Journal of Neuroimmunology 235 (2011) 56–69

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

A potential link between autoimmunity and neurodegeneration in immune-mediated neurological disease

Sangmin Lee [a,b], Lijing Xu [d], Yoojin Shin [a,b], Lidia Gardner [a,b], Anastasia Hartzes [a,b], F. Curtis Dohan [c], Cedric Raine [e], Ramin Homayouni [d], Michael C. Levin [a,b,*]

[a] Veterans Administration Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104, United States
[b] Department of Neurology, University of Tennessee Health Science Ctr, 855 Monroe Avenue, Memphis, TN 38163, United States
[c] Department of Pathology, University of Tennessee Health Science Ctr, 855 Monroe Avenue, Memphis, TN 38163, United States
[d] Bioinformatics Program, University of Memphis, 3774 Walker Ave, Rm 201 LS, Memphis, TN 38152, United States
[e] Neuropathology Department, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States

ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 11 January 2011
Accepted 8 February 2011

Keywords:
Neurodegeneration
Autoimmunity
Multiple sclerosis
Spastin
Bioinformatics

ABSTRACT

Multiple sclerosis (MS) patients make antibodies to heterogeneous nuclear ribonuclear protein A1 (hnRNP-A1), a nucleocytoplasmic protein. We hypothesized this autoimmune reaction might contribute to neurodegeneration. Antibodies from MS patients reacted with hnRNP-A1-'M9', its nuclear translocation sequence. Transfection of anti-M9 antibodies into neurons resulted in neuronal injury and changes in transcripts related to hnRNP-A1 function. Importantly, RNA levels for the spinal paraplegia genes (SPGs) decreased. Changes in SPG RNA levels were confirmed in neurons purified from MS brains. Also, we show molecular interactions between spastin (the encoded protein of SPG4) and hnRNP-A1. These data suggest a link between autoimmunity, clinical phenotype and neurodegeneration in MS.

Published by Elsevier B.V.

1. Introduction

Neurodegeneration is a major contributor to neurological disability in MS patients (Bjartmar et al., 2003; Dutta and Trapp, 2007; Lassmann, 2007; Lassmann et al., 2007). Following the relapse–remitting stage of MS, patients develop 'secondary progressive' MS, in which neurological deterioration continues in the absence of relapses (Dutta and Trapp, 2007; Lassmann et al., 2007; Noseworthy et al., 2000). Alternatively, others are diagnosed with primary progressive MS in which neurological dysfunction occurs without relapses from disease onset (Dutta and Trapp, 2007; Lassmann et al., 2007; Noseworthy et al., 2000). Thus, the majority of MS patients develop progressive neurological disease (Dutta and Trapp, 2007; Lassmann et al., 2007). Neuroradiological, neuropathological and animal studies of MS show markers for neurodegeneration manifest during the progressive phase of the illness. For example, MRI images of the brain show axonal damage, which correlates with neurological disability (Bjartmar et al., 2000; Lassmann et al., 2007). Also, accumulation of amyloid precursor protein (APP) (Ferguson et al., 1997; Kornek et al., 2000) and staining for non-phosphorylated neurofilament (SMI-32) (Trapp et al., 1998)—both markers of axonal injury—showed that axonal damage is a major component of MS lesions. Subsequent studies confirmed these observations in MS and in some experiments using experimental allergic encephalomyelitis (EAE) induced with myelin oligodendrocyte glycoprotein (MOG), a model of MS characterized by neurodegeneration in which antibodies play a significant role (Aboul-Enein et al., 2006; Brown and Sawchenko, 2007; Gold et al., 2006; Kornek et al., 2000). Importantly, there are multiple medications for the treatment of relapsing–remitting MS, but none are efficacious in progressive forms of the disease.

