Acta Ophthalmologica

Case Series

Anti-aquaporin-4 antibody-positive optic neuritis

Mineo Takagi,[1] Keiko Tanaka,[2] Tone Suzuki,[3] Atsushi Miki,[1] Masatoyo Nishizawa[2] and Haruki Abe[1]

[1]Division of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan
[2]Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan
[3]Department of Ophthalmology, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama, Japan

ABSTRACT.

Purpose: **It has recently been reported that the anti-aquaporin-4 antibody (AQP4-Ab) can be a specific marker of neuromyelitis optica. We present three cases of optic neuritis (ON) where the patients tested positive for AQP4-Ab, but showed no neurological signs.**

Methods: **Sera were obtained from 32 Japanese patients with ON and no other neurological abnormalities (mean age 46 ± 20 years). AQP4-Ab was detected by indirect immunofluorescence staining using human-AQP4-transfected HEK 293 cells.**

Results: **AQP4-Ab was positive in three female patients (aged 9, 64 and 82 years). Their illness was characterized by bilateral severe optic nerve involvement, insufficient visual recovery, and autoimmune abnormalities (such as positive antinuclear antibody). Two of these patients experienced recurrent episodes of ON. In at least two episodes, the intracranial portion of the optic nerve showed significant inflammation on magnetic resonance imaging.**

Conclusions: **These cases indicate that some ON patients have an immunological pathogenesis similar to that seen in neuromyelitis optica. In addition, examination for AQP4-Ab positivity in the initial phase of ON is important in predicting the prognosis, including the possibility of the development of transverse myelitis.**

Key words: anti-aquaporin-4 antibody – autoimmune optic neuropathy – neuromyelitis optica – optic neuritis

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Introduction

Optic neuritis (ON) is a common ophthalmological disease, but its pathophysiological mechanism is poorly understood. It is thought to be an inflammatory demyelinating disease caused by autoimmune mechanisms, and to be closely related to multiple sclerosis (MS). Multiple sclerosis has been typically classified into two subgroups in Asia – classic MS (C-MS) and optic-spinal MS – but most cases of the latter is now thought to actually be neuromyelitis optica (NMO) (Wingerchuk et al. 1999; Kira 2003; Lennon et al. 2004; Nakashima et al. 2006; Tanaka et al. 2007). Lennon et al. (2004, 2005) recently reported that the serum immunoglobulin G antibody that recognizes the aquaporin-4 water channel (AQP4) is a specific marker of NMO. In this report, we describe AQP4 positivity in three patients with ON and no neurological disturbance.

Materials and Methods

We examined sera from 32 Japanese patients with ON from June to December 2006. The diagnosis of ON was made by the authors (MT and TS, at Niigata University Hospital and Dokkyo Medical University Koshigaya Hospital, respectively), based on the subacute onset of visual loss, sluggish pupillary light reflex, central scotoma, and gadolinium (Gd)-DTPA (diethylene-triamine-penta-acetic-acid) contrast enhancement of the optic nerve under magnetic resonance imaging (MRI). Patients of all ages were included; those with a history of ON during the last 10 years (for whom detailed neuro-ophthalmological records could be reviewed) were also included. None of these patients showed signs of brain or spinal cord lesions. The patient population included 19 females and 13 males

(mean age 47.5 ± 20.0 years). Nine patients were affected in the right eye and seven in the left, and 16 had bilateral ON. The medians of worst and final best-corrected visual acuity (BCVA) were 20/400 and 20/25, respectively. Five patients experienced recurrent episodes of ON. Sera were obtained in accordance with ethical standards in line with the tenets of the Declaration of Helsinki. The study was approved by the ethical committee of Niigata University and written informed consent was acquired from all participants. Antibody testing was performed in a completely blind manner, with no clinical information provided to the examiner.

For the AQP4-Ab detection, we constructed AQP4 antigen-presenting cells as follows (Tanaka et al. 2007): total RNA was extracted from an adult human cerebellum from a donor bank and cDNA-encoding human aquaporin-4 (AQP4 M23 isoform; GenBank accession number U63623) (Lu et al. 1996) was cloned by the reverse transcription-polymerase chain reaction (RT-PCR) technique. Full-length cDNA was inserted into the *Xba*I site of a pEF-BOS expression vector and transfected to HEK 293 cells. The HEK 293 cells were then fixed in 4% paraformaldehyde in 0.1 m phosphate-buffered saline (PBS), pH 7.4. Non-specific binding was blocked with 10% goat serum/PBS, the cells were incubated with the patient serum for 60 mins at room temperature and then incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (BD Biosciences, San Jose, CA, USA). A *SlowFade* Gold anti-fade reagent (Molecular Probes, Carlsbad, CA, USA) was then applied to the slide and positivity verified under the fluorescence microscope.

Results

Positivity for AQP4-Ab was observed in three of 32 patients (Fig. 1). All positive patients were female and had episodes of severe bilateral optic nerve involvement, but no complaints of retrobulbar pain with eye movement. No neurological signs were detected by neurologist's examination.

Case 1

A previously healthy 9-year-old girl was seen at the hospital in August 2003 suffering from viral meningitis. A few weeks after her meningitis was resolved, she noticed a bilateral visual disturbance. Her BCVA was 20/400 OD and 20/800 OS. Her light reflex was sluggish OU, and her visual fields were severely impaired with central scotoma. Her anterior segment appeared normal, but funduscopy revealed bilateral disc swelling. Bilateral swelling of the optic nerves, including the intracranial portion, was noted on her MRI. Cerebrospinal fluid (CSF) findings were normal (including myelin basic protein); an oligoclonal band was not observed. The subject was treated with three cycles of i.v. methyl prednisolone pulse therapy (0.5 g/day for 3 days at 1-week intervals), followed by oral corticosteroid. Her BCVA slowly recovered, reaching 20/15 OU. In September 2004, her BCVA dropped to 20/40 OD and hand motion (HM) OS. Her left eye showed a relative afferent pupillary defect (RAPD). Her visual field was severely impaired, and funduscopy revealed left disc swelling. Her MRI showed Gd-enhancement in the left optic nerve, predominantly in the intracranial portion (Fig. 2A). Serological examination revealed high levels of antinuclear antibody (ANA) at an index of 89.6 (normal: < 20), an anti-SS-A antibody (SS-A) index of 130.0 (normal: < 30), and a rheumatoid factor (RF) of 61.31 IU/ml (normal: < 10 IU/ml). She was treated again with i.v. and then oral corticosteroids. Best corrected VA OS slowly recovered to 20/15. In March 2005, BCVA OD had dropped to light perception (LP), but disc swelling was not observed. The patient was treated again with i.v. and oral corticosteroids. Her BCVA OD slowly recovered, but only to 20/200. In September 2005, BCVA OS had dropped to 20/2000. Disc swelling was not observed. She was treated again with i.v. and oral corticosteroids. Her BCVA OS slowly recovered, but only to 20/200. AQP4-Ab positivity was detected on 6 June 2006. Levels for ANA, SS-A and RF remained similar to those seen in the second episode.

Case 2

An 82-year-old woman with mild hypertension reported bilateral visual loss in December 2005. Her BCVA was reduced to HM OU in a few days. Her light reflex was sluggish OU. Her anterior segment appeared normal and the funduscopy revealed normal discs. Her MRI revealed bilateral Gd-enhancement of the optic nerve, predominantly in the intracranial portion (Fig. 2B). She had an elevated RF of 30.4 IU/ml. She was treated with three cycles of i.v. methyl prednisolone pulse therapy (0.5 g/day for 3 days at 1-week intervals), followed by oral corticosteroid. Over several months, her BCVA slowly improved to around 20/25 OS, but reached only 20/200 OD with central scotoma. AQP4-Ab positivity was detected on 18 July 2006.

Case 3

A previously healthy 64-year-old woman reported visual loss in April 2002. Her BCVA was no LP OD and 20/30 OS with right RAPD. Her anterior segment appeared normal, but funduscopy revealed slight swelling in the right optic disc, and her MRI revealed right optic nerve swelling. The patient was not willing to start steroid pulse treatment under hospitalization, and only oral corticosteroid was administered. Visual recovery was insufficient: her BCVA in July 2002 was 20/2000 OD and 20/20 OS with right RAPD. In July 2004, her VA OS had fallen to 20/200 with central scotoma, but disc swelling was not observed. Oral steroids were immediately restarted. Over the next month, the subject's BCVA OS improved to 20/25. AQP4-Ab positivity was detected on 11 September 2006. A serological examination at that time revealed a positive ANA (1 : 80 speckled; normal < 1 : 40) and an elevated SS-A (47.8 IU/ml; normal: < 10 IU/ml).

These patients have been carefully followed, both ophthalmologically and neurologically, using neuro-imaging. Total follow-up periods so far are 4 years and 4 months, 2 years, and 5 years and 7 months for Cases 1, 2 and 3, respectively. To date, the subjects have not developed any neurological abnormalities suggesting myelitis.

