tein are not widely distributed in the mouse brain but are limited to expression in localized regions such as the midbrain, pons, hippocampus, and septum. Overall, the number of synemin-positive cells was low in the brain except in the midbrain and pons, suggesting a role for synemin in those regions. Further examination of the role of synemin-positive neurons in mammalian brain development and identification of proteins specifically interacting with synemin in those neurons will contribute to our understanding of synemin's function in the brain and help ascertain whether mutations in this gene contribute to human disease.

Supported by a Research Grant (17A-10) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare, Japan and a Grant-in-Aid for Scientific Research (C) (18590925) from the Japan Society for the Promotion of Science (to Y.M.). We thank Dr. Hart G.W. Lidov for careful reading of the manuscript.

REFERENCES

1. Blake DJ, Hawkes R, Benson MA, Beesley PW. Different dystrophin-like complexes are expressed in neurons and glia. J Cell Biol 1999;147:645–657.
2. Byers TJ, Lidov HGW, Kunkel LM. An alternative dystrophin transcript specific to peripheral nerve. Nat Genet 1993;4:77–81.
3. D'Souza VN, Nguyen TM, Morris GE, Karges W, Pillers DA, Ray PN. A novel dystrophin isoform is required for normal retinal electrophysiology. Hum Mol Genet 1995;4:837–842.
4. Hirako Y, Yamakawa H, Tsujimura Y, Nishizawa Y, Okumura M, Usukura J, et al. Characterization of mammalian synemin, an intermediate filament protein present in all four classes of muscle cells and some neuroglial cells: co-localization and interaction with type III intermediate filament proteins and keratins. Cell Tissue Res 2003;313:195–207.
5. Hugnot JP, Gilgenkrantz H, Vincent N, Chafey P, Morris GE, Monaco AP, et al. Distal transcript of the dystrophin gene initiated from an alternative first exon and encoding a 75-kDa protein widely distributed in nonmuscle tissues. Proc Natl Acad Sci U S A 1992;89:7506–7510.
6. Izmiryan A, Cheraud Y, Khanamiryan L, Leterrier JF, Federici T, Peltekian E, et al. Different expression of synemin isoforms in glia and neurons during nervous system development. Glia 2006;54:204–213.
7. Jing R, Pizzolato G, Robson RM, Gabbiani G, Skalli O. Intermediate filament protein synemin is present in human reactive and malignant astrocytes and associates with ruffled membranes in astrocytoma cells. Glia 2005;50:107–120.
8. Lederfein D, Levy Z, Augier N, Mornet D, Morris G, Fuchs O, et al. A 71-kilodalton protein is a major product of the Duchenne muscular dystrophy gene in brain and other nonmuscle tissues. Proc Natl Acad Sci U S A 1992;89:5346–5350.
9. Lidov HG, Selig S, Kunkel LM. Dp140: a novel 140 kDa CNS transcript from the dystrophin locus. Hum Mol Genet 1995; 4:329–335.
10. Mizuno Y, Thompson TG, Guyon JR, Lidov HG, Brosius M, Imamura M, et al. Desmuslin, an intermediate filament protein that interacts with alpha -dystrobrevin and desmin. Proc Natl Acad Sci U S A 2001;98:6156–6161.
11. Mizuno Y, Puca AA, O'Brien KF, Beggs AH, Kunkel LM. Genomic organization and single-nucleotide polymorphism map of desmuslin, a novel intermediate filament protein on chromosome 15q26.3. BMC Genet 2001;2:8.
12. Mizuno Y, Guyon JR, Watkins SC, Mizushima K, Sasaoka T, Imamura M, et al. Beta-synemin localizes to regions of high stress in human skeletal myofibers. Muscle Nerve 2004;30: 337–346.
13. Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates, 2nd ed. San Diego: Academic Press; 2001.
14. Peters MF, O'Brien KF, Sadoulet-Puccio HM, Kunkel LM, Adams ME, Froehner SC. beta-Dystrobrevin, a new member of the dystrophin family. Identification, cloning, and protein associations. J Biol Chem 1997;272:31561–31569.
15. Puca AA, Nigro V, Piluso G, Belsito A, Sampaolo S, Quaderi N, et al. Identification and characterization of a novel member of the dystrobrevin gene family. FEBS Lett 1998;425:7–13.
16. Sadoulet-Puccio HM, Khurana TS, Cohen JB, Kunkel LM. Cloning and characterization of the human homologue of a dystrophin related phosphoprotein found at the Torpedo electric organ post-synaptic membrane. Hum Mol Genet 1996;5:489–496.
17. Sadoulet-Puccio HM, Feener CA, Schaid DJ, Thibodeau SN, Michels VV, Kunkel LM. The genomic organization of human dystrobrevin. Neurogenetics 1997;1:37–42.
18. Sadoulet-Puccio HM, Rajala M, Kunkel LM. Dystrobrevin and dystrophin: an interaction through coiled-coil motifs. Proc Natl Acad Sci U S A 1997;94:12413–12418.
19. Sicinski P, Geng Y, Ryder-Cook AS, Barnard EA, Darlison MG, Barnard PJ. The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. Science 1989;244:1578–1580.
20. Sultana S, Sernett SW, Bellin RM, Robson RM, Skalli O. Intermediate filament protein synemin is transiently expressed in a subset of astrocytes during development. Glia 2000;30:143–153.
21. Titeux M, Brocheriou V, Xue Z, Gao J, Pellissier JF, Guicheney P, et al. Human synemin gene generates splice variants encoding two distinct intermediate filament proteins. Eur J Biochem 2001;268:6435–6448.
22. Xue ZG, Cheraud Y, Brocheriou V, Izmiryan A, Titeux M, Paulin D, et al. The mouse synemin gene encodes three intermediate filament proteins generated by alternative exon usage and different open reading frames. Exp Cell Res 2004; 298:431–444.

Reprinted from JOURNAL OF NEUROLOGY, NEUROSURGERY AND PSYCHIATRY, February 2007, Vol 78, p 196-197

PostScript

LETTERS

Hashimoto's encephalopathy presenting with progressive cerebellar ataxia

Hashimoto's encephalopathy is an autoimmune encephalopathy that came to be regarded as a new clinical entity distinct from myxo-oedema encephalopathy, associated with Hashimoto's thyroiditis.[1 2]

Hashimoto's encephalopathy has a wide clinical spectrum with various neuropsychiatric features. The detection of antithyroid antibodies in patient sera is helpful but not sufficient for the diagnosis of Hashimoto's encephalopathy because of the high prevalence of antibodies in the normal population.

Recently, we reported serum autoantibodies against the amino (NH_2) terminal region of α enolase (NAE) as a useful diagnostic marker of Hashimoto's encephalopathy.[3]

We describe here a patient with Hashimoto's encephalopathy, who presented with progressive cerebellar ataxia with mild abnormality on electroencephalography (EEG) and showed marked improvement after steroid administration. The patient was diagnosed as having Hashimoto's encephalopathy owing to the presence of the anti-NAE antibodies as well as antithyroid antibodies in the serum.

A 41-year-old woman, who had a normal dietary history, became aware of a slight unsteadiness while walking and mild dysarthria in December 2003. She had no familial history of neurological disorders or episodes of seizures. The symptoms gradually worsened, and she was admitted to the University of Fukui hospital in September 2004 because she could not stand or walk without support.

Neurological examinations showed severe gait ataxia, slurred speech and dysmetria on finger-to-nose and heel-to-knee manoeuvres. Cognitive functions and intellectual performance were normal. Ocular movement was full and smooth and without nystagmus. Deep tendon reflexes were normal and without any pathological reflex. No apparent paresis abnormal sensations including deep sensations, extrapyramidal signs or autonomic dysfunctions were found.

Magnetic resonance imaging of the brain did not detect any atrophy of the cerebellum or any abnormal signal. EEG showed diffuse slow-wave activities (7–8 Hz) without any epileptic discharge. Analysis of the cerebrospinal fluid did not show any pleocytosis or increases in protein (15 mg/dl) and immunoglobulin (Ig)G (1.2 mg/dl) levels. Peripheral blood cell counts, electrolytes, liver and kidney functions, and levels of lactate, ammonia, vitamins B_1, B_{12} and E were all normal. Serological markers specific for collagen diseases such as antinuclear, anti-DNA, anti-Sm, anti-RNP, anti-SSA, anti-SSB, anti-glutamic acid decarboxylase (GAD) antibodies, c-antineutrophil cytoplasmic antibodies and myeloperoxidase-antineutrophil cytoplasmic antibodies were either negative or in the normal range. Titters of antibodies against Herpes simplex, Varicella-zoster, Epstein-Barrvirus, cytomegalovirus and echo viruses were not raised in the serum or in the cerebrospinal fluid. Anti-Hu, anti-Yo and anti-Ri antibodies were also negative. Tumour markers such as carcinoembryonic antigen, cancer antigen (CA)19-9 and CA125 were normal. Investigation for malignancy did not detect any sign of malignant disease. Gene analyses did not detect any mutation for hereditary spinocerebellar ataxia (SCA1, SCA6, Machado–Joseph disease and dentate-rubro-pallido-luysian atrophy) or mitochondrial diseases (MELAS and MERRF).

Investigation for thyroid showed euthyroidism (thyroid-stimulating hormone (TSH), 1.7 μU/ml; normal, 0.4–4.0 μU/ml, free T_3, 3.4 pg/ml; normal, 2.4–4.3 pg/ml, free T_4, 0.9 ng/dl; normal, 0.9–1.8 ng/dl) and raised levels of antithyroid peroxidase (50.0 U/ml; normal, 0–0.3 U/ml) and antithyroglobulin antibodies (4.9 U/ml; normal, 0–0.3 U/ml). Anti-NAE antibodies in the serum—a diagnostic marker of Hashimoto's encephalopathy—were strongly positive before steroid treatment, then changed to a weak signal on an immunoblot, after treatment, determined using its recombinant protein expressed in human cultured cells, as described previously (fig 1).[3] The ethics committee of the University of Fukui approved this research. Written permission was obtained from the patient.

After intravenous administration of high-dose methylprednisolone (1 g/day) for 3 days, followed by oral administration of prednisolone (30 mg/day), the ataxia improved markedly, and the patient was able to walk unaided 3 weeks after the start of the treatment. The ataxia almost disappeared after continuous treatment for 3 months. Severity of ataxia was evaluated by the size of the estimated area when the patient stood on a stabilimeter. Estimated areas became markedly smaller on the stabilimeter after steroid treatment: pre-treatment area, $13.9 \times 8.4 = 116.76$ cm^2, post-treatment area, $4.8 \times 3.4 = 16.32$ cm^2. Slow-wave activities on EEG partially improved after the treatment.

Pure cerebellar ataxia can be caused by various reagents (alcohol or drugs), or may accompany vitamin deficiencies, viral infections, collagen diseases, autoimmune conditions (anti-GAD antibodies), spinocerebellar degenerations, neoplasm or mitochondrial diseases with or without cerebellar atrophy. Although anti-GAD antibodies were detected in the sera from patients with ataxia and type 1 diabetes,[4] the antibodies were not detected in our patient. Other possible causes of ataxia were excluded by the clinical history, and laboratory and radiological findings in the present case. Although hypothyroidism is another well-known cause of ataxia, she had normal thyroid functions.

