infection in patients (Griscelli et al., 2001; Cortez et al., 2003; Leruez-Ville et al., 2003; Li et al., 2003; Mengelle et al., 2003; Boeckh et al., 2004; Kalpoe et al., 2004); however, in Japan, the real-time PCR is not yet a common procedure in hospital laboratories because expensive equipment (a thermal cycler) is needed. Therefore, clinical samples should be analyzed in the several commercial laboratories, which result in time consuming for obtaining the final results.

Recently, Notomi et al. (2000) have reported a novel nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP), which amplifies DNA under isothermal conditions with high specificity, efficiency, and speed. The LAMP reaction requires a DNA polymerase with strand displacement activity and a set of four specially designed primers, termed inner and outer primers, which improve the assay's specificity. As a first step, a stem-loop DNA structure, in which the sequences of both DNA ends are derived from the inner primer, is constructed as the starting material. Subsequently, one inner primer hybridizes to the loop on the product in the LAMP cycle and initiates strand-displacing DNA synthesis, yielding the original stem-loop DNA and new stem-loop DNA with a stem twice as long. The final products are stem-loop DNAs with several inverted repeats of the target DNA, and cauliflower-like structures with multiple loops, amplifying the amount to 10^9 copies of the target (Notomi et al., 2000). The most significant advantage of the LAMP method is its ability to amplify specific sequences of DNA under isothermal conditions of 63–65 °C. As a result, it requires only simple and cost-effective equipment that is suitable for use in hospital laboratories. A second characteristic of the LAMP method is that it exhibits both high specificity and high amplification efficiency. Since the LAMP method uses four primers recognizing six distinct sequences on the target DNA, its specificity is extremely high. Due in part to its isothermal nature, this method also maintains extremely high amplification efficiency: there is no time lost due to changes in temperature, the reaction can be conducted at the optimal temperature for enzyme function, and the inhibition reaction that occurs at later stages of amplification (a typical problem with PCR) are less likely to take place. Thus, this method is a potentially valuable tool for the rapid diagnosis of infectious diseases in both commercial and hospital laboratories (Iwamoto et al., 2003; Kuboki et al., 2003; Hong et al., 2004; Horisaka et al., 2004; Ihira et al., 2004; Okamoto et al., 2004; Parida et al., 2004; Wakabayashi et al., 2004; Yoshikawa et al., 2004; Enomoto et al., 2005; Sugiyama et al., 2005). The goal of this study was to establish a LAMP-based CMV DNA amplification method. We found this method to be highly sensitive, specific, and sufficiently reliable for the diagnosis of CMV infection in immunocompromised patients.

2. Materials and methods

2.1. Study design

CMV (AD-169)-, herpes simplex virus (HSV) 1 (KOS)-, HSV-2 (186)-, varicella-zoster virus (Oka-vaccine)-, human herpesvirus (HHV)-6 A (U1102)-, HHV-6 B (Z29)-, and HHV-7 (RK)-infected cells were used to determine the specificity of CMV LAMP, and a plasmid containing the target sequence of CMV (pGEMCMVS12) was used to determine its sensitivity. The details of each experiment are described below.

A total of 20 post-hematopoietic stem cell transplantation (HSCT) children at the Nagoya University Hospital and the Japanese Red Cross Nagoya First Hospital were enrolled in our study. Informed consent was obtained from the parents of all children, and 2 ml of heparinized blood was collected serially from the HSCT recipients. Detection of CMV DNA in the whole blood (WB) of each sample was tested by LAMP and real-time PCR. The results obtained by CMV LAMP and real-time PCR were compared in order to assess the reliability of CMV LAMP as an indicator of CMV infection.

2.2. DNA extraction

For the experiment in which CMV LAMP was initially developed, viral DNA was extracted from CMV (AD-169)-, HSV-1 (KOS)-, HSV-2 (186)-, varicella-zoster virus (Oka-vaccine)-, HHV-6 A (U1102)-, HHV-6 B (Z29)-, and HHV-7 (RK)-infected cells using a QIAamp Blood Kit (QIAGEN, Chatsworth, CA). The same DNA extraction kit was used to extract DNA from 200 μl of WB samples collected from the recipients. After extraction, DNA was eluted in 100 μl of elution buffer and stored at −20 °C.

2.3. LAMP

LAMP reactions were conducted as described by Notomi et al. (2000) and Nagamine et al. (2002). The LAMP method requires a set of four specially designed primers (B3, F3, BIP; B1–B2c, FIP; F1c–F2) that recognize a total of six distinct sequences (B1, B2, B3, F1, F2, and F3) in the target DNA. Primers for CMV LAMP were designed against the CMV gB gene using Primer Explorer V software (Fujitsu, Tokyo, Japan). The locations and sequences of each primer are shown in Fig. 1. BIP for the CMV gB gene (CMgBBIP) consisted of the B1 direct sequence (21 nt) and the B2 complementary sequence (20 nt). Primer FIP for CMV gB (CMgBFIP) consisted of the F1 complementary sequence (20 nt) and the F2 direct sequence (20 nt). Primers B3 (CMgBB3) and F3 (CMgBF3) for CMV gB were located outside of the F2–B2 regions. Since it has been demonstrated that additional loop primers increase the amplification efficiency, loop primers for the CMV gB gene (CMgBLPB and CMgBLPF) were also synthesized. CMgBLPB consisted of the LPB sequence, and CMgBLPF consisted of LPF complementary sequence. The LAMP reaction was performed using a Loopamp DNA amplification kit (Eiken Chemical, Tochigi, Japan). Reaction mixtures (25 μl) contained 1.6 μM each of CMgBFIP and CMgBBIP, 0.2 μM each of the outer primers (CMgBF3 and CMgBB3), 0.8 μM each of the loop primers (CMgBLPF and CMgBLPB), 2× reaction mix (12.5 μl), *Bst* DNA polymerase (1 μl), and 5 μl of target DNA. A TERAMECS LA200 (Teramecs, Kyoto, Japan) was used to incubate the mixtures and measure turbidity after the LAMP reaction. The cut-off value of turbidity used to distinguish negative from positive samples was fixed at 0.1, higher than the mean + 3S.D. units of the turbidity

(A)

Nucleotide position

81181 TAGATCAAAT AAGTGATAAT GACTACGGCT ATGGCCACGA GGATGATGGT
F3

81231 GAAGGCTCCG AAGGGGTTTT TGAGGAAGGT GGCAACGCCT TCGACCACGG
F2 LPF

81281 AGGCCACCGC GCCACCCACG GCCCCAATGG CTACGCCAAC GGCCTTTCCC
F1

81331 GCGGCGCCCA GGCCGCTCAT GAGGTCGTCC AGACCCTTGA GGTAGGGCGG
B1 LPB

81381 TAGCGGGTCG ACTACCTTGT CCTCCACGTA CTTTACCCGC TGCTTGTACG
B2 B3

81431 AGTTGAATTC GCGCATGATC TCTTCGAGGT CAAAAACGTT GCTGGAACGC

(B)

Name of primers	Sequence
CMgBBIP	5'-CGCTCATGAGGTCGTCCAGAC GTGGAGGACAAGGTAGTCGA-3' (B1-B2c)
CMgBFIP	5'-CGTTGGCGTAGCCATTGGGG AGGGGTTTTTGAGGAAGGTG-3' (F1c-F2)
CMgBB3	5'-AACTCGTACAAGCAGCGG-3' (B3)
CMgBF3	5'-CACGAGGATGATGGTGAAGG-3' (F3)
CMgBLPB	5'-TTGAGGTAGGGCGGTAGCGGG-3' (LPB)
CMgBLPF	5'-AACGCCTTCGACCACGGAGG-3' (LPFc)

Fig. 1. Locations and names of target sequences used as primers for CMV LAMP of the CMV gB gene (A). Names and sequences of the primers for CMV LAMP (B). B2c, sequence complementary to B2; F1c, sequence complementary to F1; LPFc, sequence complementary to LPF.

of five negative samples. After turbidity measurements, LAMP products were subjected to 1.5% agarose gel electrophoresis for the initial validation experiments. Gels were visualized under UV light after ethidium bromide staining. Great care was taken to avoid contamination between samples; different rooms were used for DNA extraction, LAMP setup, and gel analysis. In addition, pipette tips with filters were used to minimize aerosol contamination.

2.4. Real-time PCR

Real-time PCR was used to measure the quantity of CMV DNA in each clinical sample. Conditions for real-time PCR quantifying CMV DNA, which amplifies the immediate early gene of CMV, were described elsewhere (Tanaka et al., 2000). PCR reactions were performed using a TaqMan PCR Kit (PE Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Standard curves for measuring CMV DNA were constructed using the C_T values obtained from a serially diluted plasmid, pCMVTA, which contained the target sequences. The C_T value from each sample was plotted on the standard curve, allowing the copy number to be automatically calculated using Sequence Detector v1.6 (PE Applied Biosystems).

2.5. Cloning of CMV DNA

In order to determine the sensitivity of the CMV LAMP method, a plasmid containing the target DNA sequence was constructed. First, upstream (CMS1; 5′-CTATGGCCACGAGG-ATGATG-3′) and downstream (CMS2; 5′-TCCAGCAACG-TTTTTGACCT-3′) primers spanning the sequences between the F3 and B3 primers were synthesized. CMV DNA (AD-169) was amplified with these two primers by conventional PCR, and the PCR product was cloned into a pGEM-T vector using pGEM-T Vector System II (Promega, Madison, WI) according to the manufacturer's instructions. The plasmid (pGEM-CMVS12) constructed by the system was used to make standard dilutions to evaluate the lower detection limit of the LAMP protocol.

3. Results

During assay development, we first evaluated the specificity of the CMV primers. CMV LAMP was performed on DNA extracted from CMV (AD-169)-, HSV 1 (KOS)-, HSV-2 (186)-, varicella-zoster virus (Oka-vaccine)-, HHHV-6 A (U1102)-, HHV-6 B (Z29)-, and HHV-7 (RK)-infected cells. Since the LAMP products consist of several inverted-repeat structures, positive samples reveal many bands of different sizes after agarose gel electrophoresis. Although amplified CMV demonstrated the typical ladder patterns as shown in Fig. 2, no LAMP product was detected in reactions performed with other viral DNAs.

