Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, Nishimura Y, Ochi H, Kira J.	Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders.	Tissue Antigen	73(2)	171-6	2009
Kawajiri M, Mogi M, Higaki N, Matsuoka T, Ohyagi Y, Tsukuda K, Kohara K, Horiuchi M, Miki T, Kira JI.	Angiotensin-converting enzyme (ACE) and ACE2 levels in the cerebrospinal fluid of patients with multiple sclerosis.	Mutl Scler	15(2)	262-5	2009
Doi H, Matsushita T, Isobe N, Matsuoka T, Minohara M, Ochi H, Kira JI.	Hypercomplementemia at relapse in patients with anti-aquaporin-4 antibody.	Mult Scler	15(3)	304-10	2009
Hagiwara K, Okamoto T, Shigeto H, Ogata K, Somehara Y, Matsushita T, Kira J, Tobimatsu S.	Oscillatory gamma synchronization binds the primary and secondary somatosensory areas in humans.	Neuroimage	51(1)	412-20	2010
Maekawa T, Hirano Y, Hirano S, Oribe N, Kaneko K, Kanba S, Tobimatsu S.	Decreased spatial frequency sensitivities for processing faces in male patients with chronic schizophrenia.	Clin Neurophysiol	120(8)	1525-33	2009
Maekawa T, Tobimatsu S, Ogata K, Onitsuka T, Kanba S.	Preattentive visual change detection as reflected by the mismatch negativity (MMN)--evidence for a memory- based process.	Neurosci Res	65(1)	107-12	2009
Ogata K, Okamoto T, Yamasaki T, Shigeto H, Tobimatsu S.	Pre-movement gating of somatosensory-evoked potentials by self-initiated movements: the effects of ageing and its implication.	Clin Neurophysiol	120(6)	1143-8	2009
Miyamoto K, Kusunoki S.	Intermittent plasmapheresis prevents recurrence in neuromyelitis optica.	Ther Apher Dial	13(6)	505-8	2009
Ogawa G, Kaida K, Kusunoki S, Ueda M, Kimura F, Kamakura K.	Antibodies to ganglioside complexes consisting of asialo- GM1 and GQ1b or GT1a in Fisher and Guillain-Barré syndromes.	J Neuroimmunol	214(1-2)	125-7	2009
Kureshiro J, Miyamoto K, Tanaka N, Kusunoki S.	Selective phosphodiesterase-3 inhibitor cilostazol ameliorates experimental autoimmune encephalomyelitis.	Neuroreport	20(7)	718-22	2009
Kato-Motozaki Y, Komai K, Takahashi K, Ishida C, Ueda M, Kusunoki S, Yamada M.	Polyethylene glycol interferon alpha-2b-induced immune-mediated polyradiculoneuropathy.	Intern Med	48(7)	569-72	2009
Miyamoto K, Nagaosa N, Motoyama M, Kataoka K, Kusunoki S.	Upregulation of water channel aquaporin-4 in experimental autoimmune encephalomyeritis.	J Neurol Sci	276(1-2)	103-7	2009
Guo Z, Miura K, Turin TC, Hozawa A, Okuda N, Okamura T, Saitoh S, Sakata K, Nakagawa H, Okayama A, Yoshita K, Kadowaki T, Rahoudhury S, Nakamura Y, Rodriguez B, Curb JD, Elliott P, Stamler J, Ueshima	Relationship of the Polyunsaturated to Saturated Fatty Acid Ratio to Cardiovascular Risk Factors and Metabolic Syndrome in Japanese: the INTERLIPID Study.	J Atheroscler Thromb			in press

Ohsawa M, Kato K, Itai K, Tanno K, Fujishima Y, Kondo R, Okayama A, Abe K, Suzuki K, Nakamura M, Onoda T, Kawamura K, Sakata K, Akiba T, Fujioka T.	Standardized prevalence ratios for chronic hepatitis C virus infection among adult Japanese hemodialysis patients.	J Epidemiol	20(1)	30-9	2010
Orimo H, Yaegashi Y, Onoda T, Fukushima Y, Hosoi T, Sakata K.	Hip fracture incidence in Japan: estimates of new patients in 2007 and 20-year trends.	Arch Oateoporos	4(1-2)	71-77	2009
Takahashi T, Nakamura M, Onoda T, Ohsawa M, Tanno K, Itai K, Sakata K, Sakuma M, Tanaka F, Makita S, Yoshida Y, Ogawa A, Kawamura K, Okayama A.	A. Predictive value of plasma B-type natriuretic peptide for ischemic stroke: a community- based longitudinal study.	Atherosclerosis	207(1)	298-303	2009
Makita S, Nakamura M, Satoh K, Tanaka F, Onoda T, Kawamura K, Ohsawa M, Tanno K, Itai K, Sakata K, Okayama A, Terayama Y, Yoshida Y, Ogawa A.	Serum C-reactive protein levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population.	Atherosclerosis	204(1)	234-8	2009
Miura S, Ohyagi Y, Miike T, Noda K, Motomura K, Ayabe M, Aizawa H, Taniwaki T.	Machado-Joseph disease/SCA3 and myotonic dystrophy type 1 in a single patient.	Clin Neurol Neurosurg	111(10)	791-4	2009
Miura S, Kurita T, Noda K, Ayabe M, Aizawa H, Taniwaki T.	Symmetrical brainstem encephalitis caused by herpes simplex virus.	JClin Neurosci	16(4)	589-90	2009
Miura S, Nakagawara H, Kaida H, Sugita M, Noda K, Motomura K, Ohyagi Y, Ayabe M, Aizawa H, Ishibashi M, Taniwaki T.	Expansion of the phenotypic spectrum of SCA14 caused by the Gly128Asp mutation in PRKCG.	Clin Neurol Neurosurg	111(2)	211-5	2009
Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M	Dietary fat intake and risk of Parkinson's disease: a case-control study in Japan.	J Neuro Sci	288(1-2)	117-22	2010
Murakami K, Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M	Dietary glycemic index is inversely associated with the risk of Parkinson's disease: a case-control study in Japan	Nutrition	26(5)	515-21	2010

研究成果の刊行物

（別刷）

Neural damage associated with atopic diathesis

A nationwide survey in Japan

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ABSTRACT

Background: We previously reported the occurrence of myelitis in patients with atopic disorders (atopic myelitis [AM]). To uncover the spectrum of neural damage associated with atopy, we conducted a cross-sectional nationwide survey of AM and atopy-related peripheral neuritis (APN), including Churg-Strauss syndrome (CSS), in individuals with atopic diathesis.

Method: Cases with AM diagnosed between 1996 and 2006 and cases with APN between 2000 and 2006 were collected from all over Japan. Detailed data on 109 patients with AM and 133 patients with APN were collated.

Results: Patients with APN showed a preponderance of women, higher age at onset, and greater eosinophil counts than patients with AM. Patients with AM most commonly showed cervical cord involvement, whereas patients with APN preferentially exhibited mononeuritis multiplex predominantly affecting the lower limbs. Among patients with AM, motor weakness and muscle atrophy were significantly more frequent in those with bronchial asthma than in those with other atopic disorders. Patients with APN who met the criteria for CSS showed a higher age at onset, higher frequencies of systemic organ involvement, and greater disability than those who did not. Abnormalities suggesting peripheral nervous system involvement were seen in 25.7% of patients with AM, whereas 18.8% of patients with APN had abnormalities indicating CNS involvement. Multiple logistic regression analyses revealed that atopic dermatitis increased the risk of myelitis, whereas high age at onset and bronchial asthma decreased that risk.

Conclusions: Atopy-related neural inflammation multifocally affects CNS and peripheral nervous system tissues. Both preceding atopic disorders and age seem to influence the distribution of neural damage. ***Neurology*® 2009;73:790-797**

GLOSSARY

AM = atopic myelitis; **ANCA** = antineutrophil cytoplasmic antibody; **APN** = atopy-related peripheral neuritis; **BAEP** = brainstem auditory-evoked potential; **CI** = confidence interval; **CSS** = Churg-Strauss syndrome; **EDSS** = Expanded Disability Status Scale of Kurtzke; **EP** = evoked potential; **IgE** = immunoglobulin E; **IgG** = immunoglobulin G; **MEP** = motor-evoked potential; **mRS** = modified Rankin Scale; **NCS** = nerve conduction study; **NS** = not significant; **OCB** = oligoclonal immunoglobulin G bands; **OR** = odds ratio; p^{corr} = corrected *p* value; **PNS** = peripheral nervous system; p^{uncorr} = uncorrelated *p* value; **SEP** = somatosensory-evoked potential; **SNAP** = sensory nerve action potential; **Th2** = T helper 2; **VEP** = visual-evoked potential.

Among atopy-related neurologic diseases, the best known condition is Churg–Strauss syndrome (CSS). CSS is characterized by clinical triads of preceding airway allergy, blood eosinophilia, and systemic necrotizing vasculitis involving small arteries with or without granulomas.[1] It frequently causes mononeuritis multiplex; however, the CNS has not been considered to be involved. We first reported the emergence of myelitis in patients with atopic dermatitis and other atopic disorders, and named it *atopic myelitis* (AM).[2-4] A nationwide survey of this condition in 2000 disclosed a widespread occurrence of AM in Japan.[5] A histologic study of biopsied spinal cord specimens revealed eosinophilic inflammation.[6,7] Similar cases have recently been reported in Europe,[8] including a biopsy-proven case showing marked eosinophil infiltration.[9]

Supplemental data at www.neurology.org

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We found that, in patients with AM, both CCL2, a chemokine for eosinophils, and interleukin 9, a T helper 2 (Th2) cytokine, were markedly up-regulated in the CSF, and that disease severity increased with the levels of these molecules,[10] which collectively suggest that atopy-related inflammation is operative.

A fraction of patients with AM have subclinical peripheral neuritis,[11-13] whereas a few patients with CSS show CNS vasculitis.[14] These observations led us consider the possibility that patients with an atopic constitution could multifocally develop inflammation in both CNS and peripheral nervous system (PNS) tissues. To clarify the whole spectrum of neural inflammation associated with atopy and determine what factors contribute to CNS damage, we conducted the first nationwide survey simultaneously investigating both AM and atopy-related peripheral neuritis (APN).

METHODS Survey procedures. The study was approved by the Kyushu University Ethics Committee. The survey was undertaken in 2 steps. A preliminary survey was performed from November 2006 to ascertain the approximate number of patients with AM or APN. A survey was then conducted between December 2006 and November 2007, using a questionnaire for each patient. All responses received were provided by neurologists at each facility based on the hospital discharge records of patients and the medical records of outpatients. The first survey form was mailed to 976 facilities in Japan that were educational facilities accredited by the Japanese Society of Neurology, neurology departments with 2 or more board-certified neurologists, or those in the hospitals with more than 500 beds. AM was defined as myelitis of unknown cause with either 1) hyperIgEemia (>240 U/mL) and antigen-specific immunoglobulin E (IgE) positivity or 2) coexistent or past atopic diseases following the diagnostic criteria,[5] excluding other diseases (table e-1 on the *Neurology*® Web site at www.neurology.org). APN was defined as peripheral neuritis of unknown cause, including CSS, with an atopic background, which matched the same inclusion criteria as AM mentioned above. Bronchial asthma, atopic dermatitis, allergic rhinitis, food allergy, and allergic conjunctivitis were regarded as atopic diseases in this study. We regarded patients who met the following 3 criteria as having classic CSS: existence of preceding bronchial asthma, >10% eosinophils in white blood cell counts, and more than 1 systemic symptom of vasculitis.[15] The existence of myelitis was confirmed by spinal cord MRI, motor-evoked potentials (MEPs), somatosensory-evoked potentials (SEPs), or neurologic findings of either exaggerated deep tendon reflexes or sensory levels, whereas that of peripheral neuritis was ascertained by nerve conduction study (NCS), MEPs, or SEPs.

The questionnaire used in the preliminary survey requested the number and sex of patients with AM and patients with APN diagnosed between 1996 and 2006. The second questionnaire was forwarded to the facilities reporting patients in the first survey, although the patients with APN surveyed were limited to those who were diagnosed between January 2000 and October 2006. It requested detailed clinical information on individual patients, including symptomatology, retrospectively assessed disability score using the Expanded Disability Status Scale of Kurtzke (EDSS)[16] in patients with AM and the modified Rankin Scale (mRS)[17] in patients with APN, and allergologic and electrophysiologic data (table e-1). Efficacy of treatment was also evaluated by the neurologists in attendance, based on subjective and objective clinical symptomatology.