Patients with Hashimoto's encephalopathy present with a variety of neuropsychiatric symptoms or signs such as hypertonia, tremors, myoclonus, choreoathetosis, seizures, dementia, psychiatric symptoms and strokes.[1 2] Ataxia is also reported in some patients with Hashimoto's encephalopathy.[2 5] Compared with these reported cases of Hashimoto's encephalopathy with ataxia, the clinical findings in our patient are unique: (1) absence of neurological findings other than ataxia except for mild EEG abnormality and (2) the insidious onset and slow progression of ataxia. Other reported cases of ataxia showed acute or subacute progressions accompanied by other neurological symptoms or signs.[2 5] Selim and Drachman[5] reported six patients with cerebellar ataxia associated with

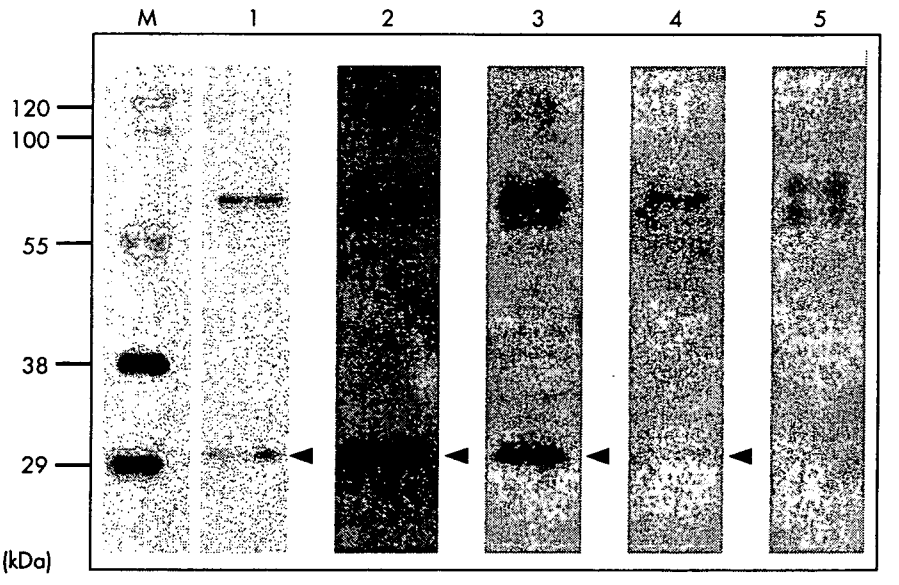

Figure 1 Immunoblot of a recombinant amino (NH_2) terminal region of α enolase (NAE) with sera from an ataxic patient with Hashimoto's encephalopathy. M, molecular weight marker; lane 1, recombinant NAE protein; lane 2, serum from a positive control (patient with Hashimoto's encephalopathy, lanes 3, serum from the present case before steroid treatment; lane 4, serum from the present case after steroid treatment; lane 5, serum from a normal control. Arrowheads indicate the position of NAE. Sera were diluted 300-fold. A strong signal against the NAE was detected in serum from the present case, and became weaker after steroid administration.

Hashimoto's thyroiditis, in most of whom cerebellar atrophy was shown on magnetic resonance imaging. One of their reported cases was treated by intravenous immunoglobulin IgG, and ataxia partially improved.[5] Although responsiveness to intravenous immunoglobulin suggested autoimmune mechanisms in the pathogenesis of ataxia in this patient, it remains uncertain whether or not ataxia and cerebellar atrophy were aetiologically associated with Hashimoto's thyroiditis. By contrast, our patient with progressive ataxia had a positive serological diagnostic marker, anti-NAE antibodies and showed an excellent response to steroid treatment, leading to a diagnosis of Hashimoto's encephalopathy.

In conclusion, this report suggests that a diagnosis of Hashimoto's encephalopathy is warranted in patients with progressive pure ataxia and anti-NAE antibodies are a useful serological marker of diagnosis. Moreover, Hashimoto's encephalopathy should be included in a differential diagnosis of a treatable ataxia.

Acknowledgements

We thank Tomomi Kame for technical assistance.

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doi: 10.1136/jnnp.2006.093005

Funding: This work was supported in part by a Neuroimmunological Disease Committee grant from the Ministry of Health, Labor and Welfare of Japan (to MY).

Competing interests: None declared.

References

1 **Shaw PJ**, Walls TJ, Newman PK, *et al.* Hashimoto's encephalopathy: a steroid-responsive disorder associated with high anti-thyroid antibody titers—report of 5 cases. *Neurology* 1991;41:228–33.
2 **Ferracci F**, Bertiato G, Moretto G. Hashimoto's encephalopathy: epidemiologic data and pathogenetic considerations. *J Neurol Sci* 2004;217:165–8.
3 **Fujii A**, Yoneda M, Ito T, *et al.* Autoantibodies against the amino terminal of α-enolase are a useful diagnostic marker of Hashimoto's encephalopathy. *J Neuroimmunol* 2005;162:130–6.
4 **Saiz A**, Arpa J, Sagasta A, *et al.* Autoantibodies to glutamic acid decarboxylase in three patients with cerebellar ataxia, late-onset insulin-dependent diabetes mellitus, and polyendocrine autoimmunity. *Neurology* 1997;49:1026–30.
5 **Selim M**, Drachman DA. Ataxia associated with Hashimoto's disease: progressive non-familial adult onset cerebellar degeneration with autoimmune thyroiditis. *J Neurol Neurosurg Neuropsychiatr* 2001;71:81–7.

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Printed in the UK by Henry Ling Limited, at The Dorset Press, Dorchester DT1 1HD

HL/JNNP/20/07

J Neurol (2007) 254:531–533
DOI 10.1007/s00415-006-0268-y

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Expression of vascular endothelial growth factor by plasma cells in the sclerotic bone lesion of a patient with POEMS syndrome

Received: 10 December 2005
Received in revised form: 8 April 2006
Accepted: 19 April 2006
Published online: 3 February 2007

Key words POEMS syndrome · VEGF · plasmacytoma · bone scintigraphy

Sirs: Bone lesions are frequently present in patients with POEMS syndrome [1, 2]. Since solitary bone lesions are often plasmacytomas, they must be aggressively treated with surgery or radiotherapy. We report a case of POEMS syndrome with a solitary bone lesion. In this patient, plasma cells in the osteosclerotic lesion were the source of vascular endothelial growth factor (VEGF).

A 42-year-old man developed distal dominant polyneuropathy two months after myocardial infarction. Following the onset of neurological symptoms, he exhibited edema, bristly skin, and swelling of the liver in the abdominal CT. Platelet counts were 822,000/mm^3, and immunoelectrophoresis demonstrated M-protein of IgA λ. Serum IL-6 and VEGF were measured using standardized ELISA (SRL, Inc., Tokyo, Japan). Although the IL-6 level was normal (2.1 pg/ml. Normal value; <4.0 pg/ml), the serum VEGF level was significantly elevated (18,500 pg/ml). He was diagnosed with POEMS syndrome. However, bone marrow aspiration from the ileum exhibited a normal appearance, and CT of the chest and the abdomen revealed no abnormal lesions, suggesting solitary or extramedullary plasmacytoma. Following steroid pulse therapy, treatment with prednisolone reduced the serum VEGF to 860 pg/ml with marked improvement of muscle weakness and skin lesions. Although the neuropathy remained clinically stable, he again exhibited edema and bristly skin 9 months after the pulse therapy, and the serum VEGF was increased to 5,120 pg/ml. Bone scintigraphy demonstrated a spot

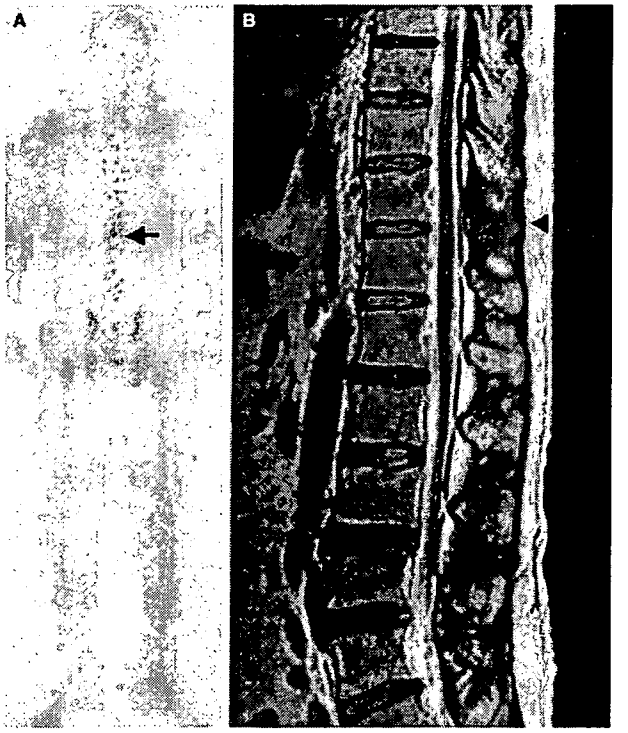

Fig. 1 (A) Bone scintigraphy showing a hot spot in Th12 (arrow). (B) T2 weighted MR image revealing a sclerotic lesion in the acantha of this vertebrae (arrowhead)

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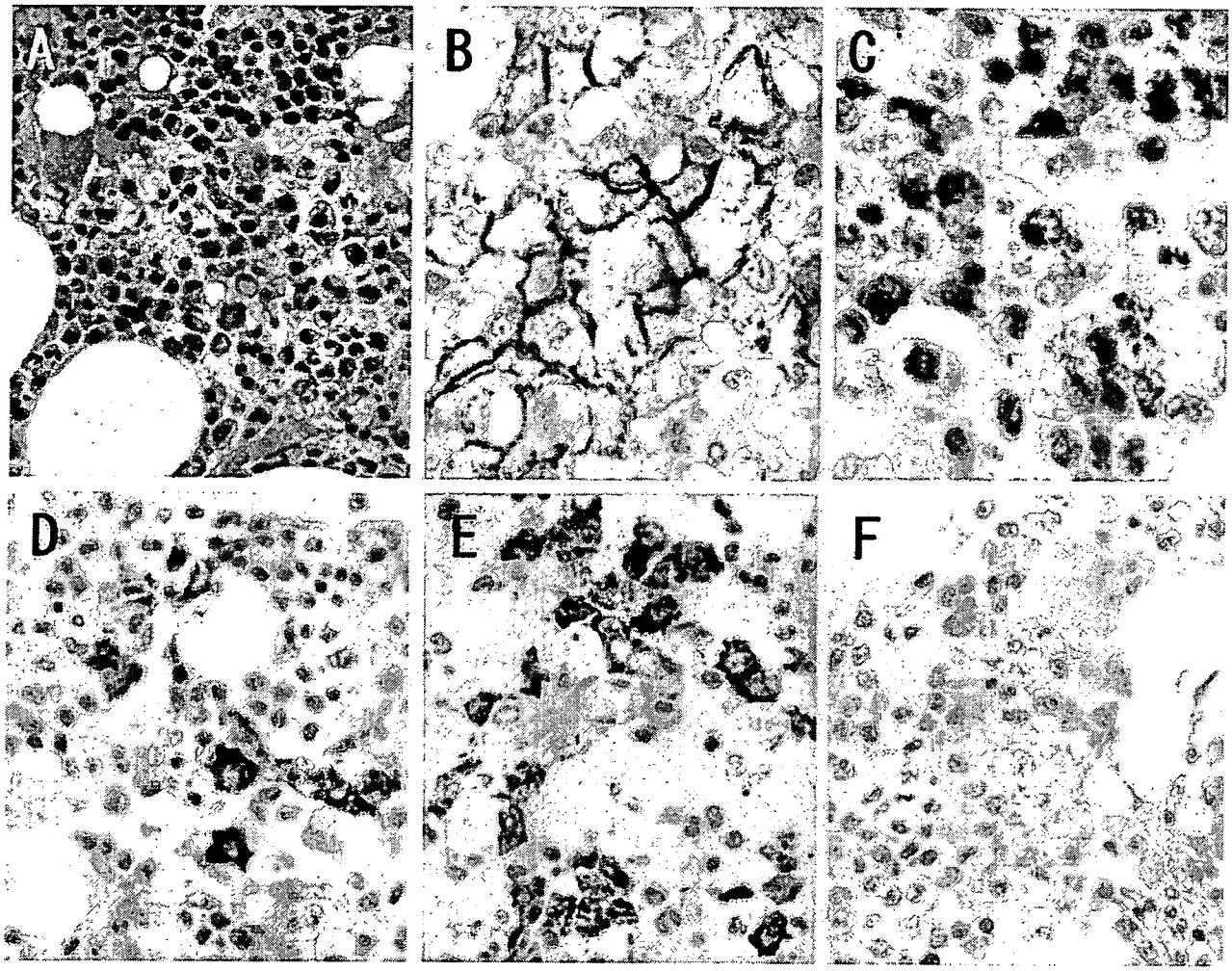

Fig. 2 (A) Hematoxylin and eosin staining exhibiting a slight increase in plasma cells in the osteosclerosic lesion. (B) Immunohistochemical stain of CD38. (C) Immunohistochemistry revealing immunoreactivity for VEGF in the cytoplasm of plasma cells. (D-F) Immunohistochemical stain of IgA (D), λ light chain (E), and κ light chain (F)

of abnormal accumulation in Th11 of the spine (Fig. 1). Bone radiography and MRI revealed a sclerotic lesion in the spinous process of the vertebra (Fig. 1). As solitary plasmacytoma was suspected, resection of the Th11 acantha was performed. After the operation, the serum VEGF levels gradually reduced to 620 pg/ml, and he has not shown the relapse of symptoms or re-elevation of the serum VEGF levels. The histological diagnosis of the resected Th11 acantha was plasmacytoma. Focal accumulation of CD38-positive plasma cells was found in the osteosclerotic lesion (Fig. 2A, B). Immunohistochemical staining for VEGF (anti-VEGF-Ab-2; Calbiochem, Darmstadt, Germany) demonstrated positive staining in the cytoplasm of most plasma cells (Fig. 2C). These cells were strongly positive for IgA and λ light chain (Fig. 2D-F).