We also determined the method's sensitivity. Serial dilutions of pGEMCMVS12 plasmid were used to determine the detection limit of CMV LAMP, which was 500 copies/tube (Fig. 3) as determined by either agarose gel electrophoresis or turbidity assay. To determine whether CMV LAMP could be used to quantify viral DNA, threshold times, defined as the time (in seconds) for reaching the threshold level (0.1), were measured for amplification of the serial dilutions of the plasmid DNA. The standard curve possessed a correlation coefficient of 0.9443 with a slope of −208.1 and a y-intercept value of 3261.4 (Fig. 4). As

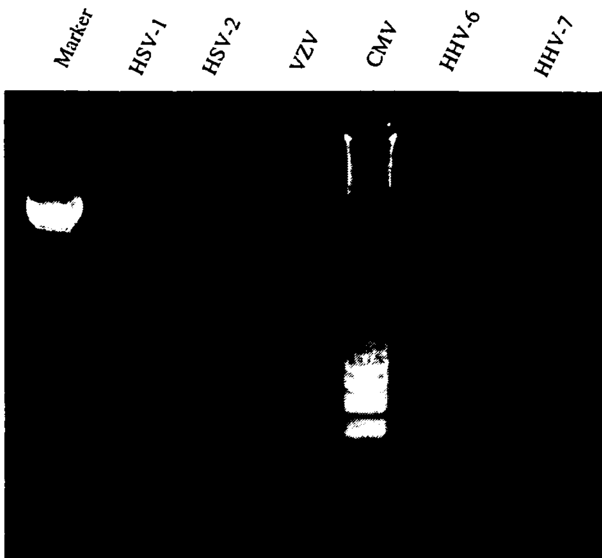

Fig. 2. DNA extracted from the six herpesviruses infected cells were amplified using CMV LAMP to determine the specificity of the method. Marker, 123-base-pair DNA ladder.

the highest threshold time for amplification of 500 copies of viral DNA was 2268 s, the LAMP reaction time to evaluate clinical specimens was set to 40 min in the subsequent experiments.

After the initial validation experiments, we analyzed 180 samples collected serially from 20 pediatric HSCT recipients. Detection of CMV DNA in WB was tested by CMV LAMP and real-time PCR. CMV DNA was detected in 34 (21%) of the 180 samples (median viral load: 1760 copies/200 μl of WB, ranging between 200 and 28,620 copies/200 μl of WB) by real-time PCR. The efficiency of CMV LAMP in the detection of CMV infection was evaluated by comparison to the real-time PCR method. The copy numbers determined by the real-time PCR were used to determine several cut-off values to distinguish between negative and positive results (Table 1). If we defined >10 copies/tube (>200 copies/200 μl of WB) as positive for CMV infection, even though the specificity of CMV LAMP was high (99.3%), the sensitivity of the method was very low (14.7%). If we defined >500 copies/tube (>5000 copies/200 μl of WB) as positive CMV infection, the sensitivity, specificity, positive predictive value, and negative predictive value of the CMV LAMP were 80.0, 98.9, 66.7, and 99.4%, respectively.

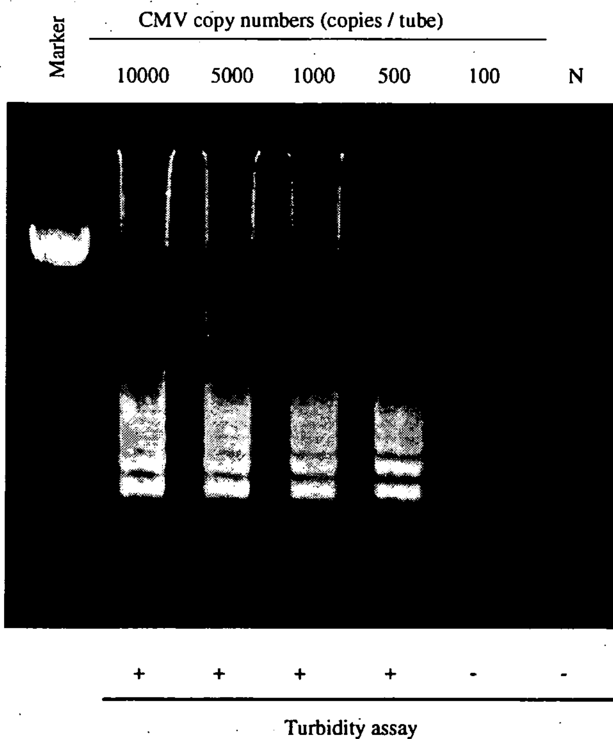

Fig. 3. Serial dilutions of pGEMCMVS12 plasmid DNA were amplified by CMV LAMP to determine the sensitivity of the assay. Marker, 123-base-pair DNA ladder.

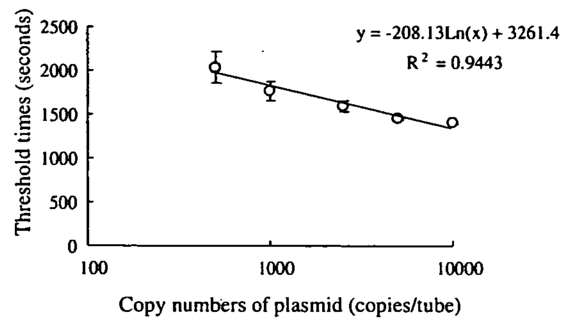

Fig. 4. Correlation of threshold time (in seconds) with serial dilutions of plasmid DNA (pGEMCMVS12) containing the target sequence. Values on the x-axis are the threshold times defined as the time to reach the threshold level of turbidity (0.1).

4. Discussion

The most popular CMV infection test in immunocompromised patients is the immunoperoxidase staining of CMV tegument protein pp65 (UL83) in peripheral blood neutrophils. The cost of technician time and the need for technical expertise, however, are major limitations of the antigen test. In Japan, this test is generally carried out in commercial laboratories, and it takes about two days to get the results of the assay. Meanwhile, real-time quantitative PCR based on TaqMan PCR or Light Cycler PCR technologies has been demonstrated to be more sensitive and easier to perform than the antigenemia assay (Cortez et al., 2003; Boeckh et al., 2004; Leruez-Ville et al., 2003; Kalpoe et al., 2004). However, its implementation in hospital laboratories has been impeded by its requirement for expensive equipment. In contrast, the LAMP method has been shown to promote the amplification of DNA under isothermal conditions with high specificity and efficiency, comparable to traditional PCR, which results in a low cost for the examination. This is a major advantage for the application of the test in hospital laboratories. Therefore, we attempted to develop CMV LAMP as a more cost-effective alternative to the antigenemia assay or real-time PCR, for future use in hospital laboratories.

Although CMV LAMP amplified CMV DNA, no cross-reactivity was observed between five other human herpesviruses, including the two β herpesvirinae (HHV-6 and HHV-7) shown in Fig. 2. The detection limit of CMV LAMP was 500 copies/tube, as determined by either agarose gel electrophoresis or the turbid-

Table 1
Test characteristics of CMV LAMP according to the copy numbers determined by real-time PCR as standard

Copy numbers[a] (copies/tube)	No. of samples with test results/no. of samples with result by standard (%)			
	Sensitivity	Specificity	PPV	NPV
>10	5/34 (14.7)	145/146 (99.3)	5/6 (83.3)	145/174 (83.3)
>50	5/26 (19.2)	153/154 (99.4)	5/6 (83.3)	153/174 (87.9)
>100	5/16 (31.3)	163/164 (99.4)	5/6 (83.3)	163/174 (93.7)
>250	4/7 (57.1)	171/173 (98.8)	4/6 (66.7)	171/174 (98.3)
>500	4/5 (80.0)	173/175 (98.9)	4/6 (66.7)	173/174 (99.4)

[a] Determined by real-time PCR. PPV, positive predictive value; NPV, negative predictive value.

ity assay (Fig. 3). These findings demonstrate that CMV LAMP has high specificity and high efficiency for the amplification of viral DNA. Moreover, as the standard curve had a high correlation efficiency (Fig. 4), this method could also be used to quantify CMV DNA, as reported elsewhere (Mori et al., 2001, 2004; Hong et al., 2004; Parida et al., 2004).

Since the turbidity assay is faster and easier than agarose gel electrophoresis, it is better suited to bedside monitoring. Moreover, as the turbidity assay can be carried out in a closed system, the risk of contamination is lower than for agarose gel electrophoresis. This is an additional advantage for clinical use (Okamoto et al., 2004; Yoshikawa et al., 2004; Enomoto et al., 2005; Sugiyama et al., 2005). Therefore, we used the turbidity assay to evaluate the reliability of CMV LAMP for the detection of viral DNA from clinical samples.

As CMV can latently infect peripheral blood mononuclear cells, it is important to discriminate between latent and active viral infections in monitoring CMV infection in immunocompromised, seropositive patients. In order to evaluate the reliability of real-time PCR for monitoring viral infection, tests have been carried out in different cell fractions and acellular fractions of blood, including peripheral blood leukocytes (Griscelli et al., 2001), WB (Cortez et al., 2003; Mengelle et al., 2003), and plasma (Leruez-Ville et al., 2003; Boeckh et al., 2004; Kalpoe et al., 2004). Theoretically, since CMV latently infects peripheral blood leukocytes, monitoring of viral DNA in these cells would be more sensitive than in other specimens. However, peripheral blood leukocytes must be separated from plasma, washed, and counted to measure viral load by molecular methods. Clearly, if WB could also be used for the monitoring of active CMV infection, this would require much less processing—a major advantage for bedside monitoring. As several investigators have demonstrated that quantification of CMV DNA from WB provides acceptable results for monitoring CMV infection in immunocompromised patients (Cortez et al., 2003; Mengelle et al., 2003), we used WB to evaluate the reliability of CMV LAMP in this study. As demonstrated by our initial validation analysis, if we use the turbidity assay for detection of LAMP products, CMV LAMP is clearly faster (40 min) and easier to perform than any other popular methods, such as real-time PCR and the antigenemia assay. Again, these are major advantages for clinical use. Thus, we recommend the use of CMV LAMP for rapid bedside monitoring system of CMV infection in immunocompromised patients. However, the sensitivity of the method is likely to be lower than real-time PCR, as demonstrated in our initial experiments. Therefore, clinical specimens containing sufficient amounts of viral DNA, such as WB, are suitable for CMV LAMP.

