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Autoantibodies and Cell-mediated Autoimmunity to NMDA-type GluRε2 in Patients with Rasmussen's Encephalitis and Chronic Progressive Epilepsia Partialis Continua

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Summary: *Purpose:* To evaluate antibody-mediated and cytotoxic T cell–mediated pathogenicity that has been implicated as the autoimmune pathophysiological mechanism in Rasmussen's encephalitis.

Methods: We examined autoantibodies against the *N*-methyl-D-aspartate glutamate receptor (NMDA-type GluR) ε2 subunit and its epitopes in serum and CSF samples from 20 patients [five histologically proven (definitive) Rasmussen's encephalitis with epilepsia partialis continua (EPC), four definitive Rasmussen's encephalitis without EPC, and 11 clinical Rasmussen's encephalitis with EPC]. We examined ^{3}H-thymidine uptake into lymphocytes after stimulation by GluRs.

Results: All nine definitive patients (five patients with EPC and four without EPC), and 10 of 11 clinical Rasmussen's encephalitis patients had the autoantibodies. In four patients, the autoantibodies were absent in early stage when epileptic seizures had already become frequent, and appeared subsequently. In two patients, the autoantibodies persisted in the serum after frontal lobe resection or functional hemispherectomy, although epileptic seizures were completely controlled. Autoantibodies to the C2 epitope predominated, while autoantibodies to the extracellular N epitope were rare. The mean ^{3}H-thymidine uptake ratios (stimulation by GluRε2-containing homogenates/stimulation by PHA) were significantly higher in definitive and clinical Rasmussen encephalitis patients than in controls. The mean ^{3}H-thymidine uptake ratios (relative to PHA) were significantly higher for GluRε2-containing homogenate than for control homogenate or GluRδ2-containing homogenate.

Conclusions: Autoantibodies against GluRε2 may be one of the diagnostic markers for Rasmussen's encephalitis with and without EPC. Patients have activated T cells stimulated by GluRε2 in peripheral blood circulation. We speculate that cellular autoimmunity and the subsequent humoral autoimmunity against GluRε2 may contribute to the pathophysiological processes in Rasmussen's encephalitis. **Key Words:** Rasmussen syndrome—EPC—GluRε2—autoantibodies—Cell-mediated autoimmunity.

Epilepsia partialis continua (EPC) is characterized by continuous myoclonic jerks of the extremities and/or the face, usually without impairment of consciousness. In patients with chronic EPC, myoclonic jerks continue for periods of days, weeks, or months, and are usually localized in one part of the body, except in serious cases (1). The clinical evolution of EPC is variable, depending on the patient's age and underlying brain diseases (1,2). Bancaud (3) classified EPC into two groups: a group with stable neurological deficit, and another group with slowly progressive neurological deficit. The latter cases are classified as chronic progressive EPC of childhood in the revised classification of epileptic syndromes (4), which may be diagnosed as Rasmussen's syndrome (5) after histological confirmation. However, biopsy of brain lesion is not generally accepted in Japan, and surgery for patients with a tentative diagnosis of Rasmussen's encephalitis is rare. Therefore, Japanese patients are generally diagnosed as

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chronic progressive EPC, even though they may have the pathology of Rasmussen's encephalitis.

Rogers et al. (6) reported glutamate receptor 3 (GluR3) as an autoantigen in Rasmussen's encephalitis, speculated to be one cause of Rasmussen's encephalitis. After their report, epilepsies causally related to autoantibodies against GluRs were highlighted. GluRs in the central nervous system (CNS) play important roles in brain function, and they are divided into ionotropic and metabotropic receptors. The ionotropic GluRs are subdivided into three major subtypes: the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate receptors. The NMDA-GluRs are heterodimers composed of GluRε (ε1-ε4) (NMDAR2A-2D), GluRζ (NMDAR1) subunits, and NR3 subunits (7,8). The molecular diversity of NMDA-GluRs contributes not only to their important physiological roles in development, synaptic plasticity, learning, and memory, but also to the pathological processes in ischemic brain injury, neurodegenerative diseases, and epilepsy (7–11).

