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Significantly increased antibody response to heterogeneous nuclear ribonucleoproteins in cerebrospinal fluid of multiple sclerosis patients but not in patients with human T-lymphotropic virus type I–associated myelopathy/tropical spastic paraparesis

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It has been reported that antibodies (Abs) against heterogeneous nuclear ribonucleoproteins (hnRNPs) are associated with human T-lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and multiple sclerosis (MS). However, these studies were done under nonmasked conditions. In order to determine whether Abs against hnRNPs associate with HAM/TSP and MS, the authors assayed Abs against two major hnRNPs, hnRNP A1 and A2/B1, in 105 cerebrospinal fluid (CSF) samples under fully masked conditions. Samples included 40 cases of HAM/TSP, 28 of MS, and 37 of other neurological diseases. Anti-hnRNP A1 Abs, and especially anti-hnRNP A2/B1 Abs, were found significantly more often in the CSF of MS patients than in other groups. However, there was no difference in the incidence of anti-hnRNP A1 Abs between HAM/TSP and other disease groups. *Journal of NeuroVirology* (2008) **14,** 130–135.

Keywords: anti-hnRNP A1 antibody; anti-hnRNP A2/B1 antibody; cerebrospinal fluid; HTLV-I–associated myelopathy/tropical spastic paraparesis; multiple sclerosis

Introduction

Multiple sclerosis (MS) is a representative autoimmune disease of the central nervous system (CNS). The pathology of MS is characterized by demyelination and mononuclear cell infiltration, but the target antigen remains unclear (Steinman, 1996; Noseworthy *et al,* 2000; Berger *et al,* 2003). Human T-lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic progressive myelopathy occurring in HTLV-I carriers. The pathological mechanisms of HAM/TSP are not yet fully understood (Osame, 1990; Jacobsen *et al*, 1990; Kuroda *et al*, 1995; Izumo *et al*, 1996).

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are the major component of the nuclear core complex in eukaryote cells. They comprise approximately 30 major hnRNPs, designated hnRNP

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A1 to U, and are involved in a variety of cellular functions, including mRNA splicing, transport, and turnover (Burd and Dreyfuss, 1994; Krecic and Swanson, 1999). Among them, the hnRNP A1 and A2/B1 proteins are the most abundant and form a distinct subgroup of highly related proteins (Burd and Dreyfuss, 1994; Krecic and Swanson, 1999). Besides these cellular functions, the potent immunogenicity of hnRNP proteins was proposed to play a pathogenic role in several autoimmune diseases (Steiner *et al*, 1992; Meyer *et al*, 1993; Hassfeld *et al*, 1995; Jones *et al*, 2004; Greidinger *et al*, 2004). In neurological diseases, Levin and colleagues suggested that HAM/TSP might be an anti-hnRNP A1–mediated autoimmune disease (Levin *et al*, 1998, 2002; Jernigan *et al*, 2003). They reported that the cerebrospinal fluid (CSF) of all 13 assayed HAM/TSP patients contained anti-hnRNP A1 antibodies (Abs) (Levin *et al*, 2002). We have found anti-hnRNP A2/B1 Abs in the CSF of the majority of tested Japanese MS patients, implicating hnRNPs in MS (Sueoka *et al*, 2004). However, the detection of anti-hnRNP Abs was not performed under masked condition in either study (Levin *et al*, 2002; Sueoka *et al*, 2004). The purpose of this work was to assay Abs in the CSF samples of HAM/TSP and MS patients under fully masked conditions to confirm the previous results.

