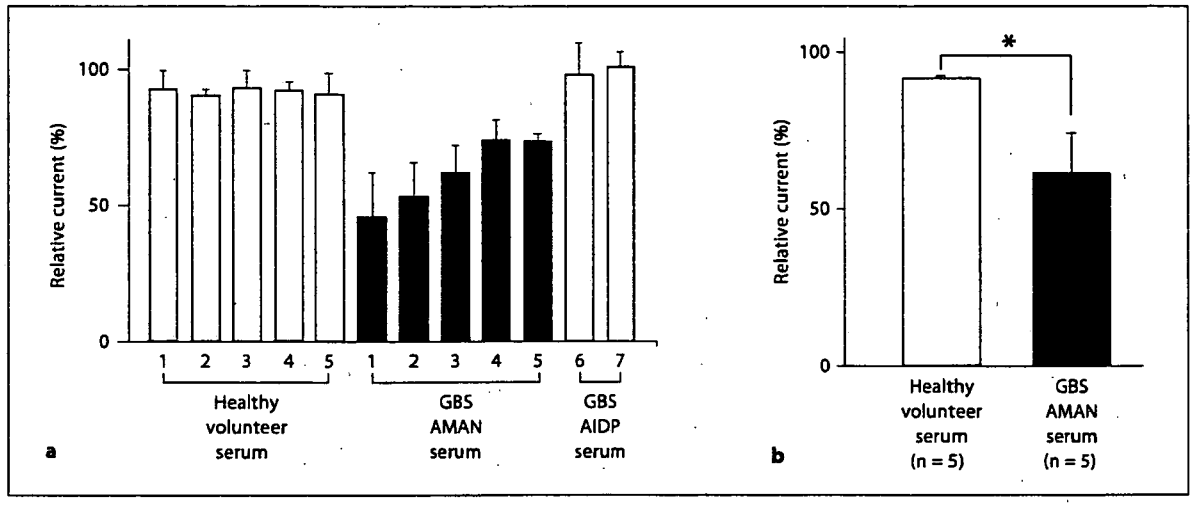

Fig. 3. Effect of serum from healthy volunteers, and patients with AMAN and AIDP, on whole-cell Ca^{2+} current in NGF-differentiated PC12 cells. **a** Inhibition of VDCC currents in PC12 cells by sera from patients with GBS (AMAN: patients 1–5, and AIDP: patients 6 and 7) and 5 healthy volunteers is shown. Each ordinate represents the Ca^{2+} current relative to that observed prior to exposure to serum, expressed as a percentage (%). Each data point represents the mean ± SD (of serum diluted to 1:100, n = 4–7). **b** Histograms representing the relative current after exposure to serum from 5 healthy volunteers (diluted to 1:50) and patients with AMAN (patients 1–5, diluted to 1:100). Mean ± SD values for the PC12 cells are shown in the columns. * $p < 0.05$ (vs. healthy volunteer serum).

action potential generation within spinal cord-muscle cocultured cells. A blockade of spontaneous muscle action potential generation at the NMJ was not observed upon exposure to serum from 5 healthy volunteers or to serum from 2 patients with GBS (patients 6 and 7). We previously reported a reversible blockade of spontaneous muscle action potential generation in innervated muscle cells by exposure to GBS serum containing IgG anti-GalNAc-GD1a antibodies [16]. Buchwald et al. [8] observed a reversible blockade at both presynaptic and postsynaptic terminals by exposure to serum or purified IgG from patients with GBS. Axonal conduction is blocked by exposure to serum from patients with high titers of anti-ganglioside antibodies [10, 17, 18]. Thus, anti-ganglioside antibodies may block neuromuscular transmission by attacking motor nerve terminals or synapses. However, inhibition of conduction of normal rat spinal root axons is not observed following local application of fresh serum from patients with GBS to tissue for 5 h [19]. Thus, there is conflicting data regarding the electrophysiological effects of GBS serum on nerve conduction [19, 20]. The discrepancy in results may be due to a requirement for supramaximal electrical stimulation of the motor nerve for an effect to be observed. In the present study, serum from patients with GBS (patients 1–5 with AMAN) blocked spontaneous muscle action potentials in the absence of electrical stimulation, while serum from 2 other patients with GBS (patients 6 and 7 with AIDP), as well as serum from healthy volunteers, did not have this effect. The mechanism by which neurotransmitter release is inhibited at the NMJ by GBS serum remains unclear; however, Ortiz et al. [10] propose that serum from patients with GBS might block calcium channels within motor nerve terminals.

Patch clamp studies show inhibition of transient sodium currents by cerebrospinal fluid from patients with GBS [5, 6]. Furthermore, serum from patients with GBS directly blocks the ion-conducting pore of the sodium channel [7]. In contrast, anti-GM1 antibodies from patients with GBS do not appear to mediate this conduction block or to block sodium channels directly [20]. In addition, IgG from patients with GBS suppresses voltage-gated potassium currents, but not sodium currents [21]. The plasma of patients with GBS significantly reduces acetylcholine-induced current amplitude, despite the fact that sodium currents detected by whole-cell voltage clamp recording do not appear to be affected [22]. The results of these electrophysiological studies suggest that serum from patients with GBS might directly or indirectly block alterations in channel function within motor nerves. We previously demonstrated that anti-asialo-GM1 (GA1) polyclonal antibodies inhibit VDCC currents in NGF-

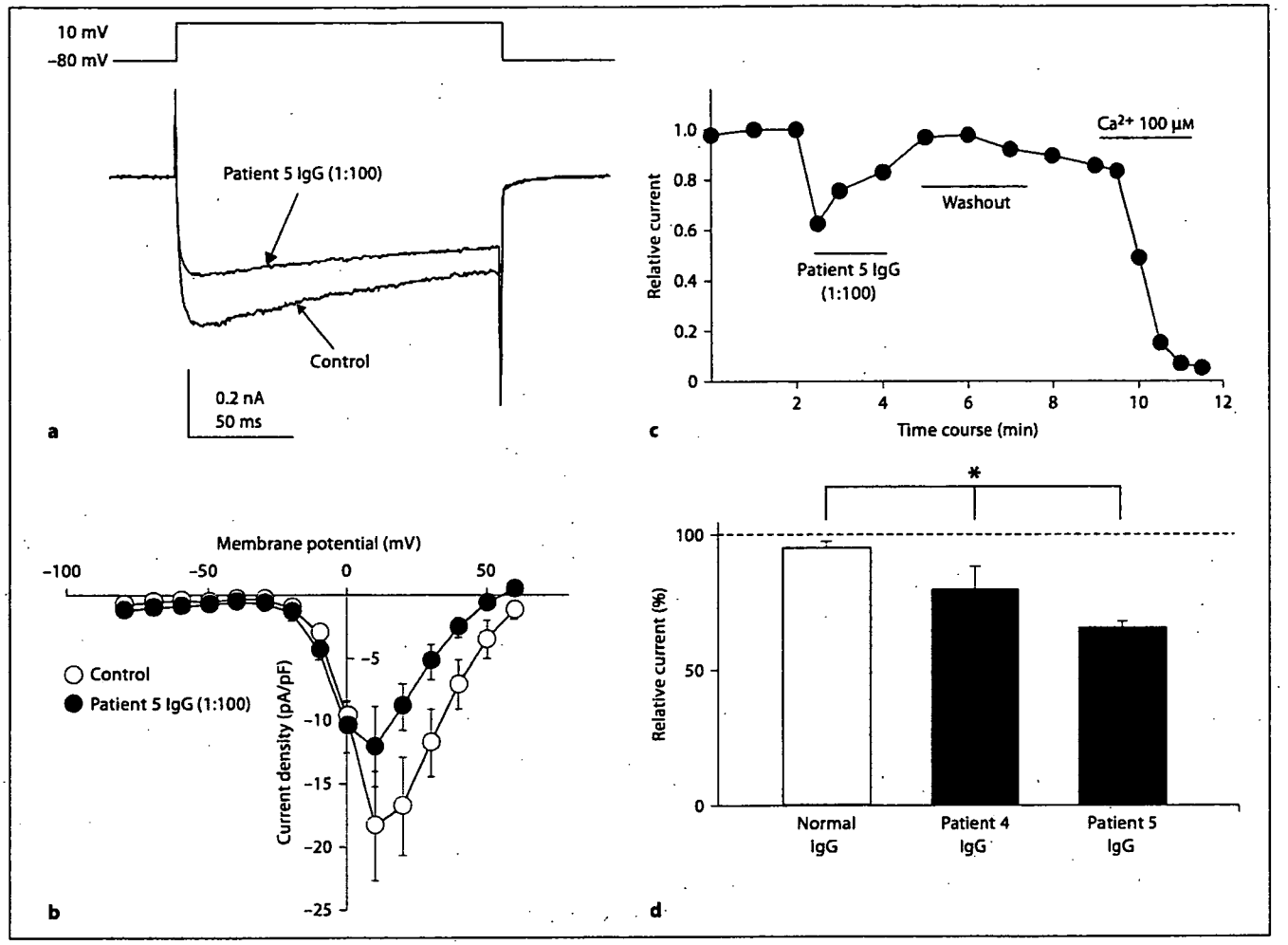

Fig. 4. Effect of purified IgG from GBS serum on whole-cell Ca^{2+} current in NGF-differentiated PC12 cells. **a** Inward Ca^{2+} current evoked by depolarization from -80 mV to +10 mV (from a holding potential of -80 mV) in controls and in the presence of purified IgG from GBS serum (patient 5; diluted to 1:100). Current traces in the presence of purified IgG from GBS serum were obtained 1 min after application. **b** Voltage-dependent activation curve of the Ca^{2+} current. The peak Ca^{2+} current density in controls (n = 5) (○) and in the presence of purified IgG from GBS serum (n = 5) (●) was plotted against each test pulse potential. Each point on the voltage curve represents the mean ± SD. **c** Time course of inhibition of the Ca^{2+} current by exposure to purified IgG from a patient with GBS (patient 5; diluted to 1:100). The amplitude of the Ca^{2+} current was measured every 1 min. Each ordinate shows the relative Ca^{2+} current compared to the control over the time course when cells were exposed to purified IgG from GBS serum and after washout of the purified IgG. **d** Histograms representing Ca^{2+} current relative to that observed in the presence of purified IgG from healthy volunteers (diluted to 1:100, n = 5), or from patients with GBS (patients 4 and 5; diluted to 1:100, n = 5). The mean values ± SD of Ca^{2+} current in PC12 cells are represented by the columns. * $p < 0.05$ (vs. healthy volunteer IgG).