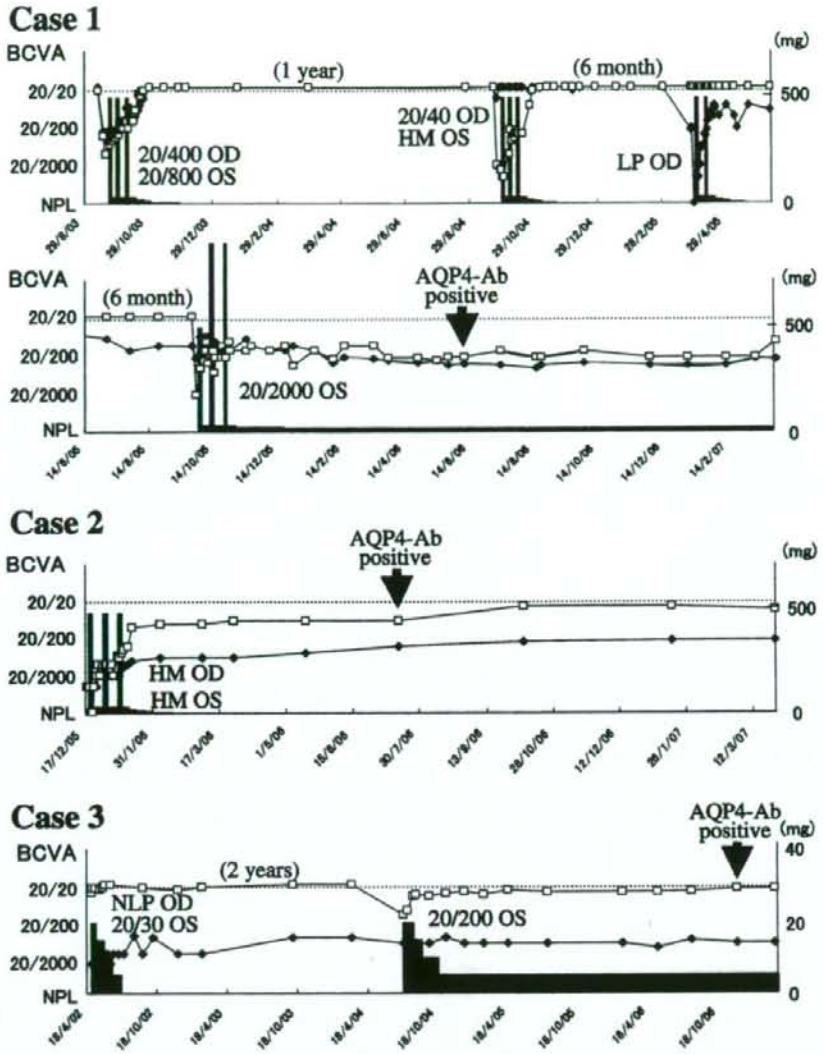

Fig. 1. Clinical course of visual acuity in aquaporin-4 antibody (AQP4-Ab) positive cases, showing dates of evaluation, best corrected visual acuity (BCVA; left ordinate) and dose of corticosteroid (in mg; right ordinate). ♦ and □ = right and left BCVA, respectively. Interval periods between attacks are indicated in parentheses. The worst BCVA in each attack is noted. NLP = no light perception; LP = light perception; HM = hand motion; OS = left eye; OD = right eye. Arrows with 'AQP4-Ab positive' indicate the dates sera were obtained.

Discussion

Our three cases of AQP4-Ab positive ON appear to have distinct clinical characteristics: all were female; all had bilateral eye involvement, and all had severe visual impairment in the acute phase and delayed, insufficient visual recovery. Two of the three patients had recurrent episodes of visual loss. These clinical characteristics could be correlated to some of the distinctive clinical features of neuromyelitis optica (Wingerchuk et al. 1999; Kira 2003; Osoegawa et al. 2005), including a high female : male ratio, high relapse frequency, and severe disability. In a comprehensive study by Lennon et al. (2004), recurrent ON was classified as a 'high-risk syndrome for NMO', and AQP4-Ab was detected in two of eight cases. Post-infection episodes like those seen in our Case 1 are also seen in NMO. However, our patients were outside the typical age range for ON (usually 15–50 years), and they lacked retrobulbar pain with eye movement. These aspects of our cases seem atypical for idiopathic ON.

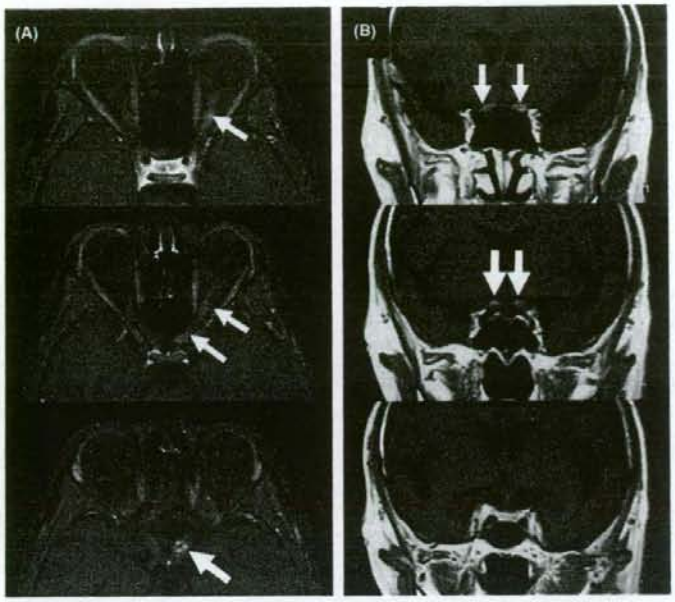

Fig. 2. Magnetic resonance images. (A) Case 1. Axial Gd-enhanced T1-weighted images (with fat suppression) obtained at the onset of the second optic neuritis episode, showing marked enhancement of the left optic nerve (arrows), especially in the intracranial portion close to the optic chiasm (bottom). (B) Case 2. Coronal Gd-enhanced T1-weighted images obtained at the onset of optic neuritis, showing marked enhancement in the intracranial portion of the bilateral optic nerves (arrows).

An autoimmune disorder involving a positive ANA or SS-A is suggested by our three cases, although no systemic diseases were observed. For recurrent episodes and positive autoantibodies, one possible diagnosis is 'autoimmune optic neuropathy' (AON) (Dutton et al. 1982), which is thought to be an entity distinct from demyelinating ON. For AON, in addition to direct inflammatory involvement of the optic nerve, an ischaemic sequel of small vessel vasculitis has been postulated (Goodwin 2006). However, AON is sometimes associated with transverse myelopathy (Goodwin 2006), and NMO is frequently associated with systemic autoimmune disorders (such as connective tissue disease), or with the presence of multiple serum autoantibodies (Wingerchuk 2006). Thus, there could be some clinical overlap between AON and NMO.

Whether the AQP4-Ab is directly related to ON in our cases remains to be elucidated. AQP4 is the predominant water channel expressed in astrocytes and ependymal cells throughout the brain and spinal cord, particularly at sites of fluid transport on the pial and ependymal surfaces in contact with CSF (Nielsen et al. 1997; Rash et al. 1998). As indicated in Fig. 2, the intracranial portion of the optic nerve adjacent to such structures was affected on at least two occasions. This inflammation of the pre-chiasmal portion of the optic nerve could explain the high frequency of bilateral involvement and lack of the retrobulbar pain with eye movement in the early stages of this condition. Preferential expression of AQP4 in the optic chiasm of rats (Venero et al. 1999) and involvement of the optic chiasm revealed by MRI in NMO (Pittock et al. 2006) support this idea.

This study indicates that a subpopulation of patients with ON may have an immunological pathogenesis similar to that seen with NMO. A recent large study (Pirko et al. 2004) revealed that 12.5% of cases of recurrent ON evolved to NMO. It is important to test for the presence of AQP4-Ab in patients with ON, because AQP4-Ab positive patients may have greater potential for developing severe transverse myelitis in the future. In addition, whether or not AQP4-Ab can be detected in mild, monophasic unilateral ON is a topic of interest. A longterm study with a large patient population would help to establish a better understanding of AQP4-Ab positive ON patients.

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Correspondence:
Mineo Takagi MD, PhD
Division of Ophthalmology and Visual Science
Graduate School of Medical and Dental Sciences
Niigata University
1-757 Asahi-machi
Chuou-ku
Niigata 951-8510
Japan
Tel: + 81 25 227 2296
Fax: + 81 25 227 0785
Email: mtakagi@med.niigata-u.ac.jp

Epitope of Autoantibodies to *N*-methyl-D-aspartate Receptor Heteromers in Paraneoplastic Limbic Encephalitis

Yukitoshi Takahashi, MD, PhD[1]

Dalmau and colleagues[1] have presented interesting data about paraneoplastic anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis associated with ovarian teratoma. They found antibodies to NR2B- and NR2A-containing heteromers of the NMDAR by ingenious technique using functional heteromeric NMDAR and its antagonists. All 12 patients' cerebrospinal fluid and serum reacted with heteromeric NMDAR composed of NMDAR1 (NR1) and NMDAR2B (NR2B), and sera and CSF from eight patients also recognized heteromeric NMDAR composed of NR1 and NMDAR2A (NR2A). Patients' sera did not react with cells transfected with individual subunits (NR1, NR2A, or NR2B), and they could not confirm the definitive epitope of antibodies to NR2B- and NR2A-containing heteromers of the NMDARs.

