2. Materials and methods

2.1. Influenza virus-associated encephalopathy (IE)

Informed consent was obtained from the parents of the patients enrolled in this study. Serum samples were obtained from 34 patients with IE, 16 with IFS, and 19 with Flu on admission to our hospital and eleven research cooperation hospitals in Japan, from December 1999 to March 2005 (Table 1). We divided the patients with IE into three groups, i.e., those who had no sequelae (Group A, $n=18$), those who had neurological sequelae (Group B, $n=10$), and those who were deceased (Group C, $n=6$). The criteria for the diagnosis of IE were: (1) clinical symptoms and signs compatible with acute encephalitis/encephalopathy (defined as a febrile disorder with alteration of consciousness and slow activity on electroencephalography lasting for more than 24 h after an acute onset), and no bacteria or fungi on CSF culture, with all other neurological, vascular, endocrine, toxic and drug-induced disorders having been excluded, and (2) isolation of the influenza virus from the throat, or a four-fold increase in the antibody titer determined by means of the hemagglutination inhibition test and/or virus antigen detection in the throat with the latex agglutination test. The day of onset of neurological symptoms was considered as the first day of illness. Serum samples were taken from the patients with IE on days 1.2±0.5 (range, 1–3) of the illness.

2.2. Influenza virus-associated febrile seizures (IFS)

Influenza virus-associated febrile seizures were defined as seizures with fever and impaired consciousness lasting less than 24 h without neurological sequelae, and influenza virus infection was proven by the above-mentioned method. Sixteen patients enrolled with IFS (10 males and 6 females, aged from 11 months to 10 years: mean, 5.2 years) (Table 1). The day of seizure onset was considered as the first day of illness. Serum samples were taken from the patients with IFS on days 1.1±0.3 (range 1–2) of the illness.

2.3. Influenza virus infection without complications (Flu)

Nineteen patients enrolled with influenza virus infections had fever and upper respiratory symptoms (9 males and 10 females, aged from 14 months to 9 years: mean, 5.4 years) (Table 1). Influenza virus infection was proven by the latex agglutination test. The day of fever onset was considered as the first day of illness. Serum samples were taken from these patients on days 2.1±1.1 (range, 1–4) of the illness.

2.4. Epstein–Barr virus (EBV) infection

The disease control subjects of viral infections were 7 patients who had EBV infection (three males and four females, aged from 2 to 12 years: mean, 5.9 years) (Table 1). The diagnosis was based on clinical presentation of a sore throat, fever, and bilateral cervical lymphadenopathy accompanied by atypical lymphocytes in the peripheral blood. All patients were positive for IgM and IgG antibodies to the EBV capsid antigen and negative for antibodies to the EBV nuclear antigen during the acute stage. The day of fever onset was considered as the first day of illness. Serum samples were taken from the patients with EBV infection on days 6.8±3.8 (range, 2–13) of the illness.

2.5. Control subjects

The control subjects were 25 healthy children (14 males and 11 females, aged from 3 months to 11 years: median, 5.4 years).

2.6. Determinations of the sCD40L concentration and platelet number

The serum concentrations of sCD40L were measured with a sandwich-type ELISA kit (R&D Systems, Minneapolis, MN). The detection limit was 4.2 pg/ml. Platelet number was assessed in the blood samples by standard automated techniques.

Table 1
Data for subjects

Group	Number	Age (years) (range)	Sex male:female	Type
IE-Group A	18	5.8±4.5 (6 months–13 years)	12:6	H1N1=1, H3N2=6, A=5, B=6
IE-Group B	10	5.9±6.6 (9 months–19 years)	4:6	H1N1=2, H3N2=4, A=3, B=1
IE-Group C	6	5.4±3.8 (2 years–10 years)	5:1	H3N1=5, A=1
IFS	16	5.2±3.0 (11 months–10 years)	10:6	A=10, B=6
Flu	19	5.4±3.1 (14 months–9 years)	9:10	A=13, B=6
EBV infection	7	5.9±3.2 (2 years–12 years)	3:4	
Controls	25	5.4±3.2 (3 months–11 years)	14:11	

IE=influenza virus-associated encephalopathy; IFS=Influenza virus-associated febrile seizures; Flu=influenza virus infection without complications; EBV=Epstein-Barr virus.
Group A=patients without sequelae; Group B=patients with sequelae; Group C=deceased patients.

2.7. Statistical analysis

All values are the means±S.D. Differences in the results between groups were analyzed by the Mann–Whitney *U*-test, with a *p*-value of less than 0.05 being taken as significant. Correlations were analyzed with Spearman's rank correlation coefficient test.

3. Results

The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 1. The serum sCD40L concentrations of IE-Group A, IE-Group B, IE-Group C, and IFS were significantly lower than those of Flu ($p=0.0015$, $p=0.0002$, $p=0.0034$, and $p=0.0123$, respectively), EBV infection ($p=0.0105$, $p=0.0047$, $p=0.00269$, and $p=0.0235$, respectively) and controls ($p=0.0003$, $p<0.0001$, $p=0.0004$, and $p=0.001$, respectively). In the IE group, the serum sCD40L concentration of Group C was significantly lower than those of Groups A ($p=0.0077$) and B ($p=0.0409$), and that of Group B was significantly lower than that of Group A ($p=0.044$). The serum sCD40L concentration of IE-Group C was significantly lower than that of IFS ($p=0.0066$). There were no significant differences in serum sCD40L concentrations among Flu, EBV infection, and controls.

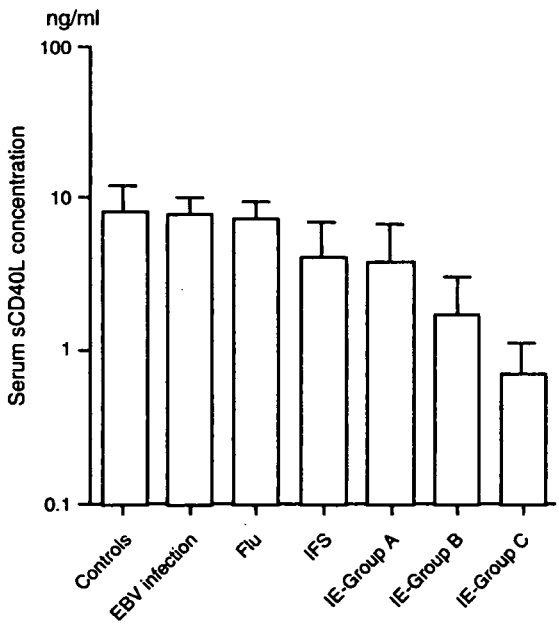

Fig. 1. The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means+1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The serum sCD40L concentration of controls was 8.23±3.61 ng/ml, EBV infection, 8.00±1.93 ng/ml, Flu, 7.49±1.96 ng/ml, IFS, 4.15±2.68 ng/ml, IE-Group A, 3.85±2.91 ng/ml, IE-Group B, 1.73±1.36 ng/ml, and IE-Group C, 0.697±0.428 ng/ml, respectively.

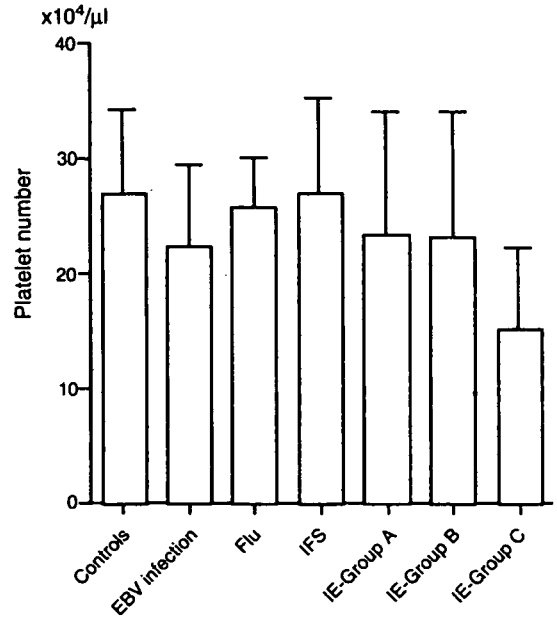

Fig. 2. The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means+1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The platelet number of controls was $27.0\pm7.5\times10^4$/μl, EBV infection, $22.9\pm6.6\times10^4$/μl, Flu, $25.9\pm4.4\times10^4$/μl, IFS, $27.0\pm8.4\times10^4$/μl, IE-Group A, $23.4\pm10.9\times10^4$/μl, IE-Group B, $23.3\pm11.0\times10^4$/μl, and IE-Group C, $15.2\pm7.2\times10^4$/μl, respectively.

The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 2. The platelet number of IE-Group C was significantly lower than those of IFS ($p=0.0078$), Flu ($p=0.0233$), and controls ($p=0.0045$) (Fig. 2). There were no significant differences in platelets numbers among IE-Group A, IE-Group B, IFS, Flu, EBV infection, and controls.