Statistical analysis. Statistical analyses of numerical variables were initially performed using the Kruskal–Wallis H test. When a significant difference was found, the Mann–Whitney U test was used to determine the significance of differences between subgroups. Uncorrelated p values (p^{uncorr}) were corrected by multiplying them by the number of comparisons (Bonferroni–Dunn correction) to calculate corrected p values (p^{corr}). Differences in ratios among groups were tested for significance by the χ^2 test or Fisher exact probability test. To clarify which background factors underlie the development of myelitis, we conducted multiple logistic regression analyses of patients with pure AM and patients with pure APN. We performed a correlation analysis of all candidate explanatory variables and then identified those variables that could better predict the existence of myelitis with manual stepwise methods, weighing the clinical importance of each variable. The subgroups and variables of interest in the statistical analyses are described in the supplementary materials (table e-1). All analyses were performed using JMP 6.0.3 (SAS Institute, Cary, NC), except for the Fisher exact probability test, which was performed using the R package (R version 2.5.1, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS Response rates and numbers of collated patients. In the preliminary survey, we received answers from 45.9% of the facilities, and 169 facilities had 1 or more patients with AM or APN. There were 186 cases of AM (96 men, 87 women, 3 unknown) and 368 cases of APN (122 men, 211 women, 35 unknown). In the second survey, 46.2% of the 169 facilities reported 137 cases of AM and 172 cases of APN. Among them, cases not fulfilling the inclusion criteria and duplicated cases were omitted; the remaining 109 cases of AM and 133 cases of APN from 76 facilities were analyzed. There was no significant difference in response rate according to the size and location of the hospitals (data not shown).

Comparison of atopic myelitis and atopy-related peripheral neuritis. The proportion of men was higher and the average age at onset was lower in patients with AM compared with patients with APN (table 1). Numbers of peripheral blood eosinophils were greater in patients with APN than in patients with AM. Specific IgEs against *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were detected with higher frequencies in patients with AM than in patients with APN, although no significant difference in total serum IgE levels was found. Patients with AM showed significantly higher frequencies of atopic dermatitis, allergic rhinitis, food allergy, and

Table 1 Comparison of demographic features between atopic myelitis and atopy-related peripheral neuritis

	AM (n = 109)	APN (n = 133)	p Value
Men:women (sex ratio)	62:47 (1:0.76)	52:81 (1:1.56)	0.006
Age at onset, y	34.0 ± 13.1	55.8 ± 14.3	<0.001
Disease duration, y	6.6 ± 4.7	4.5 ± 4.7	<0.001
Blood eosinophils, /μL*	442.4 ± 699.9	7,855.4 ± 8,744.1	<0.001
Total serum IgE, U/mL*	1,942.9 ± 3,858.1	1,765.1 ± 2,735.6	NS
Specific IgE to (%)			
D pteronyssinus	85/92 (92.4)	11/23 (47.8)	<0.001
D farinae	81/95 (85.3)	11/25 (44.0)	<0.001
Cedar pollen	50/77 (64.9)	17/29 (58.6)	NS
Preceding atopic diseases (%)			
Atopic dermatitis	48/105 (45.7)	8/111 (7.2)	<0.001
Allergic rhinitis	44/102 (43.1)	30/109 (27.5)	0.018
Bronchial asthma	31/103 (30.1)	96/130 (73.9)	<0.001
Food allergy	15/94 (16.0)	6/102 (5.9)	0.023
Allergic conjunctivitis	8/93 (8.6)	0/103 (0.0)	0.002
Mode of onset (%)			
Sudden	9/109 (8.3)	8/131 (6.1)	NS
Acute	34/109 (31.2)	31/131 (23.7)	NS
Subacute	28/109 (25.7)	54/131 (41.2)	0.012
Chronic	37/109 (33.9)	38/131 (28.8)	NS
Symptoms and signs during illness (%)			
Dysesthesia	92/108 (85.2)	112/127 (88.2)	NS
Hypesthesia	77/105 (73.3)	102/130 (92.3)	NS
Motor weakness	65/109 (59.6)	101/125 (80.8)	<0.001
Muscle atrophy	9/105 (8.6)	39/122 (32.0)	<0.001
Hyporeflexia	5/108 (4.6)	75/128 (58.6)	<0.001
Pyramidal tract sign†	89/108 (82.4)	16/129 (12.4)	<0.001
Micturition disturbance	26/103 (25.2)	11/122 (9.0)	0.001
Spinal cord MRI lesion (%)	64/105 (61.0)	3/39 (7.7)	<0.001
Brain MRI lesion (%)	8/82 (9.8)	7/67 (10.4)	NS
NCS abnormalities (%)	21/85 (24.7)	111/119 (93.3)	<0.001
Needle EMG abnormalities (%)	10/46 (21.7)	22/38 (57.9)	0.001
Evoked potential (%)			
MEP, central abnormalities	28/55 (50.9)	4/10 (40.0)	NS
MEP, peripheral abnormalities	8/55 (14.5)	6/10 (60.0)	0.001
SEP, central abnormalities	26/70 (37.1)	3/24 (12.5)	0.039
SEP, peripheral abnormalities	7/70 (10.0)	11/24 (45.8)	0.001
VEP abnormalities	5/23 (21.7)	1/3 (33.3)	NS
BAEP abnormalities	2/6 (33.3)	0/3 (0.0)	NS

The presence of serum antineutrophil cytoplasmic autoantibody was assessed in 13 patients with atopic myelitis (AM); all were negative.
*Mean ± SD.
†Hyperreflexia was seen in 77.8% of patients with AM and 9.4% of patients with atopy-related peripheral neuritis (APN) ($p < 0.001$), and pathologic reflexes were seen in 29.0% of patients with AM and 4.8% of patients with APN ($p < 0.001$).
IgE = immunoglobulin E; NS = not significant; *D pteronyssinus* = *Dermatophagoides pteronyssinus*; *D farinae* = *Dermatophagoides farinae*; NCS = nerve conduction study; MEP = motor-evoked potential; SEP = sensory-evoked potential; VEP = visual-evoked potential; BAEP = brainstem auditory-evoked potential.

allergic conjunctivitis than patients with APN, whereas the latter group showed a significantly higher frequency of bronchial asthma than the former. Both motor weakness and muscle atrophy occurred more commonly in patients with APN than in patients with AM throughout the clinical course. Pyramidal tract signs and micturition disturbance were found more commonly in patients with AM than in patients with APN, whereas hyporeflexia was more frequently detected in patients with APN than in patients with AM.

On spinal cord MRI, intramedullary lesions were detected more commonly in patients with AM than in patients with APN. NCS abnormalities and abnormal records on needle EMG, suggestive of neurogenic changes, were found more frequently in patients with APN than in patients with AM. Central conduction abnormalities in MEPs, such as no response recorded by scalp stimulation with normal elicitation by cervical or lumbar stimulation or prolonged central motor conduction times, were seen in half of the patients with AM and were also not rare in the patients with APN tested (40.0%). Peripheral nerve involvements in MEPs, such as unevoked or delayed responses to cervical or lumbar stimulation, were seen more frequently in patients with APN than in patients with AM. Central conduction abnormalities in SEPs, such as no potential recorded on the scalp with normal responses at the cervical or lumbar records or prolonged central sensory conduction times, were more frequently found in patients with AM than in patients with APN, whereas abnormalities suggestive of peripheral nerve involvement, such as unevoked potential or delayed responses in the cervical or lumbar records, were more common in patients with APN than in patients with AM. Overall, 25.7% of patients with AM (28/109) had at least 1 abnormality indicating peripheral nerve involvement by NCS or evoked potentials (EPs), whereas 18.8% of patients with APN (25/133) had overt or subclinical CNS involvement, as shown by the existence of pyramidal tract signs or abnormalities in laboratory tests, such as MRI and EPs. When the survey period was set to 2000–2006 for both AM and APN cases, essentially the same results were obtained (data not shown).

Differences in clinical features by preceding atopic disorders in patients with atopic myelitis. Patients with AM were classified according to preceding atopic disorders into 3 groups: patients with preceding bronchial asthma, those with allergic rhinitis, and those with nonairway allergies.[4,18,19] The last group included those who had atopic dermatitis, food allergy, or allergic conjunctivitis, or hyperIgEemia plus allergen-specific IgE without overt atopic diseases.

Table 2 Clinical features of patients with atopic myelitis according to preceding airway allergy

	Preceding atopic diseases		
	Bronchial asthma (n = 31)	Allergic rhinitis (n = 29)	Nonairway allergy (n = 49)
Men:women (sex ratio)	15:16 (1:1.07)[‡]	11:18 (1:1.64)[§]	36:13 (1:0.36)[‡§]
Age at onset, y	33.7 ± 15.5	35.8 ± 13.4	33.0 ± 11.1
Disease duration, y	5.5 ± 3.5	6.4 ± 3.3	7.5 ± 5.9
Symptoms and signs during illness (%)			
Dysesthesia	27/31 (87.1)	24/29 (82.8)	41/48 (85.4)
Hypesthesia	22/29 (75.9)	22/28 (78.6)	33/48 (68.8)
Motor weakness	23/31 (74.2)[¶]	20/29 (69.0)	22/49 (44.9)[¶]
Muscle atrophy	6/30 (20.0)[¶]	2/29 (6.9)	1/47 (2.1)[¶]
Hyporeflexia	2/31 (6.5)	1/29 (3.5)	2/48 (4.2)
Pyramidal tract sign	26/31 (83.9)	23/29 (79.3)	40/48 (83.3)
Hyperreflexia	24/31 (77.4)	22/29 (75.9)	38/48 (79.2)
Pathologic reflex	10/31 (32.3)	6/28 (21.4)	15/48 (31.3)
Micturition disturbance	8/29 (27.6)	8/28 (28.6)	10/46 (21.7)
Lhermitte sign	4/30 (13.3)	5/27 (18.5)	10/45 (22.2)
Painful tonic spasm	0/30 (0.0)	2/28 (7.1)	1/46 (2.2)
Girdle sensation	3/30 (10.0)	1/27 (3.7)	6/46 (13.0)
Involuntary movement*	1/30 (3.3)	3/27 (11.1)	1/47 (2.1)
EDSS at the peak of illness†	3.6 ± 2.6	3.1 ± 1.5	3.4 ± 1.9
EDSS at the last examination†	2.4 ± 1.8	2.4 ± 1.5	2.1 ± 1.6
Clinical course (%)			
Monophasic	10/28 (35.7)[¶]	10/27 (37.0)[‖]	27/41 (65.9)[¶‖]
Relapsing-remitting	3/28 (10.7)	4/27 (14.8)	8/41 (19.5)
Fluctuating	8/28 (28.6)	11/27 (40.7)[§]	3/41 (7.3)[§]
Progressive	8/28 (28.6)	2/27 (7.4)	4/41 (9.8)

*Involuntary movement included myoclonus, pseudoathetosis, and tremor.
†Mean ± SD.
‡$0.05 \leq p^{corr} < 0.1$ (bronchial asthma vs nonairway allergy).
§$p^{corr} < 0.01$ (allergic rhinitis vs nonairway allergy).
¶$0.01 \leq p^{corr} < 0.05$ (bronchial asthma vs nonairway allergy).
‖$0.05 \leq p^{corr} < 0.1$ (allergic rhinitis vs nonairway allergy).
EDSS = Expanded Disability Status Scale of Kurtzke.

Comparison of the demographic features among the 3 groups disclosed the following differences (tables 2 and e-2). The proportion of men was higher among patients with nonairway allergy than among those with allergic rhinitis ($p^{corr} = 0.006$) and those with bronchial asthma ($p^{corr} = 0.069$). Motor weakness and muscle atrophy were more common in patients with bronchial asthma than in those without airway allergy ($p^{corr} < 0.05$). A monophasic course was more commonly seen in patients with nonairway allergy than in those with bronchial asthma ($p^{corr} = 0.041$) and those with allergic rhinitis ($p^{corr} = 0.078$), whereas a fluctuating course was more frequent in patients with allergic rhinitis than in those with nonairway allergy ($p^{corr} = 0.005$). MEP abnormalities suggestive of CNS lesions were more common in the upper limbs than in the lower limbs in all patients with AM ($p = 0.016$), whereas abnormalities suggestive of PNS involvement in lower limbs were found more frequently in patients with allergic rhinitis than in those with nonairway allergy ($p^{corr} = 0.053$).