NMDARs are heterotetrameric cation channels composed of NR1 and NR2/3 subunits.[2] NMDARs are assembled early in the endoplasmic reticulum, and both NR1 and NR2 subunits are necessary for their association and their successful cell-surface targeting.[2] NR2B of rat is homologous to GluRε2 in mice. We established stable NIH3T3 transformant cell lines expressing full-length glutamate receptor ε2 (GluRε2) (NR2B) for screening of autoantibodies to GluRε2.[3] Our cell lines (transfected only with GluRε2) did not react with rabbit antibodies to GluRε2 in cell-sorter analyses. These data confirm that individual subunit of NMDARs cannot be targeted on cell surface, and that Dalmau's methods cannot determine which subunit of heterotetrameric NMDAR is autoantigen of antibodies in patients with anti-NMDAR encephalitis.

We have examined autoantibodies to full-length GluRε2 molecules by immunoblot, and N-terminal epitope of the antibodies by immunoblot using bacterial fusion proteins containing peptides from the GluRε2 (amino acid residues 1–48) (NT1).[3] We reported that autoantibodies to GluRε2 in patients with nonparaneoplastic acute limbic encephalitis

appeared usually in the early acute stages, and the autoantibodies had epitope to NT1.[4,5] Recently, we examined autoantibodies to GluRε2 with full-length GluRε2 molecules in five Japanese patients who had been proved in Dalmau's laboratory to have antibodies to NR2B- and NR2A-containing heteromers of the NMDARs. Three patients had ovarian teratoma, one had no findings of teratoma in ovary, and one had no examination of ovary. All five patients had autoantibodies to GluRε2. These data suggest that some part of autoantibodies to NMDARs in acute limbic encephalitis with and without teratoma have epitope to N terminal of GluRε2 (NR2B), and some part of antibodies detected by Dalmau's method recognize N terminal of GluRε2 (NR2B) of the heterotetrameric NMDARs.

[1]*Department of Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Urushiyama Aoi-ku Shizuoka, Japan*

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FULL-LENGTH ORIGINAL RESEARCH

A substantial number of Rasmussen syndrome patients have increased IgG, $CD4^+$ T cells, TNFα, and Granzyme B in CSF

*†Yukitoshi Takahashi, *Jyun Mine, *Yuko Kubota, *Etsuko Yamazaki, and *Tateki Fujiwara

*National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan; and †Department of Pediatrics, Gifu University School of Medicine, Gifu, Japan

SUMMARY

Purpose: We studied the immunologic molecules in cerebrospinal fluid (CSF) and discussed their evolutional changes in pediatric patients with Rasmussen syndrome (RS).

Methods: CSF samples collected from 27 patients with RS (average onset age, 7.5 ± 5.6 years) were studied. Cell count, protein, glucose, albumin, chloride, and immunoglobulin G (IgG) levels were measured by conventional methods. Surface markers of lymphocytes in CSF were examined by a cell sorter. Granzyme B, interferon γ (IFNγ), interleukin 4 (IL-4), tumor necrosis factor α (TNFα), and IL-12 in CSF were quantitated by enzyme-linked immunosorbent assay (ELISA). Autoantibodies against GluR ε2 (NR2B) were examined by immunoblot.

Results: The data of the first CSF examination showed that IgG levels (Mann-Whitney U test, $p < 0.01$), $CD4^+$ T cells ($p = 0.02$), TNFα levels ($p < 0.01$), and Granzyme B levels ($p < 0.01$) were elevated compared with disease controls. White blood cell count, IFNγ level, IL-12 level, and Granzyme B level were elevated, especially in the early stage of disease. $CD4^+$ T cells, $CD8^+$ cells, $CD3^+$ T cells, IgG levels, and TNFα levels were elevated at all stages of disease evolution. Protein levels and albumin levels were elevated in the progressed stage. Autoantibodies against GluR ε2 (NR2B) (IgG) were found in 50% of patients in the early stage, and the positive rate was low at the progressed stage.

Discussion: The present findings suggest that complex pathophysiologic mechanisms involving $CD4^+$ T cells and $CD8^+$ T cells change evolutionally during the progression of RS. A crucial cytotoxic process occurs in the early stage, and declines in the progressed stage.

KEY WORDS: Rasmussen syndrome, Granzyme B, Interferon γ, Tumor necrosis factor α, GluR ε2 (NR2B).

Rasmussen syndrome (RS) is considered to be an autoimmune disease, and is usually diagnosed by comprehensive consideration of characteristic clinical symptoms, electroencephalography (EEG), magnetic resonance imaging (MRI), and histopathologic findings (Rasmussen et al., 1958, 1978; Anderman & Rasmussen, 1991; Oguni et al., 1991; Bien et al., 2005; Takahashi, 2006). Although a correct early diagnosis is essential to the achievement of a good outcome, patients with RS are usually diagnosed with localization-related epilepsy at about onset, because they show few characteristic features of RS (including epilepsia partialis continua, hemiparesis, and focal slowing of EEG) at the onset stage.

Lymphocytic infiltration containing predominantly T cells and sparse B cells is found in surgically resected tissues from patients with RS (Farrell et al., 1995), and local central nervous system (CNS) immune responses in RS include local clonal expansion of T cells responding to discrete antigen epitopes (Li et al., 1997). Apoptosis of astrocytes by Granzyme B produced by cytotoxic T cells (CTLs) has been demonstrated in resected tissues from patients (Bien et al., 2002; Bauer et al., 2007). Peripheral blood lymphocytes in patients are sensitized to glutamate receptor (GluR) ε2 = *N*-methyl-D-aspartate (NMDA) type GluR 2B (NR2B) (Takahashi et al., 2005). Heterogeneous

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Address correspondence to Dr. Y. Takahashi at National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan. E-mail: takahashi-ped@umin.ac.jp

autoantibodies against neuronal molecules (including GluR3, GluR ε2 (NR2B), neuronal acetylcholine receptor alpha7, and munc-18) and glial cells are detected in RS (Rogers et al., 1994; Yang et al., 2000; Takahashi et al., 2003, 2005; Watson et al., 2005; Roubertie et al., 2005). Autoantibodies against GluR ε2 (NR2B) have epitopes predominantly in intracellular domains, and show epitope spreading evolutionally (Takahashi et al., 2003). In animal models of RS, anti-GluR3B T cells are found (Levite & Hermelin, 1999). From these data, we suggested that cellular autoimmunity mediated by CTLs plays a primary role in the development of RS, and that subsequent humoral autoimmunity mediated by autoantibodies also contributes to the immunopathogenesis (Takahashi et al., 2003).

Autoantibodies against GluR3 were found at first in RS (Rogers et al., 1994). Recent studies have revealed non-RS epileptic patients with the autoantibodies and RS patients without the autoantibodies against GluR3 (Wiendl et al., 2001; Watson et al., 2004; Ganor et al., 2005b). Autoantibodies against GluR ε2 (NR2B) are also detected in patients with RS and other epilepsies, and are rarely negative in patients with RS (Takahashi et al., 2003, 2006). These data suggest that RS is heterogeneous not only in clinical characteristics, but also in immunopathogenesis. Autoantibodies against GluR3 have been shown to induce currents through GluR (Rogers et al., 1994; Twyman et al., 1995) and promote death of cortical neurons by complement-dependent (He et al., 1998) and complement-independent mechanisms (Levite & Hermelin, 1999; Ganor et al., 2004a) as well as neuronal excitotoxicity. Animals immunized with the self alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) GluR3B peptide, to obtain a possible animal model for RS developed excitotoxic anti-GluR3B antibodies, anti-GluR3B T-cells, and brain damage (Levite & Hermelin, 1999; Ganor et al., 2005). Production of autoantibodies against GluR3 may depend on cleavage of GluR3B-containing fragments from the T cell receptor (TCR)-activated T cells by Granzyme B (Gahring et al., 2001; Ganor et al., 2007).

Although reports about the pathogenic role of cytokines in RS are few (Tekgul et al., 2005; Ganor et al., 2004b), cytokines are highlighted as inflammatory mediators that alter neuronal excitability and affect cell survival (Vezzani et al., 2008). We studied surface markers of T cells, cytokines, and Granzyme B in CSF samples from 27 patients with RS, and evaluated their evolutional changes to reveal their roles in immunopathogenesis, and examine their possible contribution to the diagnosis.