POEMS syndrome is a rare multisystemic disease that is associated with plasma cell dyscrasia and is characterized by elevated serum VEGF levels [3, 4]. Since the symptoms of POEMS syndrome correlate well with changes in VEGF levels, VEGF is probably involved in the pathogenesis of POEMS syndrome [5, 6]. In this condition, plasma cells, platelets, tumor cells, endothelial cells, or non-myelinating Schwann cells have been proposed as a source of VEGF [7–9]. However, it remains unclear which cell type is responsible for the increased VEGF production. The present case suggests that plasma cells in the osteosclerotic lesions could be one major source of VEGF.

Many treatment strategies, including irradiation, corticosteroids, and alkylator-based therapy, have been used for POEMS syndrome [1, 2]. Recent report indicated the therapeutic potential in treating POEMS syndrome of using autologous peripheral blood stem cell transplantation with high-dose chemotherapy [10]. Corticosteroid and a combination of melphalan and corticosteroid are effective in approximately 22% to 56% of patients [1]. The present patient responded to steroid therapy. However, he did not show continued improvement. Previously, radiation or excision of bone lesions have been the most effective treatment for POEMS syndrome [1, 2]. Therefore, intensive examination for bone lesions should contribute to improved outcome in the treatment of POEMS syndrome.

References

1. Dispenzieri A, Kyle RA, Lacy MQ, Rajkumar SV, Therneau TM, Larson DR, Greipp PR, Witzig TE, Basu R, Suarez GA, Fonseca R, Lust JA, Gertz MA (2003). POEMS syndrome: definitions and long-term outcome. Blood 101:2496–2506
2. Soubrier MJ, Dubost JJ, Sauvezie BJ (1994). POEMS syndrome: a study of 25 cases and a review of the literature. French Study Group on POEMS Syndrome. Am J Med 97:543–553
3. Watanabe O, Arimura K, Kitajima I, Osame M, Maruyama I (1996). Greatly raised vascular endothelial growth factor (VEGF) in POEMS syndrome. Lancet 347:702
4. Soubrier M, Dubost JJ, Serre AF, Ristori JM, Sauvezie B, Cathebras P, Piette JC, Chapman A, Authier FJ, Gherardi RK (1997). Growth factors in POEMS syndrome: evidence for a marked increase in circulating vascular endothelial growth factor. Arthritis Rheum 40:786–787
5. Watanabe O, Maruyama I, Arimura K, Kitajima I, Arimura H, Hanatani M, Matsuo K, Arisato T, Osame M (1998). Overproduction of vascular endothelial growth factor/vascular permeability factor is causative in Crow-Fukase (POEMS) syndrome. Muscle Nerve 21:1390–1397
6. Endo I, Mitsui T, Nishino M, Oshima Y, Matsumoto T (2002). Diurnal fluctuation of edema synchronized with plasma VEGF concentration in a patient with POEMS syndrome. Intern Med 41:1196–1198
7. Hashiguchi T, Arimura K, Matsumuro K, Otsuka R, Watanabe O, Jonosono M, Maruyama Y, Maruyama I, Osame M (2000). Highly concentrated vascular endothelial growth factor in platelets in Crow-Fukase syndrome. Muscle Nerve 23:1051–1056
8. Nakano A, Mitsui T, Endo I, Takeda Y, Ozaki S, Matsumoto T (2001). Solitary plasmacytoma with VEGF overproduction: report of a patient with polyneuropathy. Neurology 56:818–819
9. Scarlato M, Previtali SC, Carpo M, Pareyson D, Briani C, Del Bo R, Nobile-Orazio E, Quattrini A, Comi GP (2005). Polyneuropathy in POEMS syndrome: role of angiogenic factors in the pathogenesis. Brain 128:1911–1920
10. Kuwabara S, Misawa S, Kanai K, Kikkawa Y, Nishimura M, Nakaseko C, Cho RK, Hattori T (2006). Autologous peripheral blood stem cell transplantation for POEMS syndrome. Neurology 66: 105–107

Letter to the Editor

European Neurology

Eur Neurol 2007;57:238–240
DOI: 10.1159/000101289

Received: March 9, 2006
Accepted: October 16, 2006
Published online: March 26, 2007

A Chronic Progressive Case of Enteroviral Limbic Encephalitis Associated with Autoantibody to Glutamate Receptor ε2

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Dear Sir,

Enteroviruses – coxsackieviruses and echoviruses – are frequent causes of aseptic meningitis, particularly in children. These viruses occasionally cause mild diffuse encephalitis but are rarely life threatening [1]. Herein, we describe a patient with chronic progressive limbic encephalitis caused by enterovirus, who was positive for autoantibodies against glutamate receptor (GluR) IgG-ε2 in the cerebrospinal fluid (CSF), and in whom lesions were found in the bilateral hippocampus, medial temporal lobe and hypothalamus.

A 22-year-old right-handed male was referred to us in August 1995 because of fever, a 3-week history of sudden onset memory loss, repetitive questioning and subacute progressive mental confusion. He had no pyramidal or extrapyramidal disturbances or cerebellar ataxia. Fluid-attenuated inversion-recovery (FLAIR) MRI of the brain showed hyperintense lesions in the bilateral hippocampal structures (fig. 1A). No enhancement was seen after intravenous gadolinium. Routine laboratory tests, including complete blood count and blood chemistry, were normal. Serum antinuclear, anti-DNA, anti-Sm, anti-SSA/SSB, anti-RNP, anti-Scl-70 and anti-Hu antibodies were negative. Analysis of CSF showed 5 cells/mm^3 (mononuclear), protein concentration 54 mg/dl and glucose 63 mg/dl. PCR for herpes simplex virus (HSV) DNA was negative. He was treated with acyclovir (30 mg/kg/day for 14 days) and betamethasone (16 mg/day for 7 days) based on the diagnosis of limbic encephalitis. His symptoms and MRI abnormalities gradually disappeared by the second hospital month, and he was discharged without any sequelae.

In November 1995, the patient again experienced a week of fever, headache and disorientation. At second admission, the patient was disoriented with memory impairment, including anterograde and retrograde amnesia. His Mini-Mental State Examination score was 17/30. FLAIR MRI again revealed hyperintense lesions in the bilateral hippocampal structures (fig. 1B). CSF examination showed 6 cells/mm^3 (mononuclear), protein concentration 42 mg/dl and glucose 56 mg/dl. He improved again by treatment with acyclovir and betamethasone.

In January 1998, this patient first experienced a generalized seizure. In October 1998, the seizures increased in frequency, and he was admitted to our hospital. He had an amnestic syndrome and his attention was easily distracted by irrelevant environmental incidents. He also exhibited sleep apnea syndrome, and MRI showed expansion and swelling of a left temporal lobe lesion (fig. 1C). He was intubated and treated with acyclovir, betamethasone and an anticonvulsant. In February 1999, although the left temporal lobe lesion had reduced, an expanded lesion was observed in the right temporal lobe with gadolinium enhancement in the hypothalamus (fig. 1D, E). Based on the suspected diagnosis of secondary autoimmune encephalitis, mizoribine (a purine, antimetabolic, immunosuppressive agent) was added. After that, no relapse of the symptoms of encephalitis or change in MRI findings was observed. In March 2001, MRI demonstrated severe atrophy of the bilateral hippocampal gyrus and uncus (fig. 1F). He was alert and followed simple commands. However, he had severe impairment of memory retention, and he could not be extubated because of sleep apnea syndrome.

Through the course of illness, the patient was examined extensively for the causes of chronic progressive limbic en-

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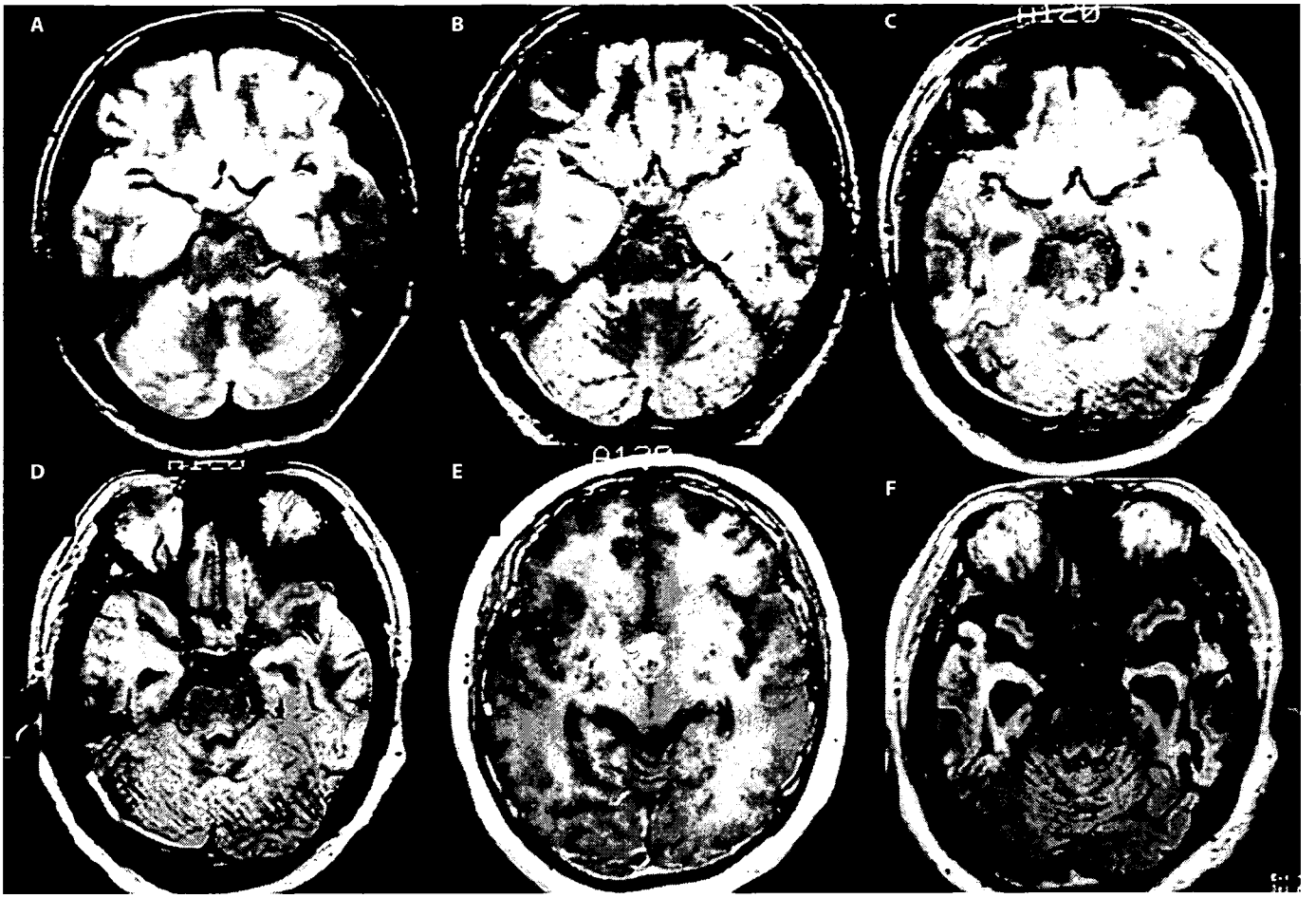