Results

Specificity of assay

The hnRNP B1 protein is identical to the A2 protein except for an additional 12 amino acids in the B1 N-terminus (Burd and Dreyfuss, 1994; Krecic and Swanson, 1999). Recombinant hnRNP B1 therefore reacted with both rabbit polyclonal anti-hnRNP A2 and anti-hnRNP B1 Abs (Figure 1A). Additionally, CSF samples tended to show stronger immunoreactivity against hnRNP B1 than hnRNP A2 in

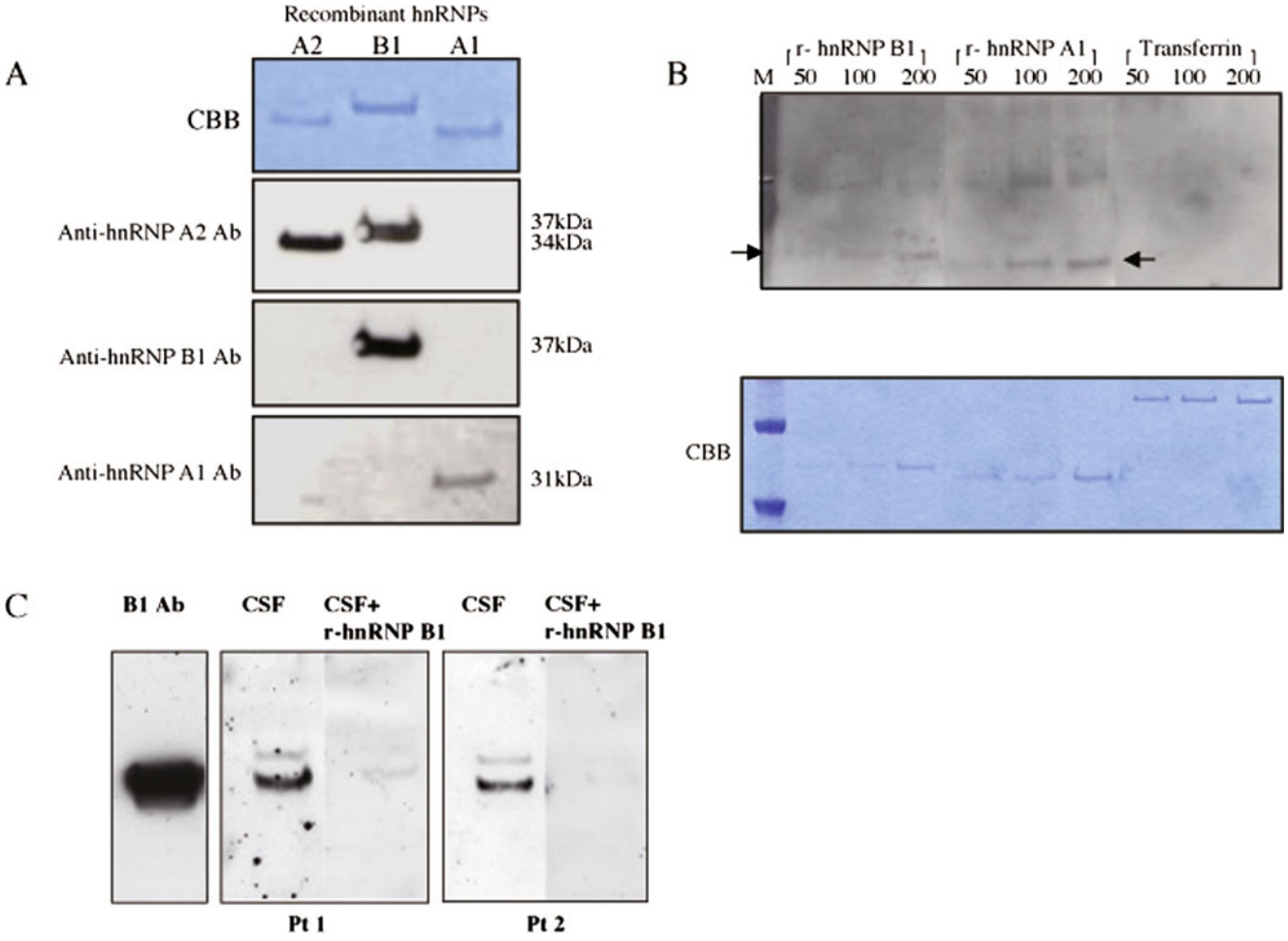

Figure 1 Purity of recombinant hnRNP A1, A2, and B1 and reactivity of the CSF with hnRNP A1 and B1. The purity of each recombinant hnRNP protein was confirmed by Western blotting with each specific antibody (**A**). The specificity of the immunoreaction was confirmed for each assay by ablation of the response with 200 ng of transferrin (**B**). The specificity of the immunoreactivity was also confirmed by experiments showing the disappearance of immunoreactivity against hnRNP B1 after pretreatment of the CSF with recombinant hnRNP B1 protein (**C**). CBB, Coomassie Brilliant Blue.

