Journal of Neuroimmunology 291 (2016) 29–38

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

MSRV envelope protein is a potent, endogenous and pathogenic agonist of human toll-like receptor 4: Relevance of GNbAC1 in multiple sclerosis treatment

Alexandra Madeira [a], Ingrid Burgelin [b], Hervé Perron [a,b], Francois Curtin [b], Alois B. Lang [b], Raphael Faucard [a,*]

[a] GeNeuro Innovation SAS, Immeuble Forum 1, Archamps Technopole, 74160 Archamps, France
[b] GeNeuro SA, 18 chemin des Aulx, 1228 Plan-Les-Ouates, Switzerland

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 3 December 2015
Accepted 10 December 2015

Keywords:
MSRV envelope
GNbAC1
HERV-W
TLR4
Monoclonal antibody
Multiple sclerosis

ABSTRACT

Multiple sclerosis associated retrovirus envelope protein (MSRV-Env) was repeatedly detected in brain lesions and blood of multiple sclerosis (MS) patients. We performed the first pharmacological characterisation of MSRV-Env on recombinant and native human TLR4. MSRV-Env is a full and highly potent TLR4 agonist of endogenous origin. MSRV-Env induces TLR4-dependent pro-inflammatory stimulation of immune cells in vitro and in vivo, and impairs oligodendrocytes precursor cells differentiation to myelinating oligodendrocytes. MSRV-Env may play a role in chronic inflammation and impaired remyelination in MS. GNbAC1, a selective monoclonal antibody, antagonizes MSRV-Env pathogenic effects and represents an innovative therapeutic approach of MS.

1. Introduction

Human endogenous retroviruses (HERV) represent approximately 8% of the human genome and result from integration of exogenous retroviruses that infected the germline of their hosts during primate evolution (Belshaw et al., 2004). HERV belong to the superfamily of repeated and transposable elements and retain properties of mobile genetic elements although most contemporary HERV copies are inactivated by mutations, deletions, or silenced by epigenetic modifications (Belshaw et al., 2005; Romanish et al., 2010). The first description of retrovirus-like particles with reverse transcriptase activity in leptomeningeal and macrophage cell cultures from multiple sclerosis (MS) patients suggested a viral origin of the disease (Perron et al., 1991). However, further molecular characterisation of this Multiple Sclerosis-associated RetroVirus (MSRV) revealed that it was not a classical infectious exogenous retrovirus but the forming member of the W family of HERV (HERV-W) (Blond et al., 1999; Komurian-Pradel et al., 1999; Perron et al., 1997). Though HERV-W elements are usually truncated or lack open reading frames (orf), several chromosomal copies retain potential orf for viral proteins (Voisset et al., 2000). Interestingly, certain exogenous viral agents can trigger the reactivation of MSRV copies, particularly Herpesviridae which have been often associated with MS epidemiology without demonstrating a causative role in the disease. Thus, reactivation of silent MSRV, which exhibits pathogenic properties, by such environmental infectious agents may be the missing link between environment and MS onset (Perron and Lang, 2010). Following the initial isolation of MSRV particles from cerebral tissues of MS patients, numerous independent studies in humans performed by two research groups have associated MS with an elevated MSRV expression, in particular its MSRV envelope protein (MSRV-Env) in serum or its corresponding RNA in human peripheral blood mononuclear cells (hPBMC) (Arru et al., 2007; Mameli et al., 2007; Perron et al., 2012). Furthermore, MSRV-Env protein was detected on the cell surface of infiltrated macrophages and activated microglia in MS brain lesions, but not in normal appearing white matter or control brains (Kremer et al., 2013; Perron et al., 2012).

MS is a devastating chronic inflammatory demyelinating disease of the central nervous system (CNS). Although its aetiology is not elucidated yet, MS probably has an autoimmune component. The presence of infiltrating T-cells, monocytes and macrophages in the brain lesions of

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; DAMP, danger-associated molecular pattern; EAE, experimental allergic encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; HERV, human endogenous retrovirus; hOPC, human oligodendrocytes precursors cells; hPBMC, human peripheral blood mononuclear cells; hTLR4, human toll-like receptor 4; Ig, Immunoglobulin; IL-6, interleukine 6; LPS, lipopolysaccharide; MS, multiple sclerosis; MSRV, multiple sclerosis associated retrovirus; MSRV-Env, MSRV envelope protein; MSRV-Env-SU, recombinant surface domain of MSRV-Env; MSRV-Env-SUG, recombinant glycosylated surface domain of MSRV-Env; MSRV-Env-T, recombinant full length MSRV-Env; OD, optical density; orf, open reading frame; PAMP, pathogen-associated molecular pattern; PMB, polymyxin B; RNA, ribonucleic acid; TNF-α, tumour necrosis factor α.