As shown in Table, if >500 copies/tube (5000 copies/200 μl of WB) are defined as positive, sensitivity (80%) and specificity (98.9) of CMV LAMP are high. However, if less than 500 copies/tube (from >10 to >250 copies/tube) is defined as positive, the sensitivity of LAMP is low despite its high specificity. As our number of samples containing high viral load was small, and there were no patients diagnosed as CMV disease in this subjects, it was difficult to precisely evaluate CMV LAMP in relation to active viral infection in HSCT recipients. Mengelle et al. (2003) recently reported that 3.4 $\log_{10}$ copies of CMV DNA in 200 μl of WB was equivalent to a threshold value of 50 pp65-positive polymorphonuclear cells per 200,000 leukocytes. Theoretically, if DNA is eluted in 50 μl of elution buffer (2× concentration of extracted DNA), which could double the sensitivity of the method, CMV LAMP product could be detected in samples containing the threshold level of CMV. Finally, as empirical treatment with effective antiviral drugs with non-negligible toxicities is an established strategy for CMV disease prevention in immunocompromised patients, the detection of CMV infection is crucial for both starting and cessation of the antiviral drugs. Therefore, in order to confirm the reliability of CMV LAMP in monitoring active CMV infection, it is necessary to compare the CMV LAMP and pp65 antigenemia assays in future analyses.

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Infections as causative factors of epilepsy

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Epilepsies bearing some relationship to infections or vaccinations are often encountered clinically. While the onset of epilepsy or aggravation of seizures may follow common infections or vaccinations, complete seizure control has also been observed after infections. However, the true mechanisms underlying the relationship between infections and epilepsies have not been fully elucidated. Recently, advances in immunology have contributed to the study of autoimmune mechanisms in Rasmussen's syndrome, a prototype of autoimmune epilepsy related to infections. The roles of autoimmunity, including cytotoxic T cells and autoantibodies against neural molecules, have been demonstrated in Rasmussen's syndrome. This review postulates the probable molecular mimicry of microbial and neural components in Rasmussen's syndrome and proposes possible autoimmune mechanisms related to the development of symptomatic epilepsies.

Epilepsy is a common neurological disease affecting 1–2% of the population and the onset of epilepsy is frequent in childhood. Childhood-onset epilepsy can begin after an infection episode, or even after vaccination, albeit rarely. Even after the onset of epilepsy, status epilepticus tends to occur under febrile conditions associated with infections or vaccinations. Parents of such pediatric patients usually suspect a causal relationship between the epilepsy (status epilepticus) and infection or vaccination. However, the question of how infections or vaccinations cause epilepsies remains largely unanswered. This review summarizes several proposed relations between infection (or vaccination) and epilepsy (Table 1) (Figure 1).

The etiologies of childhood epilepsy are classified into idiopathic (64%), prenatal (15%), perinatal (9%) and postnatal (12%) [1]. Infection of the CNS, a postnatal etiology, accounts for 4% of childhood epilepsies. In the author's epilepsy center, 12% of admitted cases of intractable partial epilepsy or generalized epilepsy were attributed to CNS infections (acute viral encephalitis, bacterial meningitis, etc.) [2]. These data suggest that CNS infections (direct infectious mechanisms) are an important cause of intractable epilepsies. Generally speaking, direct infectious mechanisms seem to include primary viral encephalitis, secondary autoimmune encephalitis and subacute sclerosing panencephalitis (Figure 1, routes 1, 2 and 4). While virus particles are found in CNS tissues in primary viral encephalitis, virus particles are absent in secondary autoimmune viral encephalitis. Strictly speaking, secondary autoimmune viral encephalitis is not classified as a direct virus infectious mechanism, but is included in parainfectious mechanisms.

Human herpes virus (HHV)-6 is an ubiquitous virus and causes exanthema subitum, a common disease in infants, and subsequently establishes latency in the CNS [3,4]. HHV-6 was detected in approximately 50% of the surgically resected brain tissues from patients with mesial temporal lobe epilepsy (mTLE), suggesting that reactivation of HHV-6 in astrocytes may have a role in the development of mTLE [5]. These latent infection and reactivation mechanisms may contribute to certain types of epilepsies (Figure 1, route 3).

Rasmussen's encephalitis is a slowly progressive disease of chronic inflammation in the CNS, resulting in hemiplegia (96%), mental deterioration (85%), visual field defects (49%) and cortical sensory defects (29%) [6]. Patients with typical Rasmussen's encephalitis manifest frequent intractable partial motor seizures in the acute phase, characteristically epilepsia partialis continua (EPC) (56%), but the frequencies of seizures decrease markedly in the residual stage [6,7]. Histological examination has revealed infiltration of T lymphocytes and microglia cells, astrocytosis and neuronal loss [6,8,9]. Although viral infection appeared to be causally related to Rasmussen's encephalitis in early investigations, Rogers and colleagues reported glutamate receptor (GluR)3 as an autoantigen in Rasmussen's encephalitis, which could be a cause of Rasmussen's encephalitis [10]. On the other hand, destruction of neurons by cytotoxic T lymphocytes (CTLs) was presented as a new

Keywords: autoantibodies, autoimmunity, cytotoxic T cell, epilepsy, glutamate receptor, HLA, infection, tacrolimus, vaccination

future Medicine

Table 1. Proposed mechanisms underlying the development of epilepsies after infections and vaccinations.

Mechanisms	Method
Direct infection mechanisms	Microbes invade the CNS and directly cause insults resulting in epileptogenesis **(Figure 1, routes 1 and 2)**
Latent infection and reactivation mechanisms	Viruses remain latent in the CNS and reactivation of the viruses causes epilepsies **(Figure 1, route 3)**
Parainfectious mechanisms	Viruses infect tissues outside the CNS and affect the CNS through immunological mechanisms **(Figure 1, routes 4 and 5)**
Modification of synaptic transmission mechanism	Febrile seizures cause long-lasting modification of synaptic transmissions, resulting in epilepsy **(Figure 1, route 6)**
Triggering mechanisms	Fever associated with infections or vaccinations incidentally triggers the onset of fever-sensitive epilepsy **(Figure 1, route 7)**

pathogenic mechanism in Rasmussen's encephalitis by Bien and coworkers [11]. The author has suggested a mechanism by which initial cellular autoimmunity is evolutionally affected by humoral autoimmunity [12,13]. These autoimmune mechanisms of epileptogenesis following infections are considered to be parainfectious mechanisms (Figure 1, routes 4 and 5). An overview of the relationship between infections and autoimmunity in Rasmussen's syndrome will be discussed in this article.

Febrile seizures are the most common convulsive events in humans, and 4–5% of the population has at least one febrile seizure during their life time [14]. Complicated febrile seizures during early childhood or infancy seem to be related to the development of mTLE with mesial temporal sclerosis [15], although it still not clear if complex febrile seizures are an epiphenomenon or a causative factor [16]. Febrile seizures result in persistent modification of neuronal excitability in limbic circuits in the developing rat brain, which lasts into adulthood [17]. This modification of synaptic transmission mechanism in the hippocampus after febrile seizures induced by infections may contribute to the development of mTLE (Figure 1, route 6).

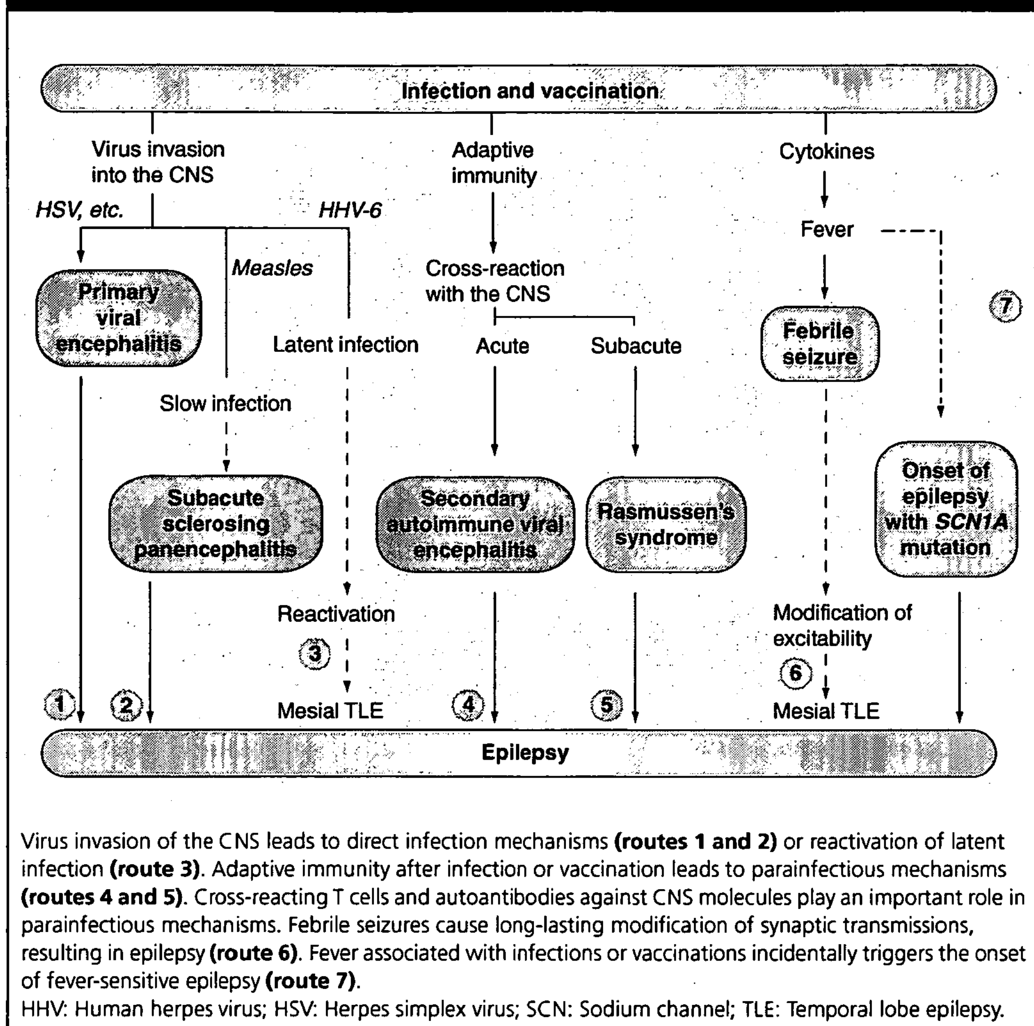
Recent advances in genetic molecular approaches have revealed several types of epilepsy related causally to gene mutations in patients with idiopathic etiologies [18]. Patients with sodium channel (SCN)1A mutations have frequent fever-induced seizures during common infections [19,20]. In patients with severe myoclonic epilepsy in infancy (SMEI), the onset of epilepsy usually follows fever-induced seizures associated with infections. In these patients, infections *per se* do not develop epilepsies but incidentally trigger the onset of epilepsies (Figure 1, route 7).