Fig. 1. Serial axial FLAIR MRI (**A–D, F**) and gadolinium-enhanced T_1-weighted image (**E**). Hyperintense lesions were seen in the bilateral hippocampus in August 1995 (**A**) and November 1995 (**B**). Expansion of the hyperintensity was seen in the left temporal lobe in January 1998 (**C**). In February 1999, hyperintensity was detected in the right temporal lobe and gadolinium enhancement in the hypothalamus (**D, E**). Severe atrophy of the bilateral hippocampus was observed in March 2001 (**F**).

cephalitis. He did not have any immunosuppressive disorders, such as HIV or agammaglobulinemia, and exhibited no abnormalities in either humoral or cell immunity. PCR studies of CSF were negative for HSV-1, HSV-2, cytomegaly, varicella-zoster and human herpes virus 6. Measles, rubella and mumps were also serologically excluded. However, RT-PCR [2] revealed enterovirus RNA in 2 CSF samples obtained in August and November 1995. Although enterovirus RNA was not detected after 1998, 3 CSF samples exhibited positivity for autoantibodies against GluR IgG-ε2 from 1998 to 1999 [3].

The nonpolio serotypes most often associated with central nervous system (CNS) infection include echoviruses 7, 9, 11 and 30; coxsackievirus B5 and enterovirus 71 [1]. Although infection with enterovirus 71 has a high mortality rate, most patients with enteroviral CNS infection exhibit a mild, generalized disease that resolves without sequelae. Severe and chronic cases of focal encephalitis have been reported [4–6], even in immunocompromised hosts [7, 8]. Hokezu et al. [9] reported an immunocompetent patient with recurrent limbic encephalitis in which RT-PCR was able to detect echovirus 7 RNA in CSF samples. The present case was remarkable because chronic progressive limbic encephalitis and severe sequelae occurred in this patient without immunodeficiency and autoantibodies against GluR IgG-ε2 being detected in the CSF of this patient. Limbic encephalitis is caused by HSV, nonherpetic viruses, paraneoplastic condition or autoimmune disorders. Recently, the presence of autoantibodies against GluR-ε2 in the CSF has been suggested to be involved in the parainfectious autoimmune pathogenesis of limbic encephalitis. Autoantibodies against GluR-ε2 may be associated with some symptoms of limibic encephalitis and sequelae (epilepsy and mental deterioration) of widespread encephalitis in childhood [10]. Moreover, they were speculated to be associated with atrophy of the hippocampus and impairment of memory. In

this case, we presumed that autoantibodies against GluR IgG-ε2 might have influenced the chronic progressive course. Enteroviral infection should be included in the differential diagnosis of limbic encephalitis. Furthermore, viral infections, such as enterovirus, can trigger the production of autoantibodies against GluR IgG-ε2 in patients with limbic encephalitis.

Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research (2006-7; 18590959) and Health and Labour Sciences Research Grants for Research on Psychiatry and Neurological Diseases and Mental Health (H17-017).

References

1 Rotbart HA: Enteroviral infections of the central nervous system. Clin Infect Dis 1995; 20:971–981.
2 Hosoya M, Honzumi K, Sato M, Katayose M, Kato K, Suzuki H: Application of PCR for various neurotropic viruses on the diagnosis of viral meningitis. J Clin Virol 1998;11:117–124.
3 Takahashi Y, Mori H, Mishina M, Watanabe M, Fujiwara T, Shimomura J, Aiba H, Miyajima T, Saito Y, Nezu A, Nishida H, Imai K, Sakaguchi N, Kondo N: Autoantibodies to NMDA receptor in patients with chronic forms of epilepsia partialis continua. Neurology 2003;61:891–896.
4 Modlin JF, Dagan R, Berlin LE, Virshup DM, Yolken RH, Menegus M: Focal encephalitis with enterovirus infections. Pediatrics 1991; 88:841–845.
5 Zifroni A, Fried M, Shalmon B, Hoffman M, Yust I: Severe encephalitis with rapid recovery. Lancet 1997;349:1328.
6 Liow K, Spanaki MV, Boyer RS, Greenlee JE, Bale JF Jr: Bilateral hippocampal encephalitis caused by enteroviral infection. Pediatr Neurol 1999;21:836–838.
7 Cree BC, Bernardini GL, Hays AP, Lowe G: A fatal case of coxsackievirus B4 meningoencephalitis. Arch Neurol 2003;60:107–112.
8 McKinney RE Jr, Katz SL, Wilfert CM: Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. Rev Infect Dis 1987;9:334–356.
9 Hokezu Y, Hashiguchi Y, Satake M, Hosoya M: A case report of recurrent limbic encephalitis caused by an enterovirus infection (in Japanese with English abstract). Rinsho Shinkeigaku 2004;44:165–170.
10 Takahashi Y: Infections as causative factors of epilepsy. Future Neurol 2006;1:291–302.

Increased Intrathecal Chemokine Receptor CCR2 Expression in Multiple Sclerosis

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Abstract: Expression of CCR2, CXCR3 and CCR4 on $CD4^+$ T or $CD8^+$ T cells in blood and cerebrospinal fluid (CSF) for multiple sclerosis (MS) was measured by 3-color flow cytometry, and compared to blood from healthy controls and CSF from patients with other inflammatory neurological diseases (INDs). $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio (representing Th1/Th2 balance) was higher in both CSF and blood of MS patients than those of IND patients or healthy controls. Percentage of CCR2-positive T cells was significantly higher in CSF from MS patients. Increased CCR2 expression on T cells in CSF and Th1/Th2 imbalance may reflect the pathological processes involved in MS.

Keywords: multiple sclerosis, CCR2, CXCR3, CCR4, chemokine receptor, chemokine

Introduction

Multiple sclerosis (MS) is a chronic and progressive inflammatory immune-mediated demyelinating disease. Recruitment of peripheral blood mononuclear cells into the central nervous system (CNS) plays a crucial role in the pathogenesis of this condition. Chemokines are low molecular weight cytokines produced in inflamed tissue, mediating the recruitment of specific leukocyte populations expressing chemokine receptors. Recent studies have shown that T cells expressing CCR5 and CXCR3 can be detected within perivascular lesions of brains with MS [Simpson et al. 1998; Balashov et al. 1999; Sorensen et al. 1999], and T cells expressing these receptors are increased in cerebrospinal fluid (CSF) compared with peripheral blood [Kivisakk et al. 2002; Sorensen et al. 1999, 2002; Teleshova et al. 2002]. Increased expression of CXCR3 on $CD4^+$ T cells in peripheral blood from MS patients compared to those of healthy individuals has also been reported [Nakajima et al. 2004a]. Since such chemokine receptors are preferentially expressed on Th1 cells, these cells are considered critical in the pathogenesis of MS. In contrast, expression of CCR4 and CCR3 (Th2-type receptors) on $CD4^+$ T cells is significantly decreased in the blood of MS patients at the time of relapse compared to healthy controls, and CCR4 expression is significantly decreased in CSF $CD4^+$ T cells compared to peripheral blood $CD4^+$ T cells [Misu et al. 2001]. These findings suggest that Th2 response is suppressed in the acute phase of MS. Th1/Th2 balance may thus play an important role in this relapsing-remitting disorder [Misu et al. 2001; Nakajima et al. 2004a].

In experimental autoimmune encephalomyelitis (EAE), CCL2 and CCR2, the main receptor for CCL2, are expressed on astrocytes, macrophages and T cells in CNS lesions during the acute phase [Jee et al. 2002]. CCR2-knockout mice are also resistant to EAE [Fife et al. 2000; Izikson et al. 2000]. These findings suggest that CCR2 and CCL2 are key susceptibility factors in EAE. Unexpectedly, CCL2 concentrations in CSF from MS patients after relapse are reduced when concentrations of other inflammatory chemokines such as CCL1, CCL5 and CXCL10 are increased [Bartosik-Psujek and Stelmasiak, 2005; Mahad et al. 2002; Nakajima et al. 2004a; Sorensen et al. 1999, 2001]. Since CCL2 has been shown to induce Th2 reactions [Gu et al. 2000; Nakajima et al. 2001], CCL2 production may be suppressed in the Th1-predominant immunological conditions of active MS. However, both CCR2 and CCL2 are abundantly detected in plaque lesions from MS patients [Mahad and Randsohoff, 2003; McManus et al. 1998]. Multiple compensatory mechanisms, particularly

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concerned with CCL2-CCR2, appear to exist in the pathogenesis of MS.

The present study examined expressions of CXCR3, CCR4 and CCR2 on $CD4^+$ T and $CD8^+$ T cells in blood and CSF from relapsing-remitting MS patients and CSF from patients with other inflammatory neurological diseases (INDs). Comparing these groups, we evaluated specific T-cell populations trafficking to the MS lesion. The results indicate that CCR2 upregulation in CSF occurs in MS.

Materials and Methods

Subjects

Paired blood and CSF samples were obtained from 10 patients with relapsing-remitting MS during the relapse phase (8 women, 2 men; mean (±SD) age, 44 ± 9 years (range, 20–56 years); mean disease duration 6 ± 5 years (range, 0.5–13 years); mean expanded disability status scale (EDSS) [Kurtzke et al. 1983] 3 ± 1.5 (range, 1.5–5.5)) (Table 1). All patients included in this study displayed definite MS, according to McDonald criteria [McDonald et al. 2001]. Samples were obtained from these patients before administration of corticosteroid pulse therapy. CSF samples were also collected from 10 patients with IND (6 women, 4 men; mean age, 39 ± 16 years (range, 18–57 years)). IND comprised viral meningitis/encephalitis (n = 8), acute disseminated encephalomyelitis (n = 1) or neuro-Behçet's disease (n = 1). All 10 patients displayed mononuclear pleocytosis (Table 1). As control samples, blood samples were collected from 10 healthy individuals (4 women, 6 men; mean age, 33 ± 9 years (range, 21–52 years)).

Flow Cytometry

Venous blood was collected in heparinized tubes and analyzed within 2 h after sampling. CSF cells were collected by centrifugation (250 × *g* for 10 min) and resuspended in PBS with 1% BSA. Whole blood and CSF cells ($\geq 5 \times 10^3$ cells/test) were labeled with directly conjugated monoclonal antibodies, according to the instructions of the manufacturer, using anti-CD3 PerCP (Becton Dickinson, San Jose, CA), anti-CD4 FITC, anti-CD8 FITC (Pharmingen, San Diego, CA), anti-CCR4 PE, anti-CXCR3 PE (Pharmingen, San Diego, CA) and anti-CCR2 PE (Dako, Kyoto, Japan), in addition to isotype-specific antibody controls. Cells were fixed in 2% paraformaldehyde and stored in the dark before analysis using a FACS flow cytometer (Becton Dickinson). Flow cytometry data were processed using CellQuest software (Becton Dickinson). Data are reported as percentages of all T cells (identified as $CD3^+$ cells) staining positively for $CD4^+CXCR3^+$, $CD8^+CXCR3^+$, $CD4^+CCR4^+$, $CD8^+CCR4^+$, $CD4^+CCR2^+$ or $CD8^+CCR2^+$.