* Corresponding author.
E-mail address: rf@geneuro.com (R. Faucard).

http://dx.doi.org/10.1016/j.jneuroim.2015.12.006

MS patients suggests that innate immune system plays a pivotal role in the mediation of cerebral damages (Weiner, 2009). Several studies show that MSRV virions or its envelope protein elicit immunopathogenic and pro-inflammatory responses on human T lymphocytes and hPBMC (Perron et al., 2001; Rolland et al., 2006). More recently, Kremer et al. (2013, 2014) showed that MSRV-Env induces the expression of pro-inflammatory cytokines and inducible NO synthase by oligodendrocyte precursors cells (OPC) in culture. Furthermore, the authors report that MSRV-Env decreases the differentiation capacity of rat OPC to mature myelinating cells. Some of these MSRV-Env effects were attributed to toll-like receptor 4 (TLR4) activation though not characterised (Kremer et al., 2013; Rolland et al., 2006). TLR signalling plays a crucial role in innate immunity through initiation of host defence responses to microbial infection (Hanke and Kielian, 2011) and these observations suggest that MSRV-Env pathogenic properties may be involved in MS.

As the aetiology of MS is unknown, there are no causal treatments of the disease. Though major progresses have been recently achieved with new oral treatments, existing therapies only alter the course of MS and once the disease develops it is lifelong. Furthermore available treatments, which are approved for the relapse-remitting form of MS, are in general strong immunomodulators associated with substantial safety risks, but they have shown only marginal or no effect in the progressive forms of the disease. There is a critical medical need for innovative and safer MS treatments, in particular dedicated to the progressive forms of the disease where remyelination impairments prevail (Curtin and Hartung, 2014). Thus, as MSRV-Env expression is associated with MS, exhibits pro-inflammatory properties and potentially impairs brain remyelination, a humanised monoclonal antibody targeting MSRV-Env, named GNbAC1, has been developed as an innovative therapeutic approach for MS (Curtin et al., 2015).

We performed the first pharmacological characterisation of MSRV-Env interaction with recombinant human TLR4 (hTLR4) and demonstrated that MSRV-Env is an endogenous and highly potent full agonist of hTLR4. Then, we performed the characterisation of MSRV-Env interaction with native hTLR4 expressed by hPBMC and human OPC (hOPC). This study confirms previous reports suggesting MSRV-Env pro-inflammatory properties in vitro and we show that this MSRV-Env feature is mirrored in vivo. Furthermore, we show that MSRV-Env impairs hOPC differentiation to mature myelinating oligodendrocytes through hTLR4 activation. GNbAC1 antagonizes all the pathogenic effects of MSRV-Env investigated, supporting the fact that GNbAC1 may provide a new and targeted therapeutic approach to the treatment of MS.

2. Materials and methods

2.1. Recombinant proteins and chemicals

Three different recombinant MSRV-Env proteins were used in this study. The full length MSRV-Env-T protein was produced from plasmid pV14 encompassing the complete orf of MSRV-Env cloned from MSRV virion RNA (58 kDa, 542 aminoacids, GenBank no. AF331500.1). MSRV-Env-SU is a 33 kDa and 293 aminoacids fraction of the extracellular domain of MSRV-Env-T. Both proteins were produced in *Escherichia coli*. MSRV-Env-SUG is the glycosylated form of MSRV-Env-SU and was produced in HEK 293 human cell line. Recombinant MSRV-Env proteins were produced by Protein'Expert (Grenoble, France) as previously described (Perron et al., 2013; Rolland et al., 2005, 2006).

GNbAC1 is a humanized monoclonal antibody of the IgG4/kappa class that selectively binds with high affinity to the extracellular domain of MSRV-Env protein (Curtin et al., 2012, 2015). GNbAC1 vehicle (20 mM histidine, 5% (*w*/*v*) sucrose, 0.01% (*w*/*v*) polysorbate 20, pH 6.0) was systematically used as a negative control in all experiments. Natalizumab (Biogen-Dompé, Zug, Switzerland), another humanized IgG4 antibody specific for the cell adhesion molecule α4-integrin, was systematically used as a GNbAC1 isotype control in this study but not in hPBMC experiments since it exerts a direct effect in this model.

