous Retrovirus and Neuroinflammation in Chronic Inflammatory Demyelinating Polyradiculoneuropathy

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ABSTRACT

Background: Human endogenous retroviruses HERV-W encode a pro-inflammatory protein, named MSRV-Env from its original identification in Multiple Sclerosis. Though not detected in various neurological controls, MSRV-Env was found in patients with chronic inflammatory demyelinating polyradiculoneuropathies (CIDPs). This study investigated the expression of MSRV in CIDP and evaluated relevant MSRV-Env pathogenic effects.

Methods: 50 CIDP patients, 19 other neurological controls (ONDs) and 65 healthy blood donors (HBDs) were recruited from two different countries. MSRV-env and -pol transcripts, IL6 and CXCL10 levels were quantified from blood samples. MSRV-Env immunohistology was performed in distal sensory nerves from CIDP and neurological controls biopsies. MSRV-Env pathogenic effects and mode of action were assayed in cultured primary human Schwann cells (HSCs).

Findings: In both cohorts, MSRV-env and -pol transcripts, IL6 positivity prevalence and CXCL10 levels were significantly elevated in CIDP patients when compared to HBDs and ONDs (statistically significant in all comparisons). MSRV-Env protein was detected in Schwann cells in 5/7 CIDP biopsies. HSC exposed to or transfected with MSRV-env presented a strong increase of IL6 and CXCL10 transcripts and protein secretion. These pathogenic effects on HSC were inhibited by GNbAC1, a highly specific and neutralizing humanized monoclonal antibody targeting MSRV-Env.

Interpretation: The present study showed that MSRV-Env may trigger the release of critical immune mediators proposed as instrumental factors involved in the pathophysiology of CIDP. Significant MSRV-Env expression was detected in a significant proportion of patients with CIDP, in which it may play a role according to its presently observed effects on Schwann cells along with previously known effects on immune cells. Experimental results also suggest that a biomarker-driven therapeutic strategy targeting this protein with a neutralizing antibody such as GNbAC1 may offer new perspectives for treating CIDP patients with positive detection of MSRV-Env expression.

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1. Introduction

Human endogenous retroviruses (HERVs) originate from ancestral integrations of exogenous retroviruses during evolution and represent 8% of the human genome, in which most copies are inactivated or silenced (Belshaw et al., 2005). However, a retroviral element expressing proteins was isolated in Multiple Sclerosis (MSRV, for Multiple Sclerosis associated RetroViral element) and unveiled a family of homologous endogenous copies (HERV-W)

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(Blond et al., 1999; Perron et al., 1997, 1991). The HERV-W family comprises multiple copies inserted in the human genome. One of them has been domesticated throughout evolution and encodes an HERV-W envelope, named Syncytin (Mi et al., 2000) for its original fusogenic properties involved in the physiological development of the syncitiotrophoblast tissue in the placenta (Frendo et al., 2003). It is selectively expressed during placentation, is transcribed from a locus (ERVWE1) within a defective HERV-W copy on chromosome 7 and has a unique molecular signature among HERV-W envelope sequences (Bonnaud et al., 2004; Mallet et al., 2004). This protein and its coding nucleotide sequences can thus be differentiated from the envelope sequences obtained from genomic RNA in purified retroviral particles from MS (Mameli et al., 2009). The latter define an MSRV-subtype of HERV-W elements that comprises multiple related defective fixed copies in the human genome such as, e.g., a partial HERV-W copy on chromosome X that potentially encodes a truncated envelope (ERVWE2 locus) and may interfere with MSRV expression (Roebke et al., 2010; do Olival et al., 2013; Garcia-Montojo et al., 2014). HERVs are not infectious viruses but human DNA sequences related to retrotransposable genetic elements, few of which have the potential to be activated by various environmental triggers, including infectious viruses on a "hit-and-run" mode (Perron and Lang, 2010; Mameli et al., 2012). HERV-W proteins are tolerated by human adaptive immune system and neither antibody nor T-cell response to HERV-W proteins can be seen, unless in rare and extreme conditions that may relate to autoimmunity (Ruprecht et al., 2008).

Beyond this fundamental research context, independent studies confirmed an association of MSRV expression with MS (Perron et al., 2012; Sotgiu et al., 2010). Its envelope protein (MSRV-Env) was shown to elicit pro-inflammatory and autoimmune responses in immune cells (Perron et al., 2001, 2013; Rolland et al., 2006) and to impair remyelination by oligodendrocyte precursor cells (OPCs) (Kremer et al., 2013), suggesting its involvement in MS pathogenesis (Perron et al., 2012; Kremer et al., 2014; Madeira et al., 2016).

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a rare immune disease of the peripheral nervous system (PNS), with multifocal inflammatory and demyelinating lesions in nerve roots also expanding to distal regions (Vallat et al., 2010). Its clinical presentation is heterogeneous and its diagnosis is challenging without known etiology or specific biomarkers (Dalakas, 2011; Anon., 2008, 2010; Koller et al., 2005). CIDP therapies are intravenous human immunoglobulins (IVIG), corticosteroids or plasma exchange. Long-term therapy is often limited by side effects and one-third of patients are refractory to existing treatments (Latov, 2014), which illustrates the unmet need for diagnostic biomarkers and innovative treatments of CIDP.