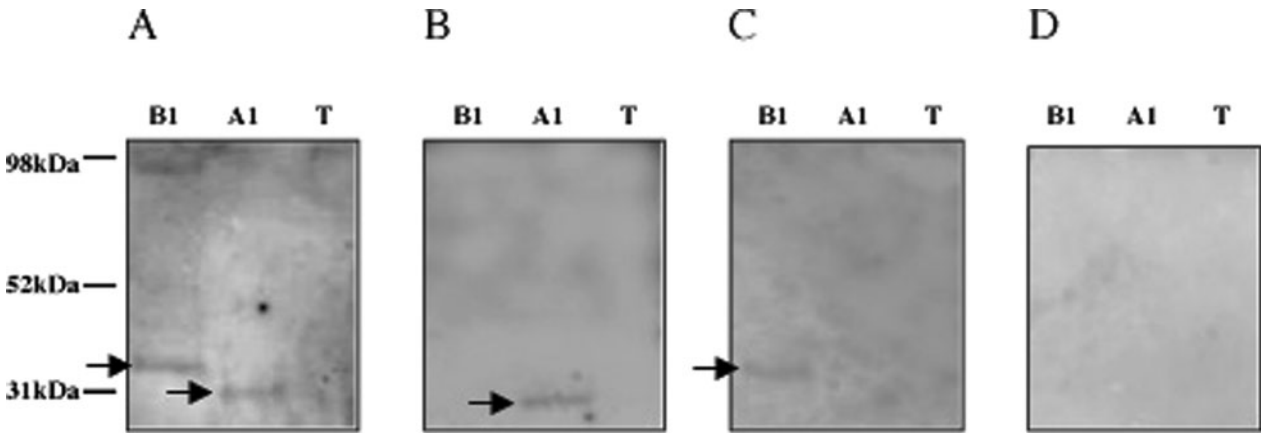

Figure 2 Immunoreactive modes of CSF with hnRNP A1 and B1. There were four patterns of immunoreactivity: reactive to both A1 and B1 (**A**), reactive to either A1 (**B**) or B1 (**C**), and no response (**D**).

preliminary experiments. Based on these findings, we used the recombinant hnRNP B1 protein as the antigen for anti-hnRNP A2 and anti-hnRNP B1 Ab assays. The specificity of immunoreactivity with hnRNP A1 and B1 was confirmed by using transferrin as a negative control in each assay (Figure 1B) and through an experiment showing the disappearance of immunoreactivity against hnRNP B1 upon pretreatment of CSF samples with hnRNP B1 (Figure 1C).

Immunoreactivity against hnRNP A1 and hnRNP B1

CSF samples showed various degrees of immunoreactivity against hnRNP A1 and B1 proteins in terms of both the intensity and the mode. There were four patterns in for immunoreactivity: reactive with both A1 and B1, reactive with either A1 or B1, or no response (Figure 2). The intensity of immunoreactivity also varied from faint to strong. Some CSF samples reacted equally to A1 and B1 and some reacted preferentially to one. Intensity levels were quantified and immunoreactive bands having an intensity ratio over 2.0 against transferrin were diagnosed as positive, as described in Materials and methods.