The relationship between vaccination and epilepsy is rarely documented, compared with the association between infections and epilepsies as described previously. Monitoring of post-vaccination adverse events for 8 years in Japan identified 269 patients manifesting neurological symptoms after diphtheria–tetanus–pertusis (DTP, 72 cases), measles (76 cases), rubella (20 cases), Japanese encephalitis (88 cases) or influenza vaccination (13 cases) [101]. These events include encephalitis (42 patients), convulsions (171 patients) and motor disturbance (5 patients). In these case reports, a causal relation with vaccination could not be denied, but confirmation of a definitive causality awaits future controlled studies. For DTP and mumps–measles–rubella (MMR) vaccinations, a transient increase in risk for febrile seizures has been reported, but not for afebrile seizures [21]. Analysis in the USA showed that inactivated influenza vaccination among children less than 2 years of age was safe, although two patients with first afebrile seizure after vaccination were reported [22]. Smallpox vaccination caused no neurological adverse events at rates above baseline estimates in the USA, although more than 20 serious adverse events were reported [23]. Owing to the small numbers of patients with neurological adverse events after vaccination, the causal relationship and hypothetic mechanisms underlying the development of epilepsy after vaccination have not been documented. However, the same mechanisms as infections might contribute to the development of epilepsy after vaccination (Figure 1). This review will focus on the frequency of vaccination as a possible causative factor of Rasmussen's syndrome.

Rasmussen's syndrome & causative factors (infections & vaccinations)

Rasmussen's syndrome is a prototype of epilepsy related causally to infections. In our epilepsy center, 38% of the patients have preceding infections, approximately 6% have vaccinations and 8.8% have head trauma as possible causative

Figure 1. Pathological routes leading from infections and vaccinations to the development of epilepsies.



Virus invasion of the CNS leads to direct infection mechanisms **(routes 1 and 2)** or reactivation of latent infection **(route 3)**. Adaptive immunity after infection or vaccination leads to parainfectious mechanisms **(routes 4 and 5)**. Cross-reacting T cells and autoantibodies against CNS molecules play an important role in parainfectious mechanisms. Febrile seizures cause long-lasting modification of synaptic transmissions, resulting in epilepsy **(route 6)**. Fever associated with infections or vaccinations incidentally triggers the onset of fever-sensitive epilepsy **(route 7)**.
HHV: Human herpes virus; HSV: Herpes simplex virus; SCN: Sodium channel; TLE: Temporal lobe epilepsy.

factors, and the frequencies are the same in patients with and without EPC (Table 2). Although vaccination was not reported as a causative factor in the study conducted at the Montreal Neurological Institute [6], two patients in the author's institute had Rasmussen's syndrome after vaccinations (Japanese encephalitis and MMR) [24]. CTLs activated by these infections or vaccinations may invade the CNS and cross-react with neurons expressing CNS molecules, resulting in neuronal apoptosis. Immunological studies are needed regarding cross-reactivity of patients' CTLs to confirm the definitive causal relationship between these possible causative factors (infections and vaccinations) and Rasmussen's syndrome [24]. Head trauma has also not been reported as a causative factor in patients of the Montreal Neurological Institute [6], but there are three patients who had a possible association with head trauma. As aseptic meningitis is sometimes observed after head trauma, this may facilitate the invasion of inflammatory T cells into the CNS. In patients with post-concussion syndrome after mild head injury, focal cortical dysfunction may occur in conjunction with the disruption of the blood–brain barrier [25].

Rasmussen's syndrome & CTLs

Lymphocytic infiltration, containing predominantly T cells and sparsely B cells, is found in surgically resected tissues from patients with Rasmussen's encephalitis [9]. Local CNS immune responses in Rasmussen's encephalitis include local clonal expansion of T cells responding to discrete antigen epitopes [26]. Destruction of neurons by granzyme B produced by CTLs has been demonstrated in resected tissues from

Table 2. Causative factors in 34 patients with Rasmussen's syndrome at the Shizuoka Institute of Epilepsy and Neurological Disorders.

	EPC type	Non-EPC type	Total
Age of onset (years)	6.3 ± 5.6	8.7 ± 8.0	7.4 ± 6.7
Preceding infections	8 (40.0%)	5 (35.7%)	13 (38.2%)
• Fever only	4	1	5
• Upper respiratory infection	2	1	3
• Influenza	1	2	3
• Mycoplasma	0	1	1
• Aseptic meningitis	1	0	1
Vaccination	1 (5.0%)	1 (7.1%)	2 (5.9%)
Head trauma	2 (10.0%)	1 (7.1%)	3 (8.8%)
None	9 (45.0%)	7(50.0%)	16(47.1%)
Total	20	14	34

EPC: Epilepsia partialis continua.

patients [11]. Peripheral blood lymphocytes in patients are sensitized to GluRε2 [13]. Heterogeneous autoantibodies against neuronal molecules (including GluR3, GluRε2, neuronal acetylcholine receptor α-7, and munc-18) and glial cells are detected in Rasmussen's syndrome [12,27]. Autoantibodies against GluRε2 have epitopes predominantly in intracellular domains and show epitope spreading evolutionally [12]. These data suggest that humoral autoimmunity mediated by autoantibodies is not the primary factor causing Rasmussen's syndrome, and that cellular autoimmunity mediated by CTLs plays a primary role in the development of this syndrome (Figure 2). For treatment, the author considers the choice of tacrolimus to suppress activation of T cells in patients with dominant hemisphere involvements [28], although functional hemispherectomy is the first choice in patients with nondominant hemisphere involvements. Autoantibodies against neural molecules seem to be produced after the onset of Rasmussen's syndrome, but those autoantibodies may affect the pathological processes after onset of Rasmussen's syndrome by the function proved in the studies of autoantibodies against GluR3 [10,29–33].

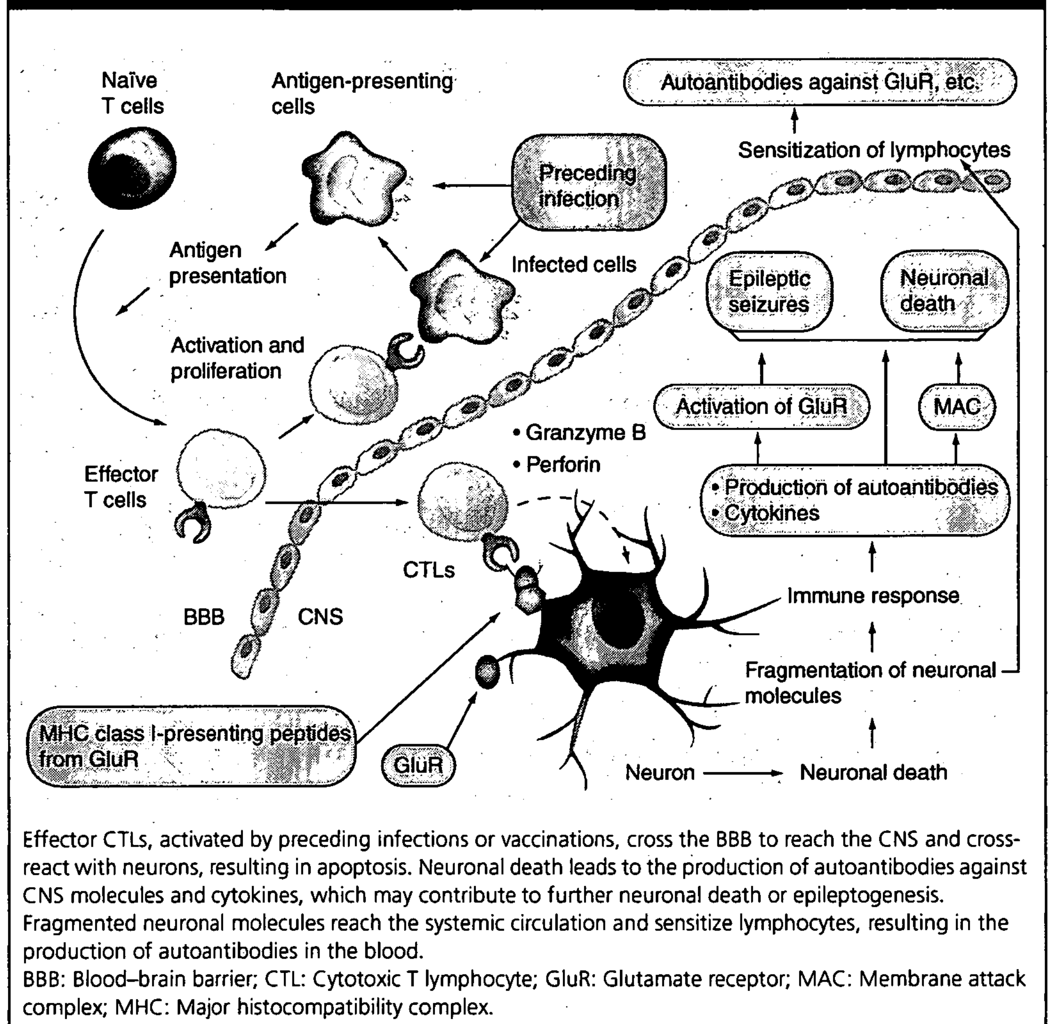
Rasmussen's syndrome & molecular mimicry determined by human leukocyte antigen

After the T-cell receptors (TCRs) on CTLs recognize both the human leukocyte antigen (HLA) class I molecule and its binding peptide expressed on antigen-presenting cells (APCs), CTLs are activated into effector CTLs that can invade the CNS easily. If, through molecular mimicry and TCR redundancy, the CTLs activated by microbial peptides are able to recognize the HLA class I molecule and its binding peptide from neuronal molecules on neurons, then the CTLs activated by infection may induce apoptosis of the neurons. Therefore, HLA class I is one of the key molecules determining the autoimmune mechanisms underlying the process from infection to the development of Rasmussen's syndrome.