Patients and Methods

Patients

We studied CSF samples from 27 patients (male, 12; female, 15), who were diagnosed with RS based on clinical characteristics at the National Epilepsy Center from 2002 to 2006. Clinical criteria include (1) intractable partial seizures, and (2) interictal symptoms and EEG suggesting progressive involvement of unilateral hemisphere at the early stage. In nine patients, histologic findings at surgical intervention also confirmed the diagnosis. Histologic criteria include microglia nodules and perivascular cuffing. In Japan, it is difficult to obtain a patient's consent to undergo brain biopsy but, for cultural and traditional reasons, it is easy to obtain consent for CSF examination. Of the 27 patients, 16 attended the National Epilepsy Center. They fulfilled the clinical criteria for RS and were included in this study after giving written informed consent (Table 1). The diagnosis was proven histologically in 5 of 16 patients. The remaining 11 patients attended other hospitals and were suspected of having RS. Their clinical data were sent to the National Epilepsy Center for evaluation, which confirmed a clinical diagnosis of RS. The diagnosis was proven by histologic findings in 4 of 11 patients. CSF samples were collected after obtaining written informed consent. CSF samples collected at other hospitals were sent to the National Epilepsy Center for analysis of autoantibodies against GluR ε2 (NR2B).

Clinical characteristics, treatment at sampling points, and surgical outcome are shown in Table 1. Sixteen patients had epilepsia partialis continua (EPC). The average onset age was 7.5 ± 5.6 years. The sampling time ranged from 1–288 months after onset. Epileptic patients without infectious etiology or progressive clinical course served as disease controls (n = 16). In disease controls, the average age at examination was 5.8 ± 4.9 years, and the average duration of epilepsy at examination was 4.5 ± 4.0 years. They had seizures at different frequencies ranging from daily to yearly.

Methods

All data analyzed except for those of patients 5, 14, and 25 were obtained before surgical interventions. Cell count, protein level, glucose level, albumin level, chloride level, and IgG in CSF were determined by conventional methods. Cell counts were examined in 29 samples from 18 patients, protein levels in 30 samples from 19 patients, glucose levels in 25 samples from 15 patients, chloride level in 22 samples from 12 patients, albumin level in 18 samples from 11 patients, and IgG levels in 20 samples from 13 patients. Surface markers of lymphocytes in CSF were examined in 17 samples from 10 patients using a cell sorter. Granzyme B (32 samples from 23 patients) were examined by Granzyme B enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. KT-078, KAMIYA BIOMEDICAL COMPANY, Seattle, WA, USA), using monoclonal antibody to human Granzyme B and Streptavidin-HRP. Interferon γ (IFNγ) (30 samples from 22 patients), interleukin 4 (IL-4) (29 samples from 22 patients), tumor necrosis factor α (TNFα) (28 samples

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Table 1. Clinical characteristics and treatment at sampling points and surgical outcome

Pt	Sex	Age at onset (year)	Age at diagnosis (year)	Sampling stage (months)	Seizure	Motor dysfunction	Mental retardation	Immunologic treatment	Age at surgical intervention and method/surgical outcome	Histologic findings	Elevated molecules in CSF	GluR ε2 (NR2B) in CSF (months)
1	F	0.2	0.9	31	D	l-HP	++	–	2Y10M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC	CD4, CD8, TNFα, GrB	8+, 17–, 31–
2	F	1.5	23.0	260	D	r-HP	–	–	–		CD4, IFN γ, TNFα, GrB	260–
3	F	2.4	2.5	288	Y	QP	+++	–	–			288–
4	M	3.6	3.9	4	D	l-HP	–	–	4.8Y: r-FH/ seizure free, MR+	MGN+VBS+EP +SD+PVC	GrB	4+
5	M	3.7	9.0	96	D	–	–	–	8Y: left temporal resection / daily EPC	FCD+MGN+VBS +EP+SD+PVC		96+
6	M	3.9	10.4	36	D	r-HP	++	–			IFNγ, TNF α, GrB	36–
				65	W	QP	+++	–			TNFα, GrB	65+
				68	D	QP	+++	–	–		CD4, CD8, TNFα, GrB	68–
7	M	4.0	5.3	18	D	–	–	–	5Y9M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC	TNFα, GrB	18+
8	M	4.1	10.0	172	D	ru-MP	+	–				172+
				184	D	ru-MP	+	Tacrolimus			CD4, CD8, TNFα,	
				186	D	ru-MP	+	Tacrolimus			CD4, CD8, TNFα,	186+
				201	D	ru-MP	+	Tacrolimus			CD4, CD8, IFNγ, TNF α, GrB	201–
				204	D	ru-MP	+	Tacrolimus	–		CD4, CD8, IFNγ, TNF α, GrB	204–
9	F	4.7	6.0	45	D	rl-MP	–	–			CD8, TNF α,	45–
				51	D	rl-MP	–	–	–		CD4, CD8,	
10	F	5.3	6.3	12	D	ll-MP	–	–			GrB	
				15	D	l-HP	–	Pulse	6Y10M: r-FH/ weekly SPS	MGN+PVC		15–
11	F	5.6	22.0	192	D	l-HP	+	–	–		CD4, CD8, TNFα, GrB	192–
12	F	5.8	18.0	31	D	r-HP	++	–			TNFα,	31+
13	F	5.9	6.0	2	D	r-HP	+	–	–		IFNγ, TNF α, GrB	2+
14	M	5.9	16.0	132	W	–	–, r-EFD	–	15Y:left frontal resection/daily SPS	FCD+PVC+gliosis, phagocyte infiltration	TNFα,	132–
15	F	6.0	10.1	36	D	QP	++	–	–		GrB	36+
16	M	6.1	7.1	12	D	–	–	–	15Y:right frontal disconnection/ seizure free	Not examined	IFNγ, TNF α, GrB	12+
17	F	6.5	12.7	168	D	r-HP	+	–	–			
18	F	6.6	6.9	4	D	l-HP	–, l-EFD	–	–		IFNγ, TNF α, GrB	4+, 5–
19	F	7.1	9.2	30	W	l-HP	+	Pulse	9Y8M: r-FH/ seizure free	MGN+VBS+EP +SD+PVC		31–

Continued

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Table 1. Continued.

Pt	Sex	Age at onset (year)	Age at diagnosis (year)	Sampling stage (months)	Seizure	Motor dysfunction	Mental retardation	Immunologic treatment	Age at surgical intervention and method/surgical outcome	Histologic findings	Elevated molecules in CSF	GluR ε2 (NR2B) in CSF (months)
20	M	7.5	7.8	4	D	r-HP	–	–			CD4, CD8, IFNγ, GrB	4+, 6+
				8	D	r-HP	–	IVIG			CD4, CD8, IFNγ, GrB	8+
				24	D	r-HP	–				IFNγ, TNF α, GrB	
				34	D	r-HP	–	Tacrolimus	–		IFNγ, TNF α, GrB	34–
21	M	7.8	10.0	50	W	l-HP	–	–	–		IFNγ, TNF α, GrB	
22	F	8.8	9.8	108	D	r-HP	++	–			TNFα,	108–
				132	D	r-HP	++	Tacrolimus	–		TNFα, GrB	132–
23	F	9.0	12.0	38	D	–	–, aphasia	–			TNFα, GrB	38+, 47+
				72	D	r-HP+ll-MP	++	Tacrolimus			CD4,	72-
				74	D	r-HP+ll-MP	++	Tacrolimus	–		CD8, GrB	74+
24	M	12.6	16.0	84	D	QP	+++	–	–		TNFα,	84–
25	M	15.0	15.4	180	W	r-HP	+, Psychosis	–	26Y: left frontal resection/weekly SPS	MGN+VBS+EP +SD+PVC	CD4,	180–
26	M	16.1	16.4	1	W	–	–	–			TNFα, GrB	1–, 2–
				3	D	lu-MP	–	Pulse	–		IFNγ, TNF α, GrB	3+
27	F	28.0	34.7	72	D	–	+	–	34Y: right frontal resection/daily CPS	MGN+VBS+EP +SD+PVC		

Sampling stage, period between onset of epilepsy and sampling point of cerebrospinal fluid (CSF); seizure, frequency of seizure; D, daily; W, weekly; Y, yearly; HP, hemiplegia; MP, monoplegia; QP, quadriplegia; l, left; r, right; ll, left lower; lu, left upper; ru, right upper; rl, right lower; mental retardation +, mild MR; ++, moderate MR; +++, severe MR; EFD, eye field defect; pulse, steroid pulse therapy; IVIG, intravenous infusion of globulin; FH, functional hemispherectomy; MGN, microglia nodule; VBS, vascular genesis on brain surface; EP, endothelial proliferation; SD, spongy degeneration; PVC, perivascular cuffing; FCD, focal cortical dysplasia. Elevated molecules in CSF show molecules beyond mean + SD of disease controls in CD4, CD8, IFNγ, TNFα, and Granzyme B (GrB); GluR ε2 (NR2B) in CSF (months), presence or absence of autoantibodies against GluR ε2 (NR2B) in CSF at each sampling stage (month), 96+ shows presence of the autoantibodies against GluR ε2 (IgG or IgM) at 96 months after the onset.

from 21 patients), and IL-12 levels in CSF (29 samples from 22 patients) were measured by ELISA (Human IFN γ ELISA kit; Endogen, Rockford, IL, U.S.A.; Human IL-4 ELISA kit; Endogen; Human IL-12 (p70) ELISA kit; Endogen; Human TNF α ELISA kit; Endogen) (Ichiyama et al., 2008). Autoantibodies against GluR ε2 (NR2B) were examined qualitatively in 46 samples from 25 patients by immunoblot (Takahashi et al., 2003, 2005). The mean number of samples per patient was 1.8 ± 1.2 (range 1–5). Some CSF data were not available from some of the 27 patients, because CSF was collected only for the examination of autoantibodies in other hospitals, and some measurements were available only recently. Data are expressed in mean ± SD.