differentiated PC12 pheochromocytoma cells [23]. In addition, the B subunit of the cholera toxin, which binds specifically to GM1 in the outer leaflet of the cell membrane on neuroblastoma N18 cells, induces a sustained elevation of intracellular calcium [11]. In the present study, serum containing anti-ganglioside antibodies (from patients 1–5 with AMAN) or purified IgG from patients with GBS (patients 4 and 5) inhibited VDCC currents in PC12 cells, while serum from some patients with GBS (patients 6 and 7 with AIDP) did not detectably alter the VDCC currents. The lack of effect of serum from some patients with GBS (patients 6 and 7) on VDCC currents might reflect the presence of anti-ganglioside antibodies among patients with GBS. Some studies demon-

strate a relationship between gangliosides and calcium channels. GM1 and GQ1b may be involved in the activation of calcium channels, including L-type and N-type calcium channels [12, 13, 24]. Based on the results of the present study, we suggest that anti-ganglioside antibodies in the serum of patients with GBS might influence calcium channel inhibition in PC12 cells. Thus, inhibition of VDCC currents by GBS serum might contribute to muscle weakness in GBS patients. Although in vitro experiments provide direction for future research, the observed effects of serum from select patients with GBS may have been due to inhibition of calcium channel function along the motor nerve terminals. Further research regarding the mechanism of inhibition of VDCC currents by GBS serum is required.

In conclusion, the present findings suggest that conduction block in some patients with GBS, particularly those with motor nerve involvement, might occur through inhibition of VDCC currents.

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INTERNAL MEDICINE

SHORT COMMUNICATION

Intermittent Intravenous Immunoglobulin Successfully Prevents Relapses of Neuromyelitis Optica

Kazumasa Okada[1], Sadatoshi Tsuji[1] and Keiko Tanaka[2]

Key words: neuromyelitis optica, IVIG

(DOI: 10.2169/internalmedicine.46.0217)

Neuromyelitis optica (NMO) is a definite clinical entity characterized by fulminant attacks of optic neuritis, acute myelitis and NMO-IgG binding selectively aquaporin-4 (AQP4) (1). As prophylactic therapy for NMO, low-dose prednisone and immunosuppressive drugs rather than β-interferon are recommended (1, 2). Although intravenous immunoglobulin (IVIG) has been reported to prevent relapses of multiple sclerosis (MS), the efficacy of IVIG for NMO has not been elucidated (3). We report an atypical case of NMO, in whom monthly IVIG prevented the relapses.

A 44-year-old woman developed symptoms and signs of dysphagia, dysarthria, right hemiplegia and urinary retention at age 32 and recovered well with intravenous methylprednisolone (IVMP). Within two years after the initial bout, she developed left optic neuritis and left hemiparesis independently. She developed paraparesis with sensory disturbance below C5 and was referred to us at age 34. MRI showed non-enhanced cervical cord lesion (C4-6) and old lesions continuing from bilateral internal capsules to the pons. Laboratory investigation was positive for SS-A antibody. She did not have sicca symptoms. Sirmer's test and unstimulated salivary flow were normal. Although minor salivary gland biopsy showed a few infiltrates of mononuclear cells, it was not diagnostic for Sjögren's syndrome. After she developed another left optic neuritis resulting in severe

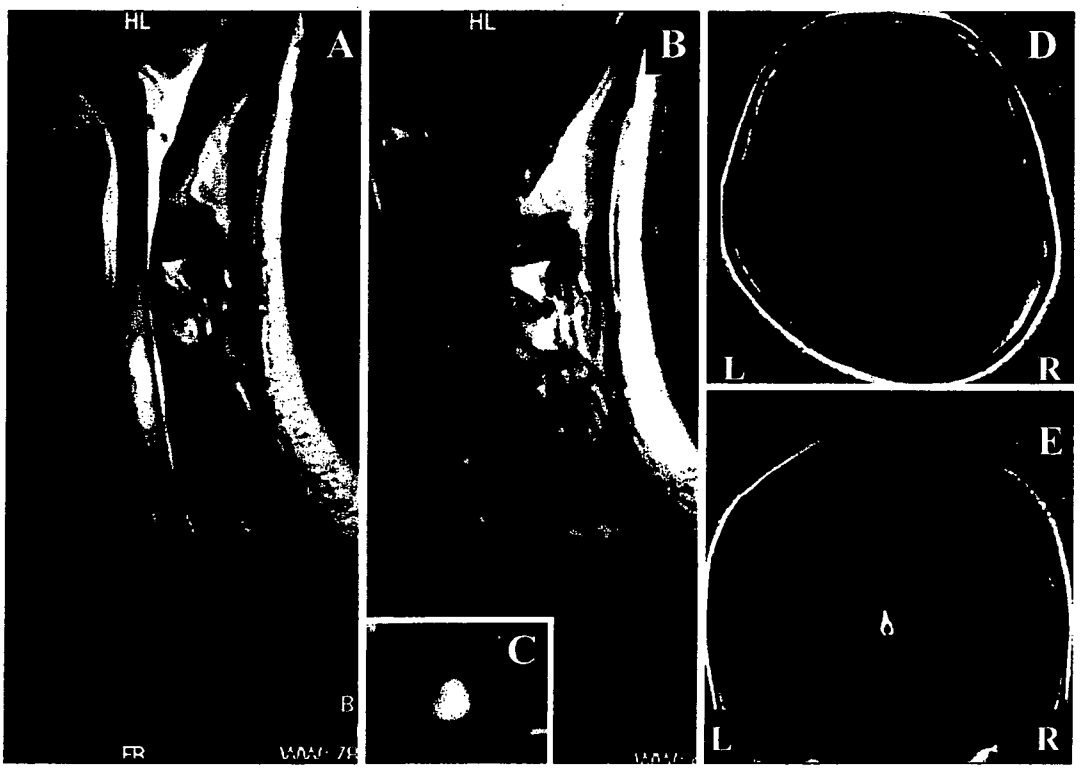

Figure 1. Cervical cord MRI showed a long extending swollen lesion with a strong contrast enhancement in C3-C7 during acute transverse myelitis (A, B). The contrast-enhanced lesion occupied the central gray matter and extending to the posterior column of the cervical cord (C). Brain MRI showed a few high signal lesions in the cerebral deep white matter, juxtacortical white matter and bilateral internal capsules extending to the upper pons on T2 images (D, E).

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visual impairment, β-interferon was introduced at age 35. Then, she developed three relapses in the spinal cord. β-interferon was ceased and azathioprine (100 mg/day) was initiated at age 36. The left optic neuritis relapsed resulting in light perception of visual acuity in her left eye at age 37 and at age 38 developed acute transverse myelitis with paraparesis, ascending to the sensory level and also urinary retention (EDSS score was 8.0). Spinal MRI showed a T2 hyperintense lesion in the cervical cord (C3-C7) with swelling (Fig. 1A, B). Contrast-enhanced lesion occupied the central gray matter and extended to the posterior column (Fig. 1C). She slowly recovered to achieve independent gait using a short brace for the right foot after IVMP. She relapsed again with paraparesis and urinary retention with a gadolinium-enhanced long swollen thoracic cord lesion (T1-T6) at age 39 (EDSS score was 8.0). Serum anti-AQP4 antibody was positive by the immunofluorescence assay, in which HEK 293 cells transfected with full-length cDNA of human AQP4 were incubated with the patient's serum and fluorescein isothiocyanate-conjugated rabbit anti-human IgG (4). After IVMP, she recovered gradually and was able to walk with a cane (EDSS score was 6.5) at the age of 40. CSF analysis had never revealed pleocytosis or oligoclonal bands. Although brain MRI fulfilled MRI criteria of McDonald for MS, the findings were atypical for MS because there were old continuous lesions from bilateral internal capsules to the pons without periventricular lesions, ovoid lesions or callosal lesions (Fig. 1D, E). We diagnosed her as NMO according to the revised criteria of NMO (1). Since monthly IVIG (0.4 g/kg/day for one day) was started at age 40 (yearly exacerbation rate is 2.0 for two years before IVIG introduction), she has never had a relapse for more than 4 years. Her EDSS score improved to 5.5 without any change of visual acuity.

IVIG seems to be effective for NMO, because NMO pathogenesis has been suggested to mediate humoral immunity involving AQP4 antibody (1, 5). IVIG might promote recovery of the damaged neuraxis, although improvement of the disability score may reflect natural recovery without relapses. It is important to prevent relapses of NMO as early from the disease onset as possible, because NMO has frequent relapses of fulminant optic neuritis and acute myelitis resulting in severe neurological sequelae (2). IVIG might be recommended for NMO and optic-spinal MS with either long spinal cord lesion or AQP4 antibody unresponsive to immunosuppressants or corticosteroid. Randomized controlled studies will be necessary to confirm the beneficial effect and dose of IVIG.