LPS is the prototypical TLR4 agonist and was used as a positive control in experiments performed with recombinant hTLR4. CD14 acts as a co-receptor of TLR4 and anti-human CD14 or TLR4 antibodies were used as selective blockers of the hTLR4/CD14 receptor complex. LPS-RS is a characterized competitive LPS antagonist extracted from *Rhodobacter sphaeroides* (Stevens et al., 2013), and Cli-095 is a selective intracellular suppressor of TLR4 signalling (Kawamoto et al., 2008; Matsunaga et al., 2011). LPS-EK Ultrapure, anti-human CD14 IgA, anti-human TLR4 IgG and corresponding isotype controls antibodies, LPS-RS, and Cli-095 were purchased from Invivogen (Toulouse, France). Polymyxin B (PMB) inhibits LPS liaison to LPS binding protein (LBP), was used as a selective LPS inhibitor and was purchased from Sigma (Buch, Switzerland). Mouse anti-CNPase (2′,3′-Cyclic nucleotide-3′-phosphohydrolase) and anti-MBP (Myelin Basic Protein) antibodies were purchased from Covance (Paris, France).

2.2. HEK-blue™-TLR4 cells culture and TLR4 signalling assays

HEK-Blue™-hTLR4 and HEK-Blue™-mTLR4 cells were obtained by co-transfection of the human or murine TLR4, MD-2 and CD14 co-receptor genes, and an inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene into HEK293 cells. The SEAP reporter gene is placed under the control of an Il-12 p40 minimal promoter fused to five NF-κB and AP-1 binding sites. Stimulation with a TLR4 agonist activates NF-κB and AP-1 which induce the production of SEAP assessed by a colorimetric assay. HEK-Blue™ Null2 is the parental negative control cell line of HEK-Blue™-hTLR4 and HEK-Blue™-mTLR4 cells. HEK-Blue™-hTLR4, HEK-Blue™ Null2 and HEK-Blue™-mTLR4 (Invivogen, Toulouse, France) culture was performed according to supplier's recommendations. In first experiments, HEK-Blue™-hTLR4 cells were classically stimulated by agonists and/or antagonists for 24 h in the recommended culture medium. In subsequent pharmacological investigations, the manufacturer's protocol was adapted to a 30 min stimulation with agonists and/or antagonists followed by a 15.5 h incubation before revelation, in Pro293A-CDM (Lonza, Basel, Switzerland), a serum-free culture medium supplemented with 50 U mL^{-1} penicillin (Life Technologies, Basel, Switzerland), 50 μg mL^{-1} streptomycin (Life Technologies, Basel, Switzerland) and 100 μg mL^{-1} Normocin (Invivogen, Toulouse, France). TLR4 activation was revealed colorimetrically with QUANTI-Blue™ detection assay (Invivogen, Toulouse, France). Optical densities were measured with a Biotek EL800 microplate reader at 650 nm with the KC Junior software (Biotek, Luzern, Switzerland).

2.3. Human PBMC cultures and cytokines ELISA

Buffy coats from healthy donors were purchased from the Centre for Blood Transfusion of Geneva (Switzerland). Accordingly, this study was not reviewed by an Institutional Review Board or Ethics Committee as all healthy donors signed a written Informed Consent Form, documented at the Centre for Blood Transfusion of Geneva, allowing the commercial use of their blood and blood components for medical research after definitive anonymization. All individual set of experiments presented here were performed with hPBMC from the same blood donor. hPBMC were isolated by Ficoll separation from buffy coats, aliquoted and frozen at −80 °C. For MSRV-Env-T concentration-response experiments, hPBMC frozen in DMSO 10% in foetal bovine serum (FBS, Biowest, Nuaillé, France) were thawed and directly stimulated for 24 h with MSRV-Env-T in 96-well plates in FBS 10%, penicillin/streptomycin 1%, MEM non-essential amino acids solution 1%, and sodium pyruvate 1 mM in RPMI culture media (Gibco, Life Technologies, Basel, Switzerland). For MSRV-Env-T inhibition experiments, hPBMC frozen in CTL-