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Original Article

Ratio of Alpha 2-Macroglobulin Levels in Cerebrospinal Fluid and Serum: An Expression of Neuroinflammation in Acute Disseminated Encephalomyelitis

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ABSTRACT

Background: Acute encephalitis and encephalopathy are life-threatening diseases in children. However, no laboratory examinations are performed for their early diagnosis and treatment. Alpha 2-macroglobulin (α2M) is a blood glycoprotein that increases during the early stages of inflammation. In the present study, we investigated the role of α2M levels in acute encephalitis and encephalopathy.
Methods: We analyzed the cerebrospinal fluid and serum samples from patients with acute disseminated encephalomyelitis, infection-related acute encephalopathy, febrile status epilepticus, and febrile seizure simplex type. Samples were collected from the pediatric department of hospitals throughout the Fukushima Prefecture between January 1, 1999, and May 31, 2012.
Results: α2M levels in the cerebrospinal fluid were 4.7 (3.8–8.4) μg/mL for acute disseminated encephalomyelitis, 2.1 (1.1–2.3) μg/mL for infection-related acute encephalopathy, 1.1 (0.9–6.4) μg/mL for febrile status epilepticus, and 1.0 (0.8–1.1) μg/mL for febrile seizure simplex type. α2M levels in patients with acute disseminated encephalomyelitis were significantly higher than those in patients with infection-related acute encephalopathy and febrile seizure simplex type ($P = 0.019$ and $P = 0.002$, respectively). The ratio of α2M level in the cerebrospinal fluid to that in the serum in patients with acute disseminated encephalomyelitis was significantly higher than the ratio in patients with febrile status epilepticus ($P = 0.04$). In patients with acute disseminated encephalomyelitis, α2M levels in the cerebrospinal fluid decreased with treatment.
Conclusions: Our results suggest that α2M levels in the cerebrospinal fluid reflect the neuroinflammatory status of patients with acute disseminated encephalomyelitis.

Introduction

Acute encephalitis and encephalopathy (AEE) can result in various sequelae, including death; hence, early intervention is required. Although AEE occurs with a low frequency, it is common in infants in East Asia.[1] It results from various causes, including viral infections and connective tissue disease; moreover, pathologies related to immune conditions are different and have not yet been clarified. Therefore AEE diagnosis is based on a comprehensive assessment of each patient's clinical course, laboratory examinations, head imaging, and electroencephalograph findings. However, in the acute phase, particularly when the patient is in a critical condition, diagnosis is often challenging because of the difficulty associated with conducting adequate examinations. In other words, these diseases are difficult to distinguish based on clinical presentations, despite the difference in pathologies.

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Acute encephalitis is classified into viral and nonviral encephalitis. The most common viral encephalitis is herpes encephalitis, whereas the most common nonviral encephalitis types include acute disseminated encephalomyelitis (ADEM) and nonherpetic acute limbic encephalitis. In viral encephalitis, viral pathogen isolation or gene detection is directly linked to its diagnosis. The herpes simplex virus is the most common cause of encephalitis in western countries. Herpes simplex encephalitis occurs at all ages during infancy and childhood, with peak incidence during the first year of life.[2] For nonviral encephalitis, diseases in which autoantibodies are involved in the pathology have recently been clarified, such as the anti–N-methyl-D-aspartic acid receptor antibody in nonherpetic acute limbic encephalitis. Nevertheless, the pathology of ADEM remains unclear.

Acute encephalopathy is classified as being either related or unrelated to infectious diseases. Infection-related acute encephalopathy (AE) is a generic term for a wide range of cerebral dysfunctions accompanying infectious diseases, although no finding has suggested pathogen invasion in the central nervous system (CNS). On the contrary, infection-unrelated acute encephalopathy is a brain dysfunction accompanying various noninfectious diseases, such as liver failure, renal failure, and hypertension. Febrile seizure simplex type (FS) is a generic term for the condition in which convulsions appear transiently during a fever. Febrile seizure is a common disease in children and has a good prognosis without clinical sequelae. As the initial clinical symptoms of ADEM, AE, and febrile seizure are similar, these diseases are difficult to differentiate in the acute phase.