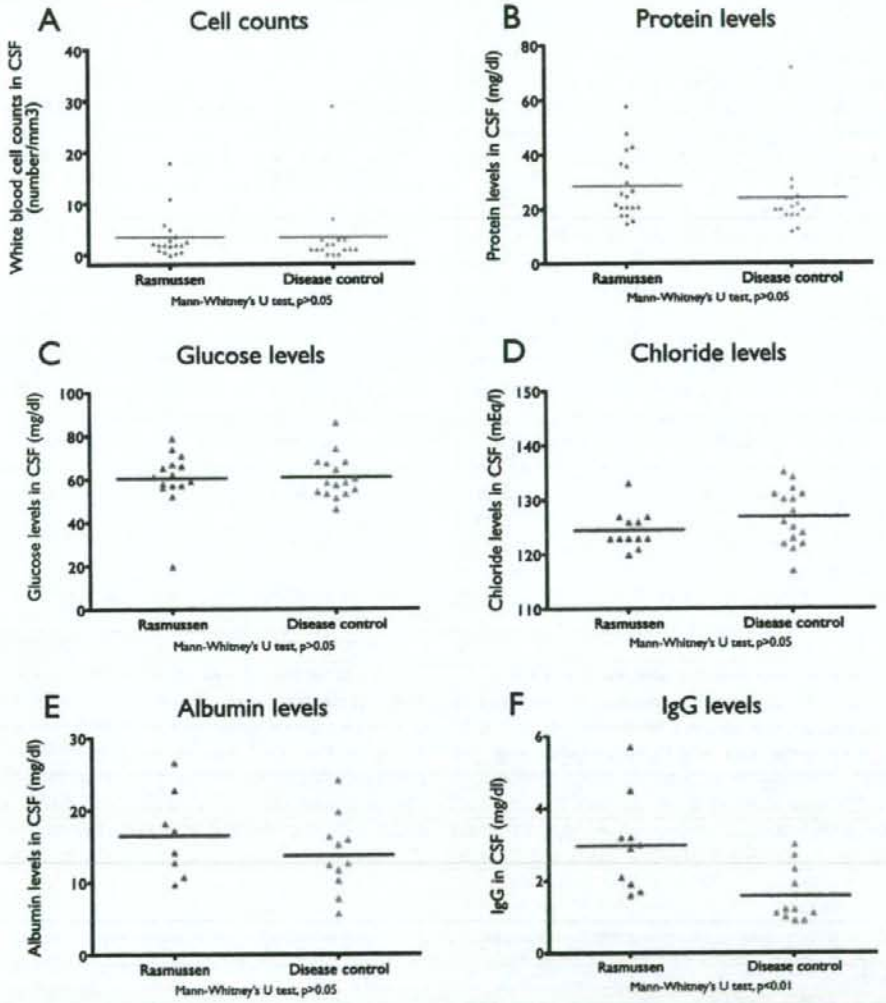

Figure 1.

Data of conventional and immunologic molecules at the first cerebrospinal fluid (CSF) examination. (**A**) White blood cell count in CSF; (**B**) protein level; (**C**) glucose level; (**D**) chloride level; (**E**) albumin level; (**F**) immunoglobulin G (IgG) level. Rasmussen: data from RS patients. Horizontal bars show mean levels. Mean interval between disease onset and first examination of white blood cell counts was 48.8 ± 46.4 months (mean ± SD).

Results

Conventional and immunologic data of the first CSF examination

Conventional and immunologic data of the first CSF examination of 19 and 24 patients before initiation of immunologic treatment were compared with those of disease controls to evaluate their roles in RS. At the first CSF examination, cell counts (3.6 ± 4.4, n = 18), protein levels (28.7 ± 12.1 mg/dl, n = 19), glucose levels (60.3 ± 13.3 mg/dl, n = 15), albumin levels (16.5 ± 5.9 mg/dl, n = 8), and chloride levels (124.6 ± 3.5 mEq/l, n = 12) in RS patients were similar to the levels in disease controls (Fig. 1). On the other hand, IgG levels were higher in RS patients (3.0 ± 1.3 mg/dl, n = 10) than in disease controls (1.6 ± 0.8 mg/dl, n = 11) (Mann-Whitney *U* test, $p < 0.01$) (Fig. 1F).

$CD4^+$ T cells were higher in RS patients (59.3 ± 21.4%, n = 7) than in disease controls (34.7 ± 15.0%, n = 12) (Mann-Whitney *U* test, $p = 0.02$) (Fig. 2). $CD8^+$ T cells were not significantly different between RS patients (34.8 ± 18.3%, n = 7) and disease controls (23.4 ± 7.0%, n = 11), whereas $CD3^+$ T cells were higher in RS patients (74.8 ± 15.5%, n = 7) than in disease controls (52.5 ± 18.4%, n = 12) ($p = 0.01$). IFNγ levels were not significantly different between RS patients (11.3 ± 6.0 pg/ml, n = 22) and disease controls (9.6 ± 9.1 pg/ml, n = 26) (Mann-Whitney *U* test, $p > 0.05$). TNFα levels were higher in RS patients (23.7 ± 34.8 pg/ml, n = 17) than in controls (4.0 ± 2.4 pg/ml, n = 13) ($p < 0.01$). Granzyme B levels were higher in RS patients (10.8 ± 15.5 pg/ml, n = 18) than in disease controls (1.2 ± 1.2 pg/ml, n = 13) (Mann-Whitney *U* test, $p < 0.01$). IL-4 and IL-12 levels were similar in RS patients and disease controls.

Clinical evolution and immune molecules in CSF

Evolutional changes of conventional laboratory data and immunologic molecules in CSF were evaluated in 27 RS patients using the data of the initial examination and subsequent follow-up examinations. White blood cell counts in CSF were elevated in two patients around onset, and were within normal limits in samples collected 5 months after onset (Fig. 3). Protein levels and albumin levels were higher in samples collected at the progressed stage, compared with those in the early stage. Half of the patients showed protein levels greater than 40 mg/dl 12.5 years after onset. Albumin levels were higher (>20 mg/dl) in half of the patients 5 years after onset. IgG levels were slightly elevated (>2.5 mg/dl) in two-thirds of patients at all stages of disease evolution.

$CD4^+$ T cell counts were elevated (>50%) in the majority of patients at all stages of disease evolution (Fig. 4). $CD8^+$ T cells were also elevated (>30%) in many patients at all stages of disease evolution, and the elevated levels declined evolutionally. $CD3^+$ T cells were elevated (~70%) in almost all patients at all stages of disease evolution. $CD4^+$ T cells and $CD3^+$ T cells were higher in samples collected at the progressed stage compared with the early stage.

IFNγ levels were elevated (>15.0 pg/ml) in many patients during the early stage, but the majority of patients had the same level as disease controls (~10.0 pg/ml) 5 years after onset (Fig. 5). IL-4 levels were higher in samples from the progressed stage compared with the early stage. IL-12 levels were elevated in several samples during the early stage. TNFα levels were elevated (>7.0 pg/ml) in many patients at all stages of disease evolution (Figure 6). Granzyme B levels were markedly elevated, especially in the early stages, and remained slightly elevated even in the progressed stage.

When patients without immunologic treatment were compared with patients after introduction of immunologic treatment, CD4, CD8, CD3, Granzyme B, IL-4, IL-12, TNFα, and IFNγ showed no statistically significant difference (data not shown, Mann-Whitney *U* test).

Clinical evolution and autoantibodies against GluR ε2 (NR2B) in CSF

Anti-GluR ε2 (NR2B) autoantibodies in the CSF samples collected from a total of 25 RS patients were examined qualitatively by immunoblot, and evolutional changes were evaluated using the positive rates at various intervals after onset. In eight samples from five patients collected within 6 months of epilepsy onset, IgG autoantibodies against GluR ε2 (NR2B) were found in half of patients (50%), and IgM autoantibodies against GluR ε2 (NR2B) were found in one of eight patients (12.5%)

Figure 2.
Cytokines and Granzyme B at the first cerebrospinal fluid (CSF) examination. **(A)** Percentage of $CD4^+$ T cells in CSF; **(B)** percentage of $CD8^+$ T cells; **(C)** percentage of $CD3^+$ T cells; **(D)** interferon γ (IFNγ) level; **(E)** tumor necrosis factor α (TNFα) level; **(F)** interleukin 4 (IL-4) level; **(G)** Granzyme B level; **(H)** IL-12 level. Rasmussen: data from RS patients. Horizontal bars show mean levels. Mean interval between disease onset and first examination of Granzyme B was 37.8 ± 42.0 months (mean ± SD).