The author studied the genotypes of HLA class I in 16 Japanese patients with Rasmussen's syndrome (9 with EPC type and 7 with non-EPC type) by polymerase chain reaction amplifications. HLA-A*2402 was a common genotype among Japanese people (36.5% of the Japanese population) and was found in 77.8% of EPC type patients ($p = 0.016$). HLA-A*0201 was found more frequently in non-EPC type patients (42.9%) compared with the Japanese population (10.7%) ($p = 0.033$). HLA-A*2601 was also found more frequently in non-EPC type patients (42.9%) compared with the Japanese population (11.3%) ($p = 0.038$). HLA-B*5201 was found more frequently in EPC type patients (33.3%) compared with the Japanese population (10.9%) ($p = 0.070$). HLA-B*4601 was found more frequently in non-EPC type patients (28.6%) compared with the Japanese population (3.4%) ($p = 0.025$).

HLA-B*4601 binds peptides with the following motif: x-[M]-x (5–7)-[Y/F] (x: free amino acid; M: methionine; Y: tyrosine; F: phenylalanine). Database analyses revealed the presence of this motif in various viral molecules and neural

Figure 2. Primary roles of cytotoxic T cells in the mechanism of developing Rasmussen's syndrome.



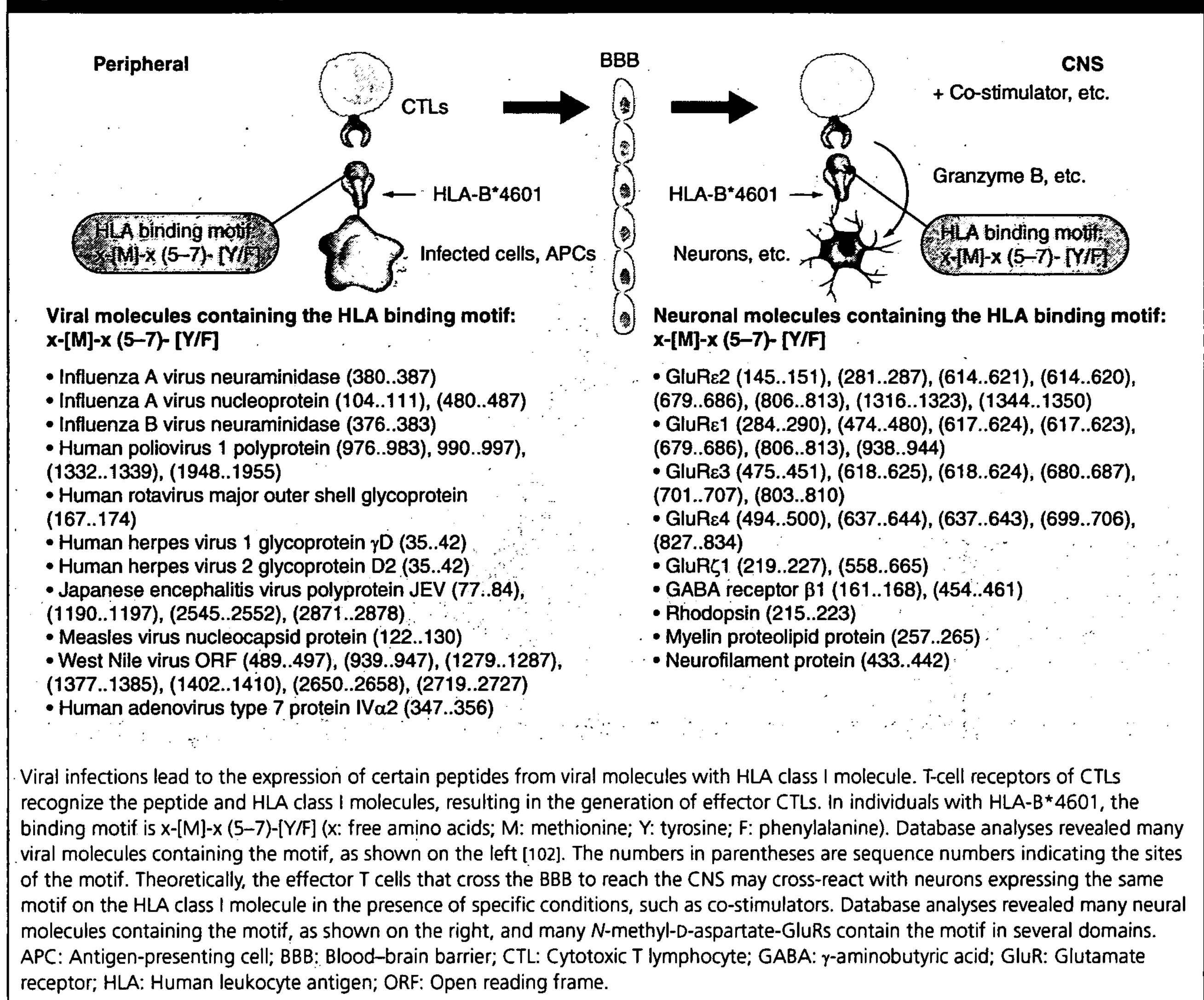
Effector CTLs, activated by preceding infections or vaccinations, cross the BBB to reach the CNS and cross-react with neurons, resulting in apoptosis. Neuronal death leads to the production of autoantibodies against CNS molecules and cytokines, which may contribute to further neuronal death or epileptogenesis. Fragmented neuronal molecules reach the systemic circulation and sensitize lymphocytes, resulting in the production of autoantibodies in the blood.

BBB: Blood–brain barrier; CTL: Cytotoxic T lymphocyte; GluR: Glutamate receptor; MAC: Membrane attack complex; MHC: Major histocompatibility complex.

molecules (Figure 3). If patients with HLA-B*4601 are infected by influenza A virus, the peptide from the neuraminidase of influenza A (AMTDWSGY) may bind to HLA-B*4601. TCRs of CTLs recognize HLA-B*4601 and the neuraminidase peptide (influenza A) on APCs, and are activated. Theoretically, the activated CTLs invade the CNS and may react with neurons possessing HLA-B*4601 and its binding peptide containing the (x-[M]-x (5–7)-[Y/F]) motif, due to degeneracy in TCR recognition, and may cause apoptosis of the neurons [34]. Peptides having the motif are found in various neuronal molecules including GluRε2 (IMEEYDWY, DMLSEHSF, IMVSVWAF etc.) and myelin proteolipid protein (FMIA ATYNF). Flexible interactions between TCRs of CTLs and various ligands on HLA-representing CNS molecules may facilitate broad CNS involvement of Rasmussen's syndrome. Alternatively, flexible interactions between CTLs and microbial molecules may enable aggravation of symptoms by infections or vaccinations. T-cell clones from Type 1 diabetes patients react with microbial mimicry peptides [34]. The cross-reactivity of CTLs from many Rasmussen's syndrome patients with microbial and neural molecules will be investigated in the future [24].

In patients with HLA-B*4601, neurons expressing *N*-methyl-D-aspartate (NMDA)-GluRs may be mainly involved in the interaction with CTLs as NMDA-GluRs possess the x-[M]-x (5–7)-[Y/F] motif in many domains (Figures 3 & 4). In patients with HLA-A*0201, the peptide from hemagglutinin of influenza A binds with the HLA-A*0201 molecule. CTLs activated by this complex may cross-react with neurons expressing NMDA-GluRs, γ-aminobutyric acid

Figure 3. Database analyses of the motif that binds to HLA-B*4601 in microbial and neural molecules.



Viral infections lead to the expression of certain peptides from viral molecules with HLA class I molecule. T-cell receptors of CTLs recognize the peptide and HLA class I molecules, resulting in the generation of effector CTLs. In individuals with HLA-B*4601, the binding motif is x-[M]-x (5–7)-[Y/F] (x: free amino acids; M: methionine; Y: tyrosine; F: phenylalanine). Database analyses revealed many viral molecules containing the motif, as shown on the left [102]. The numbers in parentheses are sequence numbers indicating the sites of the motif. Theoretically, the effector T cells that cross the BBB to reach the CNS may cross-react with neurons expressing the same motif on the HLA class I molecule in the presence of specific conditions, such as co-stimulators. Database analyses revealed many neural molecules containing the motif, as shown on the right, and many *N*-methyl-D-aspartate-GluRs contain the motif in several domains. APC: Antigen-presenting cell; BBB: Blood–brain barrier; CTL: Cytotoxic T lymphocyte; GABA: γ-aminobutyric acid; GluR: Glutamate receptor; HLA: Human leukocyte antigen; ORF: Open reading frame.

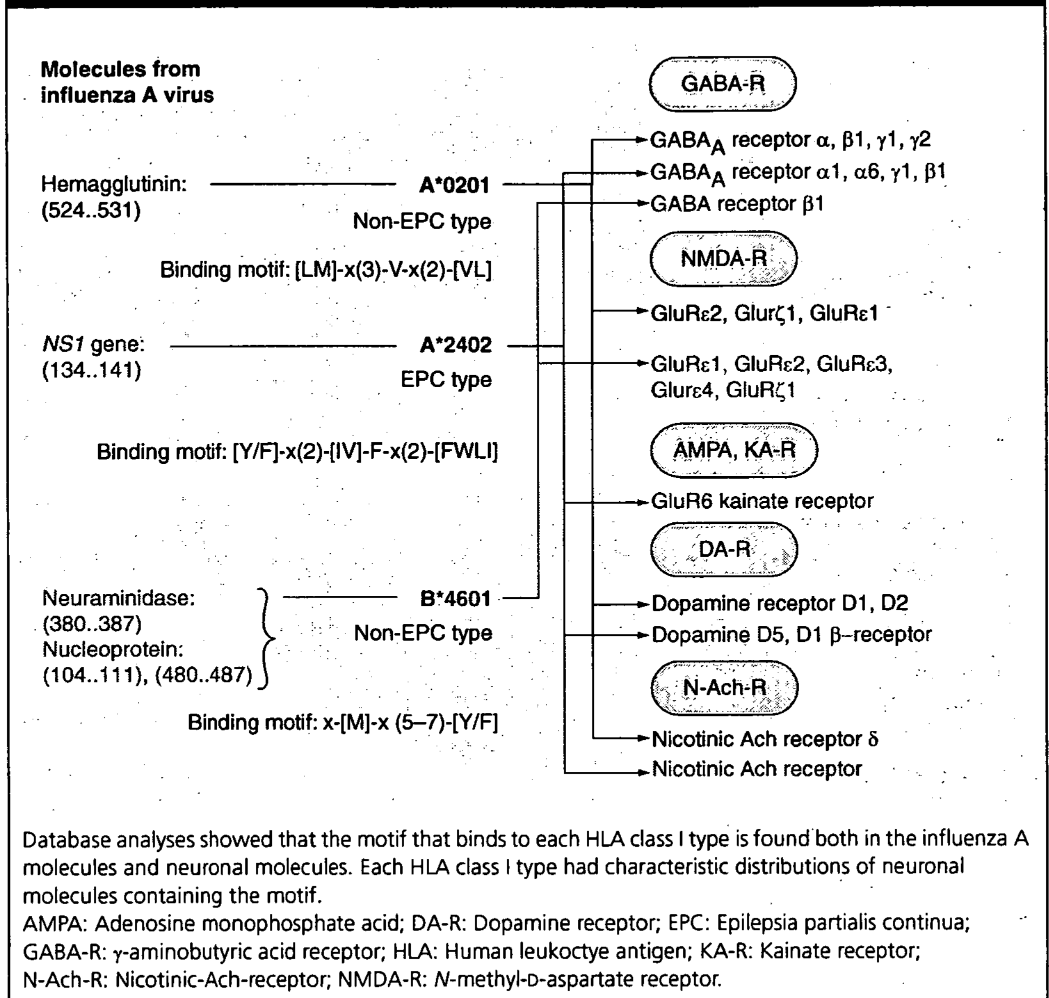
(GABA) receptors, dopamine receptors and other receptors, as these receptors have the motif that can bind with HLA-A*0201 (Figure 4). In patients with HLA-A*2402, the peptide from NS1 of influenza A binds with HLA-A*2402 and the resulting activated CTLs may cross-react with neurons expressing GABA receptors, dopamine receptors and adenosine monophosphate acid (AMPA) receptors, since these receptors, but not NMDA-GluRs, have the motif that can bind to HLA-A*2402. The HLA may explain the variable clinical symptoms manifested in patients infected by the same agent.