Statistical analysis

Comparisons between expressions of chemokine receptors in CSF and blood from MS patients, CSF from patients with IND and blood from healthy controls were performed by nonparametric Mann-Whitney U and Kruskall-Wallis tests. Values of $p < 0.05$ were considered statistically significant.

Results

Comparison of Th1/Th2-related chemokine receptor expression

The percentage of $CD4^+$ or $CD8^+$ T cells expressing these chemokine receptors among all $CD3^+$ T cells was determined by 3-color flow cytometry using anti-CD3 antibody and anti-CD4 antibody or anti-CD8 antibody and one of the following anti-chemokine receptor antibodies: anti-CXCR3

Table 1. Patient demographics and CSF findings.

	Gender (M:F)	Age median (range) (years)	CSF WBC (/mm3)	CSF protein (mg/dl)
MS (n = 10)	2:8	44 (20–56)	20 ± 6	53 ± 11
IND (n = 10)	4:6	39 (18–57)	183 ± 112	62 ± 24
Healthy (n = 10)	6:4	33 (21–52)	NA	NA

CSF WBC and protein are presented as mean ±SD.
Abbreviation: CSF: cerebrospinal fluid; WBC: white blood cells; MS: multiple sclerosis; IND: other inflammatory neurological diseases; NA: not applicable.

antibody; anti-CCR4 antibody; or anti-CCR2 antibody. In peripheral blood, percentages of $CD4^+CXCR3^+$ cells were higher for MS patients than for healthy controls ($p = 0.013$; Fig. 1A). However, no differences were observed in percentages of other subsets (Fig. 1A). When comparing CSF and blood in MS patients, percentages of all subsets other than $CD8^+CXCR3^+$ cells were significantly higher in CSF (Fig. 1B). Expressions of all chemokine receptors were also elevated in CSF from IND patients compared levels in blood for healthy controls (Fig. 1C). However, no differences were observed between percentages of $CD4^+CXCR3^+$, $CD8^+CXCR3^+$, $CD4^+CCR4^+$ or $CD8^+CCR4^+$ cells between groups (Fig. 1C). These findings suggest that activated T lymphocytes cross the blood-brain barrier (BBB) and contribute to inflammatory response in the pathogenesis of MS.

Comparison of $CD4^+CXCR3^+$/ $CD4^+CCR4^+$ ratios

As CXCR3 is expressed on Th1 cells and CCR4 is expressed on Th2 cells, $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio represents Th1/Th2 balance. $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio in blood was significantly higher for MS patients than for healthy controls ($p = 0.034$). In MS patients, $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio was similar between CSF and blood. However, $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio in CSF was significantly lower for IND patients than for MS patients ($p = 0.042$; Fig. 2). These findings suggest that Th1/Th2 imbalance indicates the pathological nature of MS.

Comparison of CCR2 expression

When comparing CSF and blood in MS patients, percentages of $CD4^+CCR2^+$ and $CD8^+CCR2^+$ cells

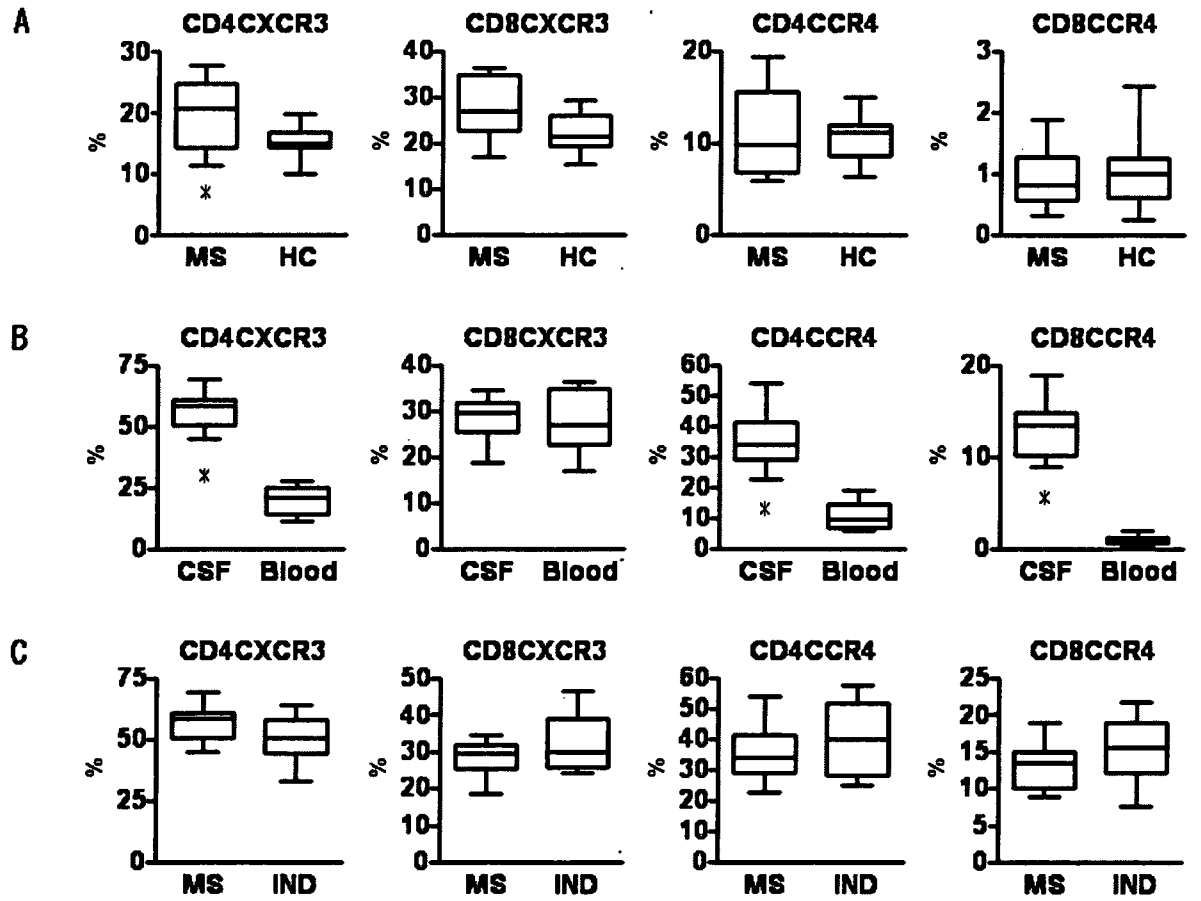

Figure 1. Comparison of chemokine receptor expression on blood T cells between MS and healthy controls (**A**), on T cells between CSF and blood from MS (**B**), and on CSF T cells between MS and IND (**C**). Data are shown as percentages of all T cells staining positive for $CD4^+CXCR3^+$, $CD8^+CXCR3^+$, $CD4^+CCR4^+$ or $CD8^+CCR4^+$.

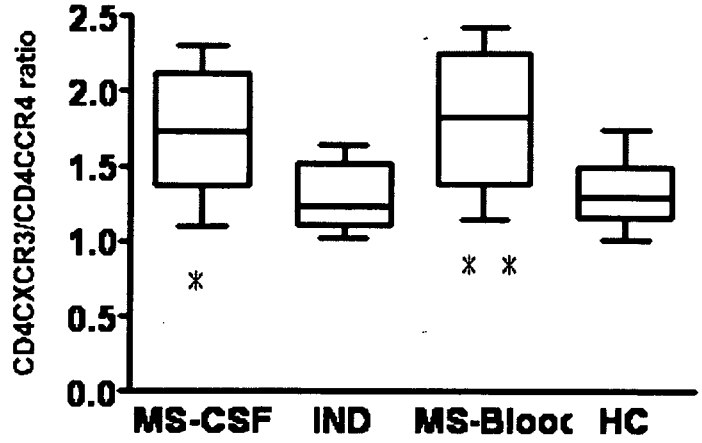

Figure 2. Comparison of $CD4^+CXCR3^+/CD4^+CCR4^+$ between CSF and blood from MS patients, CSF from patients with IND, and blood from healthy controls. As $CD4^+CXCR3^+$ cells are Th1 cells and $CD4^+CCR4^+$ cells are Th2 cells, $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio represents Th1/Th2 balance.

were significantly increased in CSF ($p = 0.002$ and 0.014, respectively; Fig. 3). In CSF, CCR2 expression on CSF $CD4^+$ and $CD8^+$ cells was also higher for MS patients than for IND patients ($p = 0.023$ and 0.028, respectively; Fig. 3). CCR2 expression was highest in CSF from MS patients (Fig. 3). These findings suggest that CCR2-expressing T cells play a pivotal role in the pathogenesis of MS.

Discussion

Recruitment of autoreactive T lymphocytes from blood to the CNS plays a crucial role in the pathogenesis of MS, as this process initiates the inflammatory response leading to demyelination and axonal degeneration. Interactions between chemokines and chemokine receptors are thought to be important in the activation and migration of activated T lymphocytes across the BBB. Investigation of chemokine receptor expression on T cells in the blood and CSF of MS patients is thus key to understanding the pathogenic processes in MS. In the present study, percentages of CXCR3-, CCR4- and CCR2-expressing T cells were higher in CSF than in blood, and this characteristic was more pronounced in CD4 T cells than in CD8 T cells. These findings suggest that activated T lymphocytes, including myelin-reactive $CD4^+$ T cells, cross the BBB and contribute to the inflammatory response (Fig. 1). No differences in percentages of CXCR3-expressing $CD8^+$ T cells were identified in this study between CSF and blood from MS patients. Previously, a significant increase in percentage of CXCR3-expressing $CD8^+$ T cells was found in CSF compared to blood from MS patients [Misu et al. 2001]. In the present study, expression of chemokine receptors was measured using 3-color flow cytometry. Expressions of each chemokine on $CD4^+$ and $CD8^+$ T cells are shown as percentages of all T cells. Since our results demonstrated a greatly increased percentage of CXCR3-expressing $CD4^+$ T cells in CSF from MS patients, the percentage of CXCR3-expressing $CD8^+$ T cells in CSF was relatively low, as in blood. Technical approaches to the detection of chemokine receptors by FACS analysis strongly affect the results [Kivisakk et al. 2002].

No difference in $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio was seen between CSF and blood from MS patients (Fig. 2). Previous studies comparing blood from MS patients and healthy individuals have reported elevations of Th1-type chemokine receptor CXCR3 and CCR5 expression and reductions in the expression of Th2-type chemokine receptors CCR4 and CCR3 in patients with MS [Misu et al. 2001; Nakajima et al. 2004; Sorensen et al. 1999]. Those findings suggest that Th1 predominance in the blood and CSF is involved in the pathogenesis of MS. Our study shows that activated T cells expressing chemokine receptors invade the CNS of IND patients, as seen in MS patients. However, $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio in the CSF was significantly higher for MS patients than for IND patients (Fig. 2). Th1/Th2 imbalance may thus reflect the pathological nature of MS and indicate the degree of CNS inflammation.