GluRε2 is the essential molecule for synaptic plasticity and development (12). In Rasmussen's encephalitis, not only epileptic seizures, but also impairment of higher brain function, such as mental retardation and visual disturbance, occur frequently. Therefore, we examined autoantibodies against NMDA-GluRε2 and their epitopes in the serum and cerebrospinal fluid (CSF) in patients with chronic EPC (13). We detected NMDA-type GluRε2 autoantibodies in histologically proven Rasmussen's encephalitis (3/3 patients), clinical Rasmussen's encephalitis (6/7 patients), acute encephalitis/encephalopathy (2/3 patients), and nonprogressive EPC (2/2 patients). The presence of autoantibodies against NMDA-GluRε2 might suggest autoimmune pathological mechanisms, but is not a hallmark of Rasmussen's encephalitis. Epitope analyses showed that the autoantibodies were predominantly against C-terminal epitopes and rarely against N-terminal epitope, with inconsistency in the profile during the course of the disease. The epitope recognition spectrum of autoantibodies was broader in CSF than in serum, and the serum or CSF profile showed an increase in number of epitopes as the disease progressed in some patients. Therefore, we speculated that the autoantibodies are produced in the CNS after cytotoxic T cell–mediated neuronal damages (13).

In this study, as an extension to our previous study in Rasmussen's encephalitis patients with EPC (13), we examined autoantibodies against NMDA-GluRε2 in 10 new Rasmussen's encephalitis patients. These new patients included four histologically proven Rasmussen's encephalitis patients without EPC (this category of Rasmussen's encephalitis was not reported in our previous paper), two histologically proven Rasmussen's encephalitis patients with EPC, and four clinical Rasmussen's encephalitis patients with EPC. Consequently, we could evaluate the autoantibodies in 20 patients with Rasmussen's encephalitis. Furthermore, as an original study, we examined lymphocyte stimulation by GluRs to estimate the cellular autoimmune pathophysiology of Rasmussen's encephalitis.

PATIENTS AND METHODS

Patients

We examined the samples from 11 clinical Rasmussen's encephalitis patients with chronic progressive EPC (Patients 1–4, 11–16, and 18), and nine histologically proven Rasmussen's encephalitis patients with EPC (Patients 5, and 7–10) or without EPC (6,17,19, and 20) (Table 1). Eleven clinical patients with chronic progressive EPC manifested intractable EPC for >1 month, and all showed neuropsychological impairment. They had progressive EPC, neuroimage findings characteristic of Rasmussen's encephalitis (14), and neuropsychological impairment (clinical Rasmussen type). Detailed clinical manifestations of Patients 1–3, 13, 14, 16, and 18 were reported in our previous article (13), and those of Patient 10 were also reported (15). Patient 14 had negative tests for anti-GluR3 autoantibodies in another hospital. Nine patients were histologically diagnosed as Rasmussen's encephalitis (definitive Rasmussen type) after functional hemispherectomy (Patient 7–10) and focal resection surgeries (Patients 5, 6, 17, 19, and 20).

Informed consent was obtained from each patient or guardian after oral or written explanation, according to the ethical principle of Declaration of Helsinki.

Establishment of stable transformant cells expressing GluRε2

Using the tetracycline-induction system (16), cDNA of GluRε2 (17), pSTneoB (18), and G418 selection (19), we established stable NIH3T3 transformant cell lines expressing full-length GluRε2 (B18) and GluRδ2 subunits (D33) (13). A1 is a control cell line without GluR expression, B18 is a cell line expressing recombinant GluRε2, and D33 is a cell line expressing recombinant GluRδ2.

Detection of autoantibodies against GluRε2 in sera and CSF

Supernatants of cell extracts prepared from B18 and A1 cultured for 48 h with doxycycline (1 μg/ml) were subjected to SDS-PAGE, and the gels were transferred to nitrocellulose membranes. Each membrane was cut into 20 strips after overnight blocking with the blocking buffer (0.02 *M* Tris HCl, 0.16 *M* NaCl, 0.05% bovine serum albumin). The strips of B18 and A1 were reacted with patients' sera (diluted 20-fold with blocking buffer) or CSF (diluted 15-fold with blocking buffer) for 48 h at 4 °C, and were stained by alkaline phosphatase-labeled second antibodies (IgG, IgA, or IgM) (Jackson ImmunoResearch, West Grove, Philadelphia, PA, U.S.A.). The presence of autoantibodies against GluRε2 was judged by a positively

