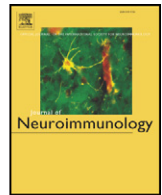
Journal of Neuroimmunology 284 (2015) 30–36

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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



Novel serum autoantibodies against talin1 in multiple sclerosis: Possible pathogenetic roles of the antibodies

Mayumi Muto [a], Masahiro Mori [a,*], Takaki Hiwasa [b], Masaki Takiguchi [b], Yasuo Iwadate [c], Akiyuki Uzawa [a], Tomohiko Uchida [a], Hiroki Masuda [a], Kazuo Sugimoto [a], Satoshi Kuwabara [a]

[a] Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan
[b] Department of Biochemistry and Genetics, Graduate School of Medicine, Chiba University, Chiba, Japan
[c] Department of Neurological Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 21 April 2015
Accepted 4 May 2015

Keywords:
Multiple sclerosis
SEREX
TLN1 protein
DDX39 protein
IgG index
Oligoclonal bands

ABSTRACT

In the pathogenesis of multiple sclerosis (MS), B cell/antibody-related mechanisms have recently received attention. To investigate the role of autoantibody in MS, we performed SEREX which can identify autoantibody cyclopedically. We identified serum antibodies against cytoskeletal protein talin1, and the levels of whom were remarkably higher in 39 MS than 43 normal controls ($P < 0.01$) and 35 disease controls ($P = 0.06$), and in MS patients without oligoclonal bands than ones with them. Moreover, we found negative-correlations between serum anti-talin1 antibody and IgG index in MS ($P = 0.03$). Anti-talin1 antibody exists in MS patients' sera, which may have some protective factor.

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1. Introduction

MS is an immune-mediated disease characterized by demyelination of the CNS. It is believed that T cell autoreactivity against antigens expressed in the brain, particularly myelin antigens, plays a pivotal role in MS pathogenesis. In contrast, there is accumulated evidence that antibodies and B cells play a substantial role in MS pathogenesis, based on the following findings, at least, in a subgroup of MS patients: (a) deposition of immunoglobulins and complement activation in acute demyelinating lesions, an immunopathological pattern type II defined by Lucchinetti et al. (2000); (b) efficacy of plasma exchange for acute exacerbations of MS patients (Weinshenker et al., 1999); (c) presence of CSF oligoclonal IgG bands (OCBs) in MS; and (d) depletion of B cells by monoclonal antibodies has a considerable effect on disease activity in MS patients (Hauser et al., 2008).

Although many components, including myelin-derived proteins and the potassium channel KIR4.1, are suspected as targets of autoantibodies, the real molecular targets which are necessary and sufficient for development of MS are unknown in MS (Lisak and Zweiman, 1977; Srivastava et al., 2012). Various antibody biomarkers for MS in the serum or CSF have been reported, but none of them are of practical use. Serological analysis of recombinant cDNA expression libraries (SEREX), an immunoscreening method that uses prokaryotically expressed cDNA libraries prepared from tissues or cell lines and sera from patients, is a very effective tool for identifying antigens recognized by spontaneous autoantibodies of patients with cancer and autoimmune diseases. Here, we elucidated whether there is a novel specific autoantibody in serum samples from MS patients using SEREX and amplified luminescence proximity homogeneous assay-linked immunosorbent assay (AlphaLISA) techniques and, if it exists, the role of the autoantibody in MS pathogenesis.

2. Material and methods

2.1. MS patient's serum analyzed by SEREX

The Local Ethical Review Board of the Graduate School of Medicine, Chiba University approved the study, and written informed consent was obtained from the patients and healthy volunteers. For immunoscreening by SEREX, serum was obtained from a 27-year-old

Abbreviations: AlphaLISA, amplified luminescence proximity homogeneous assay-linked immunosorbent assay; CSF, cerebrospinal fluid; DC, disease control; DDX39, DEAD box polypeptide 39; EDSS, Kurtzke's Expanded Disability Status Scale; MS, multiple sclerosis; NC, normal control; NDRG1, N-myc downstream regulated gene 1; OCB, oligoclonal IgG band; SEREX, serological analysis of recombinant cDNA expression libraries; TLN1, talin1.

* Corresponding author at: Department of Neurology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
E-mail address: morim@faculty.chiba-u.jp (M. Mori).

http://dx.doi.org/10.1016/j.jneuroim.2015.05.005
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Japanese MS patient. She experienced an initial attack 6 years before serum sampling and dozens of attacks thereafter. Brain MRI showed multiple T2 hyperintense lesions fulfilling Barkhof criteria (Barkhof et al., 1997). She was treated with intravenous methylprednisolone therapy at most attacks but was never treated with disease modifying drugs. Her last attack started 14 days before serum sampling. At sampling, she did not receive any treatment.

2.2. Phage cDNA library

Double-stranded cDNA library was synthesized from total RNA prepared from the human glioblastoma cell line U-87 MG (ATCC, HTB-14), as previously described. Double-stranded cDNA was synthesized through conventional procedures and ligated into the EcoRI–XhoI site of λZAP II phage. The library size was over 1.0×10^6 PFU/mL.

2.3. Immunological screening using SEREX

The screening method used in this study is the modification of a published procedure (Matsutani et al., 2012). *Escherichia coli* XL1-Blue MRF' was infected with Uni-ZAP XR phage containing a cDNA library, and cDNA expression was induced by blotting on nitrocellulose membranes pretreated with 10 mM isopropyl-β-D-thiogalactoside (IPTG; Wako Pure Chemicals, Osaka, Japan). After washing and blocking, membranes were exposed to 1:2000-diluted serum for 1 h. After washing, membranes were treated with 1:5000-diluted alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Positive reactions were developed using a color development solution containing 0.3 mg/mL of nitroblue tetrazolium chloride (Wako Pure Chemicals) and 0.15 mg/mL of 5-bromo-4-chloro-3-indolylphosphate (Wako Pure Chemicals). Positive clones were recloned twice to obtain monoclonality.