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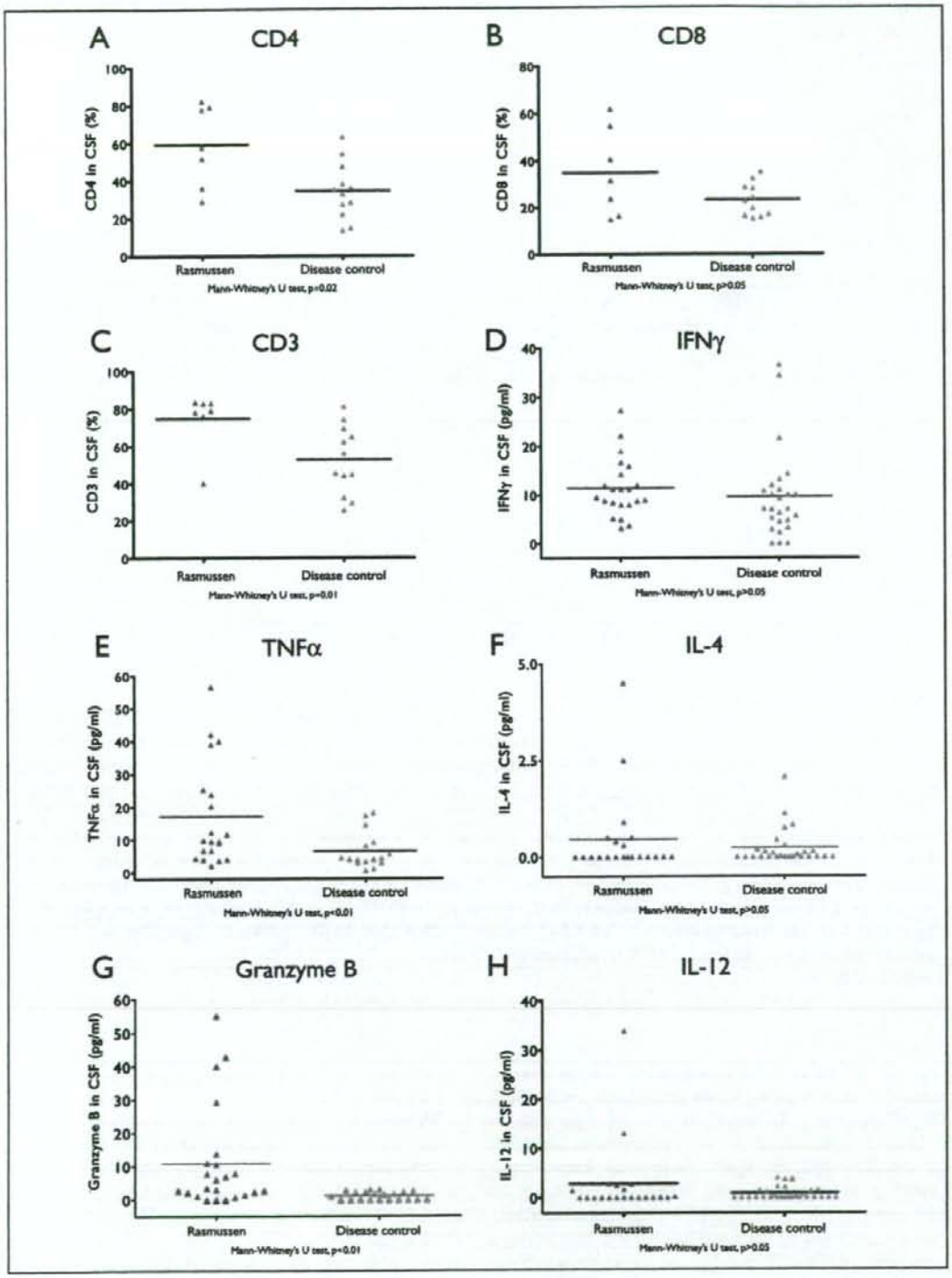
A
CD4
CD4 in CSF (%)
Rasmussen
Disease control
Mann-Whitney's U test, p=0.02
B
CD8
CD8 in CSF (%)
Rasmussen
Disease control
Mann-Whitney's U test, p>0.05
C
CD3
CD3 in CSF (%)
Rasmussen
Disease control
Mann-Whitney's U test, p=0.01
D
IFNγ
IFNγ in CSF (pg/ml)
Rasmussen
Disease control
Mann-Whitney's U test, p>0.05
E
TNFα
TNFα in CSF (pg/ml)
Rasmussen
Disease control
Mann-Whitney's U test, p<0.01
F
IL-4
IL-4 in CSF (pg/ml)
Rasmussen
Disease control
Mann-Whitney's U test, p>0.05
G
Granzyme B
Granzyme B in CSF (pg/ml)
Rasmussen
Disease control
Mann-Whitney's U test, p<0.01
H
IL-12
IL-12 in CSF (pg/ml)
Rasmussen
Disease control
Mann-Whitney's U test, p>0.05

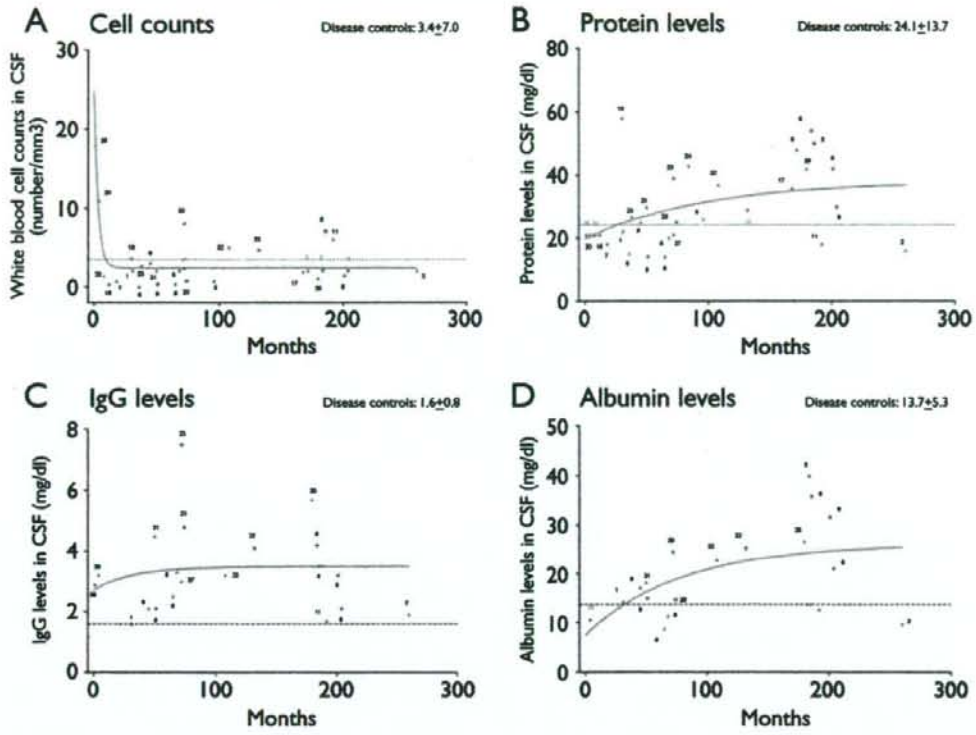

Figure 3.

Conventional and immunologic molecules in cerebrospinal fluid (CSF). (**A**) White blood cell count in CSF; (**B**) protein level; (**C**) immunoglobulin G (IgG) level; (**D**) albumin level. Horizontal axis in each panel shows time (months) from onset of epilepsy. Value (mean ± SD) for disease controls is shown above each panel. Numbers indicate patients' numbers in Table 1. Blue triangle symbols show data of patients without immunologic treatment; orange circle symbols show data of patients after introduction of immunologic treatment. Dotted lines show mean levels of disease controls. Green solid lines show nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, U.S.A.). In (**A**), nonlinear regression was done by equation of one phase exponential decay [95% confidence interval (CI) of plateau, 1.472–3.278]. In (**B**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 16.49–59.78). In (**C**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 2.328–4.659). In (**D**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 12.33–39.91).

(Fig. 6C, D). As the clinical course evolved, the proportions of patients positive for autoantibodies against GluR ε2 (NR2B) (IgG) decreased. In five of eight patients (patients 1, 6, 8, 18, and 20), autoantibodies against GluR ε2 (NR2B) (IgG or IgM) disappeared evolutionally (Table 1). In two of five patients (patients 8 and 20), the autoantibodies disappeared after the initiation of tacrolimus treatment. In all three patients without immunologic treatment (patients 1, 6, and 18), the autoantibodies disappeared in ordinary epilepsy treatment.

Discussion

We studied 27 patients who were diagnosed with RS based on clinical criteria including (1) intractable partial seizures and (2) interictal symptoms and EEG, suggesting progressive involvement of unilateral hemisphere, independent of histologic characteristics. These criteria may exclude patients with RS at an early stage before deterioration, who may be included by European consensus criteria (Bien et al., 2005).