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Review Article

Bunina bodies in amyotrophic lateral sclerosis

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Bunina bodies, which are small eosinophilic intraneuronal inclusions in the remaining lower motor neurons, are generally considered to be a specific pathologic hallmark of amyotrophic lateral sclerosis (ALS). One year before a publication by Bunina, van Reeth *et al.* described similar intracytoplasmic inclusions in the anterior horn cells in a patient with Pick's dementia with atypical ALS. At present, only two proteins have been shown to be present in Bunina bodies, one is cystatin C and the other is transferrin. Bunina bodies consist of amorphous electron-dense material surrounded by tubular and vesicular structures on electron microscopy. Although the nature and significance of Bunina bodies in ALS are not yet clear, the bodies may be abnormal accumulations of unknown proteinous materials.

Key words: amyotrophic lateral sclerosis, Bunina body, cystatin C, motor neuron disease, transferrin.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is pathologically characterized by the presence of Bunina bodies, skein-like inclusions, Lewy body-like round inclusions, and basophilic inclusions in the remaining anterior horn cells in the spinal cord. Among them, Bunina bodies, which are small eosinophilic intraneuronal inclusions in the remaining lower motor neurons, are generally considered to be a specific pathologic hallmark of ALS. It is usually said that Bunina bodies were first described by Bunina in 1962 in two cases of familial ALS,[1] and later were observed in classical ALS patients and in ALS patients in Guam.[2] Although their morphological structures are well known, their nature, origin and significance remain unclear. Here, we review the morphology of Bunina bodies based mainly on light microscopic, immunohistochemical and electron microscopic findings.[3-5]

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HISTORICAL REVIEW OF BUNINA BODIES

In 1962, Bunina,[1] in the USSR, described the presence of intracellular inclusions in the motor neurons of the spinal cord and of the brain stem in two familial ALS cases, and she suspected that the inclusions might be a neurotrophic virus. One year before publication by Bunina, van Reeth *et al.*[6] described similar intracellular inclusions in the anterior horn cells in a patient with Pick's dementia with atypical ALS. The morphology of the inclusions in their paper seemed to be identical to Bunina-type inclusions, showing clear areas in the center and forming chain-like clusters. In 1963, Zil'ber *et al.*[7] described intracellular inclusions in anterior horn cells in monkeys that were given an intracerebral inoculation prepared from the spinal cords of ALS patients. Through arrangements made by the USSR–USA Scientific Exchanges Program,[2,8] Hirano[2] examined specimens from spinal cord of two human ALS cases and two experimental monkeys reported by Zil'ber *et al.*[7]; however, he did not disclose appreciable neuronal degeneration characteristic of ALS. Furthermore, Gibbs and Gajdusek[9] failed to produce neurological symptoms after inoculations with materials derived from several types of ALS. Hirano *et al.*[10] reported that similar eosinophilic inclusions were often found in Guamanians, familiar and classical ALS, and are not limited to any particular form of ALS. While it is not clear who first called these intracellular inclusions Bunina bodies, in 1967, Hirano *et al.*[10] used this term in their review of the pathologic findings in ALS. In 1977, Hart *et al.*[11] first described the ultrastructural features of Bunina bodies, and many researchers have subsequently investigated Bunina bodies. In 1993, Okamoto *et al.*[12] reported that Bunina bodies were immunostained with anticystatin C serum, and in 2006, Mizuno *et al.*[13] described that transferrin localizes in Bunina bodies.

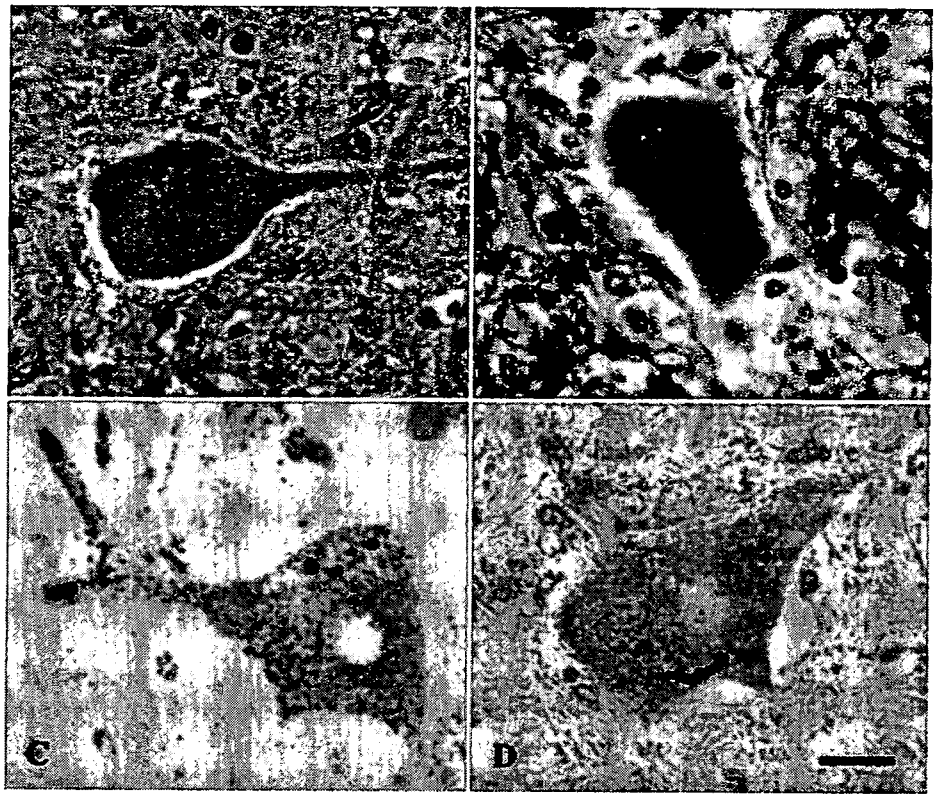

Fig. 1 Bunina bodies in the anterior horn cells in the lumbar cord of amyotrophic lateral sclerosis. (A) HE staining; (B) Klüver-Barrera staining; (C) cystatin C immunoreactivities are seen in the Bunina bodies in an anterior horn cell and its dendrites; (D) transferrin immunoreactivities are seen in the Bunina bodies. (Scale bar = 20 μm).

LIGHT MICROSCOPY

Bunina bodies are readily visualized with HE staining as bright pink, small, round, oval eosinophilic intraneuronal inclusions, sometimes showing clear areas in the center, and forming clusters[14-19] (Fig. 1A). They average 3–5 microns in diameter and the number varies in each neuron. The bodies are seen not only in the cytoplasm but also in the dendrites;[12,20] however, the bodies are not seen within the axoplasm. The bodies are bright purple granules in semi-thin sections stained with toluidine blue.[21]

Histochemical examinations showed that Bunina bodies are purple on phosphotungstic acid-hematoxylin (PTAH) staining, light blue on Klüver-Barrera staining (Fig. 1B), and red on Masson trichrome; however, they are negative for silver staining, PAS, Sudan black B and Congo red, and the bodies do not show autofluorescence and metachromasia for toluidine blue.[15,16] Tomonaga *et al.*[15] described that the bodies were red on methyl green-pyronine staining, but they were negative for methyl green-pyronine staining in our study.

Bunina bodies have been found in subtypes of ALS; however, the bodies were absent in a subset of familial ALS with posterior column involvement and in motor neuron disease with basophilic inclusions. Bunina bodies are present in almost all patients with sporadic ALS in our series, and Piao *et al.*[22] detected them in 88 cases from 102 autopsy cases of ALS. The bodies have been more frequently seen in the lumbar cord than in the cervical and thoracic cords, and are frequently seen in patients with relatively short disease duration, and ALS patients with dementia tend to have more and larger Bunina bodies in the lower motor neurons than do classical ALS patients.[3-5] Bunina bodies are mainly distributed in the motor neurons in the spinal cord and of the brain stem (nuclei ambiguus, hypoglossus, facial and motor trigeminus), and rarely in the Betz cells,[23,24] in the neurons of the oculomotor nuclei,[25] and Onuf's nuclei.[26,27] It has been reported that intracytoplasmic inclusions resembling or identical to Bunina bodies were observed in non-motor neurons of Clarke's column,[28-30] the intermediolateral nucleus[30] of the spinal cord, which consists of autonomic nerve cells, in the medullary reticular formation,[31,32] in the locus ceruleus,[33] and in neurons of the subthalamic neurons[34] in an unusual case of ALS.

IMMUNOHISTOCHEMISTRY

Despite immunohistochemical studies using many antibodies, at present, only two proteins have shown as present in Bunina bodies: one is cystatin C[12] and the other to be transferrin.[13] Other antibodies against neurofilament, tau, alpha- and beta-tubulin, microtubule-associated proteins, actin, myosin, desmin, synaptophysin, amyloid precursor protein, glial fibrillary acidic protein, alpha-synuclein[35] and p62[36] failed to demonstrate Bunina bodies.