TABLE 1. *Clinical characteristics of patients*

Patient	Onset age		Sex	Causative or underlying factors	Seizure type	Ope	Outcome			
	Epilepsy	EPC				Age	Neurological	Mental	Seizure	At age
1	2 days	1.1 yr	M	DPT vaccination, status	CPS, EPC		QP	MR+++	Daily	4.5 yr
2	2 mo	7 mo	M	Viral meningitis	CPS, EPC		QP	MR+++	Daily	4 yr
3	2 mo	4 mo	M	Polio vaccination, status	CPS, EPC		QP	MR+++	Disappeared	8 yr
4	8 mo	8 mo	M	Respiratory infection	CPS, EPC		HP	MR+++	Daily	4 yr
5	2 yr	9 yr	M		CPS, EPC	8 yr	-	MR++	Daily	9 yr
6	2.6 yr	-	M		CPS	14 yr	-	Normal	Disappeared	15 yr
7	3.6 yr	3.7 yr	M	Infection	CPS, EPC	4 yr	HP, HA	Normal	Disappeared	5yr
8	3.8 yr	3.8 yr	M	Head trauma	EPC, CPS	9 yr	HP, HA	MR+	Disappeared	11 yr
9	3.9 yr	5.3 yr	M	Influenza	CPS, EPC	5.8 yr	HP	Normal	Disappeared	5.9 yr
10	5.2 yr	6.1 yr	F	Status	CPS, EPC	6 yr	HP, HA	Normal	Daily	6 yr
11	5.8 yr	6 yr	F		CPS, EPC		MP	Normal	Daily	20 yr
12	5.9 yr	6 yr	F		CPS, EPC		HP	MR++	Daily	6.2 yr
13	6.1 yr	6.9 yr	M		EPC, CPS		HP	MR+	Daily	10 yr
14	8.9 yr	9.5 yr	F	Infection, status	CPS, EPC		HP	MR++	Daily	15 yr
15	12 yr	15 yr	M		CPS, EPC		QP	MR+++	Daily	16 yr
16	12.5 yr	12.9 yr	M		CPS, EPC		QP	MR++	Weekly	20 yr
17	16 yr	–	M	Head trauma	CPS	34 yr	MP	MR++	Weekly	34 yr
18	23 yr	31yr	F		EPC, CPS		Normal	MR+	Daily	38 yr
19	25 yr	–	F	–	CPS	29 yr	Normal	Normal	Weekly	31 yr
20	28 yr	–	F	–	CPS	34 yr	HP	MR++	Weekly	38 yr

EPC, epilepsia partialis continua; M, male; F, female; NMD, neuronal migration disorder; DPT, diphtheria-pertussis-tetanus; Status, status epilepticus; SPS, simple partial seizure; CPS, complex partial seizure; QP, quadriplegia; HP, hemiplegia; MP, monoplegia; HA, hemianopsia; MR, mental retardation.

stained band with molecular size ~180 kDa, which was found only on the B18 strip and not on the A1 strip (13).

Preparation of bacterial fusion proteins containing peptides from the GluRε2 subunit

DNA fragments encoding amino acid residues 1–48 (an amino-terminal peptide; N), 998–1074 (a carboxyl-terminal peptide; C1), 1053–1153 (a carboxyl-terminal peptide; C2), and 1353–1432 (a carboxyl-terminal peptide; C3) (17) were amplified by PCR using appropriate synthetic oligonucleotides. The positions of candidate epitopes were determined considering molecular interactions. The fragments were cloned to the bacterial fusion protein expression plasmid vector pGEX-4T-2 (Pharmacia Biotech AB, Uppsala, Sweden) or pMAL (New England Biolabs, MA, U.S.A.) for the production of fusion protein with glutathione S-transferase (GST) or maltose binding protein (MBP) (13,20). The expression plasmids were transformed into *Escherichia coli* (BL21 or TB1), and subsequent induction with isopropyl-beta-D-thiogalactopyranoside (IPTG) was performed by routine methods (21). The transformed *Escherichia coli* were sonicated to obtain supernatants of the bacterial homogenates.