Acute viral encephalitis & autoimmunity

Acute viral encephalitis is an important disease causing infection-associated epilepsies. Pathological mechanisms of acute viral encephalitis are divided into the primary viral infection mechanism and the parainfectious secondary autoimmune mechanism. In order to improve prognosis of acute encephalitis, elucidation of autoimmune mechanisms in the secondary autoimmune encephalitis is desirable.

The author analyzed autoantibodies against GluRε2 in the cerebrospinal fluid (CSF) of 46 patients with acute encephalitis or encephalopathies, categorized into localized encephalitis (24 patients) and widespread encephalitis (22 patients) by clinical symptoms in the initial stage [12,35]. Patients with localized encephalitis showed psychic symptoms (illusions, anxiety and distraction etc.), solitary seizures and/or very mild impairment of consciousness in the initial stage, and gradually evolved to severe conditions with convulsive status and a loss of consciousness. The mean age of onset was 33.0 years ± 18.4. A total of 21 out of 24 patients had a better

Figure 4. Hypothetical cross-reactions between cytotoxic T cells expressing various molecules from influenza A virus and neurons expressing various neuronal molecules in individuals with different HLA class I genotypes.



Database analyses showed that the motif that binds to each HLA class I type is found both in the influenza A molecules and neuronal molecules. Each HLA class I type had characteristic distributions of neuronal molecules containing the motif.
AMPA: Adenosine monophosphate acid; DA-R: Dopamine receptor; EPC: Epilepsia partialis continua; GABA-R: γ-aminobutyric acid receptor; HLA: Human leukoctye antigen; KA-R: Kainate receptor; N-Ach-R: Nicotinic-Ach-receptor; NMDA-R: *N*-methyl-D-aspartate receptor.

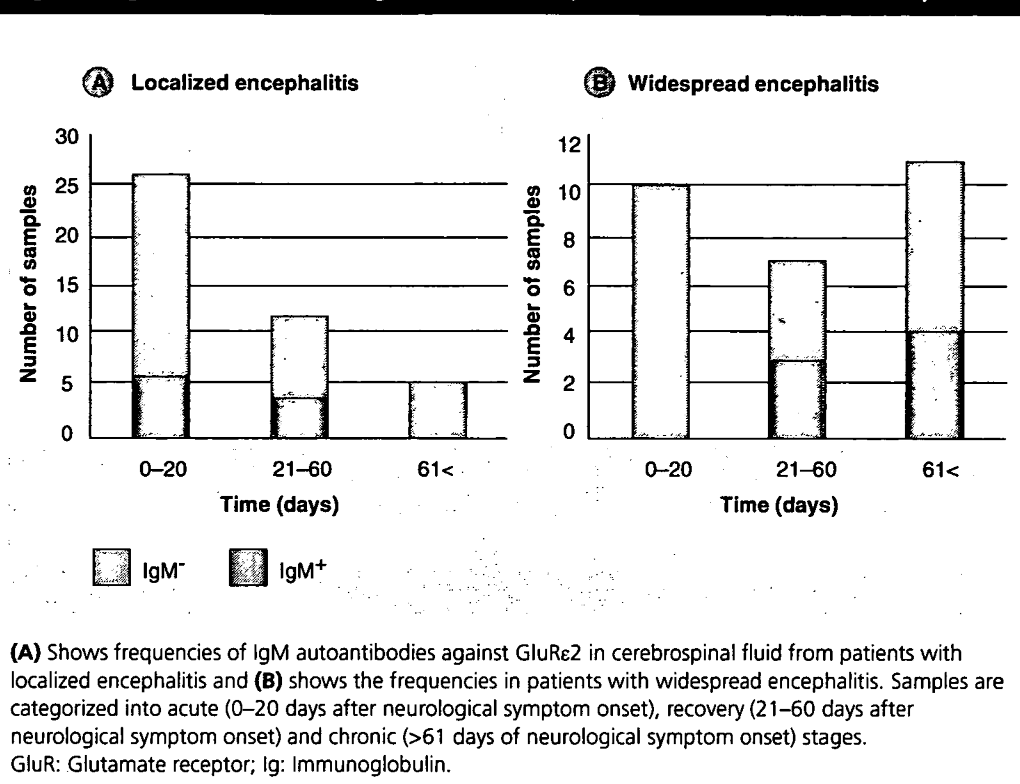
outcome, but three patients had sequelae. However, patients with widespread encephalitis showed a profound loss of consciousness and or convulsive status in the initial stage. The mean age of onset was 10.2 years ± 15.3. Six out of 22 patients were cured with better outcome, but 16 patients had sequelae.

In patients with localized encephalitis, immunoglobulin (Ig)M autoantibodies against GluRε2 tended to appear in CSF in the acute stage (0–20 days after onset of neurological symptoms) or recovery stage (21–60 days after onset of neurological symptoms) of encephalitis (Figure 5). In patients with widespread encephalitis, IgM autoantibodies against GluRε2 in CSF tended to appear in the recovery stage (21–60 days after onset of neurological symptoms) or chronic stage (>60 days after onset of neurological symptoms) of encephalitis. These data may suggest that GluR autoimmunity contributes to the onset of localized encephalitis and development of sequelae in widespread encephalitis. In patients with widespread encephalitis, the presence of autoantibodies against GluRε2 in CSF correlates significantly with onset of epilepsy after encephalitis ($p = 0.01$, Fisher's exact probability test) and with mental impairment ($p = 0.03$, Mann–Whitney U test). Therefore, autoantibodies against GluRε2 may have a causal relationship with epileptogenesis after widespread encephalitis.

Hypothetical common autoimmune processes leading from neuronal damage to neurological sequelae

In neurological diseases, irrespective of the etiological factors (infections and vaccinations etc.), common symptoms and sequelae (such as

Figure 5. IgM autoantibodies against GluRε2 in patients with acute viral encephalitis.



(A) Shows frequencies of IgM autoantibodies against GluRε2 in cerebrospinal fluid from patients with localized encephalitis and **(B)** shows the frequencies in patients with widespread encephalitis. Samples are categorized into acute (0–20 days after neurological symptom onset), recovery (21–60 days after neurological symptom onset) and chronic (>61 days of neurological symptom onset) stages.
GluR: Glutamate receptor; Ig: Immunoglobulin.

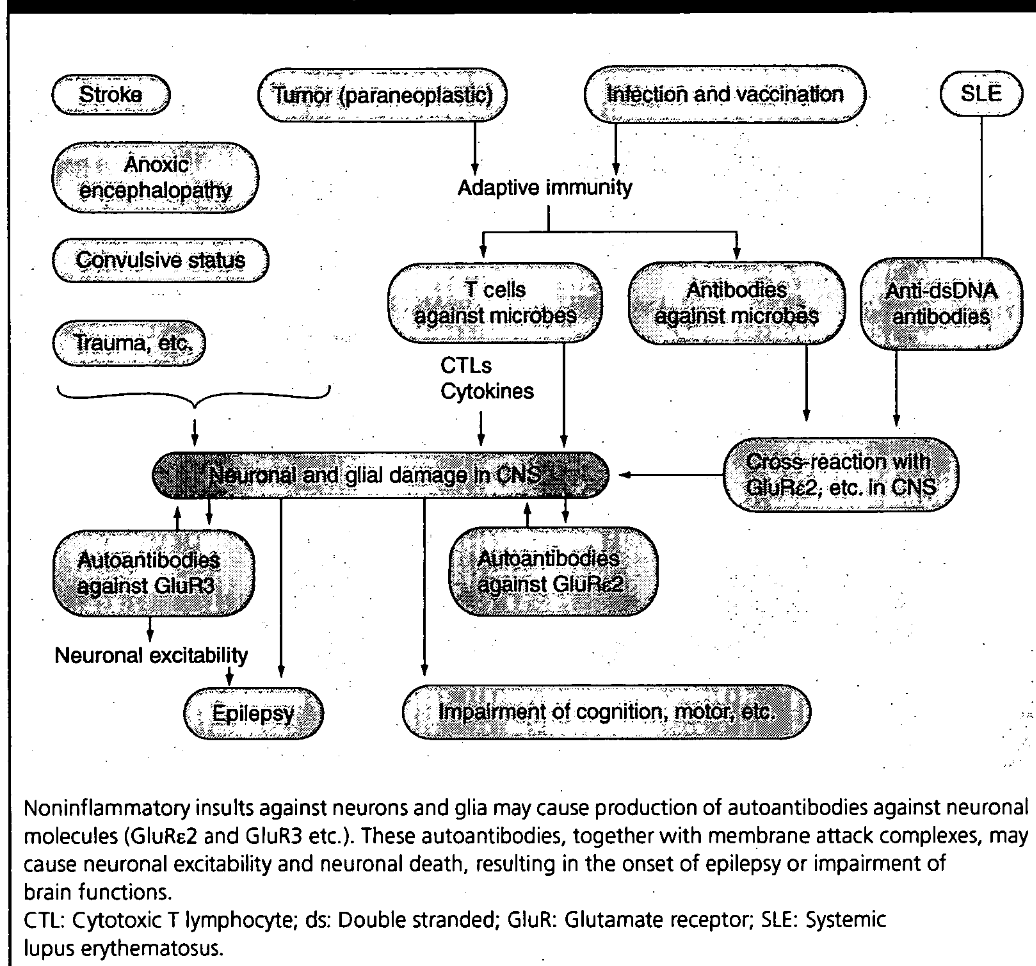
impairment of consciousness, epilepsies, cognitive impairment, and motor disturbance) tend to occur. For instance, while viral infections cause acute viral encephalitis, malignant extracerebral tumors may sometimes also cause paraneoplastic acute or subacute encephalitis. Anoxic encephalopathy in neonatal periods causes West syndrome and CNS infections also cause West syndrome as sequelae. It is postulated that autoimmune processes, including autoantibodies against neural molecules, may contribute to the subsequent processes after acute insults to the CNS (stroke, trauma, anoxic encephalopathy and convulsive status etc.), resulting in autoimmune neuronal damages (Figure 6). Autoantibodies against GluR3 and GluRε2 are found also in patients with various neurological diseases [12,36]. Several pathological roles of autoantibodies against GluR3 have been reported, including excitotoxicity [29], complement-dependent cell death [30] and complement-containing membrane attack complex (MAC) formation [31,32], although the induction of currents through GluR was controversial [33,37]. The MAC is composed of several complements and appears to induce functional pores in the cell membrane, leading to depolarization and osmotic lysis of neurons. These data show that autoantibodies can directly cause impairment of neural functions. Further studies are needed for a better understanding of autoimmune processes in neurological diseases.