Although among all patients with AM the peak EDSS scores were significantly higher than the final EDSS scores (3.4 ± 2.0 vs 2.3 ± 1.6, $p < 0.001$), the peak EDSS scores were significantly higher in those who had undergone immunotherapy than in those who had not (3.7 ± 2.2 vs 2.6 ± 1.5, $p = 0.003$), whereas there was no significant difference in final EDSS scores (2.5 ± 1.8 vs 1.8 ± 1.1) and disease duration (6.5 ± 5.1 vs 7.1 ± 3.6 years) between the 2 groups. Although the effectiveness of methylprednisolone pulse therapy and oral corticosteroids was the same among patients with different preceding atopic diseases, that of plasma exchange was much higher in patients with bronchial asthma ($p^{corr} = 0.077$) and those with allergic rhinitis ($p^{corr} = 0.027$) than in those with nonairway allergy.

Differences in clinical features according to fulfillment of classic CSS criteria. Patients with APN were classified into 2 groups: 82 patients who met the criteria for CSS[15] (classic CSS) and the remaining 51 patients who did not (nonclassic CSS) (tables 3, 4, and e-3). Patients with classic CSS had older age at onset of neurologic symptoms and shorter disease duration than those with nonclassic CSS. Chronic onset was more common in the latter group than in the former.

Lower limb onset was more common in patients with classic CSS than in those with nonclassic CSS. Throughout the entire clinical course, hypesthesia was more frequently observed in the classic CSS group than in the nonclassic group. Mononeuritis multiplex was more common than polyneuritis and asymmetrical polyneuritis in either group. Dysesthesia, pain, motor weakness, and hyporeflexia were more common in patients with classic CSS than in those with nonclassic CSS. Systemic symptoms were more common in the classic CSS group than in the nonclassic CSS group. The mRS score at the peak of illness was higher in patients with classic CSS than in those with nonclassic CSS. Peripheral eosinophilia was more prominent in the former group than in the latter. Serum antineutrophil cytoplasmic antibody (ANCA) was positive in 47.7% of patients (21/44) with classic CSS and 40.0% of patients (4/10) with nonclassic CSS. Abnormalities in NCS studies were more frequently observed in patients with classic CSS than in those with nonclassic CSS. There were no remarkable differences in either motor or sensory nerve conduction study findings between the 2

Table 3 Clinical features of patients with atopy-related peripheral neuritis

	Total (n = 133)	Classic CSS* (n = 82)	Others (n = 51)	p Value
Men:women (sex ratio)	52:81 (1:1.56)	31:51 (1:1.65)	21:30 (1:1.43)	NS
Age at onset, y†	55.8 ± 14.3	59.2 ± 11.2	49.6 ± 17.2	0.004
Disease duration, y	4.5 ± 4.7	2.9 ± 2.2	7.5 ± 6.4	<0.001
Preceding atopic diseases				
Atopic dermatitis	8/111 (7.2)	1/63 (1.6)	7/48 (14.6)	0.020
Allergic rhinitis	30/109 (27.5)	13/64 (20.3)	17/45 (37.8)	0.044
Bronchial asthma	96/130 (73.8)	82/82 (100.0)	14/48 (29.2)	<0.001
Food allergy	6/102 (5.9)	1/56 (1.8)	5/46 (10.9)	NS
Mode of onset (%)				
Sudden	8/131 (6.1)	4/82 (4.9)	4/49 (8.2)	NS
Acute	31/131 (23.7)	23/82 (28.0)	8/49 (16.3)	NS
Subacute	54/131 (41.2)	43/82 (52.4)	11/49 (22.4)	<0.001
Chronic	38/131 (28.8)	12/82 (14.6)	26/49 (53.1)	<0.001
Initial location of symptoms (%)				
Upper extremities	42/126 (33.3)	21/80 (26.3)	21/46 (45.7)	0.026
Lower extremities	84/126 (66.7)	61/80 (76.3)	23/46 (50.0)	0.003
Trunk	3/126 (2.4)	2/80 (2.5)	1/46 (2.2)	NS
Cranial nerves	5/126 (4.0)	3/80 (3.8)	2/46 (4.4)	NS
Symptoms and signs during illness (%)				
Hypesthesia	102/123 (82.9)	75/80 (93.8)	27/43 (62.8)	<0.001
Types of neuritis				
Mononeuritis multiplex	62/102 (60.8)	48/75 (64.0)	14/27 (51.9)	NS
Polyneuritis	25/102 (24.5)	18/75 (24.0)	7/27 (25.9)	NS
Asymmetrical polyneuritis	10/102 (9.8)	8/75 (10.7)	2/27 (7.4)	NS
Dysesthesia	112/127 (88.2)	76/81 (93.8)	36/46 (78.3)	0.009
Pain	86/131 (65.6)	61/81 (75.3)	25/50 (50.0)	0.003
Motor weakness	101/125 (80.8)	70/80 (87.5)	31/45 (68.9)	0.011
Muscle atrophy	39/122 (32.0)	22/79 (27.8)	17/43 (39.5)	NS
Hyporeflexia	75/128 (58.6)	56/79 (70.9)	19/49 (38.8)	<0.001
Micturition disturbance	11/122 (9.0)	4/77 (5.2)	7/45 (15.6)	NS
Pyramidal tract sign‡	16/129 (12.4)	7/79 (8.9)	9/50 (18.0)	NS
Systemic symptoms	81/131 (61.8)	58/82 (70.7)	23/49 (46.9)	0.007
Pulmonary	49/131 (37.4)	38/82 (46.3)	11/49 (22.5)	0.006
Dermal	33/129 (25.6)	20/80 (25.0)	13/49 (26.5)	NS
Renal	8/130 (6.2)	7/81 (8.6)	1/49 (2.0)	NS
Cardiac	3/130 (2.3)	3/81 (3.7)	0/49 (0.0)	NS

*Classic Churg-Strauss syndrome (CSS) indicates patients who met the criteria for CSS.[15]
†Mean ± SD.
‡9.8% of all patients with atopy-related peripheral neuritis had hyperreflexia, whereas 4.8% had pathologic reflexes. There was no significant difference in the occurrence of the 2 symptoms between the 2 groups.
NS = not significant.

groups, except for an absence of sensory nerve action potentials (SNAPs) in the lower limbs, which was more common in patients with classic CSS than in those with nonclassic CSS. EP abnormalities suggestive of CNS involvement were occasionally encountered in each subgroup. On spinal cord MRI, 3 of the 39 patients with APN examined had intramedullary lesions, whereas among 67 patients who underwent brain MRI, 10 showed brain atrophy, 3 showed an old brain hemorrhage, and 7 had other focal lesions. In the CSF, pleocytosis was present in 7 of 74 patients with APN tested, whereas a protein increase was seen in 14, more commonly in the patients with nonclassic CSS than in those with classic CSS.

Multiple logistic regression analyses. Finally, we performed multiple logistic regression analyses to identify possible factors contributing to the development of myelitis in patients with pure AM or APN (table 5). Atopic dermatitis increased the risk of developing myelitis (adjusted $p = 0.013$), whereas age at onset (adjusted $p < 0.001$) and bronchial asthma (adjusted $p = 0.006$) decreased that risk.

DISCUSSION This study had some limitations: the questionnaires were answered by many clinicians across the country, and the response rates in the surveys were not high, because of the rarity of these diseases. However, the main demographic features of patients with AM found in this study were similar to those reported in our previous studies[3,4] and the first nationwide survey on AM.[5] These features include a predilection for young adults, mild preponderance of men, predominant sensory impairment, preferential involvement of the cervical spinal cord on MRI, and scant CSF inflammatory response. The same was true for patients who met the criteria for CSS. The characteristics found in the present study, namely preferential occurrence in middle to late adulthood, mild preponderance of women, prominent eosinophilia, frequent painful dysesthesia at onset, high frequency of mononeuritis multiplex and absent SNAPs in the lower limbs, and favorable response to corticosteroids, are also described in institutional series of Japanese[20] and Western[21,22] patients with CSS. Therefore, we considered that the results of this survey were not seriously distorted by the aforementioned confounding factors.

We collected patients with APN including not only classic cases of CSS after bronchial asthma, but also those with peripheral neuritis of unknown cause subsequent to other atopic disorders. We found that patients with classic CSS showed a higher age at onset, greater disability despite the shorter disease duration, more common occurrence of acute onset, higher frequency of lower limb involvement, and higher frequencies of hypesthesia, dysesthesia, pain, motor weakness, hyporeflexia, and systemic organ involvement than patients with nonclassic CSS, in addition to having greater numbers of eosinophils and preceding bronchial asthma attributable to the inclu-

Table 4 Laboratory findings and treatment responses of patients with atopy-related peripheral neuritis

	Total (n = 133)	Classic CSS* (n = 82)	Others (n = 51)	p Value
Blood eosinophils, /μL†	7,860 ± 8,740	10,960 ± 9,250	1,930 ± 2,440	<0.001
Total serum IgE, U/mL†	1,770 ± 2,740	2,180 ± 3,070	730 ± 1,150	0.001
NCS abnormalities (%)	111/119 (93.3)	79/80 (98.8)	32/39 (82.1)	0.002
Upper extremities				
Motor nerve				
Decreased conduction velocity	16/97 (16.5)	14/68 (20.6)	2/29 (6.9)	NS
Decreased amplitude	44/97 (45.4)	31/68 (45.6)	13/29 (44.8)	NS
Not evoked	5/97 (5.2)	4/68 (5.9)	1/29 (3.4)	NS
Sensory nerve				
Decreased conduction velocity	17/98 (17.3)	12/69 (17.4)	5/29 (17.2)	NS
Decreased amplitude	37/98 (37.8)	23/69 (33.3)	14/29 (48.3)	NS
Not evoked	21/98 (21.4)	15/69 (21.7)	6/29 (20.7)	NS
Lower extremities				
Motor nerve				
Decreased conduction velocity	28/106 (26.4)	21/75 (28.0)	7/31 (22.6)	NS
Decreased amplitude	62/106 (58.5)	45/75 (60.0)	17/31 (54.8)	NS
Not evoked	25/106 (23.6)	20/75 (26.7)	5/31 (16.1)	NS
Sensory nerve				
Decreased conduction velocity	16/106 (15.1)	10/75 (13.3)	6/31 (19.4)	NS
Decreased amplitude	27/106 (25.5)	16/75 (21.3)	11/31 (35.5)	NS
Not evoked	50/106 (47.2)	41/75 (54.7)	9/31 (29.0)	0.016
Needle EMG abnormalities (%)	22/38 (57.9)	9/17(52.9)	13/21 (61.9)	NS
CSF (%)				
Pleocytosis	7/74 (9.5)	5/47 (10.6)	2/27 (7.4)	NS
<50/μL	5/7 (71.4)	4/5 (80.0)	1/2 (50.0)	NS
≥50/μL	2/7 (28.6)	1/5 (20.0)	1/2 (50.0)	NS
Increased protein	14/74 (18.9)	5/47 (10.6)	9/27 (33.3)	0.016
<100 mg/dL	12/14 (85.7)	4/5 (80.0)	8/9 (88.9)	NS
≥100 mg/dL	2/14 (14.3)	1/5 (20.0)	1/9 (11.1)	NS
OCB positive	2/23 (8.7)	2/12 (16.7)	0/11 (0.0)	NS
Increased IgG index	5/41 (12.2)	2/24 (8.3)	3/17 (17.6)	NS
mRS at the peak of illness†	3.1 ± 1.2	3.3 ± 1.1	2.6 ± 1.2	<0.001
mRS at the last examination	2.0 ± 1.0	2.0 ± 1.0	2.0 ± 1.1	NS
Treatment (%)				
Oral corticosteroids	106/132 (80.3)	80/82 (97.6)	26/50 (52.0)	<0.001
Effective	98/102 (96.1)	76/78 (97.4)	22/24 (91.7)	NS
Intravenous methylprednisolone	72/129 (55.8)	61/81 (75.3)	11/48 (22.9)	<0.001
Effective	63/64 (98.4)	53/54 (98.1)	10/10 (100.0)	NS
Immunosuppressants	12/119 (10.1)	10/72 (13.9)	2/47 (4.3)	NS
Effective	10/11 (90.9)	9/9 (100.0)	1/2 (50.0)	NS
Plasma exchange	3/117 (2.6)	1/70 (1.4)	2/47 (4.3)	NS
Effective	1/3 (33.3)	0/1 (0.0)	1/2 (50.0)	NS
Intravenous immunoglobulin	13/120 (10.8)	7/72 (9.7)	6/48 (12.5)	NS
Effective	9/12 (75.0)	5/6 (83.3)	4/6 (66.7)	NS

*Classic Churg-Strauss syndrome (CSS) indicates patients who met the criteria for CSS.[15]
†Mean ± SD.
NCS = nerve conduction study; NS = not significant; mRS = modified Rankin Scale; OCB = oligoclonal immunoglobulin G bands; IgG = immunoglobulin G.