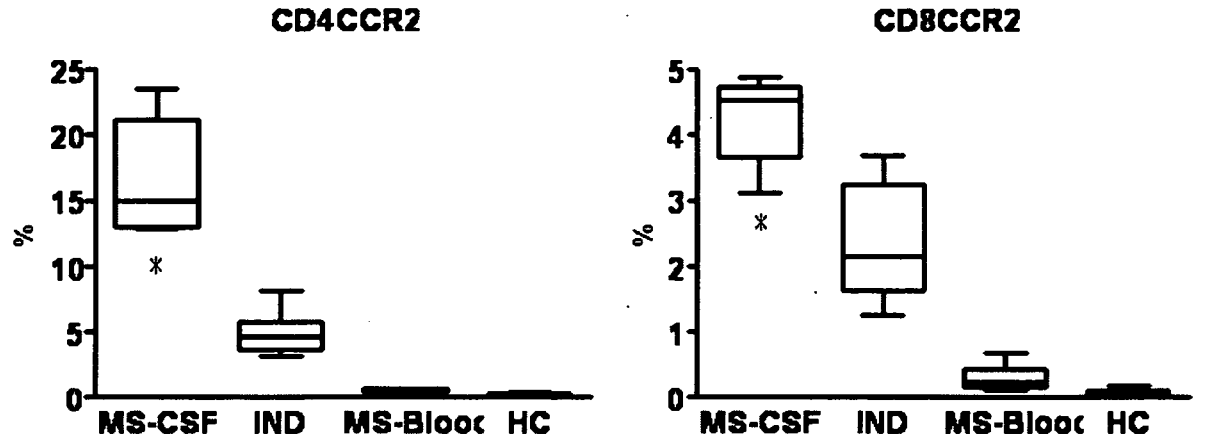

Figure 3. Comparison of CCR2 expression on $CD4^+$ and $CD8^+$ T cells between CSF and blood from MS patients, CSF from patients with IND, and blood from healthy controls. Data are shown as percentages of all T cells staining positive for $CD4^+CCR2^+$or $CD8^+CCR2^+$.

Our previous study showed that expressions of CCR2 and CD14 on monocytes in the blood of MS patients are markedly decreased, and revealed a significant negative correlation between $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio and CCR2 and CD14 expressions on monocytes [Nakajima et al. 2004b]. To elucidate whether decreases in percentage of $CD14^+CCR2^+$ monocytes in blood corresponded to those in CSF, the present study investigated CCR2-expressing monocytes in the CSF of MS patients. No $CD14^+CCR2^+$ monocytes were detected in the CSF of patients. However, the percentage of CCR2-expressing T cells was markedly increased in the CSF of MS patients. The percentage of these cells among blood T cells was extremely low. Although CCR2-expressing T cells were detectable in CSF from patients with IND, CCR2 expression was clearly decreased compared to MS patients (Fig. 1C). CCL2 and CCR2 are thought to play pivotal roles in the development and relapse of MS and EAE. However, the immune cascade, including CCL2 and CCR2, in the CNS is poorly understood. CCL2 and CCR2 expressions in the brain are closely associated with disease activity in EAE [Jee et al. 2002]. CCL-knockout mice are resistant to EAE and show significant reductions in CNS macrophage invasion [Huang et al. 2001]. Although T cells from CCL2 knockout mice transfer EAE to wild-type mice, wild-type T cells fail to cause clinical EAE in CCL-knockout mice [Huang et al. 2001]. CCR2-knockout mice likewise display decreased susceptibility to EAE [Fife et al. 2000; Izikson et al. 2000]. T cells from CCR2 knockout mice are able to induce EAE in wild-type mice, whereas CCR2-knockout recipients of wild-type T cells fail to develop EAE [Fife et al. 2000]. These findings suggest that CCL2/CCR2-induced macrophage recruitment is critical in the pathogenesis of EAE. CCL2 and CCR2 are abundantly detected in plaque lesions of MS patients [Mahad and Ransohoff, 2003; McManus et al. 1998]. An increased number of intrathecal CCR2-expressing T cells was also demonstrated in the present study. These cells may thus play a pathogenic role in acute lesions of MS.

Recent studies have shown that CCL2 concentrations in CSF and serum from early active MS patients are reduced, but increase during remission [Bartosik-Psujek and Stelmasiak, 2005; Mahad et al. 2002; Nakajima et al. 2004a; Sorensen et al. 1999, 2001, 2004]. Reduced CCL2 levels have also been seen in clinical isolated syndrome [Sorensen et al. 1999]. In contrast, elevated levels of CCL2 are present in viral meningitis and CNS vasculitis [Mahad et al. 2002] and in HIV-associated dementia [Franciotta et al. 2001]. This reduced CCL2 expression is a key to elucidating the pathogenesis of MS. As a mechanism for the low CCL2 levels in CSF, Mahad et al. suggested that CCL2 was consumed by circulating CCR2-expressing mononuclear cells, and that CCR2 was downregulated on cells migrating in response to CCL2 via the in vitro BBB model [Mahad et al. 2006]. In the present study, CCR2-expressing monocytes were not detected in CSF. This downregulation of CCR2 on monocytes in CSF would be due to internalization of CCR2 following binding of CCL2 during transmigration to the CSF. Conversely, percentages of

CCR2-expressing T cells were elevated in CSF of MS patients. Since a large amount of CCR2-expressing T cells are thought to transmigrate to the CSF in MS, these cells would be detected even after consumption of CCL2. Increases in numbers of CCR2-expressing T cells in CSF are thought to be characteristic for MS, and these cells may be associated with the relapsing-remitting autoimmune reactions of MS.

References

Balashov, K.E., Rottman, J.B., Weiner, H.L. and Hancock, W.W. 1999. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci., U.S.A*, 96:6873–6878.

Bartosik-Psujek, H. and Stelmasiak, Z. 2005. The levels of chemokines CXCL8, CCL2 and CCL5 in multiple sclerosis patients are linked to the activity of the disease. *Eur. J. Neurol.*, 12:49–54.

Fife, B.T., Huffnagle, G.B., Kuziel, W.A. and Karpus, W.J. 2000. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J. Exp. Med.*, 192:899–905.

Franciotta, D., Martino, G., Zardini, E., Furlan, R., Bergamaschi, R., Andreoni, L. and Cosi, V. 2001. Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J. Neuroimmunol.*, 115:192–198.

Gu, L., Tseng, S., Horner, R.M., Tam, C., Loda, M. and Rollins, B.J. 2000. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature*, 404:407–411.

Huang, D.R., Wang, J., Kivisakk, P., Rollins, B.J. and Ransohoff, R.M. 2001. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. *J. Exp. Med.*, 193:713–726.

Izikson, L., Klein, R.S., Charo, I.F., Weiner, H.L. and Luster, A.D. 2000. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J. Exp. Med.*, 192:1075–1080.

Jee, Y., Yoon, W.K., Okura, Y., Tanuma, N. and Matsumoto, Y. 2002. Upregulation of monocyte chemotactic protein-1 and CC chemokine receptor 2 in the central nervous system is closely associated with relapse of autoimmune encephalomyelitis in Lewis rats. *J. Neuroimmunol.*, 128:49–57.

Kivisakk, P., Trebst, C., Liu, Z., Tucky, B.H., Sorensen, T.L., Rudick, R.A., Mack, M. and Ransohoff, R.M. 2002. T-cells in the cerebrospinal fluid express a similar repertoire of inflammatory chemokine receptors in the absence or presence of CNS inflammation: implications for CNS trafficking. *Clin. Exp. Immunol.*, 129:510–518.

Kivisakk, P., Liu, Z., Trebst, C., Tucky, B., Wu, L., Stine, J., Mack, M., Rudick, R.A., Campbell, J.J. and Ransohoff, R.M. 2003. Flow cytometric analysis of chemokine receptor expression on cerebrospinal fluid leukocytes. *Methods*, 29:319–325.

Kurtzke, J.F. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, 33:1444–1452.

Mahad, D.J., Howell, S.J. and Woodroofe, M.N. 2002. Expression of chemokines in the CSF and correlation with clinical disease activity in patients with multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry*, 72:498–502.

Mahad, D.J. and Ransohoff, R.M. 2003. The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). *Semin. Immunol.*, 15:23–32.

Mahad, D., Callahan, M.K., Williams, K.A., Ubogu, E.E., Kivisakk, P., Tucky, B., Kidd, G., Kingsbury, G.A., Chang, A., Fox, R.J., Mack, M., Sniderman, M.B., Ravid, R., Staugaitis, S.M., Stins, M.F. and Ransohoff, R.M. 2006. Modulating CCR2 and CCL2 at the blood-brain barrier: relevance for multiple sclerosis pathogenesis. *Brain*, 129:212–223.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinshenker, B.Y. and Wolinsky, J.S. 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann. Neurol.*, 50:121–127.

McManus, C., Berman, J.W., Brett, F.M., Staunton, H., Farrell, M. and Brosnan, C.F. 1998. MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. *J. Neuroimmunol.*, 86:20–29.

Misu, T., Onodera, H., Fujihara, K., Matsushima, K., Yoshie, O., Okita, N., Takase, S. and Itoyama, Y. 2001. Chemokine receptor expression on T cells in blood and cerebrospinal fluid at relapse and remission of multiple sclerosis: imbalance of Th1/Th2-associated chemokine signaling. *J. Neuroimmunol.*, 114:207–212.

Nakajima, H., Kobayashi, M., Pollard, R.B. and Suzuki, F. 2001. Monocyte chemoattractant protein-1 enhances HSV-induced encephalomyelitis by stimulating Th2 responses. *J. Leukoc. Biol.*, 70:374–380.

Nakajima, H., Fukuda, K., Doi, Y., Sugino, M., Kimura, F., Hanafusa, T., Ikemoto, T. and Shimizu, A. 2004a. Expression of TH1/TH2-related chemokine receptors on peripheral T cells and correlation with clinical disease activity in patients with multiple sclerosis. *Eur. Neurol.*, 52:162–168.

Nakajima, H., Sugino, M., Kimura, F., Hanafusa, T., Ikemoto, T. and Shimizu, A. 2004b. Decreased CD14+CCR2+ monocytes in active multiple sclerosis. *Neurosci. Lett*, 363:187–189.

Simpson, J.E., Newcombe, J., Cuzner, M.L. and Woodroofe, M.N. 1998. Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. *J. Neuroimmunol.*, 84:238–249.

Sorensen, T.L., Tani, M., Jensen, J., Pierce, V., Lucchinetti, C., Folcik, V.A., Qin, S., Rottman, J., Sellebjerg, F., Strieter, R.M., Frederiksen, J.L. and Ransohoff, R.M. 1999. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest.*, 103:807–815.

Sorensen, T.L., Sellebjerg, F., Jensen, C.V., Strieter, R.M. and Ransohoff, R.M. 2001. Chemokines CXCL10 and CCL2: differential involvement in intrathecal inflammation in multiple sclerosis. *Eur. J. Neurol.*, 8:665–672.

Sorensen, T.L., Ransohoff, R.M., Strieter, R.M. and Sellebjerg, F. 2004. Chemokine CCL2 and chemokine receptor CCR2 in early active multiple sclerosis. *Eur. J. Neurol.*, 11:445–449.

Teleshova, N., Pashenkov, M., Huang, Y.M., Soderstrom, M., Kivisakk, P., Kostulas, V., Haglund, M. and Link, H. 2002. Multiple sclerosis and optic neuritis: CCR5 and CXCR3 expressing T cells are augmented in blood and cerebrospinal fluid. *J. Neurol.*, 249:723–729.

Original Paper

European Neurology

Eur Neurol 2007;57:11–18
DOI: 10.1159/000097004

Received: May 10, 2006
Accepted: July 31, 2006
Published online: November 14, 2006

Ca^{2+} Channel Currents Inhibited by Serum from Select Patients with Guillain-Barré Syndrome

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Key Words
Guillain-Barré syndrome · Acute motor axonal neuropathy · Acute inflammatory demyelinating polyneuropathy · Voltage-dependent calcium current

Abstract
We performed an electrophysiological study demonstrating inhibition of spontaneous muscle action potentials within a coculture of rat muscle and spinal cord by exposure to serum, as well as purified IgG, from patients with the acute motor axonal neuropathy (AMAN) variant of Guillain-Barré syndrome (GBS). However, exposure to serum from two patients with the acute inflammatory demyelinating polyneuropathy (AIDP) form of GBS had no effect. Using a whole-cell recording technique, we then investigated the effects of serum and purified IgG from patients with GBS on voltage-dependent calcium channel (VDCC) currents in nerve growth factor-differentiated PC12 cells. Serum from patients with GBS (AMAN) inhibited VDCC currents in PC12 cells, which was fully reversible by washing with the bath solution. Similarly, purified IgG from the serum of two patients with GBS (AMAN) also inhibited VDCC currents in PC12 cells. In contrast, sera from patients with AIDP and healthy volunteers did not affect VDCC currents in PC12 cells. These results suggest that muscle weakness in some patients with GBS might be induced by inhibition of Ca^{2+} channel currents within motor nerve terminals.