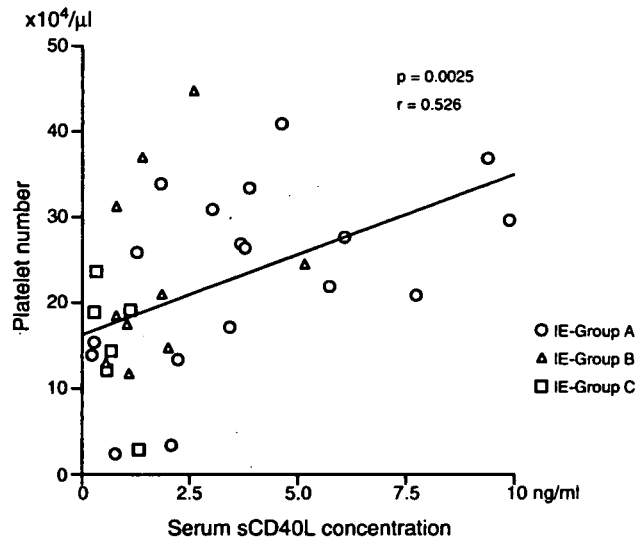

Fig. 3. Relationship between the serum concentration of sCD40L and platelets number in IE groups.

In the influenza virus infection group, including Flu, IFS, and IE, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0006$, $r=0.465$). In the entire IE group, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0025$, $r=0.526$) (Fig. 3). However, In IE groups with a poor prognosis, including Groups B and C, the serum concentrations of sCD40L were not correlated with the platelet numbers ($p=0.2273$, $r=0.312$).

4. Discussion

The CD40–CD40L interaction is pivotal in the cellular immune response. CD40L was first described as an antigen, which is expressed on activated CD4 T cells, and CD40L interacts with CD40 expressed on B cells and induces the class switch [6]. A broader role for CD40 signaling was revealed through the finding that CD40 is expressed on numerous cell types, including monocytes/macrophages, dendric cells, fibroblasts keratinocytes, endothelial cells, and vascular smooth muscle cells [18–20]. Stimulation of these cell types through CD40 induces cell functions which contribute to inflammatory responses, including the expression of adhesion molecules, and also the release of proinflammatory cytokines, such as IL-6, IL-1β, IL-8, IL-12, and TNF-α [19–24]. Serum and plasma sCD40L levels are elevated in systemic lupus erythematosus [25,26], rheumatoid arthritis and associated vasculitis [27], mixed connective tissue disease [28], systemic sclerosis [29], cystic fibrosis [30], advanced squamous cancer of the lung [31], autoimmune thrombocytopenic purpura [32], and chronic idiopathic urticaria [33]. In these disorders, the importance and immunological mechanism of the CD40–CD40L interaction have been discussed. However, >95% of plasma sCD40L is derived from platelets [34]. Therefore, it is likely that the sCD40L level depends on platelet number and activation. In fact, serum sCD40L concentrations were well correlated with platelet numbers and thrombopoiesis in patients undergoing allogenic stem cell transplantation [6].

Our present study demonstrated that serum sCD40L concentrations on the day of hospitalization were well correlated with the severity and prognosis of IE. A previous paper reported that a decrease in platelet number was correlated with a poor prognosis of IE [8]. However, the platelet number reported in the paper made no mention of the sampling time [8]. Platelet number tends to decrease gradually in IE, and therefore, the platelet numbers of patients with IE who had a poor prognosis on the day of hospitalization were often normal [12,17]. Our present study demonstrated that the platelet number of IE-Group B on the day of hospitalization was not decreased, while the serum sCD40L concentration was decreased. It is likely that the serum sCD40L concentration is decreased ahead of platelet number in IE with a poor prognosis. The condition of patients with severe IE often deteriorates rapidly within 2 days after the development of neurological signs [8]. Therefore, it is important to predict a poor prognosis in IE as soon as possible.

Why are serum sCD40L concentrations decreased ahead of platelet numbers? sCD40L is mainly released by activated platelets [28,34]. Apoptosis under hypercytokinemia has been suggested as a possible mechanism of IE [35,36]. In IE, the function of platelets may become poor and inactive, and then, the platelet number may decrease through megakaryocyte apoptosis. The definite mechanism leading to the decrease of platelet number in IE remains unclear.

In summary, serum sCD40L levels on the day of hospitalization were well correlated with the outcome of IE compared to the platelet numbers. Our findings suggest that the decreased serum sCD40L levels in IE are important for predicting a poor prognosis in the early phase.

Acknowledgments

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Analysis of cytokine levels in cerebrospinal fluid in mumps meningitis: Comparison with echovirus type 30 meningitis

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Abstract

Background: It is unclear whether or not the CSF cytokine profiles in viral meningitis differ with the kind of causative virus.
Methods: We measured the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, and IL-10 in CSF during the acute stage in 15 children with mumps meningitis (MM), and 34 with echovirus type 30 meningitis (EM).
Results: The CSF IFN-γ, IL-2, IL-6, and IL-10 levels were elevated in MM, and the CSF IFN-γ, IL-2, and IL-6 levels were elevated in EM. The CSF IFN-γ, IL-2, and IL-10 levels in MM were significantly higher than those in EM ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively). The CSF IL-6 levels in EM were significantly higher than those in MM ($p = 0.0255$). The CSF TNF-α and IL-4 levels were not elevated in MM or EM. In MM, the IL-6 level was correlated with the IL-2 and IL-10 levels in CSF ($p = 0.0347$ and $p = 0.0120$, respectively). In EM, the IFN-γ level was correlated with the IL-10 level in CSF ($p = 0.0002$).
Conclusion: CSF cytokine profiles in MM were different from those in EM. Therefore, it is likely that the pathogenesis of MM is different from that of EM.

Keywords: Cerebrospinal fluid; Cytokine; Echovirus type 30 meningitis; Mumps meningitis

1. Introduction

Aseptic meningitis is not rare in childhood. Enterovirus and mumps virus are frequently the causative agents of aseptic meningitis [1]. There have been many previous papers on cerebrospinal fluid (CSF) cytokine profiles in aseptic meningitis [2–6]. However, the causative viruses were not identified in most of the previous studies. There have been few papers on CSF cytokines in meningitis caused by a single viral agent, and all of these papers were related to enteroviruses [7–9]. Our hypothesis is that the CSF cytokine profiles in viral meningitis differ with the kind of causative virus because the immune response of a host to viral meningitis will differ with the kind of causative virus.

To determine whether or not the CSF cytokine profiles in mumps meningitis (MM) are different from those in echovirus type 30 meningitis (EM), we determined the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, and IL-10 as cytokines related to inflammation in CSF during the acute stage in children with MM and EM.

2. Patients and methods

Informed consent was obtained from the parents of the patients and controls enrolled in this study. The protocol was approved by the institutional review board

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of Yamaguchi University Hospital and the ethics committee of Sinnanyo Citizen Hospital.

2.1. Mumps meningitis (MM)

CSF samples were obtained from 15 children with MM (14 males and one female, aged from 2 to 10 years; median, 5.9 years) on admission to our hospital and Sinnanyo Citizen Hospital in Japan, from January 1994 to August 2002 (Table 1). The diagnosis of mumps was based on common clinical symptoms such as painful enlargement of the salivary glands and an increase in the antibody titer determined by enzyme-linked immunosorbent assaying (EIA). The criteria for the diagnosis of aseptic meningitis were (1) clinical symptoms and signs compatible with viral meningitis, (2) a CSF pleocytosis level of >7 leukocytes/μl, and (3) negative CSF bacterial cultures. The day of onset of symptoms associated with meningitis was considered as the first day of illness. CSF samples were taken from children with MM on days 1–2 (median, 2.0) of the illness.

2.2. Echovirus type 30 meningitis (EM)

An outbreak of EM occurred from May to August 1998 in Yamaguchi prefecture, Japan. CSF samples were obtained from 34 children with EM (25 males and nine females, aged from 1 month to 8 years; median, 5.2 years) on admission to our hospital from May to July 1998 (Table 1). The subjects in our present study were completely different from those in our previous study [7]. The criteria for the diagnosis of aseptic meningitis associated with echovirus type 30 were (1) clinical symptoms and signs compatible with viral meningitis, (2) isolation of echovirus type 30 from CSF, the throat, and/or a stool, or a four-fold increase in the antibody titer determined with the neutralization test (NT), (3) a CSF pleocytosis level of >7 leukocytes/μl, and (4) negative CSF bacterial cultures. CSF samples were taken from children with EM on days 1–5 (median, 2.0) of the illness.

2.3. Control subjects

The control subjects were 21 afebrile and noninfected children with neurological disorders (12 males and nine females, aged from 3 months to 15 years: median, 3.7 years). CSF samples were obtained from them on neurological examination and they all had normal CSF cell counts (Table 1).

2.4. Determination of cytokine concentrations

The concentrations of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA) according to the manufacturer's manual, as previously described [10–12], with modification of the data analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). Briefly, a CBA comprises a series of beads exhibiting discrete fluorescence intensities at 670 nm. Each series of beads is coated with a monoclonal antibody against a single cytokine, and a mixture of the six series of beads can detect six cytokines in one sample. A secondary phycoerythrin (PE)-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). Data were transferred to GraphPad Prism. Starting with standard dilutions, the software performed log transformation of the data, and then fitted a curve to 10 discrete points using a four-parameter logistic model. The calibration curve created for each cytokine was used to determine the cytokine concentrations in the samples. The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/ml, 2.8 pg/ml, 2.6 pg/ml, 2.6 pg/ml, 2.5 pg/ml, and 2.8 pg/ml, respectively.