2.4. Sequence analysis of identified antigens

Monoclonal phage cDNA clones were converted into pBluescript phagemids by excision *in vivo* using the ExAssist helper phage (Stratagene, La Jolla, CA, USA). Plasmid DNA was obtained from the *E. coli* SOLR strain after transformation by phagemid. cDNA insertions were sequenced by dideoxy chain termination using DNA Sequencing BigDye Terminator Kits (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Sequences were screened for homology with identified genes or proteins within the public sequence database using the NCBI-BLAST algorithm (http://www.ncbi.nlm.nih.gov/Blast.cgi/).

2.5. Expression and purification of antigenic glutathione S-transferase (GST)-fusion proteins

cDNA inserts of the clones incorporated in pBluescript were cleaved by EcoRI and XhoI and cloned into the EcoRI–XhoI site of pGEX-4T-3, pGEX-4T-2, and pGEX-4T-1 vectors (GE Healthcare Life Sciences, Pittsburgh, PA) that carry the expression of recombinant GST fusion proteins. *E. coli* JM109 cells (Nippon Gene, Toyama, Japan) containing pGEX clones ($A_{600} = 0.3$–0.5) were cultured in 200 mL of Luria broth, lysed through sonication, and centrifuged. GST fusion proteins in supernatants were purified by glutathione–Sepharose column chromatography according to the manufacturer's instructions (GE Healthcare Life Sciences) and dialyzed. Proteins were confirmed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

2.6. Western blot analysis

For Western blot analysis, three purified recombinant GST-fusion proteins, TLN1, DDX39 and NDRG1, were separated by SDS-polyacrylamide gel electrophoresis and electrically transferred onto a nitrocellulose membranes. The membrane was blocked with 0.5% non-fat dry milk in a buffer consisting of 20 mM Tris–HCl (pH 7.6), 137 mM NaCl and 0.1% Tween 20, and incubated with specific primary antibodies against TLN1 (C-20, Santa Cruz Biotechnology, Santa Cruz, CA), DDX39 (TA303418, Origene, Rockville, MD) and NDRG1 (H-60, Santa Cruz Biotechnology). After incubation with horseradish peroxidase-conjugated secondary antibody, immunoreactivity was detected with the Immobilon (Merck Millipore, Darmstadt, Germany) as described previously (Hiwasa et al., 2000).

2.7. Patients' clinical information and healthy and disease controls

Thirty-nine Japanese patients diagnosed with relapsing–remitting MS in our hospital between October 2003 and April 2014 were studied (31 women and 8 men; mean age, 40.3 years). Patients fulfilled the revised 2005 McDonald's criteria for MS (Polman et al., 2005), and their clinical courses were a relapsing–remitting type. Eight patients were treated with interferon-β, one with fingolimod, and 30 had not been treated with immunomodulators or immunosuppressants to prevent relapse.

Forty-three healthy participants (28 women and 15 men; mean age, 43.0 years) matched for sex and age were used as normal control (NC). Thirty-five patients with Bickerstaff brainstem encephalitis (17 women and 18 men; mean age 43.4 years) matched for age were used as disease control (DC) (Table 1). Clinical condition of MS patients was monitored based on the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). If the patient met the McDonald's criteria (Polman et al., 2005), clinical relapse was defined as an episode of neurological disturbance lasting at least 24 h. OCBs were determined by isoelectric focusing and were considered positive when they were only detected in CSF and comprised at least two bands. The IgG index represents (CSF IgG/serum IgG) / (CSF albumin/serum albumin) and was considered to be increased if it was >0.73. Serum samples were obtained from all 39 MS patients at the time of relapse and from 25 of these patients during remission. All the samples from patients and healthy participants were stored at −80 °C until use.

2.8. Measurement of serum antibodies against purified proteins by AlphaLISA

To evaluate serum antibody levels, AlphaLISA, was performed in 384-well microtiter plates (white opaque OptiPlate™ from PerkinElmer) containing 2.5 μL of 1:100-diluted serum and 2.5 μL of GST-fusion antigen proteins (10 μg/mL) in AlphaLISA buffer (25 mM HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/mL dextran-500, and 0.05% Proclin-300). The reaction mixture was incubated at room temperature for 6–8 h; then, anti-human IgG-conjugated acceptor beads (2.5 μL at 40 μg/mL) and glutathione-conjugated donor beads

Table 1
Clinical data of cases of multiple sclerosis, normal controls and disease controls included in this study.

	MS (n = 39)	NC (n = 43)	DC (n = 35)
Male:female	8:31	15:28	18:17
Age, mean (SD), y	40.3 (11.5)	43.0 (9.4)	43.4 (15.5)
Total disease duration, mean (SD), y	9.5 (9.6)	–	–
EDSS score, median (range)	4.4 (1.0–9.5)	–	–
OCB-positive patients (%)	39.1	–	–
Patients with high IgG index (>0.75) (%)	56.0	–	–
Disease modifying drugs		–	–
Interferon-β	8	–	–
Fingolimod	1	–	–
Nothing	30	–	–

NC, normal control; DC, disease control; OCB, oligoclonal IgG band; EDSS, Kurtzke Expanded Disability Status Scale; SD, standard deviation; –, not available.

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