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Ubiquitin-positive inclusions such as skein-like inclusions or Lewy body-like/round inclusions are another hallmark of ALS; therefore, results of immmunorectivites of Bunina bodies against ubiquitin are important. Usually, Bunina bodies were negative for ubiquitin;[37] however, a few researchers described that a small percentage of Bunina bodies show positive immunoreactivites for ubiquitin.[21,38] Murayama *et al.*[21] described that antiubiquitin antibody recognized an ill-defined structure in or around some Bunina bodies (Bunina body-related structure). Recently, the TAR DNA-binding protein of 43 kDa (TDP-43), a nuclear protein that is involved in transcriptional repression and alternative splicing, was identified as a major component of neuronal intracytoplasmic inclusions in motor neurons in ALS, as well as in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U).[39] Skein-like and Lewy body-like/round inclusions in ALS are positive for TDP-43; however, Bunina bodies are negative.[40] Bunina bodies were also negative for p62 of ubiquitin-related protein.[36]

Cystatin C, with a molecular weight of 13 260 Da, is a protein inhibitor of lysosomal cysteine protenases. Cystatin C is present in low concentrations in various extracellular fluids in humans, and is also present within human cortical neurons.[41,42] The main physiological function of cystatin C is local regulation of cystein proteinase. Immunohistochemical studies were performed with a polyclonal rabbit antiserum against human cystatin C and sections were stained by the adivin-biotin-peroxidase method.[12] Many small immunostained granules were scattered in almost all of the neurons and their dendrites in the spinal cord. Sequential staining of the same sections with HE and an anticystatin C antibody revealed that Bunina bodies were clearly labeled with anticystatin C antibody (Fig. 1C). The cytoplasmic and dendritic cystatin C-positive granules were reduced in number and size in the Bunina bodies-containing neurons compared to the normal-appearing neurons. Lewy body-like/round inclusions, skein-like inclusions and spheroids were not labeled with anticystatin C antibody.

Transferrin, an iron-binding protein, plays an important role in the transport and delivery of circulating ferric iron to the tissues. Mizuno *et al.*[13] examined transverse paraffin sections of lumbar spinal cords from 12 ALS cases, including two ALS with dementia and two ALS with basophilic inclusions, using antibodies to human transferrin. The results demonstrated that transferrin localized in Bunina bodies (Fig. 1D) and some of the basophilic inclusions. In contrast, skein-like inclusions, Lewy body-like inclusions, and round inclusions did not show obviously detectable transferrin immunoreactivities. Their findings suggest that although the mechanisms underlying transferrin accumulation in Bunina bodies and basophilic inclusions are unknown, transferrin could be involved in forming these inclusions.

ELECTRON MICROSCOPY

The fine structures of Bunina bodies have been studied by a number of investigators with somewhat variable results.[3,11,43–46] Most authors seem to agree that they consist of amorphous electron-dense material surrounded by tubular and vesicular structures, and with a few central clear areas containing cytoplasmic components (Fig. 2). Tomonaga *et al.*[44] observed two different types of cytoplasmic inclusions, Bunina bodies and laminated cytoplasmic bodies, and they emphasized that both inclusions were closely related to the endoplasmic reticulum. Takahashi *et al.*[34] also observed similar findings in the subthalamic nuclei in an unusual ALS patient.

Because of the limited number of bodies and poor fixation, their precise nature and morphogenesis have not been clarified by electron microscopy. Therefore, serial sections of the anterior horns were observed by electron microscopy to disclose the detailed structure of Bunina bodies, and Okamoto *et al.*[3,4] observed a variety of features suggesting the process of Bunina body formation. Bunina bodies, though they seem to be complicated in structure, consist mainly of two elements, one amorphous material and the other tubular and vesicular structures. Large and typical Bunina bodies consist of electron-dense amorphous material surrounded by a few tubular and vesicular structures, sometimes with a central clear area containing 10 nm filaments and other cellular organelles (Fig. 2). There was no limiting membrane. These bodies with scant tubular and vesicular structures seem to represent the advanced stage of Bunina body formation. Bunina bodies surrounded by many tubular and vesicular structures may be in the earlier

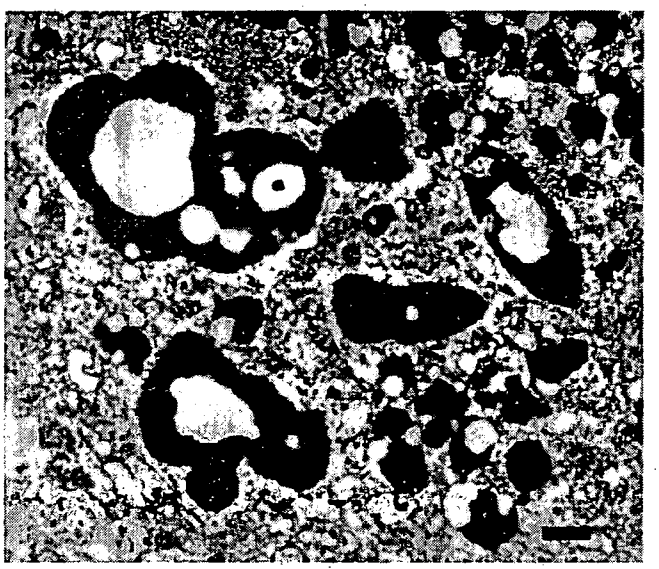

Fig. 2 Electron microscopic features of a typical Bunina body. (Scale bar = 2 μm).

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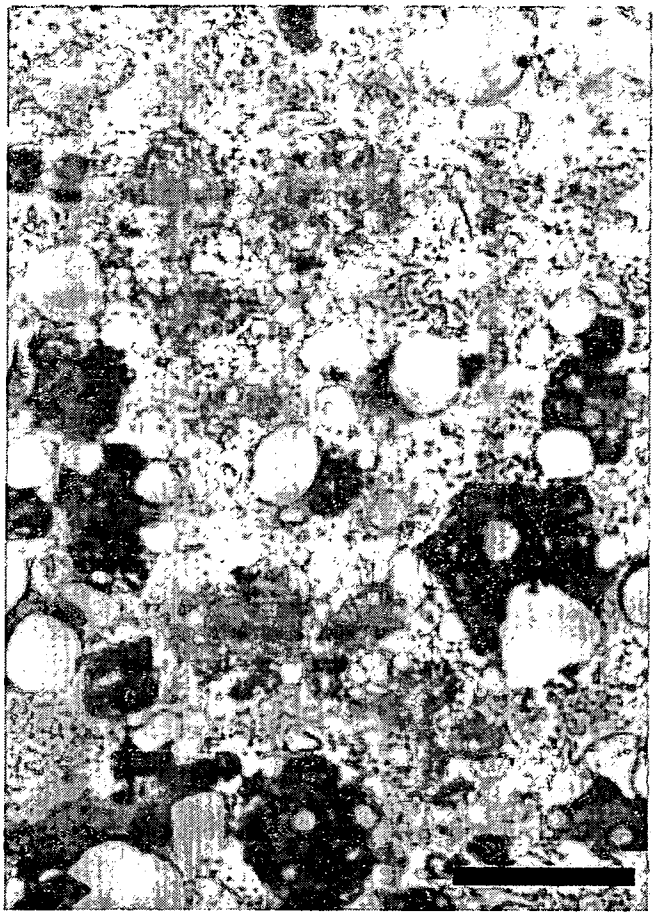

Fig. 3 Small Bunina bodies with numerous tubular and vesicular structures are seen among lipofuscin granules. (Scale bar = 2 μm).

stage (Fig. 3). Small Bunina bodies were scattered among lipofuscin granules (Fig. 3). Similar amorphous material was also deposited in association with the Golgi apparatus (Fig. 4). Okamoto *et al.*[3,4] suspect this is the earliest stage of Bunina body formation. Rarely, similar Bunina-like structures were seen around the Lewy-body like filamentous structures (Fig. 5). Laminated cytoplasmic bodies were very rarely observed in anterior horn cells, and no apparent transition between these bodies and Bunina bodies was observed.

BUNINA-LIKE INCLUSIONS

Ultrastructurally, Bunina-like inclusions were rarely observed in non-motor neurons in the olfactory bulb of aged humans,[47] in the cortical neurons in aged rats,[48] in the Betz cells of aged rhesus monkeys[49] and in the lumbar motor neurons in non-ALS patients.[50] Sasaki *et al.*[51] examined the ultrastructure of Betz cells of 17 non-ALS individuals and found electron-dense inclusion bodies in five more elderly cases,[51] and they suspected that the inclusions probably represent an age-related degenerative change. The inclusions consisted of small electron-dense inclusions with vesicles and tubular structures and they resembled figures in the earliest stage of Bunina body formation in ALS.

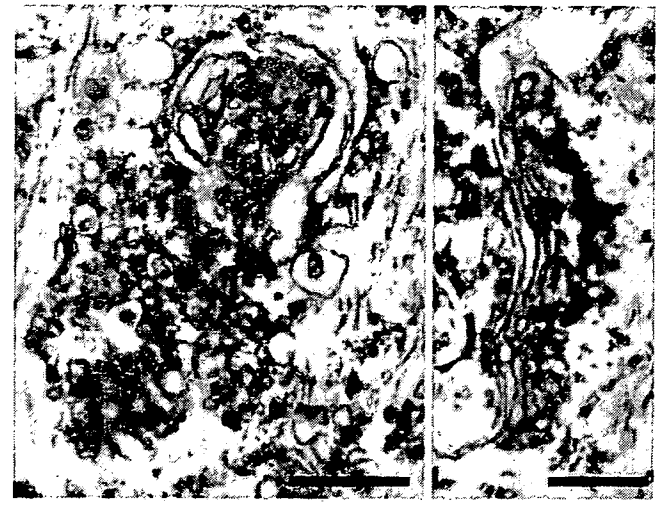

Fig. 4 Similar amorphous material are deposited in association with the Golgi apparatus (left and right). (Scale bar = 1 μm).