Antibody response against hnRNP A1 and hbRNP B1 for neurological diseases

After assaying immunoreactivity, we decoded CSF samples and determined the enrollment of the following neurological diseases: 28 cases of multiple sclerosis (MS), 40 of HAM/TSP, 16 of acute viral meningitis, and 21 cases of neurodegenerative diseases composed of 10 cases of amyotrophic lateral sclerosis (ALS) and 11 cases of hereditary spinocerebellar degeneration. Demographic data and results of the assay are summarized in Table 1. Both anti-hnRNP A1 and anti-hnRNP A2/B1 Abs were found most frequently in the CSF of MS patients, and there were significant differences in their incidence between MS and other disease groups ($P = .002$: positive for anti-hbRNP A1 Abs; $P < .001$: positive for anti-hbRNP A2/B1 Abs and positive for both Abs). The particularly high incidence (89.3%) of CSF anti-hnRNP A2/B1 Abs marked a clear delineation between MS and other disease groups (Tables 1 and 2). The pattern of immunoreactivity was also significantly different between MS and the other three disease groups. Namely, 71.4% of CSF samples from MS patients reacted with both hnRNP A1 and B1, whereas this pattern of immunoreactivity was observed in 32.5% of HAM/TSP patients, 12.5% of acute viral meningitis patients, and 9.5% of patients with neurodegenerative diseases (Table 1). When MS patients were compared to non-MS disease groups, the coexistence of anti-hnRNP A1 and anti-hnRNP A2/B1 Abs in the CSF was associated with MS, with a sensitivity of 71.4% and a specificity of 77.9% (Table 2). The presence of anti-hnRNP A2/B1 Abs in the CSF was associated with MS, with a sensitivity of 89.3%, but the specificity was 62.3% (Table 2).

In contrast, anti-hnRNP A1 Abs were positive in only 35% of the HAM/TSP patients (Table 1), and

Table 1 Incidence of CSF samples positive for anti-hn RNP A1 Abs, anti-hn RNP A2/B1 Abs, and both Abs

	MS	HAM/TSP	AM	NDD
No or cases	28	40	16	21
Mean age (range, year)	36.4 (15–74)	56.6 (9–73)	39.3 (17–65)	48.9 (13–76)
sex (F/M)	21/7	28/12	8/8	7/14
Positive for anti-A1 Abs*	20/28 (71.4%)	14/40 (35.0%)	5/16 (31.3%)	5/21 (23.8%)
Positive for anti-A2/Bl Abs**	25/28 (89.3%)	21/40 (52.5%)	5/16 (31.3%)	3/21 (14.3%)
Positive for both Abs**	20/28 (71.4%)	13/40 (32.5%)	2/16 (12.5%)	2/21 (9.5%)

Note MS: multiple sclerosis; HAM/TSP: HTLV-I–associated myelopathy/tropical spastic paraparesis; AM: acute viral meningitis; NDD: neurodegenerative diseases.

*Statistically significant $P = .002$ using chi-square test for two-by-four comparison.

**Statistically significant $P < .001$ using chi-square test for two-by-four comparison.

Table 2 Association of CSF samples positive for anti-hnRNP A1 Abs, anti-hnRNP A2/B1 Abs, and both Abs with MS

	MS ($n = 28$)	Non-MS ($n = 77$)	P value*
Anti-A1 Ab positive, *n* (%)	20 (71.4)	24 (31.2)	.003
Antl-A2/B1 Abs-positive, n (%)	25(89.3)	29 (37.7)	<.001
Both Abs-positive, *n* (%)	20 (71.4)	17 (22.1)	<.001

*Chi-square test.

there was no significant difference in the incidence between HAM/TSP and the other disease groups.

Discussion

hnRNP proteins have been suggested to be target antigens in the development of several autoimmune diseases (Steiner *et al*, 1992; Meyer *et al*, 1993; Hassfeld *et al*, 1995; Jones *et al*, 2004; Greidinger *et al*, 2004). Levin *et al* (2002) reported that anti-hnRNP A1 Abs were present in the CSF of all HAM/TSP patients examined. They also found that anti-hnRNP A1 Abs reacted with the tax protein of HTLV-I and suppressed neuronal activity (Levin *et al*, 2002), leading them to hypothesize a molecular mimicry–induced anti-hnRNP A1 Ab–mediated autoimmune mechanism for HAM/TSP (Levin *et al*, 2002; Jernigan *et al*, 2003). We have suggested that anti-hnRNP A2/B1 Abs could be a surrogate marker for MS (Sueoka *et al*, 2004) based on the presence of anti-hnRNP A2/B1 Abs in the CSF of 32 of 35 MS patients. The hnRNP A2 protein has been shown to play a critical role in the transport of myelin basic protein mRNA in oligodendroglia (Munro *et al*, 1999; Brumwell *et al*, 2002; Maggipinto *et al*, 2004), and we have suggested the possibility that immune responses against hnRNP A2 might cause demyelination in the CNS of MS patients (Sueoka *et al*, 2004).