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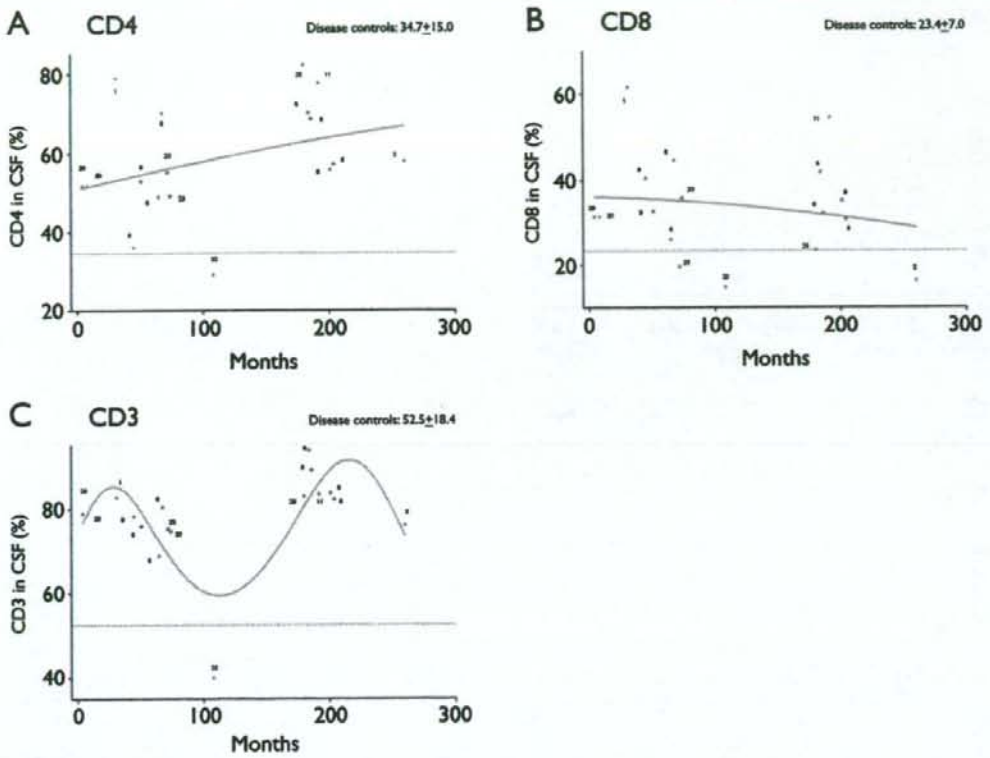

Figure 4.

Cell surface markers of lymphocytes in CSF. (**A**) Percentage of $CD4^+$ T cells; (**B**) percentage of $CD8^+$ T cells; (**C**) percentage of $CD3^+$ T cells. Horizontal axis in each panel shows time (months) from onset of epilepsy. Value (mean ± SD) for disease controls is shown above each panel. Numbers indicate patients' numbers in Table 1. Blue triangle symbols show data of patients without immunologic treatment; orange circle symbols show data of patients after introduction of immunological treatment. Dotted lines show mean levels of disease controls. Green solid lines show nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc.). In (**A**), nonlinear regression was done by equation of Gaussian distribution (95% CI of amplitude, −124.8 to 266.5). In (**B**), nonlinear regression was done by equation of Gaussian distribution (95% CI of amplitude, 8.908 to 63.63). In (**C**), nonlinear regression was done by equation of sum of two Lorentzian (95% CI of amplitude 1, 52.04–90.62).

Although an early diagnosis of RS is important to improve outcome, patients with RS are usually diagnosed with partial epilepsy at the onset of epilepsy. The possibility of RS is suspected only after epileptic seizures aggravate and brain function is impaired. We analyzed the conventional and immunologic test data of the first CSF examination, and found that IgG level, $CD4^+$ T cells, TNFα level, and Granzyme B level may contribute to a diagnosis of immune-mediated epilepsy including RS (Figs. 1 and 2). In patients with frequent partial seizures, these immune molecules should be measured for an early diagnosis of RS and the evolutional changes of these molecules should be followed to confirm the diagnosis of RS (Figs. 3–6). Clinical symptoms, MRI findings, and EEG findings are also essential for the diagnosis (Oguni et al., 1991; Bien et al., 2005).

Our cross-sectional data and nonlinear regression curves of immunologic molecules in CSF from early to progressed stages imply that white cell count and Granzyme B level are elevated in the early stage of RS around the onset of epilepsy, and decline within a few months to more or less constant levels (Figs. 3–6). $CD8^+$ T cells are

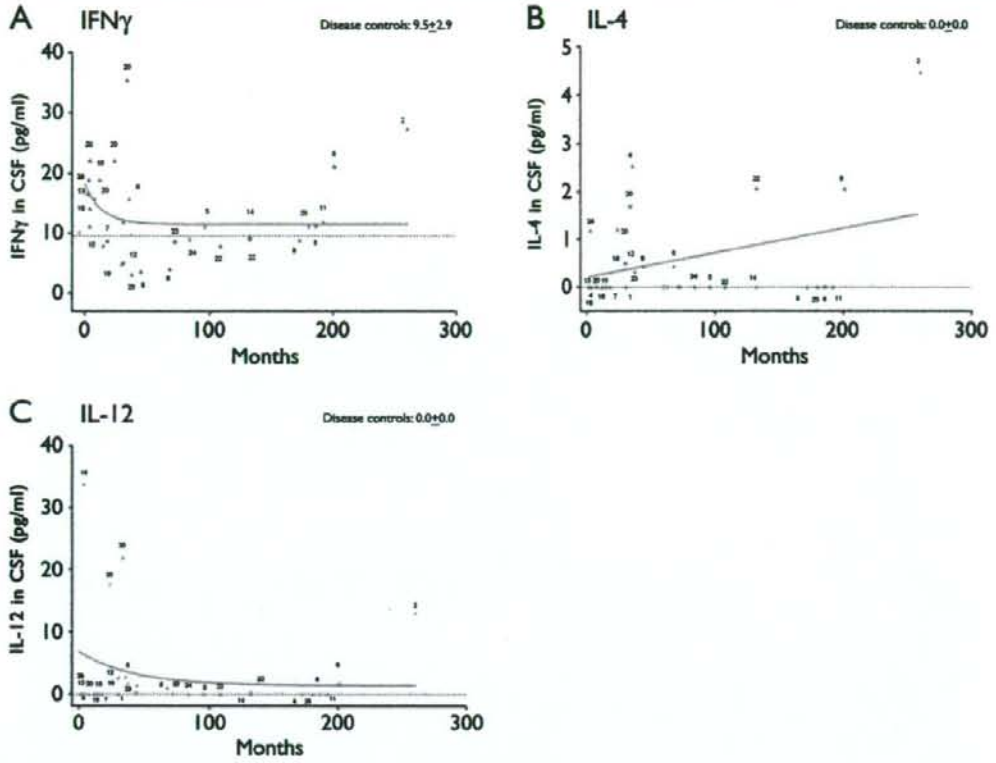

Figure 5.

Interferon γ 1 (IFNγ I), interleukin 4 (IL-4), and IL-12 in cerebrospinal fluid (CSF). (**A**) IFNγ level in CSF; (**B**) IL-4 level; (**C**) IL-12 level. Horizontal axis in each panel (**A–C**) shows time (months) from onset of epilepsy. Value (mean ± SD) for disease control is shown above each panel. Numbers indicate patients' numbers in Table 1. Blue triangle symbols show data of patients without immunologic treatment; orange circle symbols show data of patients after introduction of immunologic treatment. Dotted lines show mean levels of disease controls. Green solid lines show nonlinear or linear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc.). In (**A**), nonlinear regression was done by equation of one phase association (95% CI of plateau, 7.382–15.60). In (**B**), linear regression was done (95% CI of slope, −0.0001157 to 0.01034). In (**C**), nonlinear regression was done by equation of one phase association (95% CI of plateau, −5.106 to 7.900).

also elevated in the early stage and decline gradually toward the progressed stage. Granzyme B is usually secreted from CTLs, and sometimes from NK cells. Because the previous study of resected brain tissues has demonstrated that Granzyme B–secreting cells are not NK cells, but $CD8^+$ T cells (CTLs) in RS (Bien et al., 2002), we estimate that production of Granzyme B from $CD8^+$ T cells is activated more strongly in the early stage around onset, and continues even in the progressed stages (Figs. 4B and 6B). The cytotoxic mechanisms by Granzyme B may be very important, especially in the early stage around onset. Our data suggest that a crucial cytotoxic process contributes to the pathophysiologic mechanisms during the first few months of the disease, and declines in the progressed stage.