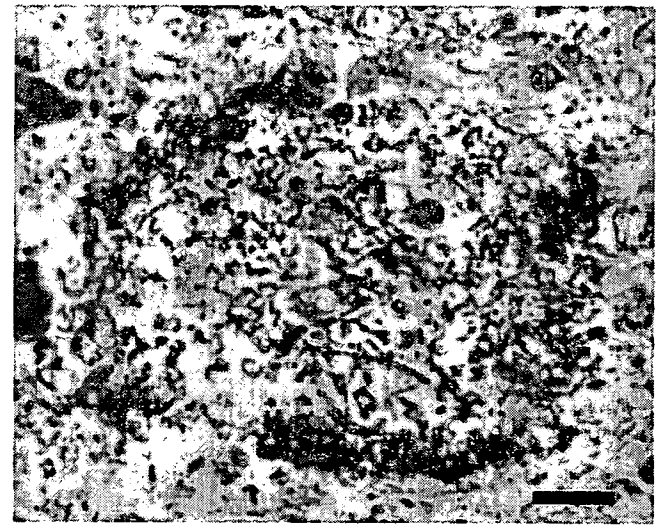

Fig. 5 Small elongated Bunina-like structures are seen around the Lewy body-like filamentous structures. (Scale bar = 2 μm).

IMMUNOELECTRON MICROSCOPY

Vibratome sections of 50-μ-thick and 10-μ-thick frozen sections, taken from 4% paraformaldehyde-fixed anterior horns of a patient with ALS and of a patient with non-ALS, were examined by immunoelectron microscopy for cystatin C[12]. Many small accumulations of immunoperoxidase reaction products were seen in the cytoplasm and dendrites of the anterior horn cells. Immunoperoxidase products were also present in Bunina bodies, especially in the

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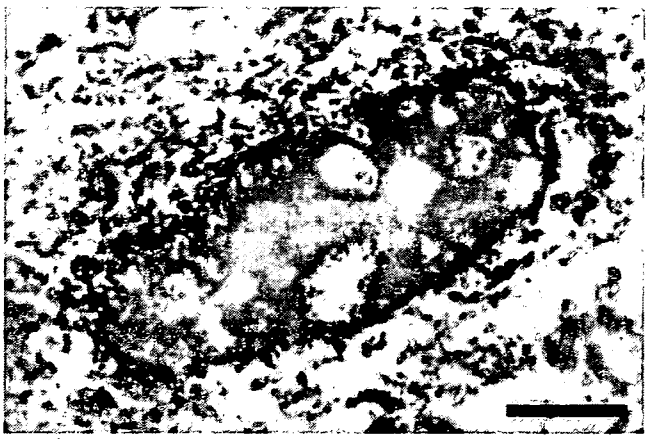

Fig. 6 Immunoelectron microscopic finding of cystatin C expression in a Bunina body. The immunoperoxidase reaction products are seen predominately in the periphery of the Bunina body. (Scale bar = 2 μm).

periphery (Fig. 6). Compared to the routine electron microscopic features of Bunina bodies, immunoperoxidase products appeared mainly to be localized in the tubular and vesicular structures in the periphery of the bodies. The exact localization of cystatin C was not clear in the autopsied human spinal cord due to artifacts; therefore, Okamoto *et al.*[12] also performed immunoelectron microscopy on the lumbar cord of an adult cat by the same methods. In an adult cat, cystatin C was mainly localized in the medial aspects of the Golgi apparatus and in the lysosomes in the anterior horn cells of the spinal cord. Therefore, Okamoto *et al.*[12] speculated that Bunina bodies might represent an abnormal accumulation of unknown proteinous materials associated with the Golgi apparatus or endoplasmic reticulum.

DISCUSSION

Bunina[1] suspected that the inclusions might be a neurotrophic virus; however, electron microscopic studies did not support the possibility of viral origin. Several theories have been proposed based mainly on electron microscopic findings. Hart *et al.*[11] suggested that Bunina bodies were a special type of autophagic vacuole, probably arising from the mitochondria in which similar electron-dense masses were encountered. However, the electron-dense masses in the mitochondria were never large enough to occupy the entire mitochondrion, and laminated cytoplasmic bodies were very rarely seen in the anterior horn cells of ALS patients. Okamoto *et al.*[3,4] could not find any signs of transition from laminated cytoplasmic bodies to Bunina bodies. Chou[14] suggested that Bunina bodies were derived from amorphous conglomerated basophilic inclusions. Murayama *et al.*[21] described Bunina body-like structures with bundles of coated filaments and speculated that they may represent ubiquitinated precursor proteins that accumulated to form Bunina bodies. Using particle-induced X-ray emission spectrometry, Yoshida *et al.*[52] showed that aluminum strongly binds to the Bunina bodies as well as the rough endoplasmic reticulum, and they speculated that the Bunina bodies may be an end-product of nucleic acid dysmetabolism at the rough endoplasmic reticulum caused by aluminum along with magnesium depletion.

From many electron microscopic features of Bunina bodies, Okamoto *et al.*[3,4] speculated that amorphous material might develop around the tubular and vesicular structures in the early stage of Bunina body formation. The deposits may increase in amount with proliferation of vesicles and tubules, which may become embedded in cellular organelles and engulfed in some areas. These structures are devoid of ribosome attachment; therefore, it is suggested that they originate from smooth endoplasmic reticulum or the Golgi apparatus. However, Okamoto *et al.*[3,4] could not identify the origin from routine ultrastructural study because they have no specific marker available for smooth endoplasmic reticulum or the Golgi apparatus.

The Golgi apparatus has important functions in processing and transporting plasma membrane, lysosomal, and secreted proteins. Gonatas *et al.*[53] reported fragmentation and atrophy of the Golgi apparatus in the motor neurons in ALS patients using anti-MG160 antibody. MG160 is a conserved membrane sialoglycoprotein of the medial cisternae of the Golgi apparatus. Immunohistochemical study using anti-MG160 antibody disclosed that almost all anterior horn cells with Bunina bodies showed fragmentation of the Golgi apparatus; however, Bunina bodies themselves were not immunostained with anti-MG160 antibody.[54] Matsumoto *et al.*[55] also showed that Bunina bodies themselves were negative for Golgi apparatus.

Recently, ubiquitin immunoreactive lesions of FTLD-U and ALS have been defined by the presence of TDP-43, and these disorders can be subsumed into a single entity under the umbrella of TDP-43 proteinopathy.[39] However, Bunina bodies, one of the specific pathologic hallmarks of ALS, are negative for TDP-43.[40] The nature and significance of the Bunina bodies in ALS are not yet clear, and the fact that Bunina bodies appeared not only in the degenerating neurons but also in the normal-looking neurons suggests that Bunina bodies are an initial change or reaction of the motor neurons. Bunina bodies may represent primary and essential pathologic changes in the anterior horn cells in ALS, such as disordered protein metabolism, rather than secondary degenerative changes. More studies are needed to clarify the relationship between the cell organelles, such as the Golgi apparatus, and Bunina bodies, and to disclose the roles of cystatin C and transferrin in the pathogenesis of ALS.

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INTERNAL MEDICINE

CASE REPORT

Paraneoplastic Limbic Encephalitis Caused by Ovarian Teratoma with Autoantibodies to Glutamate Receptor

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Abstract

We report a rare case of paraneoplastic limbic encephalitis with autoantibodies to glutamate receptor (GluR) in the cerebrospinal fluid (CSF). The 35-year-old woman with consciousness disturbance was diagnosed initially as non-herpetic encephalitis. Her signs and symptoms improved with acyclovir and steroid pulse therapy. However, after the treatment, an ovarian tumor was discovered, and we detected autoantibodies to GluR in the CSF. A possible association between the ovarian teratoma and GluR is suggested.

Key words: paraneoplastic limbic encephalitis, ovarian teratoma, glutamate receptor

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Introduction

Paraneoplastic limbic encephalitis (PLE) is a relatively rare, remote, non-metastatic neurological complication of carcinoma. PLE occurs subacutely in association with specific neuronal antibodies (1). In a Japanese survey, non-herpetic acute limbic encephalitis (non-herpetic ALE) was identified as a new subgroup of limbic encephalitis with the spectrum that includes herpes simplex encephalitis (HSE) and PLE (2, 3). Antibodies to glutamate receptor (GluR) in the central nervous system (CNS) are reported to be an important autoimmune factor in Rasmussen's encephalitis, epilepsia partialis continua, non-herpetic acute encephalitis, acute encephalitis and paraneoplastic cerebellar ataxia (4-7). Here, we describe a case of PLE associated with an ovarian teratoma and detect autoantibodies to GluR and an elevation of interleukin-6 (IL-6) in the CSF. This case illustrates a potential association between an ovarian teratoma and autoantibodies.

Case Report

A 35-year-old woman with confusion and impaired consciousness was transferred from a local general hospital to the neurology department of Kumamoto University Hospital. She had no symptoms until September 2004, she complained of headache, fever and short-term memory loss. Her symptoms gradually worsened. In the first hospital, the patient was diagnosed with viral encephalitis. Acyclovir (1.5 g per day) was administered intravenously for 11 days and 500 mg of methylprednisolone per day was added for 3 days. However, her condition did not improve, and she developed delusional thinking and auditory hallucinations.

When she was transferred from the first hospital, her temperature was 35.8℃. She showed psychiatric depression and an agitated confusional state with severe impaired attention, orientation and persistence of the depressive state. Physical examination revealed no abnormalities. Palpation of the abdomen revealed no mass. Neurological examination and systemic examination were entirely normal.