2.5. Statistical analysis

All values are medians ± 1 SD. The differences in the results between the groups were analyzed by means of

Table 1
Data for patients with mumps meningitis and echovirus type 30 meningitis, and controls

	Mumps meningitis	Echovirus type 30 meningitis	Controls
Number	15	34	21
Age	5.9 yr, 2 yr–10 yr	5.2 yr, 1 mo–8 yr	3.7 yr, 3 mo–15 yr
Sex (male:female)	14:1	25:9	12:9
Onset of symptoms to sampling of CSF (days)	2.0, 1–2	2.0, 1–5	–
Duration of fever (days)	4.0, 3–5**	2.0, 0–5	–
Hospitalization period (days)	5.0, 3–8*	3.0, 1–8	–
CSF protein (mg/dl)	30.0, 10–53	25.5, 10–84	19.0, 10–34
CSF cell count (/μl)	145, 28–648	79, 10–902	1.0, 0–6

**Significant at $p < 0.0001$ vs. echovirus type 30 meningitis. *Significant at $p = 0.0013$ vs. echovirus type 30 meningitis. (median, ranges).

the Mann–Whitney U test, a p value of less than 0.05 being taken as significant. Correlations were analyzed with use of Spearman's rank correlation coefficient test.

3. Results

The duration of fever and the hospitalization period in MM were significantly longer than those in EM ($p < 0.0001$ and $p = 0.0013$, respectively) (Table 1). There was no significant difference in age, onset of symptoms to sampling of CSF, CSF protein or cell counts between the two groups.

The concentrations of IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 in CSF of the control subjects were 13.9 ± 14.8 pg/ml, 3.0 ± 1.9 pg/ml, 2.9 ± 1.0 pg/ml, 5.2 ± 2.9 pg/ml, 3.8 ± 2.9 pg/ml, and 3.8 ± 1.5 pg/ml, respectively. The CSF IFN-γ, IL-2, IL-6, and IL-10 concentrations of MM and EM are shown in Fig. 1. The CSF IFN-γ, IL-2, IL-6, and IL-10 levels were elevated in MM, and the CSF IFN-γ, IL-2, and IL-6 levels were elevated in EM. The CSF IFN-γ levels in MM were significantly higher than those in EM (471 ± 1118 pg/ml vs. 61.2 ± 67.3 pg/ml, $p < 0.0001$). The CSF IL-2 levels in MM were significantly higher than those in EM (5.9 ± 4.7 pg/ml vs. 4.0 ± 1.2 pg/ml, $p < 0.0001$). The CSF IL-6 levels in EM were significantly higher than those in MM (1023 ± 1724 pg/ml vs. 556 ± 923 pg/ml, $p = 0.0255$). The CSF IL-10 levels were elevated in MM (84.4 ± 133 pg/ml), but not in EM (4.1 ± 4.7 pg/ml). The CSF TNF-α and IL-4 levels were not elevated in MM or EM.

In MM, the IL-6 level was correlated with the IL-2 and IL-10 levels in CSF ($p = 0.0347$ and $p = 0.0120$) (Fig. 2A and B). In EM, the IFN-γ level was correlated with the IL-10 level in CSF ($p = 0.0002$) (Fig. 3). Moreover, the protein level was correlated with the IL-6 levels in CSF of MM and EM ($p = 0.0498$ and $p = 0.0392$), and the cell count was correlated with IL-10 level in CSF of MM ($p = 0.0320$).

4. Discussion

Our previous study revealed that the numbers of macrophages in CSF were increased, and the concentrations of CSF monocyte chemoattractant protein 1 (MCP-1), which is responsible for the accumulation of macrophages at the inflammatory site [13], IFN-γ, and IL-12 were elevated in EM [7], and that the ratio of CD4/CD8 in CSF of patients with EM was high (3.0 ± 1.2). Sato et al. reported that CSF IL-6, IL-8, and IFN-γ were elevated during the acute phase, and CSF IL-10 and transforming growth factor β1 (TGF-β1) were elevated in the recovery phase in EM [8].

We revealed that the CSF cytokine profiles in MM were different from those in EM. From the clinical point of view, the duration of fever and the hospitalization period in MM were significantly longer than those in EM. MM was clinically more severe compared with EM. We suggest that the immune response of a host to viral meningitis differs with the kind of causative virus.

The previous studies demonstrated that CD8+ cytotoxic T lymphocytes (CTL) play an important role in CSF of MM [14,15]. Taking these reports in consideration, a higher level of CSF IFN-γ in MM may indicate that IFN-γ is mainly produced by CD8+ CTL in CSF in MM. In our present study, IL-2, as Th1 cytokine, levels were elevated in CSF in MM. This finding indicates the activation of CD4+ Th1 cells. Therefore, it cannot be regarded that only CD8+ CTL produce IFN-γ in CSF in MM because activated CD4+ Th1 cells produce IFN-γ [16]. With respect to cytokine levels in CSF, we suggest that CD8+ CTL and/or

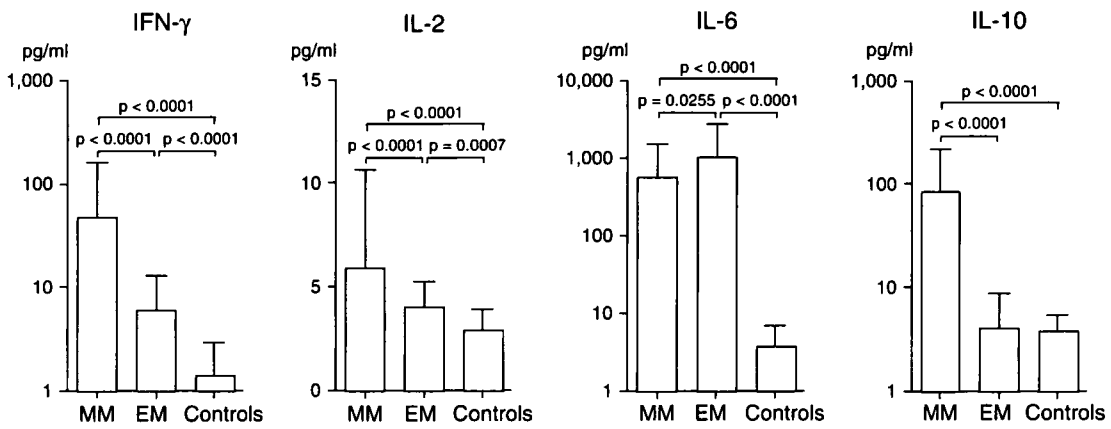

Fig. 1. CSF concentrations of IFN-γ, IL-2, IL-6, and IL-10 in patients with mumps meningitis (MM), echovirus type 30 meningitis (EM), and controls. Data are presented as medians + 1 SD. The CSF IFN-γ, IL-2, and IL-10 levels in patients with MM were significantly higher than those with EM ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively). The CSF IL-6 level in patients with EM was significantly higher than those with MM ($p = 0.0255$).

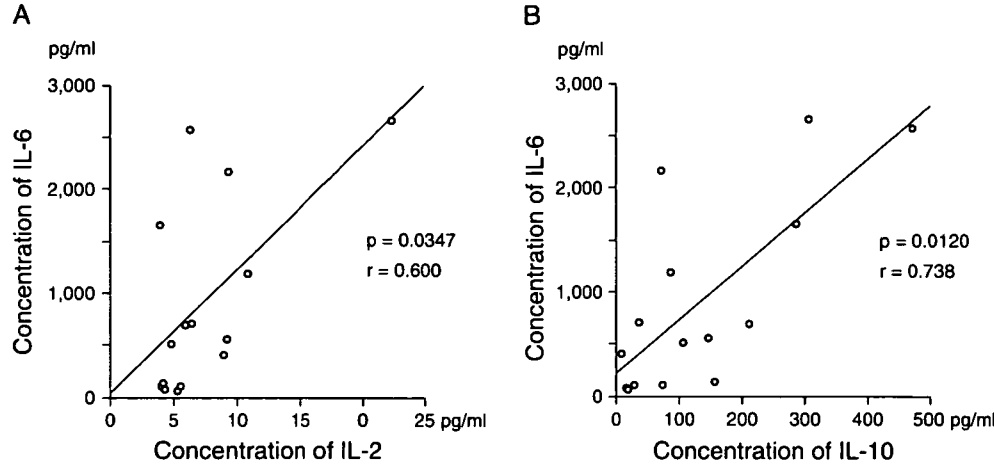

Fig. 2. Relationship between the IL-6 and IL-2 concentrations in CSF of patients with MM (A). Relationship between the IL-6 and IL-10 concentrations in CSF of patients with MM (B).

CD4+ Th1 cells play an important role in CSF in MM during the acute phase. IL-10, which is mainly produced by CD4+ Th2 cells, inhibits cytokine production by CD4+ Th1 cells [17]. Therefore, we suggest that IL-10 is induced in response to higher production of IFN-γ to modulate the balance of Th1 and Th2 in MM. IL-6 may be mainly produced by CD4+ Th2 cells because the IL-6 level is correlated with the IL-2 and IL-10 levels in CSF in MM.