Table 5 Multiple logistic regression analyses for possible factors contributing to the development of myelitis in patients with atopy-related neural damage

	AM only* (n = 66)	APN only* (n = 104)	Crude OR (95% CI)	Adjusted OR (95% CI)
Men	37/66 (56.1)	39/104 (37.5)	2.13 (1.14-3.98)	2.75 (0.95-8.68)
Age at onset, y	33.9 ± 13.2	56.3 ± 14.2	0.91† (0.88-0.93)	0.93† (0.90-0.96)
Preceding atopic diseases (%)				
Atopic dermatitis	25/64 (39.1)	4/85 (4.7)	12.98 (4.23-39.88)	5.77 (1.56-25.73)
Allergic rhinitis	24/65 (36.9)	23/84 (27.4)	1.55 (0.77-3.11)	2.33 (0.77-7.46)
Bronchial asthma	20/65 (30.8)	80/102 (78.4)	0.12 (0.06-0.25)	0.24 (0.08-0.65)
Food allergy	8/60 (13.3)	3/79 (3.8)	3.90 (0.99-15.38)	4.96 (0.93-33.74)

*Four patients with atopy-related peripheral neuritis (APN) and 15 patients with atopic myelitis (AM) who did not receive adequate examinations were not included in the analyses.
†The odds ratio (OR) presented united one.
CI = confidence interval.

sion criterion. However, other clinical and laboratory features were similar between the 2 groups, including the positivity rate for serum ANCA. It is possible that nonclassic CSS may encompass atopic patients with incidental complication of neuropathy. However, many common features between classic and nonclassic CSS cases suggest that it is potentially useful to place such nonclassic CSS cases with milder features within a spectrum of atopy-related neural damage.

A quarter of patients with AM had concomitant subclinical PNS involvement as demonstrated by the NCS or EP tests, whereas overt and subclinical CNS involvement, as shown by neurologic, MRI, or EP findings, existed in nearly 20% of patients with APN. Moreover, some patients with AM and patients with APN had visual-evoked potential or brainstem auditory-evoked potential abnormalities, in accordance with a British case report describing optic neuritis in parallel with the severity of atopic dermatitis.[23] In the literature, there are also a few reports presenting classic CSS cases with optic neuritis[24-27] and multifocal CNS vasculitis.[14] These observations and past reports indicate that a fraction of patients with AM and patients with APN develop both CNS and PNS involvement, even though the predominantly affected sites differ.

By logistic regression, preceding atopic dermatitis was shown to increase the risk of myelitis, whereas higher age at onset and preceding bronchial asthma were shown to increase the risk for peripheral neuritis. CSS was originally described in patients with bronchial asthma, whereas atopic myelitis was originally described in those with atopic dermatitis. Therefore, awareness of such associations among neurologists may in part contribute to the results. However, even among patients with AM, those with nonairway allergy tended to show sensory-predominant symptoms, such as dysesthesia and hypesthesia, whereas motor weakness and muscle atrophy were more frequent in those with airway allergy. This finding coincides with the results of previous studies.[4,5] Patients with myelitis and nonairway allergies seem to have lesions more confined to the posterior column, whereas those with airway allergies have more widespread lesions affecting the pyramidal tracts, anterior horns, and motor nerves. Th2 cytokine production in lymphocytes upon allergen stimulation is reported to be more exaggerated in patients with bronchial asthma than in those with atopic dermatitis.[28] Because Th2 cytokines are considered to be involved in both CSS and AM, such differences in Th2 cytokine production according to skin or airway allergy may in part contribute to the development of either confined or more widespread lesions, and either myelitis or peripheral neuritis.

The observation that eosinophilic inflammation is prominent in the active lesions in both patients with AM and patients with CSS supports the notion that AM and APN, at least in part, have certain common mechanisms, specifically eosinophil-mediated ones. Moreover, granuloma formation, another characteristic manifestation of CSS, was also observed in spinal cord lesions in the eldest case among the 6 patients with biopsy-proven AM.[7] However, transmural arterial necrosis with infiltration of lymphocytes and polymorphonuclear leukocytes, a distinctive feature of classic CSS, has not yet been noted in the limited biopsied materials from patients with AM. In addition, in patients with AM, no systemic multiorgan involvement due to vasculitis was observed,[3,4] and in this survey there was no case positive for ANCA, a useful marker for systemic vasculitis. Therefore, the inflammatory mechanism may not be entirely the same in AM and CSS, especially the vasculitic process involving the small arteries operative in CSS but not in AM. In general, atopic disorders become more severe and refractory in elderly patients than in younger ones after atopic diseases develop,[29] and inflammatory cytokine production is more prominent in the elderly.[30] These facts indicate a contribution of age-related immunologic changes to atopy-related neural inflammation, especially in patients with CSS.

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DISCLOSURE

Dr. Isobe, Dr. Kawamura, Dr. Ishizu, and Dr. Kawano report no disclosures. Dr. Kira serves on the editorial board of *Multiple Sclerosis, The Open Neurology Journal,* and *Autoimmune Diseases,* serves as on the editorial advisory board of *The Year in Neurology,* and serves as a journal editor of *Rinsho Shinkeigaku;* serves as a consultant for Biogen Idec Japan; received

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REFERENCES

1. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. Am J Pathol 1951;27:277–301.
2. Kira J, Yamasaki K, Kawano Y, Kobayashi T. Acute myelitis associated with hyperIgEemia and atopic dermatitis. J Neurol Sci 1997;148:199–203.
3. Kira J, Kawano Y, Yamasaki K, Tobimatsu S. Acute myelitis with hyperIgEaemia and mite antigen specific IgE: atopic myelitis. J Neurol Neurosurg Psychiatry 1998;64: 676–679.
4. Kira J, Kawano Y, Horiuchi I, et al. Clinical, immunological and MRI features of myelitis with atopic dermatitis (atopic myelitis). J Neurol Sci 1999;162:56–61.
5. Osoegawa M, Ochi H, Minohara M, et.al. Myelitis with atopic diathesis: a nationwide survey of 79 cases in Japan. J Neurol Sci 2003;209:5–11.
6. Kikuchi H, Osoegawa M, Ochi H, et al. Spinal cord lesions of myelitis with hyperIgEemia and mite antigen specific IgE (atopic myelitis) manifest eosinophilic inflammation. J Neurol Sci 2001;183:73–78.
7. Osoegawa M, Ochi H, Kikuchi H, et al. Eosinophilic myelitis associated with atopic diathesis: a combined neuroimaging and histopathological study. Acta Neuropathol 2003;105:289–295.
8. Zoli A, Mariano M, Fusari A, Bonifazi F, Antonicelli L. Atopic myelitis: first case report outside Japan? Allergy 2005;60:410–411.
9. Gregoire SM, Mormont E, Laloux P, Godfraind C, Gilliard C. Atopic myelitis: a clinical, biological, radiological and histopathological diagnosis. J Neurol Sci 2006;247: 231–235.
10. Tanaka M, Matsushita T, Tateishi T, et al. Distinct CSF cytokine/chemokine profiles in atopic myelitis and other causes of myelitis. Neurology 2008;71:974–981.
11. Horinouchi H, Inobe J, Mori T, Kumamoto T, Tsuda T. A case of atopic myelitis [in Japanese]. Rinsho Shinkeigaku 2000;40:218–221.
12. Osoegawa M, Ochi H, Yamada T, et al. High incidence of subclinical peripheral neuropathy in myelitis with hyperIgEaemia and mite antigen-specific IgE (atopic myelitis): an electrophysiological study. Intern Med 2002;41:684–691.
13. Tokunaga H, Osoegawa M, Murai H, et al. Anterior horn cell involvement in myelitis with atopic diathesis (atopic myelitis). Fukuoka Igaku Zasshi 2004;95:36–43.
14. Hagiwara K, Tominaga K, Shida N, Yamashita Y. Multifocal enhancing cortical lesions in a patient with Churg-Strauss syndrome. Neurology 2008;71:1546–1548.
15. Nagasawa T, Yoshida M. Clinical features of allergic granulomatous angiitis in Japan and propose of guideline for clinical diagnosis of this disease [in Japanese]. Nippon Naika Gakkai Zasshi 1989;78:352–356.
16. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–1452.
17. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJA, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–607.
18. Kira J, Horiuchi I, Suzuki J, et al. Myelitis associated with atopic disorders in Japan: a retrospective clinical study of the past 20 years. Intern Med 2001;40:613–619.
19. Kira J, Isobe N, Kawano Y, et al. Atopic myelitis with focal amyotrophy: a possible link to Hopkins syndrome. J Neurol Sci 2008;269:143–151.
20. Hattori N, Ichimura M, Nagamatsu M, et al. Clinicopathological features of Churg-Strauss syndrome-associated neuropathy. Brain 1999;122:427–439.
21. Guillevin L, Cohen P, Gayraud M, Lhote F, Jarrousse B, Casassus P. Churg-Strauss syndrome: clinical study and long-term follow-up of 96 patients. Medicine (Baltimore) 1999;78:26–37.
22. Solans R, Bosch JA, Pérez-Bocanegra C, et al. Churg-Strauss syndrome: outcome and long-term follow-up of 32 patients. Rheumatology 2001;40:763–771.
23. Constantinescu CS, Thomas M, Zaman AG. Atopic optic neuritis. Ocul Immunol Inflamm 2006;14:125–127.
24. Giorgi D, Laganà B, Giorgi A, et al. Ischemic optic neuritis in Churg-Strauss syndrome. Recenti Prog Med 1997;88: 273–275.
25. Vitali C, Genovesi-Ebert F, Romani A, Jeracitano G, Nardi M. Opthalmological and neuro-ophthalmological involvement in Churg-Strauss syndrome: a case report. Graefes Arch Clin Exp Ophthalmol 1996;234:404–408.
26. Shintani S, Tsuruoka S, Yamada M. Churg-Strauss syndrome associated with third nerve palsy and mononeuritis multiplex of the legs. Clin Neurol Neurosurg 1995;97: 172–174.
27. Liou HH, Yip PK, Chang YC, Liu HM. Allergic granulomatosis and angiitis (Churg-Strauss syndrome) presenting as prominent neurologic lesions and optic neuritis. J Rheumatol 1994;21:2380–2384.
28. Kimura M, Tsuruta S, Yoshida T. Differences in cytokine production by peripheral blood mononyuclear cells (PBMC) between patients with atopic dermatitis and bronchial asthma. Clin Exp Immunol 1999;118:192–196.
29. Criep LH. The march of allergy. Educational and clinical progress JAMA 1958;166:572–577.
30. O'Mahony L, Holland J, Jackson J, Feighery C, Hennessy TPJ, Mealy K. Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin Exp Immunol 1998;113:213–219.

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CSF chemokine alterations related to the clinical course of amyotrophic lateral sclerosis

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ABSTRACT

We measured the levels of 27 cytokines/chemokines and growth factors in cerebrospinal fluid (CSF) from 42 patients with sporadic amyotrophic lateral sclerosis (ALS), 12 patients with lower motor neuron disease (LMND), and 34 control patients with non-inflammatory neurological diseases (OND), using a multiplexed fluorescent bead-based immunoassay. Among cytokines/chemokines elevated in ALS, CCL2 and CXCL8 levels were negatively correlated with the revised ALS functional rating scale (ALSFRS-R) score, while CCL4 showed a positive correlation with ALSFRS-R score. CCL4 and CXCL10 showed negative correlations with disease progression rate. These chemokine alterations are assumed to somehow correlate with the clinical course of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which loss of motor neurons in the spinal cord, brainstem and motor cortex causes progressive paralysis. Studies using ALS model mice have reported that non-cell-autonomous cell death is a major contributor to motor neuron death (Boillée et al., 2006; Clement et al., 2003; Lobsiger and Cleveland, 2007; Yamanaka et al., 2008). Neuroglial inflammation is thus suggested to be crucial for motor neuron loss. Even in human sporadic ALS, increasing evidence suggests that certain cytokines/chemokines and growth factors, key mediators of both immune and neural networks, play critical roles in certain stages of ALS (Consilvio et al., 2004; McGeer and McGeer, 2002).