The results of laboratory tests including blood counts, biochemical tests, and C-reactive protein were within normal range. There were no evident endocrine or metabolic abnormalities. Tests for antinuclear antibodies were negative. Her CSF pressure was 75 mmH_2O. The fluid was clear and contained 15 cells/μl, 66 mg/dl of glucose, 34.7 mg/ml of protein, 2.96 mg/ml of IgG, 14.9 pg/ml of IL-6 (normal <9.7), 3.1 pg/ml of IL-4 (<11.6), 2.6 pg/ml of IL-2 (<4.6), 2.8 pg/ml of tumor necrosis factor-α (<6.2), 4.1 pg/ml of IL-10 (<6.1) and 7.1 pg/ml of interferon-γ (<46.6). Microscopic examinations of CSF for tumor cells and microorganism were

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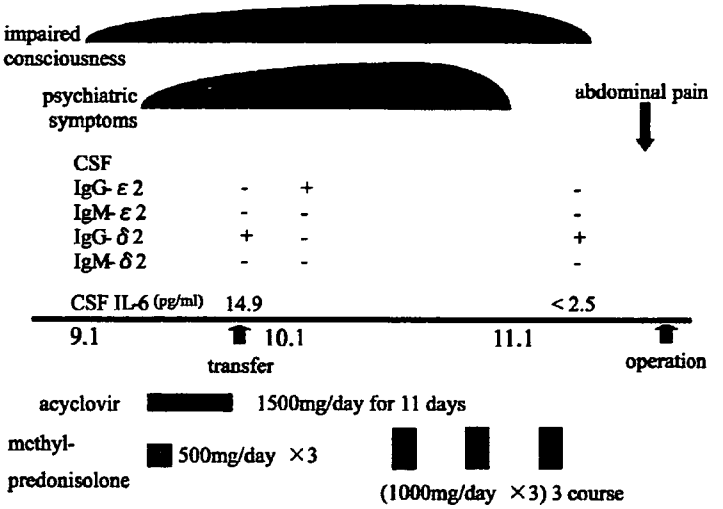

Figure 1. Clinical course. IgG-ε2, IgM-ε2, IgG-δ2, IgM-δ2: IgG and IgM autoantibodies to GluR ε2 or δ2.

negative, and cultures yielded no growth. There was no remarkable elevation of anti-viral antibody titers including mumps, rubella, echo, and varicella-zoster virus in paired serum samples. Polymerase chain reaction (PCR)-based tests for herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus-6, 7 (HHV-6, 7) in the CSF were negative. Magnetic resonance imaging (MRI) of the brain was normal.

Clinical course (Fig. 1)

The consciousness impairment progressed. The patient was restless, constantly in motion and talked incessantly and incoherently. We inferred that the limbic system was the locus for her psychiatric symptoms. A diagnosis of non-herpetic acute limbic encephalitis was made on the basis of negative findings of herpetic group (HSV, CMV, EBV) on PCR and slightly elevated IL-6 in the CSF. PLE was thought to be unlikely in the absence of a positive cytologic examination and with a normal range of tumor markers such as neuron specific enolase and soluble IL-2 receptor. In addition, a previous gynecological examination performed five months before this administration showed no significant abnormalities and the uterus and the adnexal structures were normal in size and echotexture. Methylprednisolone (1,000 mg per day) was administered intravenously for a 3 days course three times. Risperidone (2 mg per day) and olanzapine (10 mg per day) were also used to manage her confusion. After three courses of methylprednisolone, symptoms regressed and the CSF IL-6 level returned to normal range. A follow-up MRI also showed normal findings.

The patient's condition was improving, but, at three weeks after treatment, a tumor was discovered in her lower abdomen. A pelvic MRI revealed a solid tumor filling the pelvic cavity. Tumor markers associated with ovarian tumor showed 55 U/ml of CA125 and 170 U/ml of CA19-9. She had a sudden onset of high fever and abdominal pain in the lower abdomen a few days later. The resistance of the abdominal wall increased and moderate tenderness was elicited. The diagnosis was panperitonitis due to rupture of the ovarian tumor. She had an emergency right salpingo-oophorectomy. There was no apparent metastasis to the pelvic wall. The tumor was solid and included hair and cartilage. The pathologic diagnosis was an immature teratoma of grade 2 with an immature neuroepithelial component (Fig. 2). After surgical resection, she received chemotherapy. The patient is currently well at 2 years after surgical treat-

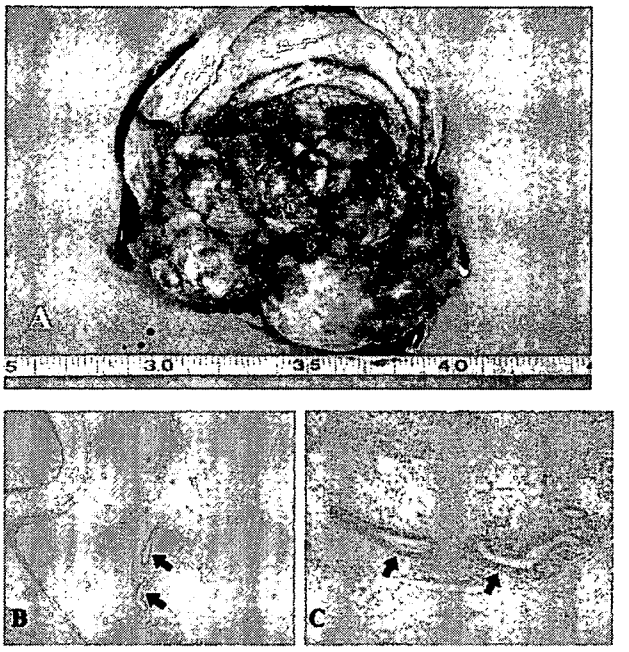

Figure 2. Pathological findings of ovarian tumor: (A) The tumor was solid and included hair and cartilage on macroscopic examination. (B), (C) Neuroepithelial cells (arrows) are shown (B,C: HE stain, B×40, C×100).

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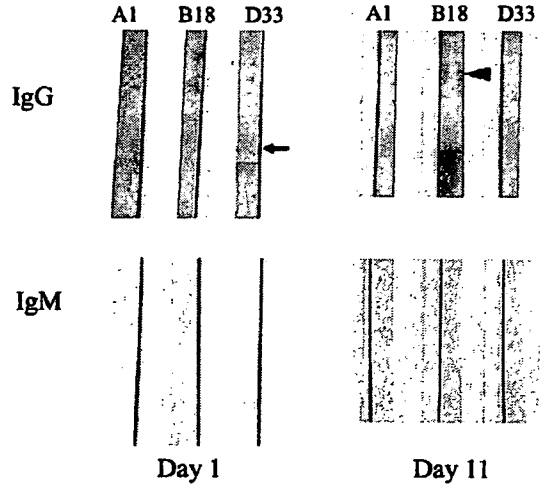

Figure 3. Detection of autoantibodies to whole molecule GluR ε2 and δ2 subunit in the cerebrospinal fluid samples from this patient. Data for IgG and IgM autoantibodies are shown. A1 = control strip. ; B18 = strip of nitrocellulose membrane containing whole GluR ε2 proteins reacted with CSF of this patient.; D33 = strip of nitrocellulose membrane containing whole GluR δ2 proteins reacted with CSF of this patient. arrowhead = whole molecule of GluR ε2 (about 180 kd), arrow = whole molecule of GluR δ2 (about 100 kd), stained with patient's autoantibodies followed by alkaline phosphate-labeled second antibodies. GluR IgG-ε2 and IgG-δ2 autoantibodies were detected in the CSF samples at the first and 11th day of admission, respectively.

ment with no evidence of recurrence of the tumor. Psychotropic drugs were gradually tapered in 4 weeks and discontinued. She has neither physical nor mental disabilities.

After surgery, to clarify the potential association between encephalitis and ovarian tumor, anti-Hu, Yo, Ri, Ma, Ta, Tr and Amphyphysin antibodies in the patient's CSF that had been stored before treatment were tested. All antibodies were negative. However autoantibodies to the glutamate receptor (GluR) subunits epsilon (ε)-2 and delta (δ)-2 were detected in the serum and CSF (Fig. 3). A sample of ovarian tumor extract was tested with immunoblotting analysis. However expression of GluR ε2 or GluR δ2 was not confirmed by immunoblot analysis using antibodies against GluR ε2 or GluR δ2.

Discussion

The present patient suffered from limbic encephalitis associated with an immature ovarian teratoma. She had autoantibodies to GluRs and elevated IL-6 level in the CSF. These findings suggest that the neurological symptoms were attributed to the paraneoplastic autoimmune mechanisms.

Recently, six cases of ovarian teratoma (mature: 2 cases, immature: 4 cases) in association with PLE have been reported. The clinical characteristics of literature cases including the present case are summarized in Table 1 (8-13). There were cases of mild CSF pleocytosis without infectious etiology and no antineural antibodies except in one case (8). Neurological symptoms of all six cases including ours improved or completely resolved after treatment.

Neurological symptoms in most patients did not improve after the first course of steroid pulse therapy, but those pa-

Table 1. Literature Cases of Paraneoplastic Encephalitis with Ovarian Teratoma

Patients	*Fadare et al.* [8]	*Nokura et al.* [9]	*Okamura et al.* [10]	*Taylor et al.* [11]	*Aydiner et al.* [12]	*Munakata et al.* [13]	*Present case*
Age (year)	33	19	15	24	39	25	35
Neurological form	limbic encephalitis	limbic and brainstem encephalitis	limbic encephalitis	encephalo -myelitis	limbic encephalitis	limbic encephalitis	limbic encephalitis
Clinical symptoms							
Impaired consciousness	-	+	+	+	+	+	-
Dysmnesia	+	+	+	+	+	+	+
Psychiatric symptom	-	+	+	+	+	-	+
Seizures	-	+	-	-	+	+	-
Cerebrospinal fluid							
Cells (μ l)	normal	34	normal	23	65	normal	15
Protein (mg/dl)	normal	19	normal	normal	64	normal	35
MRI abnormalities	N.D.	normal	normal	medulla	normal	bilateral hippocampi	normal
Treatment	operation	operation	operation	operation, IVIg steroid	operation	operation steroid	steroid operation
Pathologic diagnosis	mature	immature	immature	mature	immature	immature	immature
Sequelae	-	amnesia	-	-	affective disorder, amnesia	amnesia seizure	-

N.D.: not described, IVIg: intravenous immunoglobulin therapy

tients improved after resection of the ovarian tumor. In this patient, we continued three courses of pulse therapy because of the elevation of IL-6 in the CSF. Thereafter, symptoms in this patient improved significantly. Therefore we speculate that autoimmune mechanisms contributed to the encephalitis in this patient.