The purpose of the present study was to determine whether anti-hnRNP Abs in the CSF are linked with MS and HAM/TSP using an assay from a previous study (Sueoka *et al*, 2004), but this time under fully masked conditions. These data could provide an important insight into the pathogenesis of MS and HAM/TSP. We obtained an incidence of 71% for anti-hnRNP A1 Abs and 89% for anti-hnRNP A2/B1 Abs in 28 MS patients in this study, which was almost identical to our previous results, where the incidence was 63% for anti-hnRNP A1 Abs and 91% for anti-hnRNP A2/B1 Abs (Sueoka *et al*, 2004). The confirmation of a high incidence of anti-hnRNP A2/B1 Abs in MS patients under fully masked conditions supports the hypothesis that anti-hnRNP A2/B1 Abs could be a surrogate marker for MS (Sueoka *et al*, 2004). However, our finding that anti-hnRNP A1 Abs were found in the CSF of only 35% of HAM/TSP patients without significant differences from other disease groups does not support the hypothesis that HAM/TSP is an anti-hnRNP A1 Ab–mediated disease (Levin *et al*, 2002). Two different mechanisms have been proposed for the pathogenic mechanism of HAM/TSP: (1) The interaction of HTLV-I–specific CD8+ cytotoxic lymphocytes (CTLs) and HTLV-I–infected CD4+ T cells may cause tissue damage in the CNS (Nagai and Jacobson, 2001; Hanon *et al*, 2001; Osame, 2002). Both types of cells have been shown to produce several proinflammatory and neurotoxic cytokines such as IFN-γ; (2) Immunoglobulin G specific to HTLV-I tax, which cross-reacts with the hnRNP A1 expressed in Betz cells, could induce damage to the cell (Levin *et al*, 2002). The present data provide support for this mechanism.

In this study, Abs against hnRNP A1 and B1 were also found in the CSF of patients with acute viral meningitis and neurodegenerative diseases such as ALS. However, the immune response pattern in these disorders was different from that of MS, and the majority of CSF samples from patients with these diseases reacted with either hnRNP A1 or B1 protein, but not both. It is well known that viral infections and neurodegenerative diseases can evoke autoimmune responses (Couratier *et al*, 1998).

In conclusion, the presence of anti-hnRNP A2/B1 Abs (as well as coexistence with anti-hnRNP A1 Abs) in the CSF could be a surrogate marker for MS, but our data did not show any significant increases in anti-hnRNP A1 Abs in the CSF of HAM/TSP patients.

Materials and methods

Patients and study design

We assayed CSF samples under fully masked conditions. For this study, the Kagoshima University group prepared coded CSF samples that were stored at −80°C. Informed consent for scientific research including the present study had been obtained from all patients. The Kagoshima University group randomly selected and numbered the CSF samples, and sent them to the Saga University group without any data of clinical diagnosis. After the Saga University group finished the assay, the two groups met and opened the masked data. Standard criteria were used for clinical diagnosis including the International Panel for Multiple Sclerosis (McDonald, 2001) and the World Health Organization (WHO) criteria for HAM/TSP (Osame, 1990). None of the patients enrolled in this study received corticosteroids or any other immunosuppressive drugs at the time of sampling.