We suggest that the role of CD4+ Th1 cells is dominant compared with that of CD8+ cells as concern lymphocytes in the pathogenesis of EM during the acute phase [7]. Therefore, the level of CSF IFN-γ mainly induced by CD8+ CTL in EM was significantly lower than that in MM. IFN-γ may be mainly produced by CD4+ Th1 cells because the IFN-γ level is correlated with the IL-10 level in CSF in EM. Moreover, monocytes/macrophages will play an important role in the pathogenesis in CSF in EM [7]. The higher level of CSF IL-6 in EM than that in MM may indicate that IL-6 is mainly produced by monocytes/macrophages. Dalal et al. demonstrated that CSF IL-6 and IFN-γ were elevated in echovirus type 4 meningitis, and that the IL-6 level was correlated with the leukocyte count in CSF [9]. We demonstrated that the IL-6 level was correlated with the protein level in CSF in EM, but not with the leukocyte count. The immunological pathogenesis of echovirus type 4 meningitis may be different from that of echovirus type 30 meningitis.

In summary, the CSF cytokine profile in MM was different from that in EM. We suggest that CD8+ CTL and/or CD4+ Th1 cells in MM, and monocytes/macrophages and CD4+ Th1 cells in EM play important roles during the acute phase in the pathogenesis of meningitis. Therefore, the pathogenesis of viral meningitis will differ with the kind of causative virus.

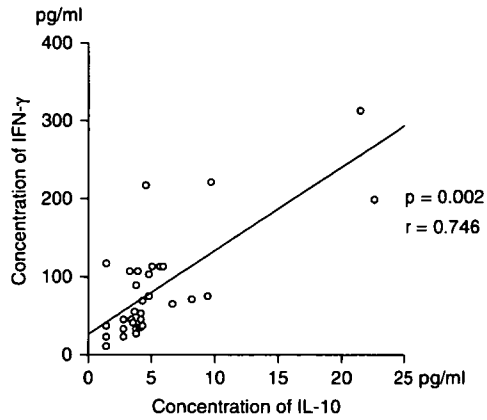

Fig. 3. Relationship between the IFN-γ and IL-10 concentrations in CSF of patients with EM.

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Analysis of serum soluble CD40 ligand in patients with influenza virus-associated encephalopathy

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Abstract

CD40 ligand (CD40L) is mainly expressed on activated platelets and CD4+T cells, and it can be cleaved from the cell surface, releasing a soluble CD40L (sCD40L). Most sCD40L is derived from activated platelets. A previous paper revealed that the platelet number of patients with influenza virus-associated encephalopathy (IE) was correlated with the outcome. We determined the utility of sCD40L as a predictor for the prognosis of IE. We measured the serum concentration of sCD40L and the platelet number on the day of hospitalization in 34 patients with IE, 16 with influenza virus-associated febrile seizures (IFS), 19 with influenza virus infection without complications (Flu), and 7 with Epstein–Barr virus (EBV) infection. The serum sCD40L concentrations in IE and IFS were significantly lower than those in controls, Flu, and EBV infections. Serum sCD40L concentrations in the IE group were 0.70±0.43 ng/ml for deceased patients, 1.73±1.36 ng/ml for those with sequelae, and 3.85±2.91 ng/ml for those without sequelae. There was no significant difference in platelet number between IE patients with and without sequelae, while the platelet number of deceased patients with IE was significantly lower than in controls, Flu, and IFS. Serum sCD40L concentration on the day of hospitalization was more correlated with the outcome of IE than platelet number. Our findings suggest that the serum sCD40L concentration during acute IE is important for predicting the prognosis at an early stage.

Keywords: Epstein–Barr virus; Influenza virus-associated encephalopathy; Platelet; Soluble CD40 ligand

1. Introduction

CD40 ligand (CD40L) is a transmembrane protein expressed on activated platelets and CD4+T cells [1,2]. CD40L can be cleaved from the cell surface, releasing a soluble CD40L (sCD40L) which is biologically active [2,3]. Several previous papers demonstrated that most serum sCD40L is derived from platelets [4–6].

Many patients with influenza virus-associated encephalopathy (IE) have been reported in Japan [7–9], and recently, some cases have been reported in Europe and the United States [10,11]. Pathological findings revealed that viral antigens and inflammatory cells were undetectable in brain tissues and suggest that direct viral invasion does not induce IE [12,13]. Serum and CSF concentrations of several proinflammatory cytokines and cytokine receptors, such as interleukin-6 (IL-6), IL-1β, and soluble tumor necrosis factor (TNF) receptor 1, are elevated and related to the clinical severity of IE [14–16]. Moreover, the platelet number tends to be correlated with the outcome of IE [8,12,17]. To evaluate the utility of sCD40L in the severity and prognosis of IE, we measured the serum sCD40L concentrations on the day of hospitalization in patients with IE, influenza virus-associated febrile seizures (IFS), influenza virus infection without complications (Flu), and Epstein–Barr virus (EBV) infection.

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2. Materials and methods

2.1. Influenza virus-associated encephalopathy (IE)

Informed consent was obtained from the parents of the patients enrolled in this study. Serum samples were obtained from 34 patients with IE, 16 with IFS, and 19 with Flu on admission to our hospital and eleven research cooperation hospitals in Japan, from December 1999 to March 2005 (Table 1). We divided the patients with IE into three groups, i.e., those who had no sequelae (Group A, $n=18$), those who had neurological sequelae (Group B, $n=10$), and those who were deceased (Group C, $n=6$). The criteria for the diagnosis of IE were: (1) clinical symptoms and signs compatible with acute encephalitis/encephalopathy (defined as a febrile disorder with alteration of consciousness and slow activity on electroencephalography lasting for more than 24 h after an acute onset), and no bacteria or fungi on CSF culture, with all other neurological, vascular, endocrine, toxic and drug-induced disorders having been excluded, and (2) isolation of the influenza virus from the throat, or a four-fold increase in the antibody titer determined by means of the hemagglutination inhibition test and/or virus antigen detection in the throat with the latex agglutination test. The day of onset of neurological symptoms was considered as the first day of illness. Serum samples were taken from the patients with IE on days 1.2±0.5 (range, 1–3) of the illness.

2.2. Influenza virus-associated febrile seizures (IFS)

Influenza virus-associated febrile seizures were defined as seizures with fever and impaired consciousness lasting less than 24 h without neurological sequelae, and influenza virus infection was proven by the above-mentioned method. Sixteen patients enrolled with IFS (10 males and 6 females, aged from 11 months to 10 years: mean, 5.2 years) (Table 1). The day of seizure onset was considered as the first day of illness. Serum samples were taken from the patients with IFS on days 1.1±0.3 (range 1–2) of the illness.

2.3. Influenza virus infection without complications (Flu)

Nineteen patients enrolled with influenza virus infections had fever and upper respiratory symptoms (9 males and 10 females, aged from 14 months to 9 years: mean, 5.4 years) (Table 1). Influenza virus infection was proven by the latex agglutination test. The day of fever onset was considered as the first day of illness. Serum samples were taken from these patients on days 2.1±1.1 (range, 1–4) of the illness.

2.4. Epstein–Barr virus (EBV) infection

The disease control subjects of viral infections were 7 patients who had EBV infection (three males and four females, aged from 2 to 12 years: mean, 5.9 years) (Table 1). The diagnosis was based on clinical presentation of a sore throat, fever, and bilateral cervical lymphadenopathy accompanied by atypical lymphocytes in the peripheral blood. All patients were positive for IgM and IgG antibodies to the EBV capsid antigen and negative for antibodies to the EBV nuclear antigen during the acute stage. The day of fever onset was considered as the first day of illness. Serum samples were taken from the patients with EBV infection on days 6.8±3.8 (range, 2–13) of the illness.

2.5. Control subjects

The control subjects were 25 healthy children (14 males and 11 females, aged from 3 months to 11 years: median, 5.4 years).

2.6. Determinations of the sCD40L concentration and platelet number

The serum concentrations of sCD40L were measured with a sandwich-type ELISA kit (R&D Systems, Minneapolis, MN). The detection limit was 4.2 pg/ml. Platelet number was assessed in the blood samples by standard automated techniques.

Table 1
Data for subjects

Group	Number	Age (years) (range)	Sex male:female	Type
IE-Group A	18	5.8±4.5 (6 months–13 years)	12:6	H1N1=1, H3N2=6, A=5, B=6
IE-Group B	10	5.9±6.6 (9 months–19 years)	4:6	H1N1=2, H3N2=4, A=3, B=1
IE-Group C	6	5.4±3.8 (2 years–10 years)	5:1	H3N1=5, A=1
IFS	16	5.2±3.0 (11 months–10 years)	10:6	A=10, B=6
Flu	19	5.4±3.1 (14 months–9 years)	9:10	A=13, B=6
EBV infection	7	5.9±3.2 (2 years–12 years)	3:4	
Controls	25	5.4±3.2 (3 months–11 years)	14:11	

IE=influenza virus-associated encephalopathy; IFS=Influenza virus-associated febrile seizures; Flu=influenza virus infection without complications; EBV=Epstein-Barr virus.
Group A=patients without sequelae; Group B=patients with sequelae; Group C=deceased patients.

2.7. Statistical analysis

All values are the means ± S.D. Differences in the results between groups were analyzed by the Mann–Whitney *U*-test, with a *p*-value of less than 0.05 being taken as significant. Correlations were analyzed with Spearman's rank correlation coefficient test.