It is impossible to exclude the possibility that the tumor was a coincidental association and that the neurological syndrome was causally related to mechanisms other than paraneoplatic mechanisms. However, we were unable to demonstrate any infectious etiology and serological data suggesting a systemic vasculitis. Neurological symptoms and increasing tumor size presented simultaneously. Taken together, her clinical presentation, CSF profile, neuroimaging, detection of autoantibodies and elevated cytokine level argues strongly in favor of a paraneoplastic etiology.

In this patient, autoantibodies against GluRε2 and GluR δ2 were detected, although the tumor expressed no detectable GluR ε2 or GluR δ2. These data suggest that autoantibodies to GluRs may be produced after neuronal injuries. Tumor immunity may induce activation of autoreactive cytotoxic T cells and produce cytotoxic cytokines etc, which may result result in neuronal damage. The data also suggest that an autoimmune mechanism against ovarian tumor may cross react with GluRs. Although the role of autoantibodies to GluRs is not clear, autoantibodies against GluR ε2 (NMDA 2B) are reported to cause neuronal apotosis in hippocampal neurons (14). Therefore, autoantibodies to GluRs, even which are produced after some neuronal injuries may affect the symptoms of paraneoplastic limbic encephalitis. Additional cases are needed to elucidate the relationship between PLE and autoantibodies to GluR.

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Case report

Refractory epilepsy accompanying acute encephalitis with multifocal cortical lesions: Possible autoimmune etiology

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Abstract

We report on a 14-year-old male suffering from acute encephalitis, whose clinical course met the criteria for acute encephalopathy with refractory, repetitive partial seizures (AERRPS). He presented with extremely refractory partial and secondary generalized seizures, and required high-dose barbiturate infusion therapy for 57 days under mechanical ventilation. Seven weeks after onset, the seizures were ameliorated by treatment with sodium bromide, carbamazepine, clobazam, and high-dose phenobarbital. Magnetic resonance imaging on day 14 of admission showed multifocal cortical legions scattered in the bilateral hemispheres; these disappeared on day 34. Diffuse and mild atrophy of the cerebral cortex, and moderate atrophy of the hippocampus, appeared by day 61. Serum anti-glutamate receptor ε2 autoantibodies were detected on day 2. The patient was discharged after 113 days of admission with intractable epilepsy, memory disability, and regression of intelligence. We discuss the etiological significance of the multifocal lesions, which are unusual findings on neuroimaging of AERRPS.
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Keywords: Refractory seizures; Repetitive seizures; Status epilepticus; High-dose barbiturate; Multifocal cortical legions; MRI

1. Introduction

Awaya et al. [1] reported on children with a "peculiar type of post-encephalitic epilepsy", presenting with extremely refractory partial seizures during a prolonged acute phase and sequelae of intractable epilepsy. Approximately 40 similar cases have been reported in Japan. Sakuma et al. [2] proposed the terminology "acute encephalitis with refractory, repetitive partial seizures (AERRPS)" for this entity, with the criteria being: (1) prolonged acute phase of more than 2 weeks; (2) partial seizures of the same symptoms persisting from the acute phase to convalescence; (3) seizures frequently evolving into convulsive status especially during the acute phase; (4) marked intractability of seizures; and, (5) exclusion of related disorders such as known viral encephalitis or metabolic disorders. Additional features including responsiveness to certain antiepileptic agents, and the presence of serum anti-glutamate receptor antibodies were reported in some cases [1,3]. AERRPS is now an accepted clinical entity in Japan, based on these characteristics; however, this disease entity has not achieved worldwide consensus, despite recent reports of cases whose clinical features meet the criteria of AERRPS [4,5]. Here, we describe a patient whose clinical course was compatible with a diagnosis of AERRPS, in which multifocal cortical lesions were detected on magnetic resonance imaging (MRI). This finding is quite

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unusual in viral encephalitis, and suggests an autoimmune basis for the pathogenesis. Such a finding has also not been described in patients with AERRPS, and we discuss the associated nosological concerns.

2. Case report

2.1. Clinical course (Fig. 1)

A 14-year-old boy presented with fever and headache persisting for 3 days. Following a 5-day remission, the symptoms reappeared in association with vomiting and eruption, as well as generalized tonic convulsion. On admission, the patient was stuporous. Body temperature was 37.9 °C. Scarlet fever-like eruption was noted on the trunk, but otherwise there were no remarkable findings. Cranial computed tomography (CT) was normal. Routine assays of blood and cerebrospinal fluid (CSF) were within normal range, and electroencephalography (EEG) showed sporadic diffuse slow waves.

On the day of admission, he developed bilateral facial twitching and eyelid fluttering, which were controlled with a bolus infusion of diazepam and continuous intravenous administration of midazolam. He remained stuporous during the interictal period. On day 2, frequent twitching in the face, hand, and foot recurred, often evolving into generalized convulsions. Treatment with a suppository of phenobarbital (PB), as well as intravenous phenytoin, pyridoxine, and lidocaine were not effective. The seizures lasted for 0.5–2 min and appeared every few minutes, sometimes culminating in secondary generalized tonic–clonic convulsions, or an epileptic status. High-dose PB with a concentration of 150 μg/ml, or continuous infusion of thiamylal at 7 mg/kg/h under mechanical ventilation, did not suppress the seizures completely. High-dose immunoglobulin and steroid pulse therapy of 30 mg/kg/day methylprednisolone for 3 days also showed no beneficial effect. Ictal EEG showed focal rhythmic 5 or 10 Hz spikes in the bilateral frontal, central or occipital areas, with occasional generalization.

On day 27, the thiamylal was replaced by thiopental sodium (TP), which controlled the seizures when administered at a dosage of 2–6 mg/kg/h. At this time, the EEG showed a burst-suppression pattern. Under infusion of these barbiturates during the acute phase, we tried several oral antiepileptic drugs: sodium valproate, zonisamide, clobazam (CLB), sodium bromide (NaBr), and carbamazepine (CBZ). Only CLB produced any improvement in this acute phase.

The seizures ceased after 3 weeks of therapy with TP, which was gradually replaced by oral PB. Brief partial seizures recurred and persisted 0–5 times per day. Thereafter, the patient gradually recovered and was able to perform normal daily activities without aid. On day 113, he was discharged under treatment with PB, CLB, NaBr, and CBZ, which were effective to some degree at this stage of the illness. He had sequelae of intellectual regression with an intelligence quotient of 66, disability

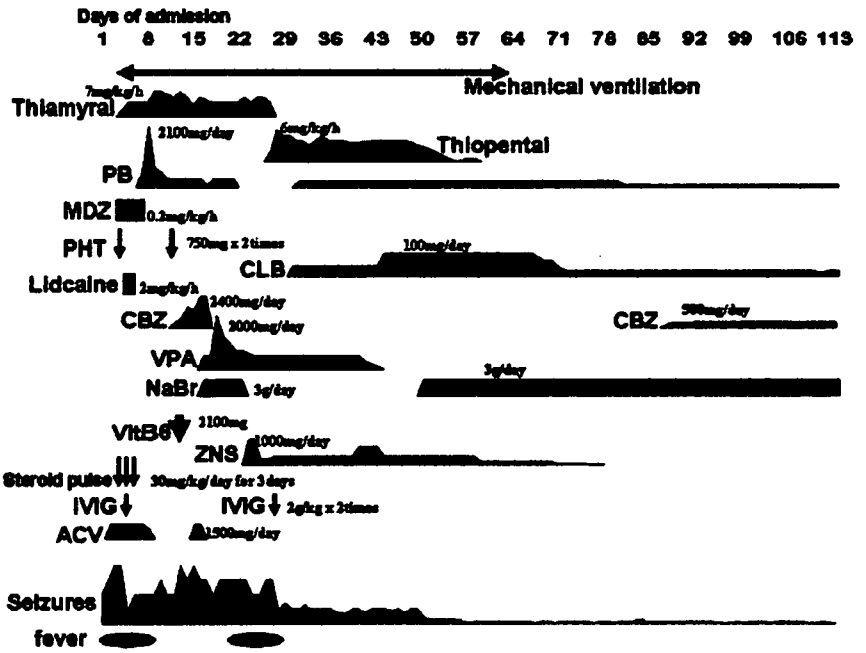

Fig. 1. Clinical course of the patient. Dosage of each drug refers to the maximum dose of continuous (thiamylal, thiopental, MDZ, and lidocaine) or one-shot (PHT, Vit.B6, steroid, IVIG, and ACV) intravenous injection or oral intake (PB, CLB, CBZ, VPA, NaBr, and ZNS). PB, phenobarbital; MDZ, midazolam; PHT, phenytoin; CLB, clobazam; ZNS, zonisamide; CBZ, carbamazepine; VPA, sodium valproate; NaBr, sodium bromide; IVIG, intravenous immunoglobulin; ACV, acyclovir.

to memorize new words and concepts at school, and intractable seizures (visual seizures, simple and complex partial seizures on extremities and face, and secondary generalized seizures).