In human ALS, the levels of CCL2 (also known as macrophage chemoattractant protein-1) (Henkel et al., 2004; Wilms et al., 2003), interleukin (IL)-6 (Sekizawa et al., 1998), tumor necrosis factor (TNF)-α (Moreau et al., 2005; Poloni et al., 2000), and transforming growth factor (TGF)-β (Ilzecka et al., 2002) have been reported to be elevated in cerebrospinal fluid (CSF). We also measured the levels of 16 cytokines and chemokines in CSF from ALS patients by multiplexed fluorescent bead-based immunoassay, and found that CCL2, IL-5, and granulocyte-colony stimulating factor (G-CSF) are significantly elevated in patients with ALS compared with the levels in patients with non-inflammatory neurologic diseases (Tanaka et al., 2006). Among these, CCL2 showed a significant negative correlation with the revised ALS functional rating scale (ALSFRS-R) score, suggesting the possibility that this chemokine is a disease-aggravating factor (Tanaka et al., 2006). Recently, Mitchell et al (2009) reported that a variety of proinflammatory cytokines and growth factors, namely, CCL2, CCL3 (macrophage inflammatory protein-1α), CCL4 (macrophage inflammatory protein-1β), IL-2, IL-6, IL-15, and IL-17, G-CSF, vascular endothelial growth factor (VEGF), granulocyte-macrophage colony stimulating factor (GM-CSF), and basic fibroblast growth factor (bFGF), were all elevated in ALS patients' CSF. They reported that none of these had any significant correlation with clinical parameters, but that non-elevated CXCL8 (IL-8) had a weak negative correlation with the ALSFRS-R score. No biomarkers related to neuroprotection in ALS are known. Therefore, in the present study, we profiled CSF cytokines/chemokines and growth factors to identify those related to the clinical parameters of ALS and lower motor neuron disease (LMND).

2. Materials and methods

2.1. Patients

A total of 42 patients with sporadic ALS (20 males and 22 females; mean age ± standard deviation [SD] at examination, 56.7 ± 13.2 years) and 12 patients with sporadic LMND (six males and six females; 55.2 ± 15.7 years) were examined (Table 1). All patients with ALS were subjected to a thorough neurological examination and diagnosed as clinically definite or probable cases of ALS based on the El Escorial diagnostic criteria (Brooks, 1994) at the Department of Neurology,

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Table 1
Demographic features of patients with sporadic amyotrophic lateral sclerosis (ALS), lower motor neuron disease (LMND), and other non-inflammatory neurological diseases (OND).

	ALS	LMND	OND
Number of patients	42	12	34
Sex (male/female)	20/22	6/6	21/13
Age at examination (mean ± SD, years)	56.7 ± 13.2	55.2 ± 15.7	54.2 ± 12.9
Disease duration (mean ± SD, months)	13.0 ± 9.3	25.9 ± 28.6	NA
Immunologic treatment (for the past years)	None	None	None
ALSFRS-R score (mean ± SD)	39.0 ± 8.1	39.6 ± 6.72	NA
CSF			
Cell count (mean ± SD, per μl)	1.15 ± 1.04	1.27 ± 0.78	1.08 ± 1.11
Total protein in CSF (mean ± SD, mg/dl)	34.0 ± 14.3	42.4 ± 27.3	37.3 ± 17.7

Abbreviations in table: ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; CSF, cerebrospinal fluid; NA, not applicable; SD, standard deviation.

Kyushu University Hospital, from 2000 to 2006. The mean disease duration at the time of CSF withdrawal was 13.0 ± 9.3 months in ALS patients and 25.9 ± 28.6 months in LMND patients. The disability level associated with the development and progression of ALS and LMND was determined using the revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999). The mean ALSFRS-R score was 39.0 ± 8.1 in ALS patients, and 39.6 ± 6.72 in LMND patients. The disease progression rate was defined as ALSFRS-R full score (48) − a patient's ALSFRS-R score/disease duration expressed in months. Thirty-four control patients with other non-inflammatory neurological diseases (OND) but no malignancies (21 males and 13 females; age at examination, 54.2 ± 12.9 years) examined during the same period were also enrolled. The OND group comprised 10 patients with cervical spondylosis, eight with sporadic spinocerebellar degeneration, four with lumbar herniation, four with metabolic neuropathy, two with hereditary spinocerebellar atrophy (SCA3 and unknown), and one each with spastic spinal paraplegia, drug-induced dystonia, peroneal nerve palsy, normal pressure hydrocephalus, Strüthers' ligament syndrome, senile blepharoptosis, and urge incontinence. No subjects were hypoxemic or undergoing any immunotherapies at the time of CSF drawing. The male-to-female ratio was not significantly different among these groups according to the chi-square test ($p>0.1$). We compared the disease duration and the CSF total protein amounts between ALS and LMND patients using the Mann-Whitney U test. The disease duration was significantly longer in LMND patients than ALS patients ($p=0.0149$), probably reflecting a slower disease course in the former, while the total CSF protein levels were not significantly different between the two groups ($p>0.1$).

2.2. Cerebrospinal fluid collection

CSF samples were obtained by lumbar puncture from all patients and immediately centrifuged at 800 rpm at 4 °C for 5 min. The liquid phase of CSF that excluded the sedimented cells was stored at −80 °C until cytokine assay. CSF findings are shown in Table 1. No patients were considered to have systemic inflammation at the time CSF was drawn, because none had elevated serum C-reactive protein level or systemic autoantibodies, such as antinuclear antibody, SS-A and SS-B.

2.3. Multiplexed fluorescent bead-based immunoassay of CSF

The CSF liquid phase samples were simultaneously analyzed for 27 cytokines and chemokines, namely, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, TNF-α, interferon (IFN)-γ, CCL2, CCL3, CCL4, CCL5 (regulated upon activation normal T-cell expressed and secreted), CCL11, CXCL8, CXCL10, G-CSF, GM-CSF, bFGF, platelet-derived growth factor-bb (PDGFbb), and VEGF, using the Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Hercules, CA), as described previously (Ishizu et al., 2005; Tanaka et al., 2006). Briefly, 50 μl of each CSF liquid and various concentrations of each cytokine standard (Bio-Rad) were added to 50 μl of antibody-conjugated beads (Bio-Rad) in 96-well filter plates (Millipore, Billerica, MA). Cytokine concentrations were calculated by reference to a standard curve for each cytokine derived using various concentrations of the cytokine standards (0.2, 0.78, 3.13, 12.5, 50, 200, 800 and 3200 pg/ml) assayed in the same manner as the CSF samples. The same batch of monoclonal antibodies for the Bio-Plex Cytokine Assay System was used throughout the experiments; the interassay and intraassay variabilities are reported to be less than 10% by the manufacturer (de Jager et al., 2003; Vignali, 2000). The detection limit for each cytokine was determined by recovery of the corresponding cytokine standard, and the lowest values with more than 70% recovery were set as the lower detection limits. The lower detection limits were as follows: 12.5 pg/ml for GM-CSF and IFN-γ, 3.13 pg/ml for IL-1ra, IL-2, IL-4, IL-6, IL-9, IL-13, IL-17, TNF-α, CCL2, CCL3, CCL11, CXCL10, G-CSF, bFGF, and VEGF, 0.78 pg/ml for IL-12(p70), CCL4, and PDGFbb, and 0.2 pg/ml for IL-1β, IL-5, IL-7, IL-10, IL-15, CCL5, and CXCL8. All samples were analyzed undiluted in duplicate.

2.4. Statistical analyses

We used the following statistical tests for appropriate applications. The non-parametric Kruskal–Wallis H test was initially employed to compare the age at CSF withdrawal and CSF cytokine/chemokine levels among the studied group. When differences were significant, the Mann–Whitney U test was used to determine the significance of differences between each group. For multiple comparisons, uncorrected P values (P^{uncorr}) were corrected by multiplying them by the number of comparisons to calculate corrected P values (P^{corr}) (Bonferroni–Dunn's correction). The disease duration and the CSF protein amounts were compared using the Mann–Whitney U test. Spearman's rank correlation analysis was used to correlate various clinical parameters and CSF cytokine/chemokine levels which were significantly different among ALS, LMND and control. The male to female ratios were compared among the groups using the chi-square test. Statistical significance was set at $P<0.05$.

3. Results

3.1. Concentrations of each cytokine/chemokine in the liquid phase of CSF

Among the cytokines/chemokines measured, G-CSF, VEGF, CCL2, CCL4, CCL5, CCL11, CXCL8, CXCL10, TNF-α, IFN-γ, IL-1β, IL-7, IL-9, IL-12 (p70), and IL-17 levels were significantly higher in ALS than in OND patients (G-CSF: 9.670 ± 0.484 vs. 7.875 ± 0.537, $P^{corr}=0.0005$; VEGF: 8.450 ± 0.676 vs. 4.855 ± 0.751, $P^{corr}=0.0039$; CCL2: 276.755 ± 11.817 vs. 199.810 ± 13.134, $P^{corr}<0.0001$; CCL4: 12.820 ± 0.974 vs. 7.700 ± 1.082, $P^{corr}=0.0048$; CCL5: 0.845 ± 0.653 vs. 0.300 ± 0.726, $P^{corr}=0.0165$; CCL11: 10.535 ± 0.551 vs. 8.395 ± 0.612 pg/ml, $P^{corr}=0.0072$; CXCL8: 35.040 ± 1.498 vs. 24.335 ± 1.665, $P^{corr}<0.0001$; CXCL10: 456.545 ± 42.442 vs. 289.760 ± 47.171, $P^{corr}<0.0001$; TNF-α: 79.850 ± 3.266 vs. 61.125 ± 3.629, $P^{corr}=0.0031$; IFN-γ: 24.370 ± 1.355 vs. 19.590 ± 1.506, $P^{corr}=0.0132$; IL-1β: 0.955 ± 0.091 vs. 0.685 ± 0.101, $P^{corr}=0.0348$; IL-7: 1.495 ± 0.075 vs. 1.125 ± 0.084, $P^{corr}=0.0135$; IL-9: 27.090 ± 1.074 vs. 20.675 ± 1.193, $P^{corr}=0.0020$; IL-12(p70): 6.900 ± 0.524 vs. 5.065 ± 0.582, $P^{corr}=0.0339$; and IL-17: 2.700 ± 0.194 vs. 2.700 ± 0.215, $P^{corr}=0.0027$) (Fig. 1). The levels of the other cytokines/chemokines did not differ significantly between the two groups. No significant difference was found between the OND and LMND groups in the levels of any of the cytokines/chemokines examined. We found no significant

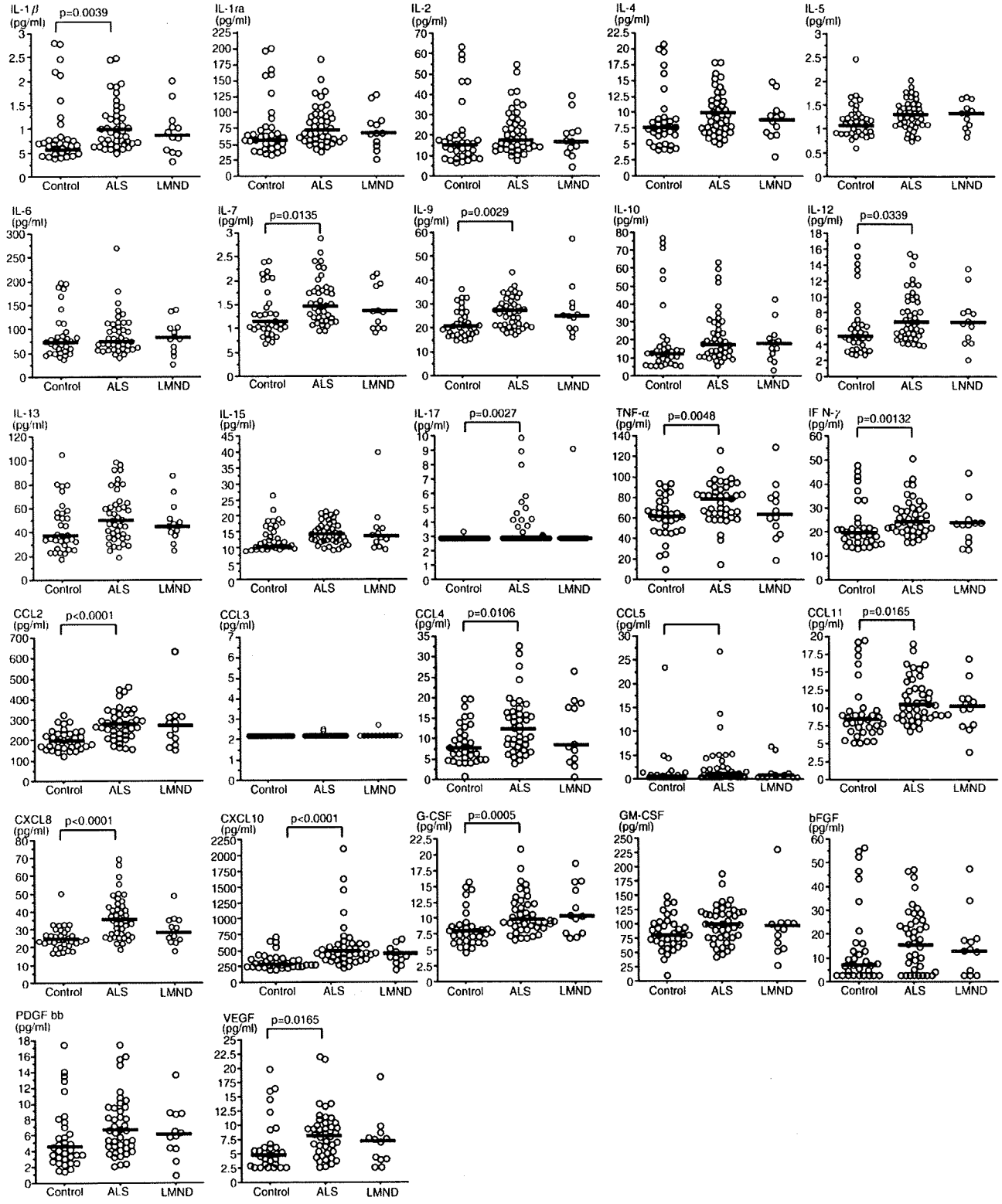

Fig. 1. Cytokine/chemokine levels in cerebrospinal fluid (CSF) supernatants from patients with amyotrophic lateral sclerosis (ALS) ($n=42$), lower motor neuron disease (LMND) ($n=12$), and other non-inflammatory neurological diseases (OND) ($n=34$) were measured using a multiplexed fluorescent bead-based immunoassay. Bars indicate the mean concentration in each group.

differences between the ALS and LMND patients, and the distributions and mean values of most cytokines/chemokines and growth factors, including VEGF in the LMND patients, showed similar trends to those in ALS patients; however, this similarity was not so obvious for some proinflammatory cytokines, namely TNF-α, CXCL8 and IL-17 (Fig. 1).