In a previous study on MS, MSRV-Env was not detected in healthy controls and in various other Neurological Diseases except for CIDP cases (5/8) (Perron et al., 2012). This observation prompted the present study to investigate a potential association of this endogenous HERV-W element with CIDP. Its results have confirmed significant MSRV expression in CIDP, have demonstrated the pathogenic effects of MSRV-Env on human Schwann cells (HSC) and their inhibition by GNbAC1, a neutralizing and highly specific humanized antibody targeting this MSRV-Env endogenous protein.

2. Patients and Methods

2.1. Origin of Samples

The overall study group consisted of 51 CIDP patients, from the Neurology Departments of Henri Mondor University Hospital (Creteil, France) and of Vaudois University Hospital (CHUV-Lausanne, Switzerland). CIDP patients fulfilled criteria of the EFNS and PNS Joint Task Force guidelines (PNS JTFOTEat, 2010). The majority of patients had symmetric sensorimotor deficits (24 patients), 9 patients had asymmetric sensorimotor deficits, MADSAM type, and one patient had pure sensory deficit. The number of patients in each category is too small to consider statistical interpretation on these symptomatologic subgroups. 19 patients with other Neurological diseases (OND) were recruited in Creteil Neurology department: type 2 diabetes associated neuropathies (n = 8), Parkinson disease (n = 3), diffuse myalgias without a known cause (n = 2), stroke, meningoradiculitis, macrophagic myofasciitis, axonal idiopathic neuropathy, Guillain–Barré syndrome, and spastic paraparesis (n = 1 each). 65 healthy blood donors (HBDS) from CHUV Lausanne or from Etablissement Français du Sang, Annemasse, France, provided samples. Written informed consent to use their blood for research analyses after anonymization was obtained from all individuals. The study protocol was approved by local research ethics committees (Créteil: ethics committees CPPIDF VI and CPPIDF IX, POLYCHROME study number ID RCB 2010-A01226–33; Lausanne: protocol 235/10).

In a first cohort (Study 1), 20 CIDP patients from Creteil and 21 HBDs from Annemasse, were enrolled. A second cohort (Study 2) included 11 additional CIDP patients and 19 OND patients from Créteil, 20 CIDP patients and 18 HBDs from Lausanne, and 26 HBDs from Annemasse, for a total of 31 CIDPs, 19 ONDs and 44 HBDs in study 2. MSRV transcript levels in PBMC were assessed separately in Studies 1 and 2. In the light of results obtained in human Schwann cells,

IL6 and CXCL10 serum levels were determined retrospectively, at the same time for both cohorts (aliquoted samples from studies 1 and 2).

For serum, 6 mL of blood was collected on a dry tube and 500 μL of serum aliquots were frozen at −80 °C. For peripheral blood mononuclear cells (PBMCs), 4 mL of blood was collected in a Cell Preparation Tube (ref. 362781, Becton Dickinson, Paris, France) and treated according to the manufacturer's instructions. PBMCs in heat-inactivated Fetal Calf Serum with 10% dimethyl-sulfoxide were stored at −80 °C.

2.2. Quantification of MSRV-env and -pol Transcripts in PBMC by Real-time RT-PCR (qRT-PCR)

Thawed PBMCs were washed with PBS (1700 g for 20 min at 10 °C). Total RNA was isolated with QIAamp RNeasy Mini Kit (Qiagen, Courtaboeuf, France) and treated with Turbo DNA-Free™ (Life Technologies, Saint-Aubin, France) according to the manufacturer's instructions. RNA concentration was assessed with a Nanodrop 2000 (Fisher Scientific, Illkirch, France) before adjustment to 10 ng/μL with RNase-free water. First-strand cDNA was synthesized with i-script select cDNA-synthesis kit using oligonucleotide dT (18) (BioRad, Marnes-La-Coquette, France) at 42 °C for 60 min, inactivated at 85 °C for 5 min and adjusted to 10 ng μL^{-1} with RNase-free water. 50 ng of cDNA was used with iQ supermix (BioRad, Marnes-La-Coquette, France) and corresponding sets of primers/probes for qRT-PCR. The internal control was glucuronidase beta gene, GUS B (Taqman gene expression assay GUS B, Life Technologies, Saint-Aubin, France) and specific sets of primers and probes for MSRV-env as described (Mameli et al., 2009). MSRV-pol transcripts were quantified with a FAM™ fluorescent reporter (forward primer: 5′-CCTGTACGTC CTGACTCTC-3′; reverse primer: 5′-CTTGGGCTAATGCCTGGCC-3′; probe: FAM-CCAACGTCTCAACTCACCTGG-TAMRA). PCR was performed with a C1000 thermal cycler and a CFX96 real-time system (BioRad, Marnes-La-Coquette, France), with an initial denaturation step (95 °C, 10 min) followed by 45 cycles of successive denaturation (95 °C for 10 s) and annealing/extension (60 °C, 1 min) steps. For each sample, the expression of MSRV transcripts and GUS B was calculated as the cycling threshold (Ct), assessed in triplicates, and MSRV transcript level was expressed as relative expression to GUS B, according to the ΔCt method with reference gene (Real-Time Application Guide, BioRad, Marnes-La-Coquette, France). For each sample, a control without reverse transcriptase (No RT) was performed to detect