Many cytokines and biochemical proteins have been studied as biomarkers of AEE. Reportedly, levels of inflammatory cytokines in the cerebrospinal fluid (CSF) increase during the acute phase, and studies have reported the role of interferon-γ in acute encephalitis; the role of interleukin-6 (IL-6) and IL-8 in bacterial meningitis; and the role of tumor necrosis factor α, soluble tumor necrosis factor receptor-1, and IL-6 in AE.[3,4] On the contrary, regarding biochemical proteins in the CSF, S-100B, glial fibrillary acidic protein, and tau protein have been reported as brain injury biomarkers of AE.[5-8] These biomarkers represent the repair process of damaged nerve cells and axons. Therefore they are not suitable for expressing neuroinflammation in the acute phase.

Alpha 2-macroglobulin (α2M) can be an indicator of acute inflammation, which is a biological defense response, unlike the previously reported damaged substances resulting from cytotoxicity, such as S-100B, glial fibrillary acidic protein, and tau protein. We focused on α2M, whose blood level increased in a short time at the onset of inflammation. An increase in α2M levels in the CSF in bacterial meningitis has been reported.[3,9,10] Extensive studies have been conducted on the relationship between α2M levels in the CSF and CNS diseases in patients with bacterial meningitis, showing that α2M levels in the CSF reflected α2M produced in the serum during systemic inflammation because of the damage to the blood-brain barrier (BBB).[3,10-12] However, there have been no reports on α2M levels in patients with ADEM.

In this study, we aimed to measure α2M levels in the CSF and serum samples of patients with ADEM, AE, febrile status epilepticus (FSE), or FS in the acute phase and to examine the association between neuroinflammation and α2M.

Materials and Methods

Sample collection

Participants

We analyzed preserved samples collected from 35 patients with ADEM, AE, FSE, and FS who were treated in the pediatric departments of hospitals throughout Fukushima Prefecture between January 1, 1999 and May 31, 2012. These samples were stored to assess cytokine levels and conduct viral polymerase chain reactions. The first diagnosis was provided based on the findings on admission, and it was confirmed by the clinical course. A presumable diagnosis of ADEM was clinically made based on alterations to or a loss of consciousness lasting more than 24 hours, presenting with acute polysymptomatic neurological signs, and magnetic resonance imaging showing one or multiple lesions suggestive of demyelination. A presumable diagnosis of AE was clinically established based on the acute onset of impaired consciousness accompanied by brain dysfunction, usually preceded by infection.[1,13] The FS group consisted of children who presented with fever and seizures; however, they were later found to be free from acute neurological damage based on the course of clinical events, laboratory data, and brain imaging, where available. FSE was diagnosed in patients with febrile seizure who presented with seizures lasting for more than 30 minutes. CSF samples from patients with bacterial meningitis were used as positive control subjects, and patients with leukemia in remission with no neurological abnormality confirmed by regular examinations were used as negative control subjects. Patients with viral meningitis, myelitis, or vascular, metabolic, endocrine, or toxic disorders were excluded.

Preservation of CSF and serum samples

CSF and serum samples were all collected within 24 hours after disease onset. These samples were immediately centrifuged after collection and stored at −80°C. Patients who provided samples in which macroscopic hemolysis was observed were excluded from the analysis.

Ethics

Preserved samples were used in this study. This study was conducted with the approval of the Ethics Committee of the Fukushima Medical University (approval no. 1684). Information related to this research was released on our university web site, and patients were included in the study using an opt-out methodology.

Immunoblotting

The protein concentration of clinical samples was quantified by bicinchoninic acid method using a BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA, USA). CSF samples were diluted with a sample-loading buffer and heated at 95°C for 5 minutes, and then electrophoresed at 200 mV, 20 mA per sheet, for 73 minutes using a 5% to 20% gradient polyacrylamide gel (Wako, Osaka, Japan). Clinical specimens with a total protein content of 0.4 μg and 2 ng of human plasma α2M (Sigma-Aldrich, St. Louis, MO, USA) as a standard were electrophoresed. The gel of <15 kDa was cut out from the main gel and subjected to silver staining (Wako) to detect transthyretin as a banding control. It was then transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) at 300 V, 350 mA, for 45 minutes by wet Western blotting. After blocking for 1 hour at room temperature under 3% milk phosphate-buffered saline containing Tween-20 (PBST), the membranes were reacted with 3 μg/mL of anti-α2M antibody (ICN/Cappel, Aurora, OH, USA) and diluted with 3% milk PBST for two hours. After washing three times with PBST, the membranes were reacted with horseradish peroxidase–labeled anti-goat IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), diluted to 0.08 μg/mL with 3% milk PBST for 1 hour, and then washed in the same way with PBST. Immunoreactive bands were detected using a SuperSignal West Dura Extended Duration Substrate (ThermoFisher). The band intensity was calculated using an Image Saver 6 luminescence detector (ATTO, Tokyo, Japan).