What causes MS is unknown, but evidence suggests that interactions between environmental agents, auto-antigens and the immune response in genetically susceptible people contribute to its cause (Dutta and Trapp, 2007; Lassmann et al., 2007; Noseworthy et al., 2000). Because no environmental agents have been rigorously proven to cause MS, we use human T-lymphotropic virus type 1 (HTLV-1) associated myelopathy/tropical spastic paraparesis (HAM/TSP) as a model to study MS (Lee et al., 2005; Levin et al., 2002a). HAM/TSP is caused by infection with HTLV-1, which allows for direct comparison between an environmental agent and auto-antigens. HAM/TSP patients develop spastic paraparesis and sensory abnormalities that can be clinically indistinguishable from progressive forms of MS, particularly primary progressive MS (Levin et al., 1997; Levin and Jacobson, 1997). Furthermore, both diseases are associated with neurodegeneration, particularly of corticospinal tracts and posterior columns (Ganter et al., 1999; Lee et al., 2005; Lovas et al., 2000;

* Corresponding author at: University of Tennessee Health Science Center, Link Building Room 415, 855 Monroe Avenue, Memphis, TN 38163, United States. Tel.: +1 901 523 8990X6809, +1 901 448 2243; fax: +1 901 577 7486.
E-mail address: mlevin@uthsc.edu (M.C. Levin).

0165-5728/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jneuroim.2011.02.007

Umehara et al., 2000). HAM/TSP patients make antibodies to HTLV-1 that cross-react with hnRNP A1 implicating molecular mimicry in the pathogenesis of HAM/TSP. Molecular mimicry is strongly associated with other immune-mediated neurological diseases such as the axonal form of Guillain–Barre Syndrome and also thought to contribute to the pathogenesis of MS (Kalume et al., 2004; Lee and Levin, 2008; Lee et al., 2006b, 2005; Levin et al., 1998, 2002a, 2002b; Yuki et al., 2004). hnRNP A1 is an RNA binding protein that plays a crucial role in mRNA transport, metabolism and translation and is required for normal cellular functioning (Dreyfuss et al., 2002). Antibodies from HAM/TSP patients reacted specifically with the 'M9' shuttling domain of hnRNP A1, the sequence required for its transport into and out of the nucleus (also known as the nuclear export sequence (NES) and nuclear localization sequence (NLS)) (Lee et al., 2006b; Levin et al., 2002a; Michael et al., 1995). Notably, MS patients make antibodies to hnRNP A1 as well as to neuronal and axonal antigens (Norgren et al., 2005; Rawes et al., 1997; Sadatipour et al., 1998; Sueoka et al., 2004). Further, MS patients make antibodies to oligodendrocytes as well as myelin, and these antibodies may contribute to the pathogenesis of the disease (Abramsky et al., 1977; Edgington and Dalessio, 1970; Genain et al., 1999). Therefore, we hypothesized that antibodies from MS patients would also recognize 'M9' and that antibodies to 'M9' might play a role in neurodegeneration in an in vitro model of antibody-mediated autoimmunity.

2. Methods

2.1. Sera and tissue

For sera, informed consent is on record from participating institutions. Tissue samples included brain samples from three patients with MS, two patients with HAM/TSP and one normal control. The clinical and autopsy data are contained within supplement 2.

2.2. Samples and IgG purification

IgG was harvested from the serum of MS and HAM/TSP patients and normal control participants using the Melon Gel IgG Purification Kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. Resulting IgG was dialyzed three times against phosphate buffered saline (PBS) buffer. Purified IgG was quantified using the BCA method.

2.3. Cell lines and cloning of hnRNP A1

NT-2 and SK-N-SH cells (American Type Culture Collection) were cultured under standard conditions as previously described (Lee et al., 2006b). Cloning of hnRNP A1 and its recombinant fragments was completed as previous described (Lee et al., 2006b; Levin et al., 2002a).

2.4. Antibodies and additional reagents

The antibodies to human transportin (ab10303), hnRNP A1(ab 4791) and KLH (ab 34766) were purchased from Abcam. Anti-Spastin mouse monoclonal antibodies (54443) were purchased from Santa Cruz Biotechnology. Secondary antibodies were as follows. Anti-rabbit–TxRed antibodies (TI-1000) and anti-mouse FITC antibodies (TI-2000) were from Vector laboratories, and anti-goat-FITC antibodies (sc2024) were from Santa Cruz Biotechnologies.