Epitope analyses of autoantibodies against GluRε2 in sera and CSF

Supernatants of bacterial homogenates containing induced GluRε2 polypeptide fusion proteins synthesized by the methods described previously were subjected to SDS-PAGE, and the proteins in gels were transferred to nitrocellulose membranes. Each membrane was reacted with serum or CSF (diluted 20- or 15-fold with blocking buffer) for 48 h at 4°C, and stained by alkaline phosphatase-labeled second antibodies (IgG or IgM). A positive reaction with an epitope was judged by obtaining a stained band at the appropriate molecular weight (13).

Lymphocyte stimulation test by GluRs

Peripheral venous blood was collected in heparinized sterile vacutainer tubes (Becton Dickinson, Oxford, U.K.). Blood cells and serum were separated by centrifugation at 1,800 rpm for 5 min. Blood cells were diluted with PBS (-) and applied on Ficoll-Conray (d = 1.077), and centrifuged at 1800 rpm for 20 min to separate the lymphocytes. The lymphocytes were washed twice in PBS by centrifugation at 2,000 rpm for 5 min. Then lymphocytes were diluted to 1×10^6 cells/ml in complete RPMI-1640 supplemented with 20% autologous serum. These cells were cultured in triplicate in 96-well microplates for 3 days with the corresponding antigens. Then ^{3}H-thymidine (0.25μCi/well) was added to each well. After incubation for 16 h, the cells were harvested using an automatic cell harvester and the incorporated radioactivity was measured by liquid scintillation counter. The supernatants of homogenates from A1, D33, and B18, and phytohemagglutinin (PHA) were used as antigens.

Statistical analysis

For the comparison of LST data, we used Mann–Whitney's U test or Wilcoxon signed-ranks test, using statcel software. The numbers indicate mean ± SD (standard deviation).

TABLE 2. *Autoantibodies against glutamate receptors*

			EPC				Whole GluRε2			Epitope analysis			
Pt	Age at exam	Latency	Ext	Face	CPS	Sample	IgG	IgM	IgA	N	C1	C2	C3
1	0.8 yr	0.8 yr	−	−	D	Serum	−	−					
	0.9 yr	0.9 yr	−	−	D	Serum	+	+	−	−	−	+	−
2	1.1 yr	0.9 yr	+	−	D	Serum	+	+	−	−	−	−	−
	1.1 yr	0.9 yr	+	−	D	CSF	+	+	−	+	+	+	+
	4.0 yr	3.8 yr	+	−	W	Serum	+	−	−	−	−	+	−
3	3.8 yr	3.7 yr	−	−	C	Serum	−	−	−	−	−	−	−
4	3.5 yr	2.8 yr	+	+	−	CSF	−	−					
	4.0 yr	3.3 yr	+	+	−	CSF	+	−					
5	9 yr	7 yr	−	+	D	CSF	+	−					
6	11 yr	7 yr	−	−	D	Serum	+	+		−	−	+	−
	15 yr	12 Y	−	−	C	Serum	+	+					
7	3.9 yr	0.2 yr	+	−	M	Serum	+	−		−	+	+	+
	3.9 yr	0.2 yr	+	−	M	CSF	+	−		−	+	+	+
8	3.9 yr	0.1 yr	+	−	M	Serum	−	−	−				
	4.7 yr	0.9 yr	+	−	M	Serum	+	+		−	−	−	−
	5.2 yr	1.4yr	−	−	Y	Serum	−	−					
	8.1 yr	4.3 yr	+	+	M	Serum	+	−		−	−	−	−
	10 yr	6.2 yr	−	−	C	Serum	+	+					
9	5.4 yr	2.5 yr	+	−	W	Serum	+	−					
	5.4 yr	2.5 yr	+	−	W	CSF	+	−					
10	6.1 yr	0.9 yr	+	−	W	Serum	+	−		−	+	−	+
	6.7 yr	1.5 yr	+	−	D	Serum	+	−		−	+	−	+
11	20 yr	14 yr	+	−	C	Serum	+	+					
12	6.1 yr	0.2 yr	−	+	D	Serum	+	−					
	6.1 yr	0.2 yr	−	+	D	CSF	+	−					
13	7.1 yr	1 yr	+	+	C	Serum	−	−		−	−	+	−
	7.1 yr	1 yr	+	+	C	CSF	−	+		−	−	+	−
	11.3 yr	5.2 yr	−	+	Y	Serum	+	+					
14	12 yr	3 yr	+	−	D	Serum	+	−		−	−	+	−
15	15 yr	3 yr	+	−	D	Serum	+	+					
	16 yr	4 yr	+	−	D	Serum	−	−					
16	18.5 yr	6 yr	−	−	W	Serum	+	−		+	−	+	+
	19.5 yr	7 yr	−	−	W	CSF	+						
17	34 yr	18 yr	−	−	W	Serum	+	+					
18	38 yr		−	−	D	Serum	+	+		−	+	−	+
19	29 yr	4 yr	−	−	D	Serum	+	+		+	+	−	+
20	36 yr	8 yr	−	−	W	Serum	−	−					
	38yr	10 yr	−	−	W	Serum	+	+					