3. Results

The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 1. The serum sCD40L concentrations of IE-Group A, IE-Group B, IE-Group C, and IFS were significantly lower than those of Flu ($p=0.0015$, $p=0.0002$, $p=0.0034$, and $p=0.0123$, respectively), EBV infection ($p=0.0105$, $p=0.0047$, $p=0.00269$, and $p=0.0235$, respectively) and controls ($p=0.0003$, $p<0.0001$, $p=0.0004$, and $p=0.001$, respectively). In the IE group, the serum sCD40L concentration of Group C was significantly lower than those of Groups A ($p=0.0077$) and B ($p=0.0409$), and that of Group B was significantly lower than that of Group A ($p=0.044$). The serum sCD40L concentration of IE-Group C was significantly lower than that of IFS ($p=0.0066$). There were no significant differences in serum sCD40L concentrations among Flu, EBV infection, and controls.

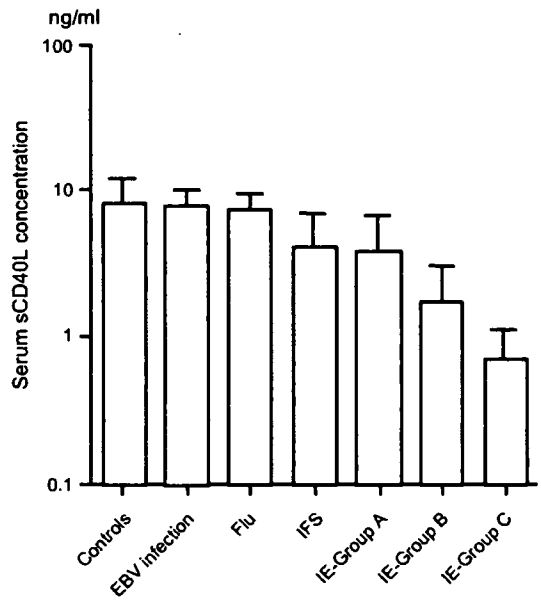

Fig. 1. The serum sCD40L concentrations of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means + 1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The serum sCD40L concentration of controls was 8.23 ± 3.61 ng/ml, EBV infection, 8.00 ± 1.93 ng/ml, Flu, 7.49 ± 1.96 ng/ml, IFS, 4.15 ± 2.68 ng/ml, IE-Group A, 3.85 ± 2.91 ng/ml, IE-Group B, 1.73 ± 1.36 ng/ml, and IE-Group C, 0.697 ± 0.428 ng/ml, respectively.

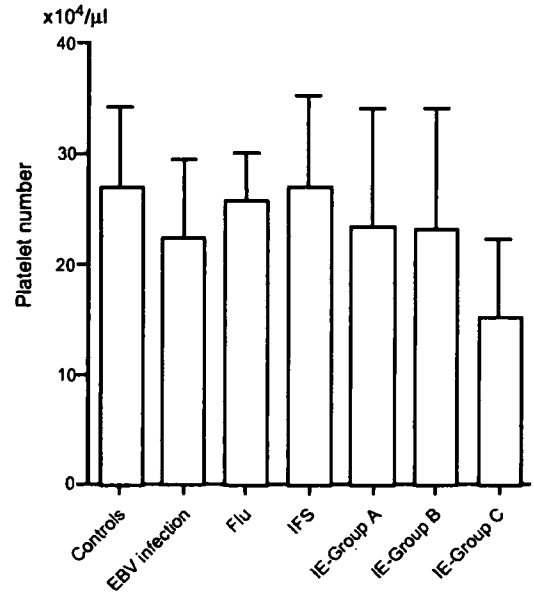

Fig. 2. The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization, and controls. Data are presented as means + 1 S.D. IE-Group A, IE without sequelae; IE-Group B, IE with a poor prognosis; IE-Group C, the deceased patients with IE. The platelet number of controls was $27.0 \pm 7.5 \times 10^4/\mu l$, EBV infection, $22.9 \pm 6.6 \times 10^4/\mu l$, Flu, $25.9 \pm 4.4 \times 10^4/\mu l$, IFS, $27.0 \pm 8.4 \times 10^4/\mu l$, IE-Group A, $23.4 \pm 10.9 \times 10^4/\mu l$, IE-Group B, $23.3 \pm 11.0 \times 10^4/\mu l$, and IE-Group C, $15.2 \pm 7.2 \times 10^4/\mu l$, respectively.

The platelet numbers of patients with IE, IFS, Flu, and EBV infection on the day of hospitalization are shown in Fig. 2. The platelet number of IE-Group C was significantly lower than those of IFS ($p=0.0078$), Flu ($p=0.0233$), and controls ($p=0.0045$) (Fig. 2). There were no significant differences in platelets numbers among IE-Group A, IE-Group B, IFS, Flu, EBV infection, and controls.

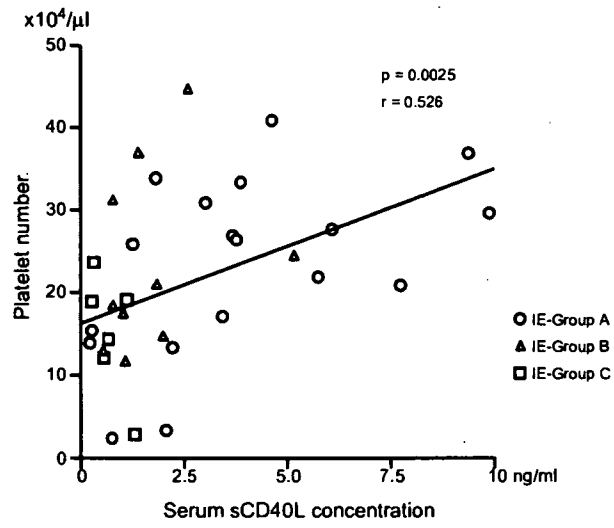

Fig. 3. Relationship between the serum concentration of sCD40L and platelets number in IE groups.

In the influenza virus infection group, including Flu, IFS, and IE, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0006$, $r=0.465$). In the entire IE group, the serum concentrations of sCD40L were correlated with the platelet numbers ($p=0.0025$, $r=0.526$) (Fig. 3). However, In IE groups with a poor prognosis, including Groups B and C, the serum concentrations of sCD40L were not correlated with the platelet numbers ($p=0.2273$, $r=0.312$).

4. Discussion

The CD40–CD40L interaction is pivotal in the cellular immune response. CD40L was first described as an antigen, which is expressed on activated CD4 T cells, and CD40L interacts with CD40 expressed on B cells and induces the class switch [6]. A broader role for CD40 signaling was revealed through the finding that CD40 is expressed on numerous cell types, including monocytes/macrophages, dendric cells, fibroblasts keratinocytes, endothelial cells, and vascular smooth muscle cells [18–20]. Stimulation of these cell types through CD40 induces cell functions which contribute to inflammatory responses, including the expression of adhesion molecules, and also the release of proinflammatory cytokines, such as IL-6, IL-1β, IL-8, IL-12, and TNF-α [19–24]. Serum and plasma sCD40L levels are elevated in systemic lupus erythematosus [25,26], rheumatoid arthritis and associated vasculitis [27], mixed connective tissue disease [28], systemic sclerosis [29], cystic fibrosis [30], advanced squamous cancer of the lung [31], autoimmune thrombocytopenic purpura [32], and chronic idiopathic urticaria [33]. In these disorders, the importance and immunological mechanism of the CD40–CD40L interaction have been discussed. However, >95% of plasma sCD40L is derived from platelets [34]. Therefore, it is likely that the sCD40L level depends on platelet number and activation. In fact, serum sCD40L concentrations were well correlated with platelet numbers and thrombopoiesis in patients undergoing allogenic stem cell transplantation [6].

Our present study demonstrated that serum sCD40L concentrations on the day of hospitalization were well correlated with the severity and prognosis of IE. A previous paper reported that a decrease in platelet number was correlated with a poor prognosis of IE [8]. However, the platelet number reported in the paper made no mention of the sampling time [8]. Platelet number tends to decrease gradually in IE, and therefore, the platelet numbers of patients with IE who had a poor prognosis on the day of hospitalization were often normal [12,17]. Our present study demonstrated that the platelet number of IE-Group B on the day of hospitalization was not decreased, while the serum sCD40L concentration was decreased. It is likely that the serum sCD40L concentration is decreased ahead of platelet number in IE with a poor prognosis. The condition of patients with severe IE often deteriorates rapidly within 2 days after the development of neurological signs [8]. Therefore, it is important to predict a poor prognosis in IE as soon as possible.

Why are serum sCD40L concentrations decreased ahead of platelet numbers? sCD40L is mainly released by activated platelets [28,34]. Apoptosis under hypercytokinemia has been suggested as a possible mechanism of IE [35,36]. In IE, the function of platelets may become poor and inactive, and then, the platelet number may decrease through megakaryocyte apoptosis. The definite mechanism leading to the decrease of platelet number in IE remains unclear.

In summary, serum sCD40L levels on the day of hospitalization were well correlated with the outcome of IE compared to the platelet numbers. Our findings suggest that the decreased serum sCD40L levels in IE are important for predicting a poor prognosis in the early phase.