Our cross-sectional data and their nonlinear regression curves imply that IFNγ and IL-12 are produced especially in the early stage around onset, and $CD4^+$ T cells and TNFα level are elevated from the early stage to the progressed stage. IFNγ activates macrophage to secrete IL-12 and TNFα, and IL-12 facilitates the proliferation of Th1 cells in $CD4^+$ T cells. Therefore, differentiation and proliferation of autoreactive Th1 cells induced by cytokines in the early stage increase the ratio of $CD4^+$ T cells in CSF,

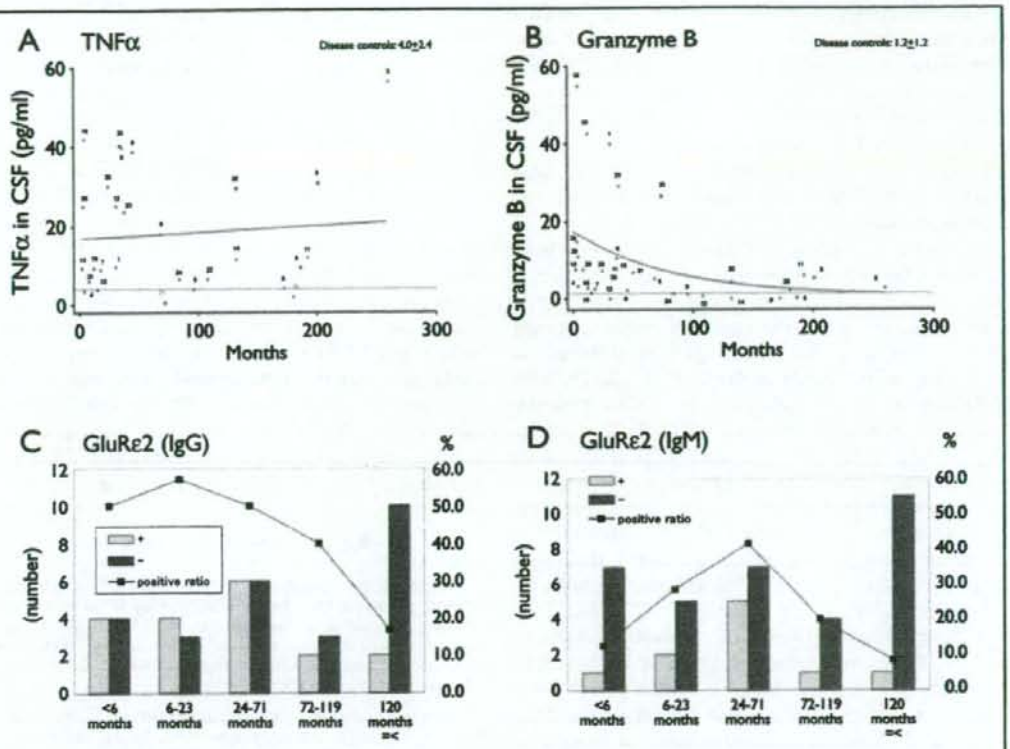

Figure 6.

Tumor necrosis factor α (TNFα), Granzyme B, and autoantibodies against GluR ε2 (NR2B) in cerebrospinal fluid (CSF). (**A**) TNFα level in CSF; (**B**) Granzyme B level; (**C**) immunoglobulin G (IgG) autoantibodies against GluR ε2 (NR2B); (**D**) IgM autoantibodies against GluR ε2 (NR2B). In panels **C** and **D**, yellow columns show the number of samples with antibodies against GluR ε2 (NR2B), and blue-green columns show the number of samples without autoantibodies against GluR ε2 (NR2B). Horizontal axis in each panel (**A–B**) shows time (months) from onset of epilepsy. Value (mean ± SD) for disease control is shown above each panel. Numbers indicate patients' numbers in Table 1. Blue triangle symbols show data of patients without immunological treatment; orange circle symbols show data of patients after introduction of immunological treatment. Dotted lines show mean levels of disease controls. Green solid lines show linear or nonlinear regression curves produced by computer software, GraphPad Prism 5 (GraphPad Software Inc.). In (**A**), linear regression was done (95% CI of slope, −0.06487 to 0.09824). In (**B**), nonlinear regression was done by equation of one phase association (95% CI of plateau, −20.17 to 21.29).

and initiate the subsequent autoimmune mechanisms. Prolonged production of TNFα from the early stage to the progressed stage leads to elevation of IL-6 in CNS. IL-6 may contribute to the inflammatory process and inhibition of regulatory T cells, resulting in augmentation of the autoimmune process. TNFα is reported to modulate AMPA-induced excitotoxicity (Bernardino et al., 2005), and to reduce GABA receptor (Stellwagen et al., 2005). Furthermore, transgenic mice of TNFα came to show epileptic seizures (Probert et al., 1995). These findings suggest that TNFα may contribute directly to epileptogenesis. Adding to the cytotoxic T-cell–mediated immune mechanisms, the autoimmune process mediated by Th1 cells and the epileptogenic effect of TNFα seem to be important also in RS. On the other hand, IFNγ induces the expression of major histocompatibility complex (MHC) (class I+ II) and Inter-Cellular Adhesion Molecule 1 (ICAM-1), and production of TNFα in microglia. These effects of IFNγ lead to the enhancement of the autoimmune cytotoxic process in CNS by $CD8^+$ T cells. TNFα induces the expression of MHC class I on astrocytes, and loosens capillary endothelial junctions. These effects of TNFα may also lead to

apoptosis of astrocytes by $CD8^+$ T cell and inflammation. Brain tissues of patients with RS are reported to show characteristic astrocytic apoptosis (Bauer et al., 2007).

Tacrolimus, an inhibitor of cytokine production, is effective in preserving neurologic function and delaying the progression of cerebral hemiatrophy in RS (Bien et al., 2004). Because IFNγ and Granzyme B are elevated, especially in the early stage, early initiation of tacrolimus may improve the outcome of RS. In patients 8, 20, and 22, development or progression of mental retardation was not observed after introduction of tacrolimus. However, in patient 23, progression of mental retardation was observed and higher levels of Granzyme B were sustained. Although our study showed no significant difference of CD4, CD8, CD3, Granzyme B, IL-4, IL-12, TNFα, or IFNγ between patients without immunologic treatment and patients after introduction of immunologic treatment, this may be attributed to the variety of clinical stage at the introduction of immunologic treatment and the variety of immunologic treatment. Further investigation is required to examine the effects of early initiation of tacrolimus in RS patients, which will depend on a correct early diagnosis using immunologic data in CSF and other methods.

Detrimental effects of autoantibodies against GluR mimicking excess of glutamate are reviewed in epilepsy, encephalitis, and other diseases (Levite & Ganor, 2008). Autoantibodies against GluR ε2 (NR2B) (IgG) were detected in half of patients within 6 months of epilepsy onset, and the autoantibody positive rate was lower in the progressed stage (Figure 6), and in all three patients without immunologic treatment, the autoantibodies disappeared evolutionally (Table 1). These data suggest that autoantibodies against GluR ε2 (NR2B) may be involved in the pathologic mechanisms from the early stage up to several years after onset of epilepsy. In a previous study in which we examined the effect of IgG antibodies against GluR ε2 (NR2B) in CSF from RS patients on excitatory postsynaptic current (EPSC) using patch clamp methods, both IgG in CSF from RS patients and rabbit IgG antibodies against mouse GluR ε2 (NR2B) had no effect on EPSC (Takahashi et al., 2006). However, anti-dsDNA antibodies in CSF from patients with systemic lupus erythematosus have been shown to cross-react with the N terminus of GluR ε2 (NR2B) and cause neuronal apoptosis in the rat hippocampus (DeGiorgio et al., 2001). It is possible that autoantibodies against GluR ε2 (NR2B) may also cause apoptosis in the rat hippocampus. Furthermore, autoantibodies against the N terminus of GluR ε2 (NR2B) have been reported to cause hippocampal neuron damage with ensuing memory impairment (Kowal et al., 2006), and amygdala neuron damage with emotional behavior impairment (Huerta et al., 2006). Therefore, autoantibodies against GluR ε2 (NR2B) may contribute to the cognitive and behavioral changes in RS through inducing apoptosis. A recent study has reported a causal relationship between autoantibodies against the N terminus of NMDA-type GluR hetero complexes and paraneoplastic encephalitis in patients with ovarian teratoma (Dalmau et al., 2007; Takahashi, 2008). Further investigations and a more suitable assay for the quantitative measurement of antibodies to NMDA receptors are required to elucidate the involvement of autoantibodies against GluR ε2 (NR2B) in the pathophysiologic mechanisms of RS.

In the later stages of RS, nonlinear regression curves of albumin levels in CSF imply that the CSF albumin level (protein level) increases evolutionally (Fig. 3). Direct brain exposure to serum albumin results in albumin uptake into astrocytes through transforming growth factor-beta receptors (TGF-β R), and induces NMDA-receptor–mediated neuronal hyperexcitability and subsequently epileptiform activity (Ivens et al., 2007). Therefore, elevated albumin levels in CNS may contribute to the pathologic mechanisms of intractable epilepsy, and TGF-β R may be a candidate therapeutic target for epilepsy in RS also.

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We have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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