3.2. Correlations between individual cytokine/chemokine levels and between each cytokine/chemokine level and various clinical parameters

The concentration of CCL2 in CSF was negatively correlated with the ALSFRS-R score ($r=-0.390$, $P=0.0126$) and positively correlated with the CSF total protein level ($r=0.420$, $P=0.0071$) (Fig. 2). The concentration of CCL4 was positively correlated with the ALSFRS-R score ($r=0.354$, $P=0.0235$) and disease duration ($r=0.315$, $P=0.0435$), and negatively correlated with the disease progression rate ($r=-0.475$, $P=0.0024$). The concentration of

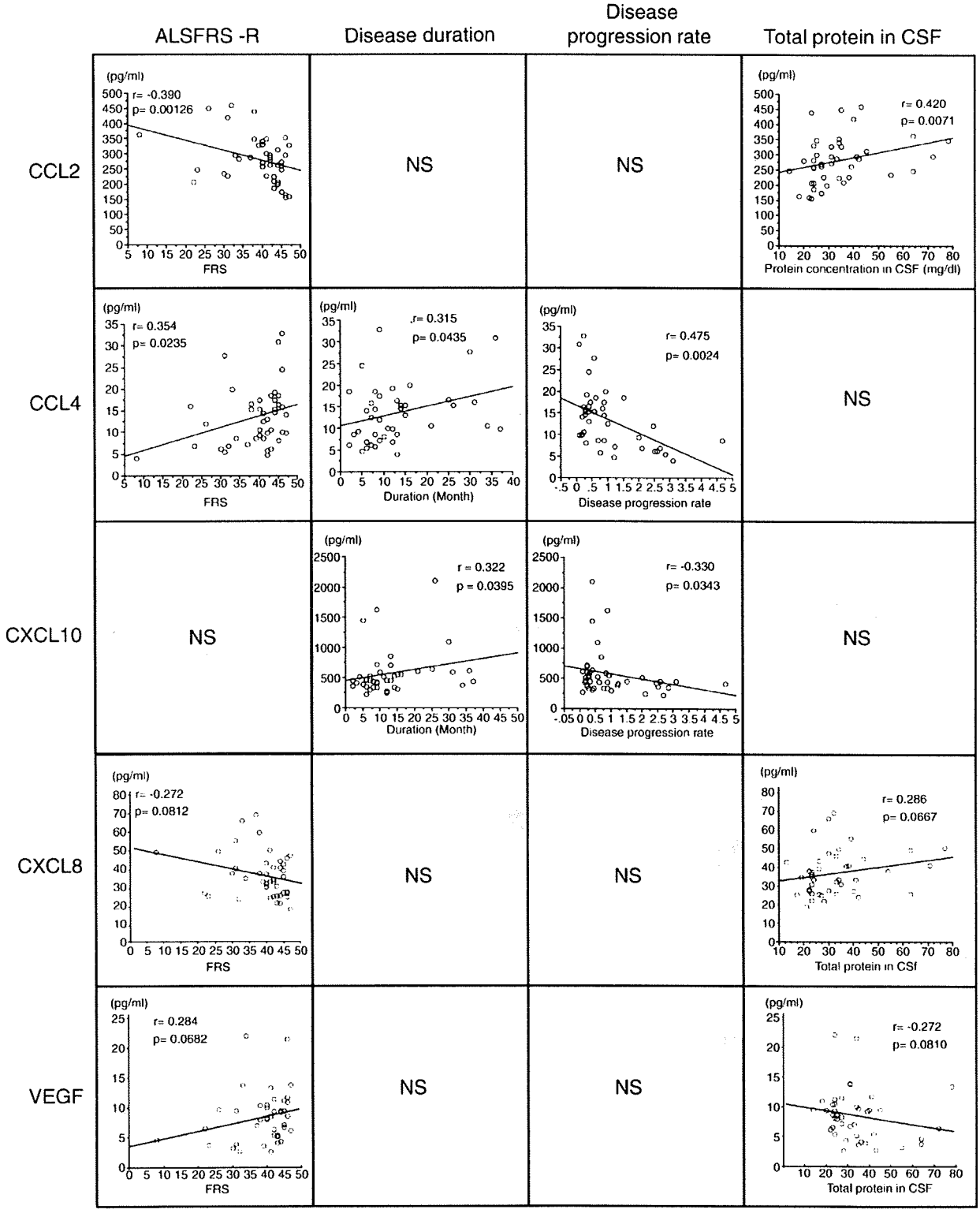

Fig. 2. Correlations between the concentrations of individual cytokines/chemokines in cerebrospinal fluid (CSF) and various clinical parameters. ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale. Abbreviations: CSF, cerebrospinal fluid; NS, not significant; VEGF, vascular endothelial growth factor.

CXCL10 was positively correlated with disease duration ($r=0.322$, $P=0.0395$) and negatively correlated with the disease progression rate ($r=-0.330$, $P=0.0343$). CXCL8 showed a tendencies for a negative correlation with the ALSFRS-R score ($r=-0.272$, $P=0.0812$) and for a positive correlation with the CSF total protein level ($r=0.286$, $P=0.0667$). VEGF tended to have a positive correlation with the ALSFRS-R score ($r=0.285$, $P=0.0682$) and a negative correlation with the CSF total protein level ($r=-0.272$, $P=0.0810$). CCL2 levels correlated positively with the levels of CXCL8 ($r=0.586$, $P=0.0002$), IL-9 ($r=0.490$, $P=0.0017$), IL-7 ($r=0.451$, $P=0.0039$), IFN-γ ($r=0.434$, $P=0.0055$), and CCL11 ($r=0.417$, $P=0.0076$), while CCL4 levels correlated positively with CXCL10 ($r=0.535$, $P=0.0006$) and VEGF ($r=0.440$, $P=0.0049$) levels (Table 2).

4. Discussion

In the present study we found that VEGF, G-CSF, IFN-γ, CCL2, CCL4, CCL5, CCL11, CXCL8, CXCL10, TNF-α, IL-1β, IL-7, IL-12, and IL-17 levels in CSF were elevated in ALS patients compared with OND patients. The main new findings concerning clinical correlation were that among cytokines/chemokines and growth factors elevated in ALS CSF, CCL2 and CXCL8 levels were negatively correlated with the ALSFRS-R score, whereas CCL4 and VEGF levels showed positive correlations with the ALSFRS-R score, and both CCL4 and CXCL10 levels showed negative correlations with disease progression rate.

Among the chemokines up-regulated in ALS CSF, CCL2 and CCL4 showed distinct patterns of correlation with clinical parameters: CCL2 had a positive correlation with disease severity whereas CCL4 had negative correlations with disease severity and progression rate. Furthermore, CCL2 and CCL4 showed distinct association patterns with proinflammatory cytokines in ALS CSF, suggesting that CCL2 and CCL4 interact with distinct cytokine/chemokine networks. Although both chemokines act on macrophages and microglia, their receptors are different: CCR2 is the main receptor for CCL2, while CCR5, a marker for type 1 helper T (Th1) cells, is also present on macrophages and is the main receptor for CCL4. Because the expression of both CCR2 and CCR5 is differentially regulated upon differentiation and activation in monocytes/macrophages (Kaufmann et al., 2001), these chemokines are thought to act on distinct subsets of monocyte lineage cells (Tacke and Randolph, 2006; Ubogu et al., 2006). Classical $CD14^+CD16^-$ monocytes express CCR2, while non-classical $CD14^{+/low}CD16^+$ ones express CCR5 and target Th1 immune responses (Tacke and Randolph, 2006; Weber et al., 2000).

We (Tanaka et al., 2006) and others (Baron et al., 2005; Henkel et al., 2004; Wilms et al., 2003) have reported increased levels of CCL2 in the CSF of ALS patients. Using distinct ALS patients in their early course, we confirmed our previous finding that CCL2 level is associated with disease severity (Tanaka et al., 2006). The significant positive correlation of CCL2 with disease progression rate found in our previous study was not obvious in the present one, probably because only ALS patients in the early course were enrolled this time. Observations that the CCL2 level was higher in CSF than in sera, that the levels in the two compartments were not correlated (Baron et al., 2005; Tanaka et al., 2006; Wilms et al., 2003), and that CCL2 production was enhanced in glial cells (Henkel et al., 2004), suggest that the main source of CCL2 is likely to be glial cells. CCL2 activates microglia, which then produce abundant proinflammatory cytokines/chemokines and inducible nitric oxide synthase, leading to the production of neurotoxic nitric oxide (Possel et al., 2000; Zhao et al., 2004). The positive correlation of CCL2 level with CSF protein amounts reflects a breakdown of the blood-brain barrier, supporting its role in glial inflammation. Therefore, it is reasonable to assume that CCL2 acts as a disease-aggravating factor in ALS. In addition, a tendency toward CXCL8 having a negative correlation with the ALSFRS-R score is consistent with the findings of Mitchell et al. (2009). Because CCL2 and CXCL8 levels were significantly positively correlated, these chemokines may constitute a neurotoxic cytokine network.

On the other hand, CCR5, a receptor for CCL4 and CCL5, is not only expressed on microglia, but also on neurons and astrocytes (Kaul and Lipton, 1999). CCL4 has been shown to be produced intrathecally by glial cells and to delay progression of HIV-associated dementia (Kaul and Lipton, 1999). The HIV envelope glycoprotein gp120 induces activation of macrophages and microglia (Kaul et al., 2001) and neuronal apoptosis in HIV-associated dementia (Brenneman et al., 1988); considered essential for its pronounced neurodegenerative effects (Kaul and Lipton, 1999). CCL4 together with CCL5 protects neurons from gp120-induced neuronal apoptosis (Kaul and Lipton, 1999) via an Akt-dependent signaling pathway in which neuronal Akt protects against excitotoxic insults (Kaul et al., 2007). Since excitotoxicity is also postulated to be operative in ALS (Heath and Shaw, 2002), intrathecally up-regulated CCL4 and CCL5 in ALS may represent a host defense mechanism. IFN-γ has recently been reported to enhance neurogenesis and neuroprotection in a mouse model of Alzheimer's disease (Baron et al., 2008), and to mediate neuroprotection against excitotoxic neural damage (Lee et al., 2006). Thus up-regulation of IFN-γ and the downstream molecule CXCL10 in ALS CSF may also represent a host's neuroprotective action in ALS. This may partly explain the significant negative correlation of CXCL10 with disease progression rate observed in the present study. A positive correlation of CCL4 and CXCL10 concentrations in CSF may thus be a reflection of a concerted host defense mechanism.

In the present study, a variety of proinflammatory cytokines/chemokines and growth factors were found to be up-regulated intrathecally in the early course of ALS. Patients with LMND also showed milder but similar trends for increases of proinflammatory cytokine/chemokines in CSF to those seen in ALS patients. It is possible that similar mechanisms may be partly operative in LMND, or alternatively, that some ALS patients might have been inappropriately included in the LMND group.