Acknowledgments

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Case report

A case of acute encephalitis with refractory, repetitive partial seizures, presenting autoantibody to glutamate receptor Gluε2

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Abstract

An 11-year-old male was admitted to our hospital because of high-grade fever, repetitive seizures, and prolonged impairment of consciousness (Glasgow coma scale E1, M5, V1). His seizures were repetitive complex partial seizures that expanded from the unilateral face to the corresponding side of the body. He sometimes developed secondary generalized seizures. While most seizures lasted 1 or 2 min, intractable seizures also frequently (about 5 times/h) occurred. We diagnosed him as encephalitis/encephalopathy, and treated him with artificial respiration, thiamylal sodium, mild hypothermia therapy, steroid pulse therapy, massive γ-globulin therapy, etc. Afterwards, he had sequelae, such as post-encephalitic epilepsy (same seizures continued to recur), hyperkinesia, impairment of immediate memory, change in character (he became sunny and obstinate), dysgraphia, and mild atrophy of the hippocampus, amygdala, and cerebrum. However, he could still attend a general junior high school. He was diagnosed as acute encephalitis with refractory, repetitive partial seizures (AERRPS). In this case, he was positive for autoantibody to glutamate receptor Gluε2 IgG or IgM in an examination of blood and spinal fluid, and we presumed that this may have influenced his sequelae. In this case, a combination of mild hypothermia therapy, steroid pulse therapy, and massive γ-globulin therapy was effective.

Keywords: Autoantibody to glutamate receptor Gluε2; Acute encephalitis with refractory, repetitive partial seizures (AERRPS); A peculiar type of post-encephalitic/encephalopathic epilepsy; Mild hypothermia therapy; Massive γ-globulin therapy; Steroid pulse therapy

1. Introduction

A peculiar type of post-encephalitic/encephalopathic epilepsy was first reported by Awaya et al. [1]. It is characterized by epilepsy with the same repetitive intractable partial seizures from the acute phase to the convalescence phase. However, it is not known when epileptogeneity is acquired. Soon thereafter, Shiomi et al. reported a similar case of encephalitis accompanied by frequent seizures in Japan. Sakuma et al. proposed the terminology acute encephalitis with refractory, repetitive partial seizures (AERRPS), which satisfied the following five criteria: (1) a prolonged acute phase of more than 2 weeks, (2) partial seizures with the same symptoms persisting from the acute phase to the convalescence phase, (3) seizures frequently evolving into status convulsivus, especially during the acute phase, (4) marked intractability of seizures, and (5) exclusion of related disorders such as known viral encephalitis or metabolic disorders [2], based on these two previous reports. On the other hand, it was reported that autoantibody to glutamate receptor Gluε2 was often positive in Rasmussen's encephalitis [3] and in acute encephalitis/encephalopathy. It is possible that autoantibody to glutamate receptor Gluε2 may cause persistent excitation of glutamate receptor Gluε2 and may be associated with seizures and impairment of the central nervous system. We report here a case of AERRPS, presenting autoantibody to glutamate receptor Gluε2. To the best of our knowledge,

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this is the first report of AERRPS presenting autoantibody to glutamate receptor Gluε2. It is possible that autoantibody to glutamate receptor Gluε2 may be associated with the pathophysiology of AERRPS.

2. Case report

An 11-year-old male was admitted to our hospital because of high-grade fever, repetitive seizures, and prolonged impairment of consciousness (Glasgow coma scale E1, M5, V1). His seizures were repetitive complex partial seizures that expanded from the unilateral face to the corresponding side of the body. He sometimes developed secondary generalized seizures. While most seizures lasted 1 or 2 min, intractable seizures also frequently (about 5 times/h) occurred. The family history and past history were not marked. On admission, he showed no abnormal neurological findings except for impairment of consciousness and intractable seizures. On blood examination, he showed no abnormality except for FDP and ALT (16 μg/ml (1–12 μg/ml), 53 U/l (5–40 U/l), respectively). On spinal fluid examination, leukocyte count was 25 mm^3. Brain computed tomography (CT) and magnetic resonance imaging (MRI) with T2-weighted imaging (T2-WI) showed no abnormality (Fig. 1(A)). There was no significant increase in any virus antibody titer. His clinical course after admission is described in Fig. 2. On day 1 of admission, he was administered glycerol (5 ml/kg × 4 times/day), acyclovir (5 mg/kg × 3 times/day), γ-globulin (250 mg/kg/day for 3 days), steroid pulse therapy (methylprednisolone 25 mg/kg/day for 3 days), and midazolam (0.1 mg/kg/h) for his encephalitis and seizures. On day 2, since he had repetitive seizures, the dose of midazolam was increased and he was administered lidocaine hydrochloride. On day 3, artificial respiration was begun along with thiamylal sodium at 3 mg/kg/h because of intractable seizures. Afterwards, we treated him with thiamylal sodium at 8 mg/kg/h because of intractable seizures and mild hypothermia therapy. He was given an intravenous injection of phenytoin (5 mg/kg × 2 times/day) during treatment with thiamylal sodium, but this was not effective. Interictal electroencephalogram (EEG) on day 8 showed slow spike and wave predominantly in the frontal and central region (Fig. 3(A)). Ictal EEG on day 8 showed rhythmic spikes in the left frontal–central–temporal region with antecedent spikes (Fig. 3(B)). At this time, he was treated with thiamylal sodium at 6 mg/kg/h because of repetitive seizures. Interictal EEG on day 10 showed a burst-suppression pattern and spikes were present during the burst phase (Fig. 3(C)). He was then treated with mild hypothermia therapy and thiamylal sodium at 8 mg/kg/h, and the seizures stopped. On day 10, the leukocyte count was 3 mm^3 and IgG was 10.2 mg/dl (reference value, 0.2–0.6 mg/dl) on spinal fluid examination. Since we thought that a mechanism of abnormal immunity may be involved in his encephalitis because of the increase in IgG on spinal fluid examination, massive γ-globulin therapy (400 mg/kg/day over 5 days) was performed again on day 12. On day 12, we discontinued treatment with thiamylal sodium and began treatment with massive phenobarbital suppository therapy (20 mg/kg/day) because of an impairment of liver function on blood examination. In association with a decrease in thiamylal sodium, his EEG findings worsened. However, EEG spikes almost disappeared following treatment with

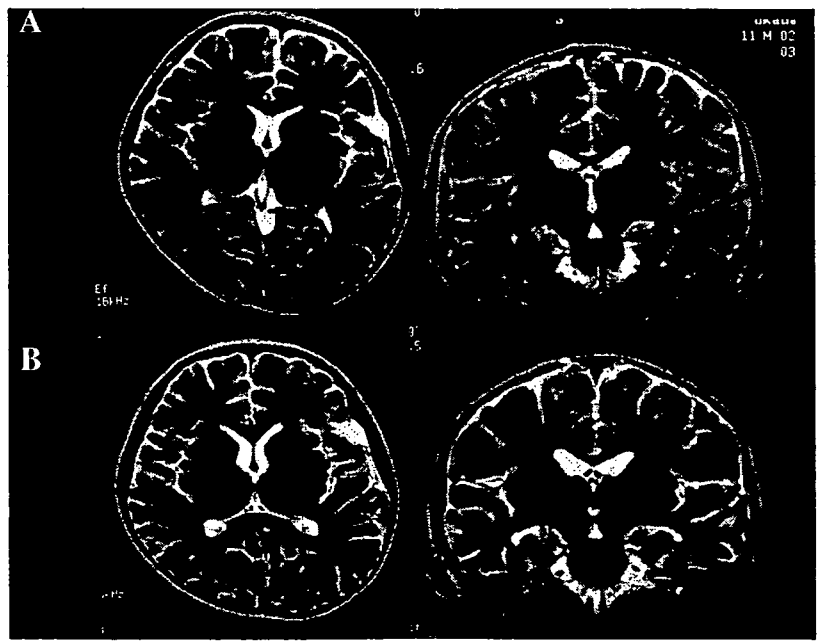

Fig. 1. (A) Brain MRI T2-WI on admission showed no abnormality. (B) Brain MRI on day 36 showed mild atrophy of the hippocampus, amygdala, and cerebrum.