2.5. Western blotting

Western blotting was performed as described previously (Lee et al., 2006b; Levin et al., 2002a). Briefly, purified hnRNP A1, GST-hnRNP A1 fragments, human tissues, human neurons and dNT2 cells were separated on 8–16% gradient gels to improve resolution of the high molecular weight bands and transferred to PVDF (polyvinylidene difluoride) membranes (Amersham Biosciences), followed by Western blotting. For human tissues, neurons and cell lines, 30 mcg of protein was added per lane. Human tissues were extracted with T-PER tissue extraction reagent containing Halt protease inhibitor mixture (Pierce Technology). Samples were homogenized (4 °C), and tissue debris was removed by centrifugation (10,000 rpm, 5 min). The supernatant was reserved and its protein concentration was determined by the BCA protein assay (Pierce Technology). For epitope analyses, 0.2 mcg of each fusion protein was added per lane. For screening of multiple IgG samples for immunoreactivity to hnRNP A1-M9, 40 mcg of GST-M9 was added to a single broad center lane (allowing for simultaneous detection of up to 33 samples) using a SURF-BLOT system (Idea Scientific Company). Western blot analysis was performed using 1:100-diluted MS IgG and normal IgG. Initial epitope analysis was performed using IgG isolated from two MS patients. Goat anti-human IgG linked to horseradish peroxidase was utilized as a secondary antibody at a dilution of 1:25,000. Western blots were visualized using a chemiluminescent substrate (ECLplus, GE Healthcare). The first patient was female and had secondary progressive MS, with symptoms for 15 years, an expanded disability status scale (EDSS) of 4.0 with both pyramidal and posterior column signs. The second patient was male and also had secondary progressive MS, with symptoms for 41 years, an EDSS of 6.0 with both pyramidal and posterior column signs. The clinical characteristics of the study population are presented in Supplement 2, Table A. An inhibition assay was performed using MS IgG pre-incubated with hnRNP A1-M9$^{293–304}$ and hnRNP A1$^{185–196}$ (control) fragments in sequential concentrations of 0 μg/μl, 1 μg/μl, 10 μg/μl, and 50 μg/μl per lane.

2.6. Transfection and detection of antibodies in NT-2 cells

Unlabeled (for microarray experiments) and FITC (for immunocytochemistry experiments) anti-hnRNP A1 or anti-KLH antibodies were transfected using a liposomal-based protein delivery kit, per manufacturer's instructions (Bioporter, Genlantis). Twenty-four hours after transfection, cells were prepared for immunohistochemistry using standard procedures.

2.7. Immunohistochemistry

NT-2 cells were grown in Poly-D-lysine covered chambers, treated with retinoic acid and mitotic inhibitors prior to the experiment. Cells were fixed in 4% paraformaldehyde for 10 min at room temperature (RT), then washed in 1× PBS, and permeabilized in 0.2% Triton X-100, for 30 min at RT.

All slides were blocked in 6% Milk-TBST and washed in PBS prior to antibody application. Primary antibodies were applied for 1.5 h at RT. Each slide was double stained with rabbit polyclonal antibodies to hnRNP A1 and transportin or spastin antibodies. Slides were washed 5× in 1× PBS. Secondary antibodies were: FITC (transportin, spastin) and TxRed (hnRNP A1) labeled. Slides were incubated for 1.5 h with secondary antibodies, then washed and mounted using DAPI mounting media (Millipore). Labeling with Fluoro Jade C was performed as described (Schmued et al., 2005).

2.8. Microarray and quantitative real-time PCR

Each experimental group (untouched control, anti-hnRNP A1 and anti-KLH) consisted of three separate 75 cm^2 flasks of NT2 neurons. Unlabeled antibody (50 mcg/2 × 10^7 NT2 cells) was transfected into each of the flasks for 24 h per the manufacturer's instructions. Following transfection of antibodies into the NT-2 neurons, total RNA