Introduction

Guillain-Barré syndrome (GBS) is an acute immune-mediated peripheral neuropathy, which includes acute motor axonal neuropathy (AMAN), acute inflammatory demyelinating polyneuropathy (AIDP), and related disorders. Anti-GM1 IgG antibodies, which are frequently found in the serum of patients with GBS, are thought to interfere with axonal function. In fact, GM1 ganglioside is found in the axolemma, nodes of Ranvier, as well as distal motor nerve terminals [1–3], where voltage-gated sodium channels exist [4]. Furthermore, neuromuscular junctions (NMJs), which are rich in gangliosides, lie outside the blood-nerve barrier, and are an important site of many antibody-mediated autoimmune diseases. Electrophysiological studies demonstrate the presence of factors which block sodium channels in the cerebrospinal fluid of patients with GBS, thus impairing the conduction of nerve impulses, resulting in muscular weakness and sen-

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Table 1. Relationship between anti-ganglioside antibodies, SMAP and Ca^{2+} current in GBS patient sera

Diagnosis	Age/ sex	Titer of anti-ganglioside antibodies						SMAP	Relative Ca^{2+} current, %
		GA1	GM1	GM2	GalNAc-GD1a	GD1a	GD1b		
Patient 1 (AMAN)	25/M	16,000	16,000	NT	NT	NT	4,000	+	46.00 ± 16.00
Patient 2 (AMAN)	65/F	>64,000	4,000	4,000	32,000	2,000	0	+	53.11 ± 12.52
Patient 3 (AMAN)	44/M	0	>32,000	0	0	0	0	+	62.20 ± 10.05
Patient 4 (AMAN)	52/M	400	6,000	400	3,000	400	0	+	74.17 ± 7.20
Patient 5 (AMAN)	58/F	4,000	2,000	1,000	1,000	1,000	1,000	+	73.57 ± 2.80
Patient 6 (AIDP)	33/F	0	0	0	0	0	0	-	98.09 ± 11.64
Patient 7 (AIDP)	57/M	0	0	0	0	0	0	-	100.74 ± 5.54
Healthy volunteer 1	40/M	0	0	0	0	0	0	-	92.30 ± 7.19
Healthy volunteer 2	23/M	0	0	0	0	0	0	-	90.03 ± 2.36
Healthy volunteer 3	50/M	0	0	0	0	0	0	-	93.00 ± 6.29
Healthy volunteer 4	45/M	0	0	0	0	0	0	-	92.23 ± 3.11
Healthy volunteer 5	20/F	0	0	0	0	0	0	-	90.86 ± 7.74

NT = Not tested; SMAP = spontaneous muscle action potential; response of SMAP of rat spinal-muscle coculture system to the application of patients' sera is expressed as inhibition (+) or no effect (-).

sory disturbances in patients with GBS [5, 6]. Patch clamp studies further show direct inhibition of the ion-conducting pore of voltage-gated sodium channels by exposure to serum from patients with GBS [7]. Thus, a number of studies suggest that inhibition of voltage-gated ion channels might be a mechanism by which nerve conduction is impaired in patients with GBS.

The quantal release of neurotransmitter is inhibited by serum from patients with GBS and Miller-Fisher syndrome following electrical stimulation of the phrenic nerve [8]. Transmitter release evoked by nerve stimulation is dependent on calcium influx through voltage-gated calcium channels. In addition, voltage-gated calcium channels play a major role in neuromuscular transmission by enabling calcium ion influx into motor nerve terminals, thereby affecting acetylcholine release. Electrophysiological studies demonstrate a number of types of voltage-gated calcium channels in rat peripheral nerve terminals [9]. Several studies suggest a possible relationship between gangliosides and different voltage-gated calcium channels [10–13]. This led us to investigate whether Ca^{2+} channels, which mediate neurotransmission at the NMJ, might be affected by serum from patients with GBS.

In the present study, we evaluated the effects of serum and purified IgG from patients with GBS on spontaneous muscle action potentials using a rat spinal cord-muscle coculture system by examining voltage-dependent calcium channel (VDCC) currents in nerve growth factor (NGF)-differentiated PC12 cells.

Materials and Methods

Patient Serum

Serum samples were collected from patients with GBS (5 with AMAN and 2 with AIDP) and 5 healthy volunteers. GBS was diagnosed using commonly accepted clinical criteria [14], and the patients were classified as having AMAN or AIDP based on electrophysiological criteria [15]. Each serum sample was subjected to enzyme-linked immunosorbent assay (ELISA) to determine IgG anti-ganglioside antibody titers (table 1). The serum samples were then analyzed for their effects on spontaneous muscle action potential generation and VDCC current. All samples were refrigerated before use (stored at -80°C).

ELISA for the Detection of Anti-Ganglioside Antibodies

ELISA was used to investigate the reactivity of serum IgG to each of the major glycosphingolipid antigens, such as GM1, GM2, GA1, GD1a, GD1b, and GalNAc-GD1a. Each well of the microtiter plates was coated with 10 pM of ganglioside in methanol. Serially diluted serum samples in 0.5% casein phosphate-buffered saline (PBS) were left to incubate overnight. After washing the plates three times with PBS (pH 7.4), secondary antibodies, including peroxidase-conjugated goat anti-human or anti-rabbit IgG antibodies, were added and incubated for 2 h. After washing the plates with PBS, the colors were developed using *o*-phenylenediamine as the substrate. Titers were calculated using the most diluted serum sample for each patient at which the OD_{490} was 0.1 or greater.

Spinal Cord-Muscle Coculture

Pregnant Wistar rats were individually housed under automatically controlled environmental conditions using a 12-hour light cycle with free access to food and water. Experiments were carried out in accordance with the guidelines for animal care of Showa Pharmaceutical University, as well as the guidelines for animal use published by the National Institute of Health.

The muscle-spinal cord coculture was established according to the method of Taguchi et al. [16]. Muscle was excised from prenatal day 17 fetal rats and cut into small pieces in Tyrode's solution containing 100 μg/ml of streptomycin and 100 μg/ml of penicillin. The pieces were then incubated at 37°C for 20 min in Ca^{2+}- and Mg^{2+}-free Tyrode's solution containing 1 mg/ml collagenase. For the innervation experiments, explants of whole transverse slices of fetal spinal cord, including the dorsal root ganglia, from prenatal day 17 fetal rats were placed on the bottom of a collagen-coated 35-mm petri dish. Individual muscle cells isolated by trituration were placed on the slices of spinal cord and cultured. Muscle cells and spinal cord were cocultured in 67% Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, N.Y., USA), 23% medium 199 (Gibco BRL), and 10% fetal calf serum (Boehringer Mannheim Biochemica, Germany), and supplemented with 25 ng/ml fibroblast growth factor (Sigma, St. Louis, Mo., USA) and 20 μg/ml insulin (Gibco BRL). The muscle-spinal cord cocultures were kept in a CO_2 incubator (5% CO_2, 95% O_2, at 37°C). In this experiment, cultured cells were maintained for up to 1 week in the same medium.

Measurement of Spontaneous Muscle Action Potentials

After 1 week of coculture, the innervated muscle specimens were placed in an experimental chamber (a 35-mm petri dish) on the stage of an inverted microscope. The experimental chamber (volume: 1 ml) was continuously perfused with medium (containing 67% Dulbecco's modified Eagle's medium and 23% medium 199) at a rate of 1–2 ml/min, with continuous bubbling using 5% CO_2/95% O_2. Glass microelectrodes filled with 3 M KCl with a tip resistance of 20–40 MΩ were used to record spontaneous muscle action potentials. Electrical activity was recorded by each electrode, connected to a microelectrode amplifier and displayed on an oscilloscope. Data were transferred to and stored in a computer. The action potentials were low-pass filtered at 1 kHz. All experiments were carried out at 33 ± 1°C.

Measurement of Calcium Current Recorded in NGF-Differentiated PC12 Cells

PC12 cells obtained from the Health Science Research Bank (HSRRB, Osaka, Japan) were maintained in RPMI-1640 supplemented with 5% fetal calf serum, 10% horse serum, 50 U/ml penicillin and 50 μg/ml streptomycin, and incubated at 37°C in 5% CO_2 in air. Cells were seeded into 35-mm plastic dishes covered with collagen-coated glass coverslips and cultured for 4–7 days in culture medium supplemented with 100 ng/ml 7S NGF. The cells were grown on coverslips placed in an experimental chamber on the stage of an inverted microscope. Currents were recorded at room temperature (20–26°C) using the whole-cell mode of patch clamping and a Multiclamp 700A device. Data were analyzed using pCLAMP 8.2 software. Currents were sampled at 2 kHz after low-pass filtering at 1 kHz (–3 dB). The bath solution contained (in mM): NaCl (115), HEPES (20), TEA-Cl (20), $CaCl_2$ (10), $MgCl_2$ (2) and glucose (10). The pH was adjusted to 7.35 with 1 M NaOH. The bath solution was bubbled with 5% CO_2/95% O_2. The pipette solution contained (in mM): CsCl (120), HEPES (10), TEA-Cl (20), $CaCl_2$ (1), $MgCl_2$ (2) and EGTA (11). The pH was then adjusted to 7.35 with 1 M CsOH. Patch electrodes were made from borosilicate glass using a vertical two-stage patch pipette puller. Pipette resistance ranged from 5.0 to 7.0 MΩ when filled with solution.

Purification of IgG

ImmunoAssist MG-PP (Kanto Chemical Co., Inc., Tokyo, Japan) was used to purify IgG from the serum of patients with GBS in accordance with the manufacturer's instructions. Briefly, 2 g of ImmunoAssist were packed into a column (1.5 × 1.0 cm) and equilibrated with 10 mM phosphate buffer (pH 6.8). Five hundred microliters of serum were applied and the albumin fraction was eluted with 10 mM phosphate buffer (pH 6.8). The IgG fraction, eluted with 10 mM phosphate buffer containing 300 mM NaCl (pH 6.8), was pooled and concentrated with a Centricon device (Millipore Corporation, Bedford, Mass., USA).

Statistical Analysis

All data are expressed as means ± SD. To compare the effects of serum and purified IgG from patients with GBS on VDCC currents within PC12 cells, statistical analysis was performed using a paired t test or Dunnett's multiple comparison test; p values <0.05 were considered significant.