Preparation and purification of recombinant hnRNP A1 and B1 proteins

The methods of preparation and purification of hnRNP A1, A2, and B1 proteins have been described in detail previously (Sueoka *et al*, 2001). Briefly, cDNAs were obtained by reverse transcriptase–polymerase chain reaction (RT-PCR) using primers

representing the following coding regions: sense, 5′-AGAGCTAGCTCTAAGTCAGAGTCTC-3′ and antisense, 5′-TTTCCCGGGAAATCTTCTGCCACTG-3′ for hnRNP A1; sense 5′-ATGGAGAGAGAAAAGGAA CAGTTCCGTAAGCTCTT-3′ and antisense, 5′-TACCCCGGGGTATCGGCTCCTCCCAC-3′ for hnRNP A2; and sense, 5′-CTCGCTGAGAAAACTTTAGAAA CTG-3′ and antisense, 5′-TACCCCGGGGTATCGGCT CCTCCCAC-3′ for hnRNP B1. The PCR products were purified and then subcloned into the pTYB2 vector of the IMPACT-CN system (New England BioLab, MA). Purity and product size were confirmed by both sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using a monoclonal Ab against hnRNP A1 (Santa Cruz Biotechnology, CA) and polyclonal Abs against hnRNP A2 and B1, which were obtained from rabbits immunized with 19-mer synthetic peptides derived from hnRNP A2 and B1, as previously described (Sueoka *et al*, 2001).

Western blotting

The immunoreactivity of the CSF with hnRNP A1 and B1 proteins was analyzed by Western blotting. CSF was used after 1:50 dilution with a blocking buffer. In the present study, based on the results of preliminary experiments, 100 ng/lane of recombinant hnRNP A1 and B1 were blotted on to BA-S-83–reinforced nitrocellulose membranes (Schleicher & Schuell, Keene, NH). After blocking with 5% skim milk, membranes were incubated with each specimen for 1.5 h, and then with second antibodies for an additional 1 h. The enhanced chemiluminescence (ECL) kit (Amersham, Arlington Heights, IL) was used for the detection of immunoreactive bands. Immunoreactivity against transferrin was also assayed as a negative control for each assay. The three authors scored the immunoreactivity as positive or negative independently. Then, the intensity of immunoreactive bands was analyzed using ImageJ software (http://www.rsb.info.nih.gov/ij, version 1.37v). Intensity levels were quantified and immunoreactive bands having an intensity ratio over 2.0 against transferrin were diagnosed as positive.

Statistical analysis

We used the chi-square test for two-by-two or two-by-four contingency tables to compare differences of the incidence of anti-hnRNP Abs in the CSF among the disease groups.

References

Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, Dilitz E, Deisenhammer F, Reindl M (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N Engl J Med* **349:** 139–145.

Brumwell C, Antolik C, Carson JH, Barbarese E (2002). Intracellular trafficking of hnRNP A2 in oligodendrocytes. *Exp Cell Res* **279:** 310–320.

Burd CG, Dreyfuss G (1994). Conserved structures and diversity of functions of RNA-binding proteins. *Science* **265:** 615–621.

Couratier P, Yi FH, Preud'homme JL, Clavelou P, White A, Sindou P, Vallat JM, Jauberteau MO (1998). Serum autoantibodies to neurofilament proteins in sporadic amyotrophic lateral sclerosis. *J Neurol Sci* **154:** 137–145.

Greidinger EL, Gazitt T, Jaimes KF, Hoffman RW (2004). Human T cell clones specific for heterogeneous nuclear ribonucleoprotein A2 autoantigen from connective tissue disease patients assist in autoantibody production. *Arthritis Rheum* **50:** 2216–2222.

Hanon E, Goon P, Taylor GP, Hasegawa H, Tanaka Y, Weber JN, Bangham CR (2001). High production of interferon but not interleukin-2 by human T-lymphotropic virus type I-infected peripheral blood mononuclear cells. *Blood* **98:** 721–726.

Hassfeld W, Steiner G, Studnicka-Benke A, Skriner K, Graninger W, Fischer I, Smolen JS (1995). Autoimmune response to the spliceosome. An immunologic link between rheumatoid arthritis, mixed connective tissue disease and systemic lupus erythematosus. *Arthritis Rheum* **38:**777–785.

Izumo S, Goto I, Itoyama Y, Okajima T, Watanabe S, Kuroda Y, Araki S, Mori M, Nagataki S, Matsukura S, Akamine T, Nakagawa M, Yamamoto I, Osame M (1996). Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicenter, randomized, double-blind, controlled trial. *Neurology* **46:** 1016–1021.