Latency, latency from onset to examination; EPC, epilepsia partialis continua; Age at exam, age at examination of autoantibodies; Ext, EPC involving extremities; Face, EPC involving facial muscles, tongue, or throat; CSF, cerebrospinal fluid; GluR, glutamate receptor; NT, not tested. In the column of CPS, D indicates daily frequency of CPS, W indicates weekly, M indicates monthly, Y indicates yearly, C indicates controlled. In the column of whole GluRε2 and epitope analysis, + indicates presence of autoantibodies, and – indicates absence of autoantibodies.

RESULTS

Autoantibodies against GluRε2 molecules in sera and CSF

Autoantibodies against full-length GluRε2 molecule were studied in the sera and or CSF of 20 patients with definitive or clinical Rasmussen encephalitis (Table 2). All nine patients with definitive Rasmussen's encephalitis (five with EPC and four without EPC), and 10 of 11 patients (except Patient 3) with clinical Rasmussen's encephalitis had the autoantibodies in serum or CSF. Although Patient 3 had no autoantibodies against GluRε2, the test was done at the residual stage (22) when EPC and CPS had both remitted. Nineteen patients had IgG autoantibodies against the GluRε2 molecule during the course of disease, and 11 patients had IgM autoantibodies. None of the patients we examined had IgA autoantibodies against GluRε2. In Patients 1, 4, 8, and 13, the IgG and IgM autoantibodies against GluRε2 were absent in the early stage when epileptic seizures already became frequent, and appeared subsequently. In Patient 8, after intravenous high-dose gamma-globulin infusion therapy (200 mg/kg weight, 5 days), EPC remitted and CPSs decreased to a yearly occurrence, accompanied by negative autoantibodies at the age of 5.2 years. At age 7.3 years, EPC recurred and the autoantibodies again became positive. Thus, Patient 8 showed fluctuation of autoantibodies in relation to the severity of clinical symptoms modulated by immunotherapy. In Patient 15, the autoantibodies became negative after steroid pulse therapy, but epileptic seizures persisted. In Patients 6 and 8, the autoantibodies persisted in the serum after frontal lobe resection or functional hemispherectomy, although epileptic seizures were controlled.