Conclusion

Mechanisms leading from infection or vaccination to the development of epilepsy are not fully understood, but the following mechanisms are proposed: direct infection, reactivation of latent infection, parainfectious mechanism, modification of synaptic transmission and triggering mechanism.

In Rasmussen's syndrome, a prototype of parainfectious autoimmune epilepsy, 44% of patients have preceding infections or vaccinations and 8.8% of patients have head trauma as possible causative factors; thus, the important roles of CTLs and autoantibodies against neural molecules are unveiled. Several HLA class I genotypes are found at significantly higher rates in patients with Rasmussen's syndrome, compared with the Japanese population. Motif analyses of HLA-binding peptides in microbial molecules and neural molecules postulated that CTLs activated by infections may cross-react with neuronal molecules due to molecular mimicry and TCR degeneracy. HLA type may affect the clinical phenotypes of autoimmune-mediated epilepsies.

Figure 6. Hypothesis of common autoimmune processes leading from neuronal damage to neurological sequelae.



Noninflammatory insults against neurons and glia may cause production of autoantibodies against neuronal molecules (GluRε2 and GluR3 etc.). These autoantibodies, together with membrane attack complexes, may cause neuronal excitability and neuronal death, resulting in the onset of epilepsy or impairment of brain functions.
CTL: Cytotoxic T lymphocyte; ds: Double stranded; GluR: Glutamate receptor; SLE: Systemic lupus erythematosus.

In acute viral encephalitis, GluR autoimmunity may contribute to the onset of localized encephalitis as well as the development of sequelae in widespread encephalitis. Autoantibodies against GluRε2 may have a causal relation with epileptogenesis following widespread encephalitis.

Noninflammatory insults, such as stroke and anoxic encephalopathy, may precipitate the onset of symptomatic epilepsies due to neuronal injuries. Autoimmune mechanisms, including autoantibodies against neuronal molecules and complement MAC, may contribute to these processes.

Future perspective

Recently, brain inflammation in epilepsy has been highlighted as a common factor in epilepsies [38]. Elucidation of the autoimmune mechanisms and verification of molecular mimicry between microbial and neuronal molecules in Rasmussen's syndrome may facilitate understanding of the autoimmune process, by which infections or vaccinations lead to the development and aggravation of symptomatic epilepsies other than Rasmussen's syndrome. Prevention of epilepsy onset after CNS infections and novel 'pinpoint' immunological treatment of epilepsies after onset may become possible in the future. Antigen-specific immuno-potentiating or -suppressive therapy with altered peptide ligands may become available for the treatment of some symptomatic epilepsies [39]. For the treatment of West syndrome, adrenocorticotropic hormone is currently used, but other specific immunological treatments may be possible after elucidation of its autoimmune mechanisms in the future.

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Executive summary

Introduction

- Childhood-onset epilepsy may begin after an infection episode or following vaccination, albeit rarely. After the onset of epilepsy, status epilepticus tends to occur under febrile conditions triggered by infections or vaccinations.
- A total of 12% of admitted cases of intractable epilepsy were attributed to CNS infections (acute viral encephalitis and bacterial meningitis etc.).
- The mechanisms by which infections or vaccinations cause epilepsies are not fully understood, but the following mechanisms are possible: direct infection, reactivation of latent infection, parainfectious mechanism, modification of synaptic transmission and triggering mechanism.
- Few data are available on the relationship between vaccination and epilepsy.

Rasmussen's syndrome & causative factors (infections & vaccinations)

- A total of 44% of patients have preceding infections or vaccinations and 8.8% have head trauma as possible causative factors.
- Two patients were encountered who developed Rasmussen's syndrome after vaccination (Japanese encephalitis vaccine and mumps–measles–rubella vaccine).

Rasmussen's syndrome & cytotoxic T cells

- Cytotoxic T lymphocytes (CTLs) may play a primary role in the development of Rasmussen's syndrome.

Rasmussen's syndrome & molecular mimicry defined by human leukocyte antigen

- T-cell receptors (TCRs) on CTLs recognize both human leukocyte antigen (HLA) class I and its binding peptide to induce apoptosis of targeted cells.
- The genotypes of HLA class I were studied in 16 Japanese patients with Rasmussen's syndrome (9 with epilepsia partialis continua, and 7 without epilepsia partialis continua). HLA-A*2402, HLA-A*0201, HLA-A*2601, HLA-B*5201 and HLA-B*4601 were found at significantly higher rates in the patients compared with the healthy Japanese population.
- Motif analyses of HLA-binding peptides in microbial and neural molecules suggest that cytotoxic T cells activated by infections may cross-react with neurons owing to molecular mimicry and TCR degeneracy.
- HLA type may affect the pattern of CNS involvement in CTL activity and subsequent clinical phenotypes in autoimmune-mediated epilepsies.

Acute viral encephalitis & autoimmunity

- Pathological mechanisms of acute viral encephalitis are divided into the primary viral infection mechanism and the secondary parainfectious autoimmune mechanism.
- Clinically, acute encephalitis is divided into two categories: localized encephalitis and widespread encephalitis. In the initial stage, patients with localized encephalitis showed psychic symptoms, solitary seizures and/or very mild impairment of consciousness. Patients with widespread encephalitis showed profound loss of consciousness and/or convulsive status.
- In patients with localized encephalitis, immunoglobulin (Ig)M autoantibodies against glutamate receptor (GluR)ε2 tended to appear in cerebrospinal fluid (CSF) in the acute or recovery stage and may contribute to disease onset.
- In patients with widespread encephalitis, IgM autoantibodies against GluRε2 tended to appear in CSF in the chronic or recovery stage and may contribute to epileptogenesis and mental retardation as sequelae.

Hypothesis of common autoimmune processes leading from neuronal damage to neurological sequelae

- The author postulates that autoimmune processes, including autoantibodies against neuronal molecules and membrane attack complex, may contribute to the development of sequelae following acute insults (both inflammatory and noninflammatory) to the CNS, including the development of epilepsy.

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Vaccination and infection as causative factors in Japanese patients with Rasmussen syndrome: Molecular mimicry and HLA class I

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Abstract
Rasmussen syndrome is an intractable epilepsy with a putative causal relation with cellular and humoral autoimmunity. Almost half of the patients have some preceding causative factors, with infections found in 38.2%, vaccinations in 5.9% and head trauma in 8.9% of Japanese patients. In a patient with seizure onset after influenza A infections, cross-reaction of the patient's lymphocytes with GluRε2 and influenza vaccine components was demonstrated by lymphocyte stimulation test. Database analyses revealed that influenza A virus hemagglutinin and GluRε2 molecules contain peptides with the patient's HLA class I binding motif (HLA − A*0201). The relative risks of HLA class I genotypes for Rasmussen syndrome are 6.1 (A*2402), 6.4 (A*0201), 6.3 (A*2601) and 11.4 (B*4601). The relative risks of HLA class I-A and B haplotypes are infinity (A*2601 + B*5401), 21.1 (A*2402 + B*1501), 13.3 (A*2402 + B*4801) and 5.1 (A*2402 + B*5201). Some alleles and haplotypes of HLA class I may be the risk factors in Japanese patients. Cross-reactivity of cytotoxic T lymphocytes may contribute to the processes leading from infection to the involvement of CNS.