The delicate network of cytokines/chemokines and growth factors will already have been disturbed in the early course of motor neuron degeneration, with some up-regulated cytokines/chemokines such as CCL2 possibly neurotoxic, and others such as CCL4 possibly neuroprotective. Deciphering the complex actions of these altered cytokine/chemokine and growth factor networks may help the future elucidation of the pathogenesis of ALS.

Table 2
Summary of altered cytokines/chemokines in ALS CSF.

Cytokines examined	Alteration	p value
IL-1β	Increase	$p=0.0348$
IL-7	Increase	$p=0.0135$
IL-9	Increase	$p=0.0020$
IL-12(p70)	Increase	$p=0.0339$
IL-17	Increase	$p=0.0027$
TNF-α	Increase	$p=0.0031$
IFN-γ	Increase	$p=0.0132$
CCL2	Increase	$p<0.0001$
CCL4	Increase	$p=0.0048$
CCL5	Increase	$p=0.0165$
CCL11	Increase	$p=0.0072$
CXCL8	Increase	$p<0.0001$
CXCL10	Increase	$p<0.0001$
G-CSF	Increase	$p=0.0005$
VEGF	Increase	$p=0.0039$

The following cytokines/chemokines showed no significant alteration: IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, CCL3, GM-CSF, and PDGFbb.

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References

Baron, P., Bussini, S., Cardin, V., Corbo, M., Conti, G., Galimberti, D., Scarpini, E., Bresolin, N., Wharton, S.B., Shaw, P.J., Silani, V., 2005. Production of monocyte chemoattractant protein-1 in amyotrophic lateral sclerosis. Muscle Nerve 32, 541–544.

Baron, R., Nemirovsky, A., Harpaz, I., Cohen, H., Owens, T., Monsonego, A., 2008. IFN-gamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease. FASEB J. 22, 2843–2852.

Boillée, S., Yamanaka, K., Lobsiger, C.S., Copeland, N.G., Jenkins, N.A., Kassiotis, G., Kollias, G., Cleveland, D.W., 2006. Onset and progression in inherited ALS determined by motor neurons and microglia. Science 312, 1389–1392.

Brenneman, D.E., Westbrook, G.L., Fitzgerald, S.P., Ennist, D.L., Elkins, K.L., Ruff, M.R., Pert, C.B., 1988. Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. Nature 335, 639–642.

Brooks, B.R., 1994. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. J. Neurol. Sci. 124, 96–107 Suppl.

Cedarbaum, J.M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., Nakanishi, A., 1999. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J. Neurol. Sci. 169, 13–21.

Clement, A.M., Nguyen, M.D., Roberts, E.A., Garcia, M.L., Boillée, S., Rule, M., McMahon, A.P., Doucette, W., Siwek, D., Ferrante, R.J., Brown Jr., R.H., Julien, J.P., Goldstein, L.S., Cleveland, D.W., 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302, 113–117.

Consilvio, C., Vincent, A.M., Feldman, E.L., 2004. Neuroinflammation, COX-2, and ALS—a dual role? Exp. Neurol. 187, 1–10.

de Jager, W., te Velthuis, H., Prakken, B.J., Kuis, W., Rijkers, G.T., 2003. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin. Diagn. Lab. Immunol. 10, 133–139.

Heath, P.R., Shaw, P.J., 2002. Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. Muscle Nerve 26, 438–458.

Henkel, J.S., Engelhardt, J.I., Siklos, L., Simpson, E.P., Kim, S.H., Pan, T., Goodman, J.C., Siddique, T., Beers, D.R., Appel, S.H., 2004. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann. Neurol. 55, 221–235.

Ilzecka, J., Stelmasiak, Z., Dobosz, B., 2002. Transforming growth factor-Beta 1 (tgf-Beta 1) in patients with amyotrophic lateral sclerosis. Cytokine 20, 239–243.

Ishizu, T., Osoegawa, M., Mei, F.J., Kikuchi, H., Tanaka, M., Takakura, Y., Minohara, M., Murai, H., Mihara, F., Taniwaki, T., Kira, J., 2005. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain 128, 988–1002.

Kaufmann, A., Salentin, R., Gemsa, D., Sprenger, H., 2001. Increase of CCR1 and CCR5 expression and enhanced functional response to MIP-1 alpha during differentiation of human monocytes to macrophages. J. Leukoc. Biol. 69, 248–252.

Kaul, M., Garden, G.A., Lipton, S.A., 2001. Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 410, 988–994.

Kaul, M., Lipton, S.A., 1999. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. Proc. Natl Acad. Sci. USA 96, 8212–8216.

Kaul, M., Ma, Q., Medders, K.E., Desai, M.K., Lipton, S.A., 2007. HIV-1 coreceptors CCR5 and CXCR4 both mediate neuronal cell death but CCR5 paradoxically can also contribute to protection. Cell Death Differ. 14, 296–305.

Lee, J., Kim, S.J., Son, T.G., Chan, S.L., Mattson, M.P., 2006. Interferon-gamma is up-regulated in the hippocampus in response to intermittent fasting and protects hippocampal neurons against excitotoxicity. J. Neurosci. Res. 83, 1552–1557.

Lobsiger, C.S., Cleveland, D.W., 2007. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat. Neurosci. 10, 1355–1360.

McGeer, P.L., McGeer, E.G., 2002. Inflammatory processes in amyotrophic lateral sclerosis. Muscle Nerve 26, 459–470.

Mitchell, R.M., Freeman, W.M., Randazzo, W.T., Stephens, H.E., Beard, J.L., Simmons, Z., Connor, J.R., 2009. A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. Neurology 72, 14–19.

Moreau, C., Devos, D., Brunaud-Danel, V., Defebvre, L., Perez, T., Destee, A., Tonnel, A.B., Lassalle, P., Just, N., 2005. Elevated IL-6 and TNF-alpha levels in patients with ALS: inflammation or hypoxia? Neurology 65, 1958–1960.

Poloni, M., Facchetti, D., Mai, R., Micheli, A., Agnoletti, L., Francolini, G., Mora, G., Camana, C., Mazzini, L., Bachetti, T., 2000. Circulating levels of tumor necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. Neurosci. Lett. 287, 211–214.

Possel, H., Noack, H., Putzke, J., Wolf, G., Sies, H., 2000. Selective upregulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharide (LPS) and cytokines in microglia: in vitro and in vivo studies. Glia 32, 51–59.

Sekizawa, T., Openshaw, H., Ohbo, K., Sugamura, K., Itoyama, Y., Niland, J.C., 1998. Cerebrospinal fluid interleukin 6 in amyotrophic lateral sclerosis: immunological parameter and comparison with inflammatory and non-inflammatory central nervous system diseases. J. Neurol. Sci. 154, 194–199.

Tacke, F., Randolph, G.J., 2006. Migratory fate and differentiation of blood monocyte subsets. Immunobiology 211, 609–618.

Tanaka, M., Kikuchi, H., Ishizu, T., Minohara, M., Osoegawa, M., Motomura, K., Tateishi, T., Ohyagi, Y., Kira, J., 2006. Intrathecal upregulation of granulocyte colony stimulating factor and its neuroprotective actions on motor neurons in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 65, 816–825.

Ubogu, E.E., Callahan, M.K., Tucky, B.H., Ransohoff, R.M., 2006. CCR5 expression on monocytes and T cells: modulation by transmigration across the blood-brain barrier in vitro. Cell. Immunol. 243, 19–29.

Vignali, D.A., 2000. Multiplexed particle-based flow cytometric assays. J. Immunol. Methods 243, 243–255.

Weber, C., Belge, K.U., von Hundelshausen, P., Draude, G., Steppich, B., Mack, M., Frankenberger, M., Weber, K.S., Ziegler-Heitbrock, H.W., 2000. Differential chemokine receptor expression and function in human monocyte subpopulations. J. Leukoc. Biol. 67, 699–704.

Wilms, H., Sievers, J., Dengler, R., Bufler, J., Deuschl, G., Lucius, R., 2003. Intrathecal synthesis of monocyte chemoattractant protein-1 (MCP-1) in amyotrophic lateral sclerosis: further evidence for microglial activation in neurodegeneration. J. Neuroimmunol. 144, 139–142.

Yamanaka, K., Boillée, S., Roberts, E.A., Garcia, M.L., McAlonis-Downes, M., Mikse, O.R., Cleveland, D.W., Goldstein, L.S., 2008. Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. Proc. Natl Acad. Sci. USA 105, 7594–7599.

Zhao, W., Xie, W., Le, W., Beers, D.R., He, Y., Henkel, J.S., Simpson, E.P., Yen, A.A., Xiao, Q., Appel, S.H., 2004. Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. J. Neuropathol. Exp. Neurol. 63, 964–977.

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Reappraisal of brain MRI features in patients with multiple sclerosis and neuromyelitis optica according to anti-aquaporin-4 antibody status

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ABSTRACT

Brain lesions are not uncommon in neuromyelitis optica (NMO) patients with anti-aquaporin-4 (AQP4) antibody; however, the appearance of these lesions is said to be different from that of those in Western patients with multiple sclerosis (MS). To clarify the similarities and dissimilarities of brain lesions in anti-AQP4 antibody-positive and -negative MS and NMO patients, we examined the presence of anti-AQP4 antibody in the sera of 148 consecutive patients fulfilling Poser's criteria for clinically definite MS, of whom 38 also met the revised NMO criteria, using an immunofluorescence method, and analyzed brain lesions by magnetic resonance imaging (MRI). Brain lesions fulfilling the Barkhof criteria were significantly more common in 121 patients without anti-AQP4 antibody than in 27 patients with anti-AQP4 antibody (57.0% vs. 33.3%, $P=0.033$), while the frequency of those that met the Paty criteria was not different between the two groups (74.4% vs. 73.5%). Ovoid lesions were detected more commonly in patients without anti-AQP4 antibody than in those with the antibody (72.3% vs. 48.2%, $P=0.022$). The anti-AQP4 antibody-positive patients had significantly more atypical brain lesions, such as extensive brain lesions, than the anti-AQP4 antibody-negative ones (18.5% vs. 1.7%, $P=0.0023$). Thus, although MS-like brain lesions are more common in anti-AQP4 antibody-negative patients than anti-AQP4 antibody-positive patients, approximately 30 to 50% of patients with anti-AQP4 antibody harbour brain MRI lesions indistinguishable from those present in typical MS patients, such as periventricular ovoid lesions, suggesting the existence of considerable overlap in brain MRI features between anti-AQP4 antibody-positive and -negative Asian patients. In the present study, NMO patients with brain lesions showed a significantly higher annualized relapse rate ($P^{corr}=0.017$) and higher frequency of anti-AQP4 antibody ($P^{corr}<0.0001$) than typical NMO patients without brain lesions, suggesting that development of brain lesions in NMO may reflect high disease activity and thus be a warning sign.

1. Introduction

Neuromyelitis optica (NMO) is characterised by severe and selective involvement of the optic nerves and spinal cord, which frequently shows longitudinally extensive spinal cord lesions (LESCLs) extending over three or more vertebral segments. NMO was originally defined as a disease involving only the optic nerves and spinal cord with a monophasic course [1]. However, the concept of NMO has changed along with the collection and analysis of samples from many NMO patients, and a relapsing form of NMO is widely accepted in Western countries [2,3]. Additionally, a highly specific IgG against NMO, designated NMO-IgG, has been described [4], and its relevant antigen was reported to be aquaporin-4 (AQP4) [5]. The presence of NMO-IgG/anti-AQP4 antibody has also influenced the concept of NMO. Some patients with NMO-IgG also show atypical brain lesions, such as large confluent lesions (>3 cm) and diencephalic lesions, during their clinical course [6]. In fact, 60% of patients fulfilling the 1999 criteria for NMO show brain lesions on MRI [6]. Thus, based on such evidence, the 2006 revised criteria for NMO include the presence of NMO-IgG and do not preclude patients with brain lesions [3].

The distribution of atypical brain lesions on MRI reflects the distribution of high expression of AQP4, and histopathological analyses of NMO show perivascular IgM and IgG deposition with complement activation and loss of immunoreactivity to AQP4 [7–9]. These findings suggest a role for humoral immunity in the pathogenesis of NMO and a direct etiological role for NMO-IgG.