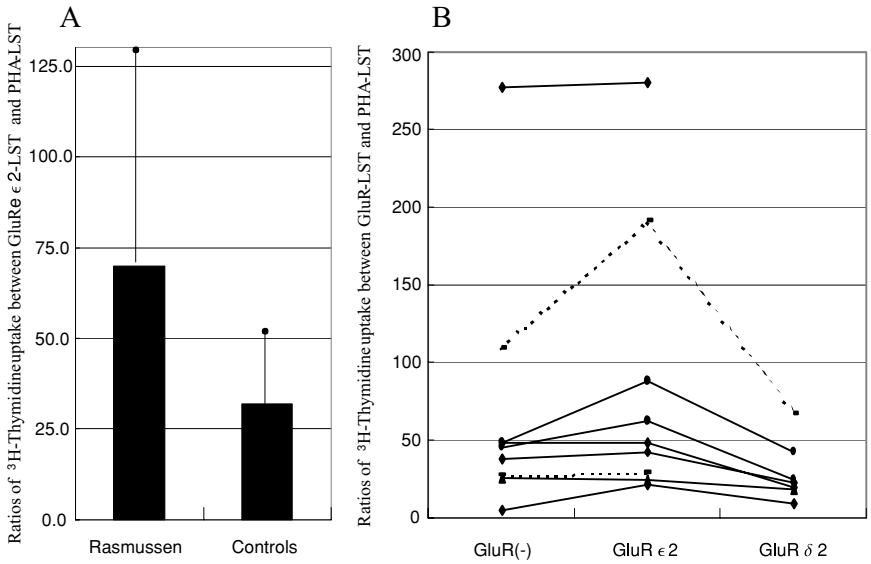

FIG. 1. A: Mean ^{3}H-thymidine ratios of uptake stimulated by GluRε2 relative to PHA in lymphocyte stimulation test (LST) using lymphocytes from patients with Rasmussen syndrome and healthy controls. GluRε2-LST and PHA-LST were conducted using supernatants of cell culture homogenates containing recombinant GluRε2 (B18) and PHA. Vertical axis indicates ratios of uptake stimulated by supernatant of B18 homogenate containing recombinant GluRε2 to that stimulated by PHA (GluRε2/PHA $\times$1000). **B:** Ratios of uptake stimulated by GluRε2 relative to PHA, those stimulated by GluRδ2 relative to PHA, and those stimulated by control homogenate [GluR (-)] relative to PHA for each patient. GluR (-): Uptake stimulated by control homogenates (A1)/uptake stimulated by PHA $\times$1000. GluRε2: Uptake stimulated by B18 homogenate containing recombinant GluRε2/uptake stimulated by PHA $\times$1000. GluRδ2: Uptake stimulated by D33 homogenate containing recombinant GluRδ2/uptake stimulated by $\times$1000 PHA.

Epitope analyses of autoantibodies against GluRε2 in sera and CSF

IgG autoantibodies against the putative epitopes (N, C1, C2, and C3) of the GluRε2 molecule were examined using bacterial fusion proteins containing the corresponding GluRε2 polypeptides (13). Of 12 patients with IgG autoantibodies against the GluRε2 molecule, three patients had serum autoantibodies to N epitope, four had autoantibodies to C1, seven had autoantibodies to C2, and six had autoantibodies to C3 (Table 2). Autoantibodies to C2 epitope predominated, while autoantibodies to the extracellular N epitope were rare. The epitope pattern in patients without EPC was not different from that in patients with EPC. The spectra of epitope recognition of the autoantibodies were broader in the CSF than in the serum in almost all patients, judging from simultaneously collected samples.

Lymphocyte stimulation by GluRs

In a preliminary study, we examined ^{3}H-thymidine uptake after stimulation with B18 homogenates at different protein concentrations in six definitive patients, and the uptake was highest at the concentration of 40 μg/ml. Subsequently, we conducted lymphocyte stimulation tests (LSTs) using homogenates of D33 (cell line expressing GluRδ2 subunits), B18 (cell line expressing GluRε2), and A1 (control cell line without GluR expression) at protein concentrations of 40 and 400 μg/ml. The mean ^{3}H-thymidine uptake ratios for B18 stimulation (uptake stimulated by B18 divided by uptake stimulated by PHA $\times$ 1000) were 69.3 ± 60.7 (n = 23) in patients with definitive and clinical Rasmussen encephalitis and 23.1 ± 19.7 (n = 7) in controls ($p < 0.05$, Mann–Whitney's U test) (Fig. 1A). In patients with definitive Rasmussen encephalitis, the mean ^{3}H-thymidine uptake ratios were 69.3 ± 82.8 (n = 9) for A1 stimulation (uptake stimulated by A1 divided by uptake stimulated by PHA $\times$1000), 87.6 ± 89.4 (n = 9) for B18 stimulation (uptake stimulated by B18 stimulation divided by uptake stimulated by PHA $\times$1000), and 29.0 ± 19.7 (n = 7) for D33 stimulation (uptake stimulated by D33 divided by uptake stimulated by PHA $\times$1000). The uptake ratios for B18 stimulation were significantly higher than those for A1 ($p < 0.05$, Wilcoxon signed-ranks test) and D33 stimulation ($p < 0.05$, Wilcoxon signed-ranks test). The uptake ratios of GluRε2 to PHA were relatively low in Patients 6 and 8, after seizures were controlled after epilepsy surgeries, comparing with those in patients with seizures.