Jacobson S, Shida H, McFarlin DE, Fauci AS, Koenig S (1990). Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* **348:** 245–248.

Jernigan M, Morcos Y, Lee SM, Dohan FC Jr, Raine C, Levin MC (2003). IgG in brain correlates with clinicopathological damage in HTLV-1 associated neurologic disease. *Neurology* **60:** 1320–1327.

Jones DA, Yawalkar N, Suh KY, Sadat S, Rich B, Kupper TS (2004). Identification of autoantigens in psoriatic plaques using expression cloning. *J Invest Dermatol* **123:** 93–100.

Krecic AM, Swanson MS (1999). hnRNP complexes: composition, structure, and function. *Curr Opin Cell Biol* **11:** 363–371.

Kuroda Y, Matsui M, Yukitake M, Kurohara K, Takashima H, Takashima Y, Endo C, Kato A, Mihara F (1995). Assessment of MRI criteria for MS in Japanese MS and HAM/TSP. *Neurology* **45:** 30–33.

Levin MC, Krichavsky M, Berk J, Foley S, Rosenfeld M, Dalmau J, Chang G, Posner JB, Jacobson S (1998). Neuronal molecular mimicry in immune-mediated neurologic disease. *Ann Neurol* **44:** 87–98.

Levin MC, Lee SM, Kalume F, Morcos Y, Dohan FC Jr, Hasty KA, Callaway JC, Zunt J, Desiderio D, Stuart JM (2002).

Autoimmunity due to molecular mimicry as a cause of neurological disease. *Nat Med* **8:** 509–513.

Maggipinto M, Rabiner C, Kidd GJ, Hawkins AJ, Smith R, Barbarese E (2004). Increased expression of the MBP mRNA binding protein hnRNP A2 during oligodendrocyte differentiation. *J Neurosci Res* **75:** 614–623.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* **50:** 121–127.

Meyer O, Tauxe F, Fabregas D, Gabay C, Goycochea M, Haim T, Elias A, Kahn MF (1993). Anti-RA33 antinuclear autoantibody in rheumatoid arthritis and mixed connective tissue disease: comparison with antikeratin and antiperinuclear antibodies. *Clin Exp Rheumatol* **11:** 473–478.

Munro TP, Magee RJ, Kidd GJ, Carson JH, Barbarese E, Smith LM, Smith R (1999). Mutational analysis of a nuclear ribonucleoprotein A2 response element for RNA trafficking. *J Biol Chem* **274:** 34389–34395.

Nagai M, Jacobson S (2001). Immunopathogenesisi of human T cell lymphotropic virus type I-associated myelopathy. *Curr Opin Neurol* **14:** 381–386.

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000). Multiple sclerosis. *N Engl J Med* **343:** 938–952.

Osame M (1990). Review of WHO Kagoshima Meeting and Diagnostic guidelines for HAM/TSP. In: *Human retrovirology: HTLV*, 1st ed. Blattner WA (ed). New York: Raven Press, pp 191–197.

Osame M (2002). Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (HAM/TSP). *J NeuroVirol* **8:**359–364.

Steiner G, Hartmuth K, Skriner K, Maurer-Fogy I, Sinski A, Thalmann E, Hassfeld W, Barta A, Smolen JS (1992). Purification and partial sequencing of the nuclear autoantigen RA33 shows that it is indistinguishable from the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. *J Clin Invest* **90:** 1061–1066.

Steinman L (1996). Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* **85:** 299–302.

Sueoka E, Sueoka N, Goto Y, Matsuyama S, Nishimura H, Sato M, Fujimura S, Chiba H, Fujiki H (2001). Heterogeneous nuclear ribonucleoprotein B1 as early cancer biomarker for occult cancer of human lungs and bronchial dysplasia. *Cancer Res* **61:** 1896–1902.

Sueoka E, Yukitake M, Iwanaga K, Sueoka N, Aihara T, Kuroda Y (2004). Autoantibodies against heterogeneous nuclear ribonucleoprotein B1 in CSF of MS patients. *Ann Neurol* **56:** 778–786.