However, either NMO-IgG or anti-AQP4 antibody is detected in around 10% of MS patients who fulfil the established clinical criteria

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for MS [4,10,11]. We have also reported that anti-AQP4 antibody-positive patients occasionally have MS-like brain lesions, such as periventricular ovoid lesions [11]. Thus, it remains to be elucidated whether brain MRI lesions are distinguishable between MS and NMO patients, and between those with and without anti-AQP4 antibody, or if considerable overlaps exist between these conditions. Because the opticospinal form of MS (OSMS) in Asians is now claimed to be the same as NMO in Westerners, it is especially problematic to differentiate NMO from MS in Asian patients, who frequently show severe and selective involvement of the optic nerve and spinal cord, irrespective of the presence or absence of anti-AQP4 antibody [11]. Therefore, it is critical to compare brain MRI lesions in a large unbiased series of Asian MS and NMO patients. In the present study, we aimed to compare the frequencies of typical MS-like brain lesions and atypical ones between Japanese MS and NMO patients, and between those with and without anti-AQP4 antibody.

2. Methods

2.1. Patients

For the present study, we enrolled 148 consecutive patients with clinically definite relapsing–remitting or secondary-progressive MS based on the Poser criteria [12], seen at the MS clinic of the Department of Neurology, Kyushu University Hospital during 1987–2007, and whose sera were available for anti-APQ4 antibody assays. From a retrospective review of the medical records of all patients, we recorded demographic and imaging data. There were 137 relapsing–remitting and 11 secondary-progressive MS patients. All patients were residents of Kyushu Island, the southernmost part of mainland Japan. None were seropositive for human T cell leukaemia virus type I. No patients with primary progressive MS were included in the present study. Patients with monophasic NMO without subsequent relapse were also excluded to avoid including patients with acute disseminated encephalomyelitis. Among the 148 patients, 27 (18.2%, 24 female and 3 male) were positive for anti-AQP4 antibody, and 38 also met the revised Wingerchuk's criteria for NMO [3]. We then classified these NMO patients into "typical NMO" and "NMO with brain lesions" based on whether they had brain lesions meeting the Paty criteria [13]. We adopted the Paty criteria [13] because in the Wingerchuk's revised criteria for NMO [3] the Paty criteria were recommended for determining the presence of MS-like brain lesions. Therefore, "typical NMO" patients were those who showed only optic neuritis and myelitis without brain lesions fulfilling the Paty criteria [13]. "NMO patients with brain lesions" were those who had only optic neuritis and myelitis and also had brain lesions fulfilling the Paty criteria [13]. "MS patients" were those who met the Poser [12] and the revised McDonald criteria [14] and did not meet the definition of either "typical NMO" or "NMO with brain lesions".

2.2. Magnetic resonance imaging

All MRI studies were performed using 1.5-T Magnetom Vision and Symphony units (Siemens Medical Systems, Erlangen, Germany) as described previously [15]. The typical imaging parameters for the brain were as follows: axial T2-weighted turbo spin-echo imaging using TR/TE = 2800/90 ms, flip angle = 180°; axial turbo-fluid-attenuated inversion recovery (FLAIR) imaging using TI/TR/TE = 2200/9000/110 ms, flip angle = 180; and sagittal and axial pre-contrast and axial and coronal post-contrast T1-weighted spin-echo imaging using TR/TE range = 400–460/12–17 ms, and flip angle range = 80–90°. One excitation, with a matrix of 256 × 256, slice thickness of 5 mm, and slice gap of 2.5 mm, was used for all brain studies. Gadopentetate dimeglumine at 0.1 mmol/kg body weight was administered intravenously for contrast-enhanced studies.

MRI scans were taken at the time of clinical relapse (within 30 days of the onset of acute exacerbation) or in the remission phase. Brain MRI scans from 87 patients at relapse and 136 patients in remission were examined. Brain MRI lesions were evaluated according to the Barkhof criteria [16] and Paty criteria [13] for MS. Atypical brain lesions, such as extensive brain lesions (>3 cm), bilateral diencephalic (thalamic/hypothalamic) lesions, cavity formation and extension from the cervical cord into the brainstem were defined based on previous reports [6,11]. At the time of brain MRI, treatment was being received by the patients being imaged in 108 out of 223 scans (58 on IFNβ-1b, 42 within one month of steroid pulse therapy and eight on both).

2.3. Anti-AQP4 antibody assay

Green fluorescence protein (GFP)-AQP4 fusion protein–transfected human embryonic kidney cells (HEK-293) were produced as previously described [11]. AQP4-expressing cells were initially incubated with human serum samples diluted 1:4 with DMEM for 1 h at 37.0 °C without cell fixation, washed in phosphate-buffered saline, and then visualized with an Alexa 594-conjugated goat anti-human IgG antibody (Invitrogen). The fluorescence of non-fixed cells was observed using a confocal laser-scanning microscope (FLUOVIEW FV300; Olympus Optical Co., Tokyo, Japan). The anti-AQP4 antibody assay was carried out at least twice for each sample, and those that gave a positive result twice were deemed to be positive.

2.4. Statistical analysis

Statistical analyses of numerical variables were performed using the Mann–Whitney *U* test. Differences in frequencies between the two subgroups were tested for significance using Fisher's exact probability test. When multiple comparisons were performed, uncorrected *P* values (P^{uncorr}) were corrected by multiplying them by the number of comparisons (Bonferroni–Dunn's correction) to calculate corrected *P* values (P^{corr}).

Table 1
Comparison of demographic features between patients with NMO and those with MS.

	NMO patients ($n=38$)	MS patients ($n=110$)
No. of females/males	32/6 (5.3:1)	75/35 (2.1:1)
Age at onset (years)a	35.4 ± 14.2	31.3 ± 12.4
Disease duration (years)[a]	11.8 ± 8.7	11.9 ± 9.9
Annualized relapse rate[a]	0.99 ± 0.58*	0.68 ± 0.58*
EDSS score[a]	5.2 ± 2.6*	3.4 ± 2.6*
Anti-AQP4 antibody	24/38 (63.2%)*	3/110 (2.7%)*
Frequency of symptoms:		
Optic neuritis	38/38 (100.0%)*	62/110 (56.7%)*
Bilateral optic neuritis	6/38 (15.8%)	13/110 (11.9%)
Severe optic neuritis (≥FS 5)	28/38 (73.7%)*	38/110 (34.5%)*
Myelitis	38/38 (100.0%)	91/110 (82.7%)
Acute transverse myelitis	23/38 (60.5%)*	22/110 (20.0%)
Secondary progression	0/38 (0.0%)	11/110 (10.0%)
CSF:		
Marked pleocytosis (≥50/μl)	4/35 (11.4%)	6/101 (5.9%)
Neutrophilia (≥5/μl)	4/35 (11.4%)	4/94 (4.3%)
OB	5/34 (14.7%)*	37/90 (41.1%)*
IgG index (≥0.658)[b]	14/33 (42.4%)	42/83 (50.6%)
LESCLs during the entire course	32/38 (84.2%)*	28/110 (25.5%)*

AQP4 = aquaporin-4; CNS = central nervous system; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.

[a] Means ± SD.

[b] The upper normal range of IgG index was derived from our previous study [24].

* $P<0.05$.

Table 2
Comparison of demographic features among patients with typical NMO, patients with NMO with brain lesions, and patients with MS.

	Typical NMO patients ($n=21$)	NMO with brain lesions ($n=17$)	MS patients ($n=110$)
No. of females/males	18/3 (5.7:1)	14/3 (5:1)	75/35 (2.1:1)
Age at onset (years)[a]	33.4 ± 14.5	37.8 ± 14.0	31.3 ± 12.4
Disease duration (years)[a]	11.6 ± 8.8	12.1 ± 8.7	11.9 ± 9.9
Annualized relapse rate[a]	0.77 ± 0.43*	1.26 ± 0.62*,**	0.68 ± 0.58**
EDSS score[a]	5.6 ± 2.3*	4.7 ± 2.9	3.4 ± 2.6*
Anti-AQP4 antibody	7/21 (33.3%)*,***	17/17 (100.0%)*,**	3/110 (2.7%)**,***
Frequency of symptoms:			
Optic neuritis	21/21 (100.0%)*	17/17 (100.0%)**	62/110 (56.6%)*,**
Bilateral optic neuritis	4/21 (19.0%)	2/17 (11.8%)	13/110 (11.9%)
Severe optic neuritis (≥FS 5)	14/21 (66.7%)*	14/17 (82.3%)**	38/110 (34.5%)*,**
Myelitis	21/21 (100.0%)	17/17 (100.0%)	91/110 (82.7%)
Acute transverse myelitis	15/21 (71.4%)*	8/17 (47.1%)	22/110 (20.0%)*
Secondary progression	0/21 (0.0%)	0/17 (0.0%)	11/110 (10.0%)
CSF:			
Marked pleocytosis (≥50/μl)	1/18 (5.6%)	3/17 (17.6%)	6/101 (5.9%)
Neutrophilia (≥5/μl)	1/18 (5.6%)	3/17 (17.6%)	4/94 (4.3%)
OB	3/18 (16.7%)	2/16 (12.5%)	37/90 (41.1%)
IgG index (≥0.658)[b]	8/17 (47.1%)	6/16 (37.5%)	42/83 (50.6%)
LESCLs during the entire course	17/21 (81.0%)*	15/17 (88.2%)**	28/110 (25.5%)*,**

AQP4 = aquaporin-4; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.
', '', ''' Corrected $P<0.05$.
[a] Means ± SD.
[b] The upper normal range of IgG index was derived from our previous study [24].

3. Results

3.1. Demographic features

The demographic features of the 148 patients are summarized in Table 1. The disease duration was similar between NMO and MS patients. Although relapse rate, Kurtzke's Expanded Disability Status Scale (EDSS) scores [17], and frequencies of severe optic neuritis, ATM, and LESCLs during the entire course were significantly greater in the 38 patients who satisfied the revised NMO criteria [3] than in the remaining 110 MS patients, the frequency of oligoclonal bands (OB) was significantly higher in MS patients than that in NMO patients. Among MS patients, 65.2% had OBs and/or an elevated IgG index (OB/high IgG index). Although none of the four MS patients with CSF neutrophilia had anti-AQP4 antibodies, all of them had LESCLs.

When clinical features were compared among patients with typical NMO, NMO with brain lesions and MS, EDSS score and frequencies of severe optic neuritis, ATM and LESCLs during the entire course were significantly greater in typical NMO patients than in MS patients ($P^{corr}=0.0024$, $P^{corr}=0.023$, $P^{corr}<0.0001$, and $P^{corr}<0.0001$, respectively) (Table 2). Annualized relapse rates were significantly higher in NMO patients with brain lesions than in MS patients ($P^{corr}<0.001$) and typical NMO patients ($P^{corr}=0.017$). Frequencies of severe optic neuritis and LESCLs were also significantly higher in NMO patients with brain lesions than in MS patients ($P^{corr}<0.001$ and $P^{corr}<0.001$, respectively). Anti-AQP4 antibody positivity rate was highest in NMO patients with brain lesions and the rate was significantly higher in NMO patients with brain lesions (100%) than typical NMO patients (33.3%, $P^{corr}<0.0001$) and MS patients (2.7%, $P^{corr}<0.0001$). It was also significantly higher in

Table 3
Comparison of demographic features between anti-AQP4 antibody-positive and -negative patients with NMO and MS.

	Anti-AQP4 antibody-positive patients ($n=27$)	Anti-AQP4 antibody negative patients ($n=121$)
No. of female/male patients	24/3 (8.0:1)*	83/38 (2.2:1)*
Age at onset (years)[a]	36.3 ± 13.8	31.4 ± 12.7
Disease duration (years)[a]	13.7 ± 9.2	11.5 ± 9.6
Annualized relapse rate[a]	1.0 ± 0.62*	0.71 ± 0.57*
EDSS score[a]	4.7 ± 2.6	3.7 ± 2.7
Frequency of symptoms:		
Optic neuritis	27/27 (100.0%)*	72/121 (59.5%)*
Bilateral optic neuritis	4/27 (14.8%)	15/121 (12.4%)
Severe optic neuritis (FS ≥ 5)	21/27 (77.8%)*	45/121 (37.2%)*
Myelitis	26/27 (96.3%)	103/121 (85.1%)
Acute transverse myelitis	12/27 (44.4%)	33/121 (27.3%)
CSF:		
Marked pleocytosis (≥50/μl)	3/25 (12.0%)	7/111 (6.3%)
Neutrophilia (≥5/μl)	3/25 (12.0%)	5/104 (4.8%)
OB	5/24 (20.8%)	37/100 (37.0%)
IgG index (≥0.658)[b]	9/23 (39.1%)	47/93 (50.5%)
LESCLs during the entire course	20/27 (74.1%)*	40/121 (33.1%)*

AQP4 = aquaporin-4; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.
[a] Means ± SD.
[b] The upper normal range of IgG index was derived from our previous study [24].
* $P<0.05$.