2.2. Laboratory data (Table 1)

On admission, the blood analysis was unremarkable other than a mild decrease in the platelet count and a mild elevation in liver enzymes. Lactate and pyruvate in serum (on day 1) and CSF (on day 2) were normal. CSF showed transient pleocytosis and a mild elevation of protein content. Extensive tests for viral infection yielded negative results other than mumps virus-IgM, which was weakly positive on day 3 but negative on day 10, and parotitis did not appear clinically. Serum anti-glutamate receptor (GluR) ε2 IgM and anti-GluRε2 IgG autoantibodies were positive on day 2. Serum anti-GluRδ2 IgM and IgG (on day 2), CSF anti-GluR ε2 and GluRδ2 autoantibodies were negative.

2.3. Neuroimaging (Fig. 2)

MRI was normal on day 3. On day 14, multiple high-intensity lesions appeared in the bilateral cerebral cortex with frontal and parietal predominance on fluid-attenuated inversion recovery (FLAIR) imaging (Fig. 2A and B). This distribution was consistent with the epileptic foci on EEG at this time. These lesions were not Gadolinium-enhanced, and no abnormality was noted on T1, T2 or diffusion-weighted images. White matter, basal ganglia, brainstem, and cerebellum were spared. These high-intensity lesions were almost gone by day 34 (Fig. 2C). Generalized cerebral cortical atrophy, predominantly in the bilateral frontal lobes and hippocampi, appeared on day 61 (Fig. 2D and F). On day 117, the frontal lobe atrophy was vastly improved but the hippocampal atrophy persisted (Fig. 2E and G). On day 10, single photon emission-computed tomography (SPECT) showed hyperperfusion in the left frontal and parietal lobes (Fig. 2H), which was consistent with the high-signal lesions observed on FLAIR imaging (Fig. 2B). There was a simultaneous reduced blood flow with widespread distribution including the hippocampus (not shown). Blood flow recovered throughout the brain on day 80, and hyperperfusion was apparent in parietal and occipital lobes (not shown) and bilateral hippocampus (Fig. 2I).

3. Discussion

We reported herein on a young male patient presenting with seizures who showed a prodromal period with fever and eruption. The clinical symptoms and course met the criteria of AERRPS [2,3]. Based on these symptoms and the mild pleocytosis in CSF, a diagnosis of encephalitis was made for this patient. Together with the parenchymal cellular infiltration [5], such findings support the validity of the term of 'encephalitis' also in AERRPS, although no infective agents have been identified thus far in this entity [1–3].

Table 1
Laboratory data

WBC		3840/μl
PLT		117×10^3/μl
AST		51 IU/L
ALT		54 IU/L
Serum lactate		16.1 mg/dl
Serum pyruvate		0.88 mg/dl
CSF lactate		16.1 mg/dl (day 2)
CSF pyruvate		0.89 mg/dl (day 2)
CSF cell count		47/3 (day 2) and 19/3 (day 15)
CSF Protein		32 mg/dl
Viral culture (pharynx, blood, stool, and CSF) for rhinovirus, coxsackievirus, parainfluenza virus, influenza virus, RS virus, echovirus, enterovirus 71, poliovirus, and rotavirus		All negative
Blood titer for:		
Mycoplasma	(day 1)	All negative
Herpes simplex virus (HSV), *Varicella zoster* virus (VZV)	(day 3)	
Cytomegalovirus, adenovirus, Japanese encephalitis virus, *Rubella* virus, measles virus	(day 11)	
PCR for HSV and VZV in CSF	(day 6)	Negative
Serum antinuclear antibody, C-ANCA, P-ANCA, lupus anticoagulant		Negative
Serum mumps virus IgM	(day 3)	Weakly positive
	(day 11)	Negative
Mumps virus IgG	(day 70)	Negative
Serum IgG-GluRε2, IgM-GluRε2	(day 2)	Positive
IgG-GluRδ2, IgM-GluRδ2	(day 2)	Negative

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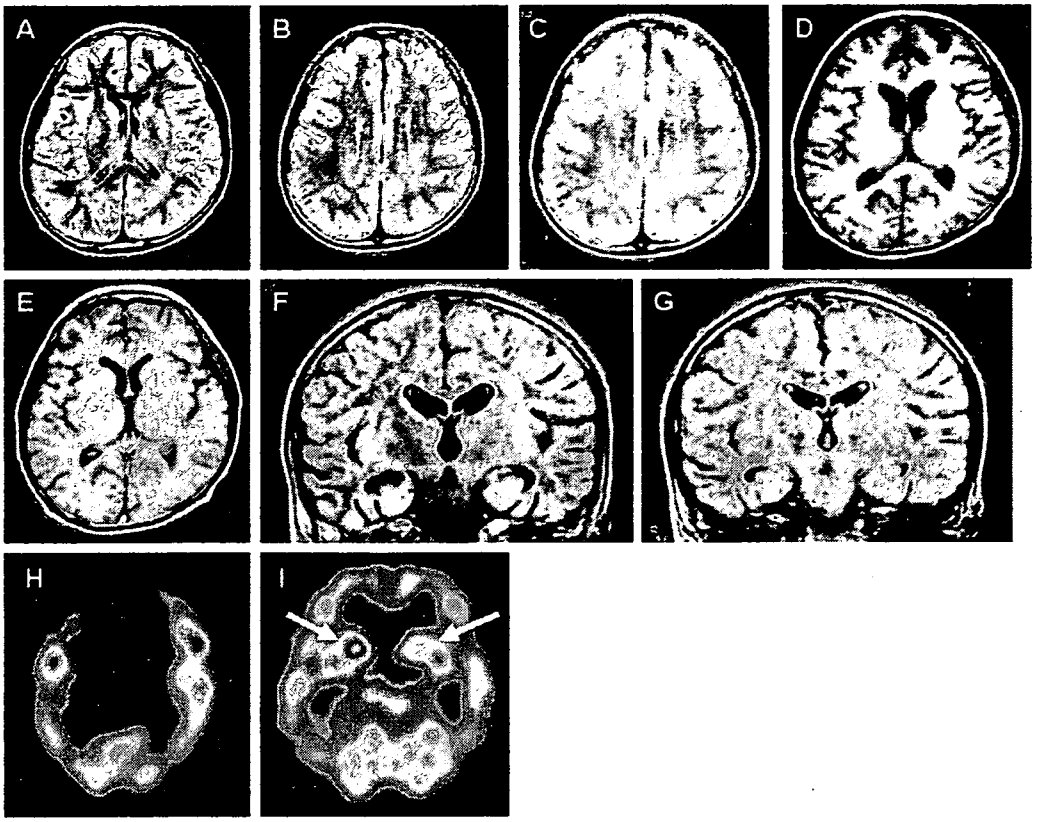

Fig. 2. Neuroimaging of the patient. Magnetic resonance imaging (MRI) [A–C, F, and G: fluid-attenuation inversion recovery (FLAIR) images; (D) and (E), T1-weighted images] and single photon emission-computed tomography (SPECT) (H and I) of the patient. On day 14 of illness (A and B), multiple cerebral cortical lesions were distributed in the cerebral cortex, predominantly in the left parietal and bilateral frontal lobes. White matter, basal ganglia, thalamus, cerebellum (not shown), and brainstem were spared. On day 34 (C), cortical lesions (B) almost disappeared on FLAIR imaging. On day 61 (D and F), diffuse cerebral cortical atrophy with predominance in the frontal lobes (D) and hippocampus (F) was noted. On day 117 (E and G), the cortical atrophy had almost disappeared (E) but the hippocampal atrophy remained (G). On day 10, hyperperfusion was apparent in the left frontal, parietal, and right frontal lobes (H), consistent with high-signal lesions (B). On day 80, hyperperfusion appeared in the bilateral hippocampus (I, arrows).

Encephalitis with multifocal cortical cerebral lesions on MRI, without involvement of other brain regions, is extremely rare. Such findings have been reported in presumed autoimmune conditions, including neuro-Behçet disease and Rasmussen's encephalitis [6,7]. In addition, autoantibodies against GluRε2 were seen in the present patient, as is reported in Rasmussen's encephalitis [8], acute limbic encephalitis [8], and occasionally in AERRPS [3]. The GluRε2 autoantibodies in the present patient might be involved in the pathogenesis of the multifocal cortical lesions and refractory seizures, as suggested by similar findings of GluR autoantibodies in an animal model of Rasumussen's encephalitis [9]. The lack of motor sequelae and the mild degree of brain atrophy observed in the current case are common to many cases with AERRPS, in contrast to the considerable intractability of the epilepsy [1,2]. This suggests the presence of a nondestructive, but excitatory action, caused by autoantibodies binding to cortical neurons in this entity. In the present patient, however, GluRε2 autoantibodies were absent from the CSF of the patient. We therefore speculate that other GluR autoantibodies are present and involved in the pathogenesis in this case.

Interestingly, SPECT studies on this patient showed a hyperperfusion in the areas with high-signal lesions on FLAIR imaging during the acute phase, and later in the bilateral hippocampus during the chronic phase. This delayed hyperperfusion of the hippocampus may have resulted from secondary epileptogenesis induced by the refractory seizures, or from direct binding of unidentified GluR autoantibodies to hippocampal neurons [10]. These mechanisms might also be related to the hippocampal atrophy and the intellectual sequelae of the patient.

Definitive diagnosis of the present patient with AERRPS still warrants caution, despite the intriguing hypothesis of an immunological pathomechanism in this case, as the decision was based on clinical characteristics alone. Multifocal lesions are rare and GluRε2 autoantibodies are not necessarily present in AERRPS patients. The heterogeneity of this entity warrants further exploration for a better understanding of its