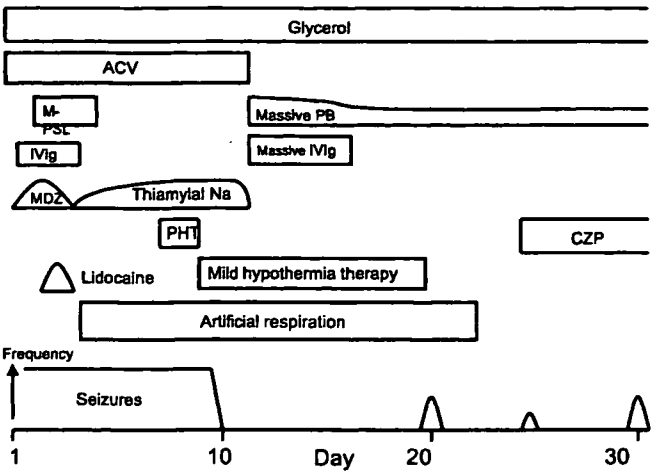

Fig. 2. Clinical course after admission. Abbreviations: ACV, acyclovir; M-PSL, methylprednisolone (steroid pulse therapy); IVIg, intravenous γ-globulin; PB, phenobarbital; MDZ, midazolam; PHT, phenytoin; CZP, clonazepam.

massive γ-globulin therapy and massive phenobarbital suppository therapy. On day 12, acyclovir was also stopped because polymerase chain reaction of herpes simplex virus DNA was negative on spinal fluid examination. On day 20, we stopped mild hypothermia therapy and on day 22 he was extubated. After extubation, his level of consciousness gradually improved. However, since the same seizures appeared several times per week, we started clonazepam (0.075 mg/kg/day) on day 24. The frequency of seizures then gradually decreased. Brain MRI on day 36 showed mild atrophy of the hippocampus, amygdala, and cerebrum (Fig. 1(B)). EEG on day 39 showed a disappearance of spikes. However, the same seizures continued to recur at about once per month under the oral administration of phenobarbital and clonazepam. Afterwards, he had sequelae, such as post-encephalitic epilepsy (same seizures continued to recur), hyperkinesia, impairment of immediate memory, change of character (he became sunny and obstinate), and dysgraphia. However, he could still attend a general junior high school. He was positive for autoantibody to glutamate receptor Glue2 IgG or IgM in an examination of blood and spinal fluid on day 10, but negative on day 80.

3. Discussion

For the treatment of seizures in AERRPS, barbiturate and benzodiazepine are often effective in the acute phase, and phenytoin, zonisamide, and potassium bromide in addition to barbiturate and benzodiazepine are often effective in the convalescence phase [2]. In this case, midazolam, lidocaine hydrochloride, and phenytoin were not effective, and a complete suppression-burst suppression pattern on EEG and thiamylal sodium at 8 mg/kg/h were necessary to stop his seizures; thus, he showed inveterate epileptogeneity. Massive phenobarbital suppository therapy (20 mg/kg/day)

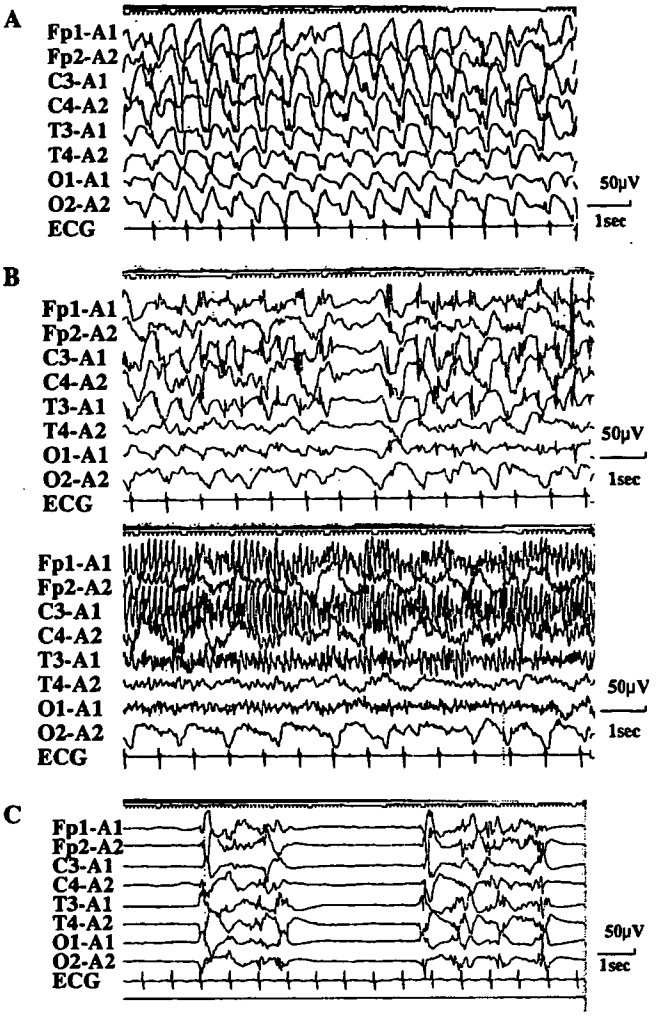

Fig. 3. (A) Interictal EEG on day 8 showed slow spike and wave predominantly in the frontal and central lobe. He was treated with thiamylal sodium at 6 mg/kg/h because of repetitive seizures. (B) Ictal EEG on day 8 showed rhythmic spikes in the left frontal–central–temporal region with antecedent spikes in the same region. (C) Interictal EEG on day 10 showed a burst-suppression pattern and spikes were present during the burst phase. He was treated with mild hypothermia therapy and thiamylal sodium at 8 mg/kg/h, and the seizures stopped.

was partly effective and made it possible to break away from thiamylal sodium. Sakuma et al. reported that massive phenobarbital therapy or phenytoin was useful for the discontinuation of barbiturate [2]. In our case, phenobarbital was most effective at stopping seizures after the early phase of treatment. Sakuma et al. reported that phenobarbital was effective in 2/15 cases in the acute phase, and in 4/12 cases in the convalescence phase [2]. Hamano et al. reported a case of AERRPS that showed the transient disappearance of seizures with the occurrence of choreo-ballistic involuntary movements [4]. That case showed secondary generalized seizures that originated in the face. It was thought that epileptic discharge from the lateral motor cortex was transmitted to basal ganglia or the brain stem, and resulted in secondary generalized seizures. Thus, it is possible that

impairment of the basal ganglia associated with involuntary movement may have blocked epileptic discharge from the motor cortex, and convulsions decreased accompanied by a worsening of involuntary movement [5]. It is possible that hyperexcitability in the subcortex may have blocked epileptic discharge from the cortex [4]. These results may be useful for the treatment of AERRPS.

For the treatment of encephalitis/encephalopathy in AERRPS, we used steroid pulse therapy, massive γ-globulin therapy, and mild hypothermia therapy. A combination of mild hypothermia therapy with steroid pulse therapy is recommended for encephalopathy [6,7]. In encephalitis, since cytokine [8] and neopterin [9] are both increased on spinal fluid examination, it is thought that inflammation and immunoreaction are present in the central nervous system. The aims of hypothermia therapy and steroid pulse therapy are (1) to suppress brain edema, (2) to suppress secondary impairment of nerve cells due to the transmission of excitatory amino acids and neurotoxic materials, and (3) to suppress an abnormal increase in cytokine [6]. In this case, the aims of massive γ-globulin therapy were (1) to immunize against contagions, (2) to suppress an abnormal increase in cytokine, and (3) to suppress abnormal immunity and the generation of antibody (autoantibody to glutamate receptor Gluε2, etc.) because of an increase in IgG on spinal fluid examination. Sandstedt et al. reported that γ-globulin therapy was more effective in cases with a high level of IgG on spinal fluid examination in intractable post-encephalitic epilepsy [10].

In this case, autoantibody to glutamate receptor Gluε2 was positive on blood and spinal fluid examination. It has been reported that autoantibody to glutamate receptor is often positive in Rasmussen's encephalitis [3] and acute encephalitis/encephalopathy [11]. On the other hand, patients with West syndrome or Lennox–Gastaut syndrome, or control subjects are negative for autoantibody to glutamate receptor [11]. In acute encephalitis/encephalopathy, it has been speculated that severe cases tend to generate autoantibody to glutamate receptor Gluε2 [11]. Patients with status convulsivus in the acute phase generated autoantibody to glutamate receptor Gluε2 significantly more often than those without status convulsivus [11]. Furthermore, patients who were positive for autoantibody to glutamate receptor Gluε2 had sequelae such as developmental delay, motor paralysis, or epilepsy significantly more often than those who were negative for autoantibody to glutamate receptor Gluε2 [11]. It is possible that autoantibody to glutamate receptor may persistently adrenalize glutamate receptor, and this may be associated with the generation of seizures and impairment of the central nervous system. Also, since the glutamate receptor plays an important role in the genesis of memory and learning in the hippocampus, it has been speculated that autoantibody to glutamate receptor may be associated with atrophy of the hippocampus and impairment of memory. To the best of our knowledge, there has been no previous report in which autoantibody to glutamate receptor Gluε2 was positive in AERRPS. In general, AERRPS shows a neurologically poor prognosis, and the appearance of autoantibody to glutamate receptor also reflects a neurologically poor prognosis. If it can be demonstrated that an abnormal immune state is involved in AERRPS based on an examination of autoantibody to glutamate receptor Gluε2, it may be possible to reduce the incidence of sequelae through the use of immunotherapy. In this case, the patient could attend a general junior high school. These results suggest that active therapy such as with a combination of mild hypothermia therapy, steroid pulse therapy, and γ-globulin therapy may be effective in AERRPS.

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PAPER

Evaluation of combination therapy using aciclovir and corticosteroid in adult patients with herpes simplex virus encephalitis

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See Editorial Commentary, p 1469

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Objective: Herpes simplex virus encephalitis (HSVE) is associated with significant morbidity and mortality, even with appropriate antiviral therapy. In the present investigation, the first to assess efficacy of corticosteroid treatment with aciclovir therapy in HSVE, multiple logistic regression analysis was performed of predictors of outcome in adult patients with HSVE.