TABLE 1.
Patient Characteristics

Classification of Disease	ADEM	AE	FSE	FS
n	5	7	12	11
Age (y), median (interquartile range)	4.4 (1.0-11.4)	2.3 (0.7-7.0)	1.1 (0.6-4.4)	2.6 (0.3-7.3)
Sex (B:G)	1:4	4:3	6:6	6:5

Abbreviations:
ADEM = acute disseminated encephalomyelitis
AE = infection-related acute encephalopathy
B = boys
FS = febrile seizure simplex type
FSE = febrile status epilepticus
G = girls

Enzyme-linked immunosorbent assay

For enzyme-linked immunosorbent assay (ELISA), 100 μL of anti-human α2M antibody (2.0 μg/mL; ICN/Cappel Pharmaceuticals) diluted with 0.05 M carbonate-bicarbonate (pH 9.6) was added to each well of a 96-well C8 MaxiSorp immunomodule plate (Nunc, Roskilde, Denmark) and incubated at 4°C overnight. After washing once with Tris-buffered saline containing Tween-20 (TBST), 300 μL of 10% Block Ace (DS Pharma Biomedical, Osaka, Japan) was added to each well and incubated as a blocking step for one hour. After washing five times with TBST, 0.75 μL of each CSF sample was applied to each well and incubated at 37°C for one hour. After washing again five times with TBST, the plate was incubated with 100 μL of horseradish peroxidase–conjugated anti-human α2M antibody (2.0 μg/mL; GeneTex, San Antonio, TX, USA) at room temperature for one hour. After further washing five times with TBST, the plate was reacted with 3, 3′, 5, 5′-tetramethylbenzidine peroxidase substrates (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). The reaction was quenched using 1 M hydrochloric acid, and the absorbance at 450 nm was measured with a microplate reader (Bio-Rad Laboratories).

Immunohistochemistry

Formalin-fixed paraffin-embedded brain tissue infected with herpes encephalitis was cut into 5-μm-thick sections using a microtome and mounted on glass slides. Deparaffinized sections were incubated with or without anti-human α2M antibody (goat IgG, AF1938, R&D Systems) followed by biotinylated rabbit anti-goat IgG (Nichirei, Tokyo, Japan). The antigens were visualized with avidin-biotin peroxidase complex and peroxidase diaminobenzidine enzyme histochemistry. Sections were then counterstained with cresyl violet and cover-slipped.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 21 (IBM Japan, Ltd., Tokyo, Japan). Continuous variables were expressed as medians (interquartile range). Comparisons between groups were made using the Kruskal-Wallis test. Thereafter, comparisons between two groups were made using the Mann-Whitney *U* test. A *P* value <0.05 was considered statistically significant.

Results

Patient characteristics

Table 1 shows the characteristics of patients according to the diagnosis. In total, 35 patients (17 boys, 48.6%, and 18 girls, 51.4%) were enrolled. The age at disease onset ranged from infancy to school aged. No significant differences were found in their age at diagnosis.

General examination findings

The number of cells in the CSF and the white blood cell count in blood, as well as the C-reactive protein levels in the blood samples, are shown in Table 2. The median number of cells in the CSF was 41.5 (27.8 to 49.0)/μL for patients with ADEM, 2.0 (1 to 2.5)/μL for those with AE, 1.5 (1.0 to 2.8)/μL for those with FSE, and 5.0 (1.0 to 7.0)/μL for those with FS. The number of cells in the CSF samples from patients with ADEM was significantly higher than those from patients with AE, FSE, and FS (P = 0.02, 0.01, and 0.04, respectively). On the contrary, no significant differences were found in the number of cells in the white blood cell count or C-reactive protein levels among the blood samples.

Detection of α2M in CSF by Western blotting

A 180-kDa α2M band (Fig 1) was detected in all CSF samples from patients with AE, ADEM, FSE, and FS. Strong bands were detected in two samples (nos. 1 and 2) from patients with ADEM. Samples from patients with bacterial meningitis, in whom α2M levels in the CSF were reported to increase markedly, were used as positive control subjects (no. 11). A strong band was also detected in the other samples from patients with AE, but most other samples had weak bands. In addition, the bands of the control samples were weak (nos. 9 and 10).