Keywords: *Rasmussen syndrome, HLA, cytotoxic T cells, influenza, vaccination, epilepsy*

Introduction

Rasmussen's encephalitis is a slowly progressive, autoimmune-mediated chronic inflammatory disease of the CNS. The mean age of onset is 7.4 years. The disease may be preceded by some causative factors including infection, and the initial seizure episode manifests various forms such as partial onset generalized tonic-clinic (pGTC) seizures (30%), focal motor seizures (26%) and complex partial seizures (CPS) (26%) (Andermann 1991). One third of the patients have preceding infections within 1 month before onset. Patients with typical Rasmussen's encephalitis manifest frequent intractable partial motor seizures in the acute phase, characteristically epilepsia partialis continua (EPC) (56%). Patients begin to manifest EPC 1.8 years after the onset of epilepsy, but the seizure frequency decreases markedly in the residual stage (Andermann 1991, Bien et al. 2002b) (Figure 1). Patients in the residual stage are affected by hemiplegia (96%), mental deterioration (85%), visual field defect (49%), and cortical sensory defect (29%) (Andermann 1991). Histological examination reveals infiltration of T lymphocytes and microglia cells, astrocytosis, and neuronal loss in the lesion (Aguilar and Rasmussen 1960, Andermann 1991, Farrell et al. 1995). Functional hemispherectomy is the only reliable therapy when the non-dominant side is involved, but hemiparesis and hemianopsia are

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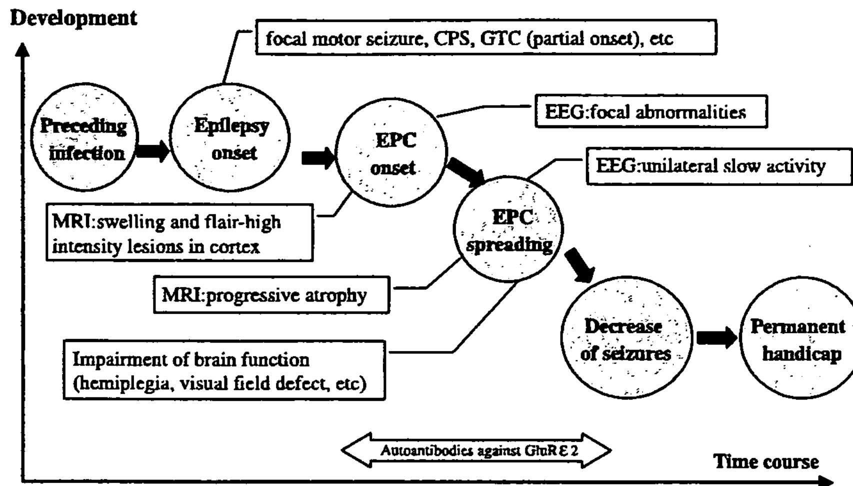

Figure 1. Schematic presentation of the typical clinical course of Rasmussen syndrome. After preceding infections, epileptic seizures (focal motor seizures or partial onset generalized tonic clonic convulsions, etc.) appear, followed by progressive deterioration of clinical symptoms and aggravation of EEGs and MRI abnormalities. In the residual stage, permanent handicap is observed, but epileptic seizures decrease. GTC, generalized tonic clonic seizure; GluR, glutamate receptor; EPC, epilepsia partialis continua.

unavoidable after operation. When the dominant side is affected, there is no effective therapy.

Viral infections were implicated as the causal agent of Rasmussen's encephalitis in early investigations (Andermann 1991), and direct infection by several candidate viruses (CMV, tick-borne encephalitis virus, etc.) has been postulated as one of the possible mechanisms causing the disease (Takahashi 2006) (Figure 2).

Rogers et al. (1994) reported glutamate receptor 3 (GluR3) as an autoantigen in Rasmussen's encephalitis, and proposed autoantibodies against this molecule to be one cause of Rasmussen's encephalitis (humoral autoimmune hypothesis). Their report

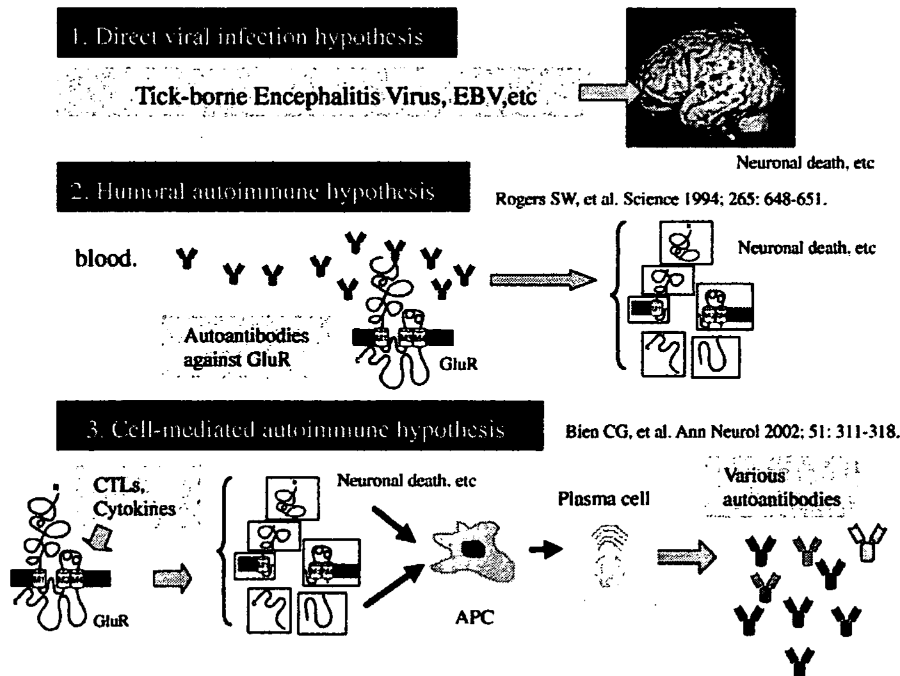

Figure 2. Hypotheses of autoimmune pathologies in Rasmussen syndrome. Direct viral infection hypothesis presumes that viral particles invade the brain and cause neuronal death, etc. Humoral autoimmune hypothesis supposes that autoantibodies against neural molecules (GluR3, etc.) play the primary roles to develop Rasmussen syndrome. Cell-mediated autoimmune hypothesis speculates that CTLs and/or cytokines from $CD4^{+}T$ cells play the primary roles. EBV, Ebstein bar virus; GluR, glutamate receptor; APC, antigen-presenting cells.

introduced a new perspective of autoimmune-mediated mechanism to the field of epilepsy. Several pathological roles of autoantibodies against GluR3 have been demonstrated, including excitotoxicity (Levite and Hermelin 1999), complement-dependent cell death (He et al. 1998) and membrane attack complex (MAC) (Xiong and McNamara 2002, Xiong et al. 2003) have been shown, although induction of currents through GluR remains controversial (Twyman et al. 1995, Watson et al. 2004). The MAC is composed of several complements, and appears to induce functional pore in cell membrane, leading to depolarization and osmotic lysis of neurons. These data indicate that autoantibodies can directly cause impairment of neural functions.

On the other hand, Bien et al. (2002a) proposed the destruction of neurons by cytotoxic T cells (CTLs) as a new pathogenic mechanism in Rasmussen's encephalitis (cell-mediated autoimmune hypothesis). Lymphocytic infiltration containing predominantly T cells and sparsely B cells can be observed in surgically resected tissues from patients with Rasmussen's encephalitis (Farrell et al. 1995), and local CNS immune responses in Rasmussen's encephalitis include clonal expansion of T cells responding to discrete antigen epitopes (Li et al. 1997). Peripheral blood lymphocytes from patients are sensitized to GluRε2 (Takahashi et al. 2005). Heterogeneous autoantibodies against neuronal molecules (including GluR3, GluRε2, neuronal acetylcholine receptor alpha7, and munc-18) (Yang et al. 2000, Watson et al. 2001, Takahashi et al. 2003) and glial cells (Roubertie et al. 2005) are detected in Rasmussen syndrome. Autoantibodies against GluRε2 have epitopes predominantly in intracellular domains, and show epitope spreading evolutionally (Takahashi et al. 2003). We postulated that the autoimmune-mediated mechanism for the development of Rasmussen syndrome involves primarily cellular autoimmunity mediated by cytotoxic T cells, and evolutionarily involves humoral autoimmunity mediated by autoantibodies (Takahashi et al. 2003, 2005). These autoimmune mechanisms of epileptogenesis after infections can be classified as parainfectious mechanisms (Figure 3) (Takahashi 2006).

Causative factors of in patients with Rasmussen syndrome

In our epilepsy center, 44% of Japanese patients with Rasmussen syndrome had prior infections or vaccinations, and approximately 8% had head trauma as preceding causative factors, and the frequencies are almost same in patients with EPC and in those without EPC (Table I) (Takahashi 2006). The microbes causing infections were not identified in the majority of patients, except in three patients infected by influenza virus and one patient by mycoplasma. Likewise, in the study conducted at Montreal Neurological Institute, the causative microbes were not documented except measles (encephalitis) and varicella (Andermann 1991).

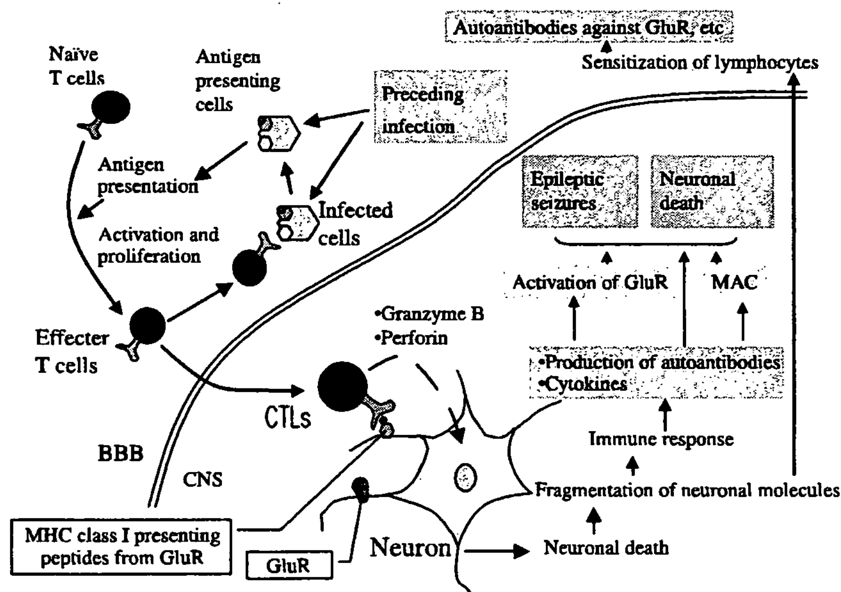

Figure 3. Involvements of CTLs and autoantibodies in the hypothetic mechanisms of the development of Rasmussen syndrome. Effecter T cells activated by preceding infections or vaccinations reach the CNS by crossing blood brain barrier, and cross-react with neurons, etc. resulting in apoptosis. Neuronal death leads to production of autoantibodies against CNS molecules and cytokines, which might contribute to the further neuronal death or epileptogenesis. Fragmented neuronal molecules reach systemic circulation and sensitize lymphocytes, resulting in production of autoantibodies in the blood. BBB, blood brain barrier; CNS, central nervous system; CTLs, cytotoxic T cells; GluR, glutamate receptor; MAC, membrane attack complex.