Methods: A non-randomised retrospective study of 45 patients with HSVE treated with aciclovir was conducted. The patients were divided into poor and good groups based on outcome at three months after completion of aciclovir treatment. The variables evaluated were: clinical variables (sex, age, days after onset at initiation of aciclovir, Glasgow Coma Scale (GCS) at initiation of aciclovir, initial and maximum values for the cell numbers and protein concentration in the cerebrospinal fluid, and corticosteroid administration); neuroradiological variables (detection of lesions by initial cranial computed tomography and by initial magnetic resonance imaging); and one neurophysiological variable (detection of periodic lateralised epileptiform discharges on the initial electroencephalogram). Single variable logistic regression analysis was performed followed by multiple logistic regression analysis. The best set of predictors for the outcome of HSVE was estimated by stepwise logistic regression analysis.

Results: A poor outcome was evident with older age, lower GCS score at initiation of aciclovir, and no administration of corticosteroid. Patient age, GCS at initiation of aciclovir, and corticosteroid administration were found to be significant independent predictors of outcome on multiple logistic regression analysis, and these three variables also formed the best set of predictors ($R^2 = 0.594$, $p < 0.0001$).

Conclusion: Combination therapy using both aciclovir and corticosteroid represents one of the predictors of outcome in HSVE.

Herpes simplex virus encephalitis (HSVE) is associated with significant morbidity and mortality, even when appropriate antiviral therapy is administered in the acute stage of the illness.[1–3] Although antiviral therapy in HSVE is highly effective in reducing mortality, only fewer than half of patients with HSVE are able to return to normal health.[1–3] This finding indicates the need for further improved therapeutic regimens for HSVE.

Age over 30 years, initiation of antiviral therapy more than four days after onset, a Glasgow Coma Scale (GCS) score of 6 points or less, and the detection of focal lesions by cranial computed tomography (CT) at the initiation of therapy have been reported to be predictors of a poor outcome in HSVE.[2 4 5] These previous investigations used single variable analysis and did not assess the significance of each variable independently. The present study achieved this by employing multiple logistic regression analysis. Single variable analyses test the significance of each variable separately, whereas multiple logistic regression analysis tests the independent effect of each variable after considering the associations among the variables. There has been only one previous report estimating the outcome in HSVE by multivariable analysis.[6]

However, the effect of corticosteroid treatment along with administration of aciclovir on the outcome of HSVE has not yet been assessed. The percentage of patients administered corticosteroid treatment along with antiviral therapy has been given as 60–70% in prospective randomised studies estimating the efficacy of adenine-arabinoside and aciclovir treatments.[2 3] In these previous reports, the percentage of administered corticosteroids was established to be equivalent in the two groups of different antiviral drugs, but the efficacy of corticosteroid treatment was not evaluated. The present investigation is the first study employing multiple logistic regression analysis to assess the efficacy of corticosteroid treatment with aciclovir administration in the outcome of HSVE.

METHODS

We evaluated a series of 45 patients with HSVE (19 women; mean age 46.0 years, median age 45.0 years, range 17–77) from a total of 146 patients with acute encephalitis who were initially suspected of having HSVE. The patients were admitted to six hospitals (Nihon University Itabashi Hospital, Tohoku University Hospital, and four affiliated hospitals) between 1996 and May 2004. To perform evaluations with predictors of outcome in adult HSVE patients, we established diagnostic and therapeutic protocols in 1996, before starting the present study. In our diagnostic protocol the aetiological diagnosis of HSVE was based on positive results being obtained in the following three laboratory tests: the nested polymerase chain reaction (PCR), chemiluminescence assay,[7] and specific intrathecal HSV antibody synthesis, as has been described previously.[7] All of the 45 patients participating in this study fulfilled this diagnostic protocol. HSVE due to HSV-1 was further confirmed by Southern blot hybridisation in all patients. The patients were treated

Abbreviations: CT, computed tomography; GCS, Glasgow Coma Scale; HSVE, herpes simplex virus encephalitis; MRI, magnetic resonance imaging

according to our therapeutic protocol which consisted of intravenous aciclovir (30 mg/kg bodyweight per day) for 14 days at the time of admission. The therapeutic protocol permitted the use of corticosteroids at the discretion of the treating physician, but it did not specify the dosage or duration of corticosteroid treatment. When used, corticosteroids were started at the initiation of aciclovir treatment. The present study therefore provides non-randomised retrospective data on the efficacy of corticosteroid with aciclovir treatment. All of the patients gave informed consent to participate in the study.

We included the following clinical parameters:

(1) sex
(2) age
(3) number of days after onset at initiation of aciclovir
(4) GCS score at initiation of aciclovir
(5) initial (maximum) leucocyte cell count in the cerebrospinal fluid (CSF)
(6) initial (maximum) CSF protein
(7) administration of corticosteroid at the acute stage

and the following neuroradiological and neurophysiological parameters:

(8) evidence of focal lesions detected by initial cranial CT
(9) evidence of focal lesions detected by initial magnetic resonance imaging (MRI)
(10) detection of periodic lateralised epileptiform discharges (PLEDs) on initial electroencephalogram (EEG).

Initial CT examinations in all patients were performed within 24 hours of admission. The initial MRI examinations were performed and the initial EEGs of all patients were examined within two days of admission. CSF samples from each patient were transferred immediately after collection to two laboratories for measurement by chemiluminescence assay (S Kamei, Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo) and by nested PCR (T Morishima, Department of Health Science, Nagoya University School of Medicine, Nagoya). No information about the serological data for all patients was available at the time of measurement at these laboratories; hence all CSF samples were blinded with regard to the aetiological diagnosis before they were assayed. We assessed the patients with HSVE for clinical outcomes three months after the completion of aciclovir treatment in the same way as described previously.[8] The morbidity was classified into five groups as reported previously[8]:

- normal
- mild sequelae—for patients with minor neuropsychological deficits
- moderate sequelae—for patients with limitations due to motor, speech, memory, or seizure disorders
- severe sequelae—for patients requiring supportive care
- death.

Statistical analysis

In September 2004, a statistical analyst (K Hirayanagi, Department of Hygiene and Public Health, Nihon University of Physical Education, Tokyo) at another independent institute evaluated the data for the clinical, neuroradiological, and neurophysiological parameters. The 10 variables among the three sets of parameters were evaluated as follows:

- Clinical independent variables: These were categorised as (1) sex (male = 0, female = 1), (2) age (years; real numbers), (3) days after onset at initiation of aciclovir (days), (4) GCS score at initiation of aciclovir, (5) initial (maximum) leucocyte cell count in the CSF (/μl) (0–10, 11–100, 101–300, and ≥301), (6) initial (maximum) CSF protein (mg/dl) (≤50, 51–100, and ≥101), and (7) administration of corticosteroid at the acute stage (given = 0, not given = 1). Concerning (4), values for the subtraction GCS score from 16 (1–13; value increasing according to the severity of consciousness disturbance) were entered in the statistical analysis.
- Neuroradiological and neurophysiological independent variables: These were categorised as (8) evidence of focal lesions detected by initial cranial CT (absent = 0, present = 1), (9) evidence of focal lesions detected by initial cranial MRI (absent = 0, present = 1), and (10) detection of PLEDs on the initial EEG (absent = 0, present = 1).

Based on the outcomes the patients were divided into two groups: a poor outcome (moderate to severe sequelae to death) and a good outcome (normal and mild sequelae). Dichotomous dependent variable recovery was assigned a value of 1 when the outcome was poor and 0 when the outcome was good.

SAS/STAT software (Version 8) was used for the statistical analysis. The correlation of each variable against outcome was also evaluated by Spearman's rank correlation test. Single variable logistic regression analysis was employed to examine the significance of the independent variables in relation to prognosis. Variables (5) and (6), in this single logistic regression analysis, were examined as categorical variables. Then multiple logistic regression analysis was undertaken for the variables which in the single variable logistic regression analysis had been found to be associated with a poor outcome at $p<0.05$. This is in accordance with the statistical design that has been used in previous studies for outcome predictors in limited numbers of patients with neuroinfections.[9] Furthermore, the interactions between age and the extracted significant variables were assessed by the multiple logistic regression analysis including the products of age and the extracted significant variables. The best set of predictors for the outcome of HSVE was also estimated by stepwise logistic regression analysis of the above 10 variables. In addition, in October to November 2004, we retrospectively collected the details of the corticosteroid treatment in the acute stage from the patients' medical records. The details included the type of corticosteroid, initial dosage of corticosteroid (converted to dosage of prednisolone), and the duration of its administration. We used Fisher's exact probability test and the Mann–Whitney U test to statistically analyse for each baseline clinical characteristic the differences between the patients who were treated with and without corticosteroid. The same tests were also used to analyse the differences in the manner of corticosteroid administration between the two hospital groups.

RESULTS

The clinical and other findings of the 45 patients with HSVE included in the present study are given in table 1. A poor outcome was noted in 19 patients (42%). The rate of poor outcome was not significantly different between the two hospital groups (13/30 patients at Nihon University Itabashi Hospital and two affiliated hospitals *v* 6/15 patients at Tohoku University Hospital and two affiliated hospitals) ($p>0.99$, Fisher's exact probability test). The baseline clinical characteristics of the two groups of patients under aciclovir treatment with and without corticosteroid administration are given in table 2. The results of the correlation, and the single