DISCUSSION

Rasmussen's encephalitis is divided into two clinical subtypes by the existence of EPC. In our previous article (13), we reported that autoantibodies against NMDA-type GluRε2 were detected in Rasmussen's encephalitis patients with EPC (definitive Rasmussen's encephalitis patients, 3/3 patients, and clinical Rasmussen's encephalitis patients, 6/7 patients). Subsequently, the present study demonstrated the autoantibodies also in patients with definitive Rasmussen's encephalitis without EPC

(4/4 patients). This suggests that autoantibodies against GluRε2 are important for the diagnosis of both subtypes of Rasmussen's encephalitis, independent of EPC. Because EPC is a typical clinical diagnostic marker of Rasmussen's encephalitis, presurgical diagnoses of Rasmussen's encephalitis are usually difficult in patients without EPC, compared with those with EPC. Detection of autoantibodies against GluRε2 can contribute to the presurgical diagnosis and may facilitate immunological treatment in patients with dominant side involvement.

In these 19 patients with Rasmussen's encephalitis and autoantibodies against the NMDA GluRε2 subunit, the epitope pattern in patients with EPC was not different from that in patients without EPC. Clinical subtypes might be determined by some factors other than difference of epitopes of the autoantibodies. We need to investige further into the distribution of immunological lesions to decide the factors.

In this study, we could reveal the existence of peripheral lymphocytes selectively stimulated by GluRε2 in patients with Rasmussen's encephalitis using LST. SD in patients' data of the mean ^{3}H-thymidine uptake ratios is slightly large. This might be attributed to the effect of treatments and the stage of Rasmussen's encephalitis. The ^{3}H-thymidine uptake ratios were lower mainly in patients with steroid therapy, and the ratios tended to become lower in the progressed stage (data not shown). Lymphocytes stimulated by LST are usually T cells. Although we could not confirm a subset of stimulated T cells ($CD4^+$ or $CD8^+$) by GluRε2, activated effector T cells that could invade the CNS beyond the blood brain barrier definitively exist in peripheral blood circulation. We estimate that these activated T cells are produced by cross-reaction using molecular mimicry after an infection and play an important role in the subsequent onset of Rasmussen's encephalitis.

In previous reports, lymphocytic infiltration containing predominantly T cells and sparsely B cells were reported in resected tissues from Rasmussen's encephalitis patients (23), and local CNS immune responses in Rasmussen's encephalitis included local clonal expansion of T cells responding to discrete antigen epitopes (24). Our proof about the existence of peripheral lymphocytes stimulated by GluRε2 can explain the infiltration of T cells in the CNS. $CD8^+$ T cell cytotoxicity was reported to contribute to the pathogenesis of Rasmussen's encephalitis, and neuronal loss resulting from cytotoxic T cell attack and concomitant release of antigens such as GluR3 were speculated to generate secondary antibody-mediated damage (25,26). The probable heterogeneity of autoantibodies in Rasmussen's encephalitis, epitope spreading and autoantibodies directed predominantly against intracellular epitopes of NMDA GluRε2, also suggests an important role of T cell-mediated autoimmunity (13). Mice immunized with the GluR3B peptide produced autoantibodies but exhibited no epilepsy, even by facilitated entry of the autoantibodies into the brain (27). These data have suggested that the primary cause of Rasmussen's encephalitis is not autoantibodies, but T cell–mediated autoimmunity. In four patients, the autoantibodies against GluRε2 were absent in the early stage, even when epileptic seizures had already become frequent, and appeared later. This delayed appearance of autoantibodies against GluRε2 also supports the hypothesis about primary cause of Rasmussen's encephalitis.

After epilepsy surgeries, Patients 6 and 8 became seizure free, but the autoantibodies persisted in the serum. However, the uptake ratios for GluRε2 to PHA in LST were relatively low in Patients 6 and 8, when seizures were controlled after epilepsy surgeries. These data suggest that autoantibodies against the GluRε2 in sera are not causally related to active pathophysiological processes, and is not a marker of activity of Rasmussen's encephalitis, while the uptake ratio of GluRε2 to PHA in LST might be a marker of disease activity.

International collaborating studies on the contribution of multiple autoantibodies and cellular immunity to the pathophysiology of Rasmussen's encephalitis are expected to elucidate the pathophysiological mechanisms mediated by autoimmunity in Rasmussen's encephalitis and other neurological diseases.

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