Results

Effects of Serum and Purified IgG from Patients with GBS on Neuromuscular Transmission

After 5–7 days of coculture, asynchronous contraction of several individual muscle fibers occurred at the newly developed NMJs. Spontaneous action potentials within innervated muscle cells were recorded, with a frequency of 9.1 ± 2.8/5 s and an amplitude of 52.7 ± 8.5 mV (experiment number of times (n) = 25). The mean resting membrane potential of the muscle cells was –50.3 ± 6.4 mV (n = 25). Patient characteristics, their diagnoses, and their anti-ganglioside antibody titers are shown in table 1. Figure 1 shows the effects of serum from a patient with GBS (patient 1) on spontaneous muscle action potential generation at the NMJ. The addition of serum from this patient with GBS (patient 1, diluted to 1:100) completely blocked spontaneous muscle action potential generation at the NMJ within 1 min (fig. 1a). This blockade was fully reversed by washing with medium (fig. 1b). The addition of serum from other patients with GBS (patients 2–5, n = 5–6) to the bath solution also blocked the frequency of spontaneous muscle action potentials (table 1). Serum from patients 4 and 5 (diluted to 1:100, n = 5) had the same blocking effect as that from patient 1 (table 1). Spontaneous muscle action potential generation at the NMJ was unaffected by exposure to serum diluted to 1:100 from patients 2 and 3 with GBS, but was blocked by dilutions of 1:50. This blockade was fully reversed by washing with medium for 2–3 min. The resting membrane potential of the muscle cells and the amplitude of spontaneous muscle action potentials were unchanged by the addition of serum from patients with GBS (patients 1–5, n = 5–6). The ap-

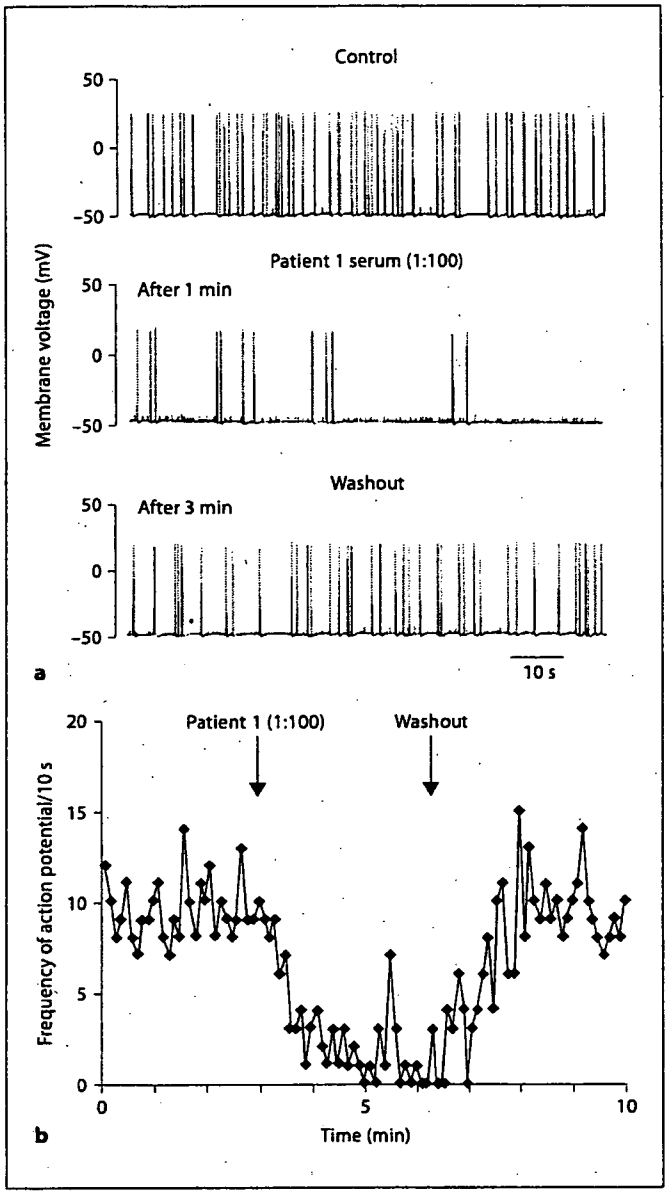

Fig. 1. Effect of GBS serum on spontaneous muscle action potential generation in a rat spinal cord-muscle coculture system. **a** Blockade of spontaneous muscle action potentials by GBS serum (patient 1; serum diluted to 1:100). **b** Time course of inhibition of spontaneous muscle action potential by GBS serum (patient 1; diluted to 1:100). The number of spontaneous muscle action potentials was measured every 10 s. Arrows indicate when GBS serum was applied and when the bath solution was exchanged. After washing out the serum, recovery from inhibition was observed.

plication of purified IgG from select patients with GBS (patients 4 and 5, n = 4–6, diluted to 1:100) blocked the frequency of spontaneous muscle action potential generation at the NMJ (data not shown). This effect at the NMJ was not observed upon exposure to serum (diluted to 1:50, n = 4–6) from 5 healthy volunteers (table 1).

Effect of GBS Serum on VDCC Current in NGF-Differentiated PC12 Cells

We used the whole-cell recording technique on NGF-differentiated PC12 cells to test the effects of serum from patients with GBS (patients 1–7, n = 4–7) on VDCC currents. PC12 cells in 4- to 7-day cultures with evidence of neurite outgrowth were selected for recording of the Ca^{2+} current.

Figure 2a shows the effects of serum from 1 patient with GBS (AMAN, patient 5, diluted to 1:100) on Ca^{2+} current evoked by depolarization from a holding potential of -80 mV to a testing potential of +10 mV in NGF-differentiated PC12 cells. Figure 2b demonstrates the current-voltage relationship of the Ca^{2+} current. In this figure, the voltage dependence of inhibition is demonstrated by the current-voltage curve in the presence of serum from a patient with GBS (from patient 5). In cells demonstrating peak current at +10 mV, GBS serum-induced inhibition started at around 0 mV and reached a maximum at +10 mV, after which inhibition persisted with further depolarization. The Ca^{2+} current elicited by a +10-mV command pulse was significantly inhibited (by 26.43 ± 2.80%) when serum from this patient with GBS (patient 5, n = 5) was added to the bath solution (fig. 2c). In addition, serum from patients 1–5 with GBS (AMAN, diluted to 1:100, n = 4–7) inhibited the amplitude of the Ca^{2+} current observed in PC12 cells (fig. 3a). The Ca^{2+} current was significantly inhibited (by 38.19 ± 12.41%) by the addition of serum from patients with GBS (patients 1–5, 1:100, n = 5) to the bath solution, compared with healthy volunteers (fig. 3b). However, sera from healthy volunteers (diluted to 1:50, n = 4–7), as well as from patients 6 and 7 with GBS (AIDP, 1:100, n = 4–5), did not affect the Ca^{2+} current in PC12 cells (fig. 3a).

Effect of Purified IgG from the Serum of a Patient with GBS on VDCC Current in NGF-Differentiated PC12 Cells

Purified IgG from the serum of a patient with GBS inhibited the Ca^{2+} current of PC12 cells. The Ca^{2+} current was significantly inhibited (by 34.29 ± 2.39%) by the addition of purified IgG from the serum of a patient with GBS (patient 5, 1:100, n = 5) to the bath solution (fig. 4a,

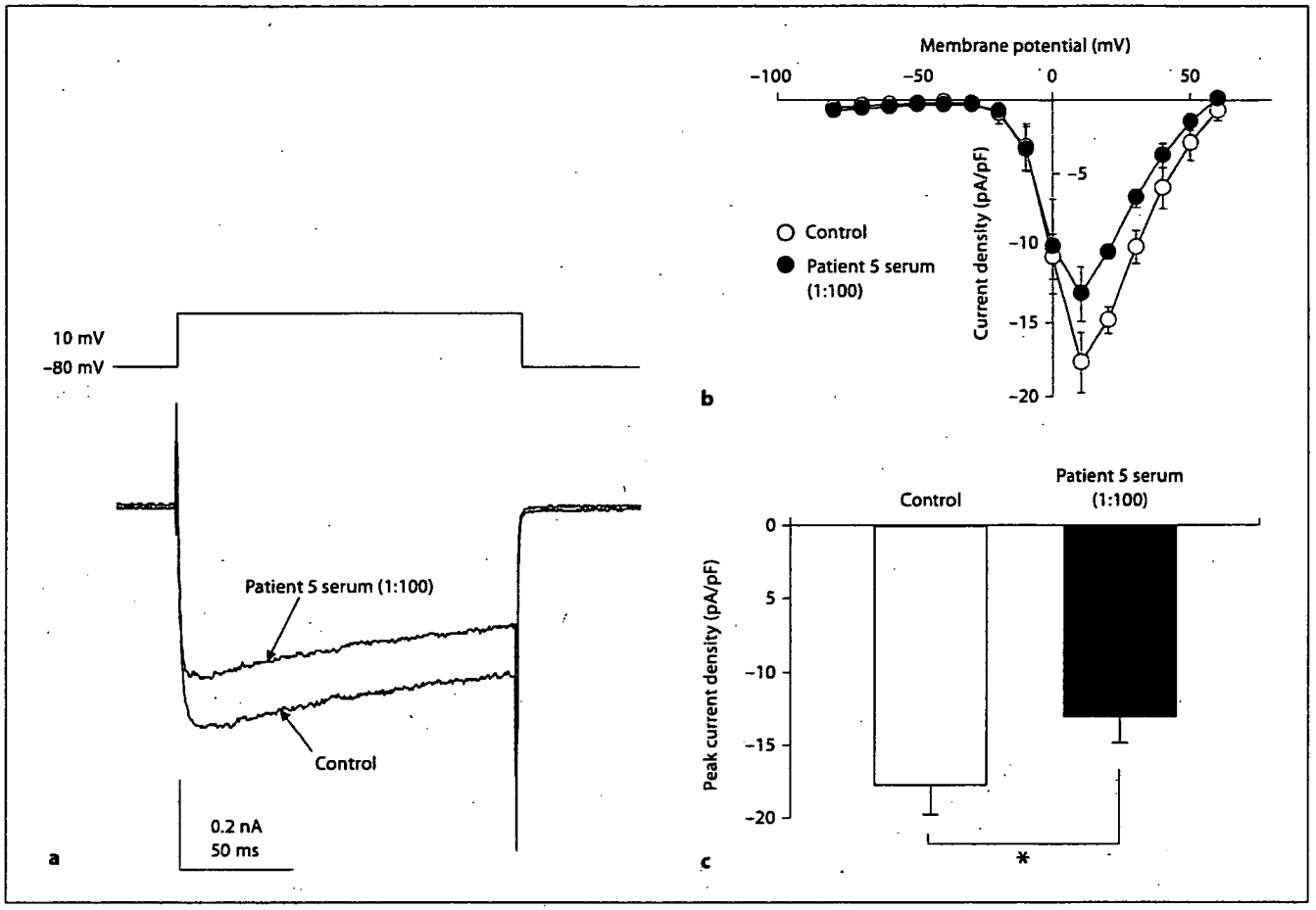

Fig. 2. Effect of GBS serum on whole-cell Ca^{2+} current in NGF-differentiated PC12 cells. **a** Inward Ca^{2+} current evoked by depolarization from -80 mV to +10 mV (from a holding potential of -80 mV) in control specimens and in the presence of GBS serum (patient 5; diluted to 1:100). Current traces in the presence of GBS serum were obtained 1 min after application. **b** Voltage-dependent activation curve of the Ca^{2+} current. The peak Ca^{2+} current in controls (○) and in the presence of GBS serum (●) was plotted against each test pulse potential. Each data point on the voltage curve represents the mean ± SD (n = 5). **c** Histograms representing the peak Ca^{2+} current density (pA/pF) to that observed in controls (n = 5) and in the presence of GBS serum (diluted to 1:100, n = 5). The mean values ± SD in PC12 cells are represented by the columns. * $p < 0.05$ (vs. control).

d). Figure 4b describes the current-voltage relationship of the Ca^{2+} current. In figure 4b, the voltage dependence of inhibition is demonstrated by the current-voltage curve in the presence of purified IgG from the serum of a patient with GBS (patient 5). In NGF-differentiated PC12 cells demonstrating peak current at +10 mV, purified GBS IgG-induced inhibition started at around 0 mV and reached a maximum at +10 mV, after which inhibition persisted with further depolarization. The time course and reversibility (washout) of the blockade are shown in figure 4c. Purified IgG from a patient with GBS (patient 5) inhibited the Ca^{2+} current within 1 min, and this inhibition was fully reversed by washing with the bath solution. In addition, the application of purified IgG from the serum of a patient with GBS (patient 4, diluted to 1:100, n = 4) significantly inhibited Ca^{2+} current (by 20.30 ± 8.62%) in PC12 cells (fig. 4d).

Discussion

In the present study, serum (patients 1–5 with AMAN) and IgG (patients 4 and 5) from select patients with GBS reversibly blocked the frequency of spontaneous muscle