TABLE 2.
CSF Cells, WBC, and CRP Levels in Serum Samples

Classification of Disease	ADEM	AE	FSE	FS
CSF cells (/μL)	41.5* (27.8-49.0)	2.0 (1.0-2.5)	1.5 (1.0-2.8)	5.0 (1.0-7.0)
WBC (/μL)	13,000 (10,200-15,400)	10,700 (8750-13,750)	10,900 (8300-16,538)	12,900 (—)
CRP (mg/dL)	0.97 (0.53-1.26)	0.16 (0.10-0.67)	0.96 (0.37-1.57)	1.2 (—)

Abbreviations:
ADEM = acute disseminated encephalomyelitis
AE = infection-related acute encephalopathy
CSF = cerebrospinal fluid
CRP = C-reactive protein
FS = febrile seizure simplex type
FSE = febrile status epilepticus
WBC = white blood cell
The number of CSF samples: ADEM (n = 5), AE (n = 7), FSE (n = 12), and FS (n = 11). The number of serum samples: ADEM (n = 3), AE (n = 7), FSE (n = 10), and FS (n = 1). All concentrations are expressed as median level (interquartile range).
* *P* values (<0.05) for CSF cells are detected versus AE, FSE, and FS.

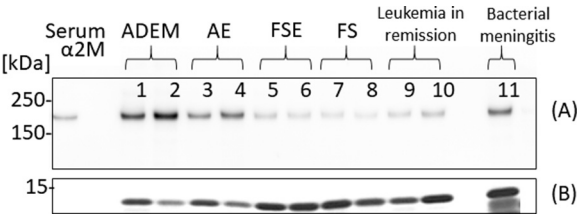

FIGURE 1. Western blotting of CSF samples. (A) The same quantity of CSF protein (0.4 μg) was applied to each lane. Western blotting was performed using anti-sheep α2M antibody. Nos. 1 and 2 are for patients with ADEM, 3 and 4 are for patients with AE, 5 and 6 are for patients with FSE, and 7 and 8 for patients with FS. Migrating positions of molecular weight markers (150 and 250 kDa) are indicated on the far left of the figure. The bands for leukemia in remission (nos. 9 and 10) and that for bacterial meningitis (no. 11) are shown on the far right side of the figure. (B) Silver staining was performed and a gel <15 kDa was used as a loading control. α2M, alpha 2-macroglobulin; ADEM, acute disseminated encephalomyelitis; AE, acute encephalopathy; CSF, cerebrospinal fluid; FS, febrile seizure; FSE, febrile status epilepticus.

Quantification of α2M in CSF and serum by ELISA

To confirm the results of the Western blot analysis, we performed sandwich ELISA for α2M using the CSF and serum samples (Table 3). The median α2M level (interquartile range) in the CSF was 4.7 (3.8 to 8.4), 2.1 (1.1 to 2.3), 1.1 (0.9 to 6.4), and 1.0 (0.8 to 1.1) μg/mL in patients with ADEM, AE, FSE, and FS, respectively. The α2M level in patients with ADEM was significantly higher than those in patients with AE and FS ($P = 0.019$ and $P = 0.002$, respectively). For reference, the α2M level in the CSF from patients with bacterial meningitis (n = 4) and control patients (n = 18) was 24.0 (6.9 to 25.2) and 1.54 (1.3 to 2.0) μg/mL, respectively (data are not shown). On the contrary, the median α2M level in the serum of patients was 363.9 (352.3 to 380.8) mg/dL in ADEM, 353.8 (328.7 to 429.7) mg/dL in AE, 356.4 (335.1 to 392.3) mg/dL in FSE, and 263.6 (174.7 to 284.9) mg/dL in FS. No significant differences were observed in α2M levels in the serum among the diseases. The ratio of the α2M level in the CSF to that in the serum was examined, and the median value of this ratio in patients with ADEM was significantly higher than those in patients with AE and FSE ($P = 0.016$ and $P = 0.04$, respectively).

Scatter diagram of α2M levels in CSF and serum

To investigate the correlation between α2M levels in the CSF and serum, we plotted α2M levels in 10 paired samples of the CSF and serum from five patients with ADEM and five patients with AE (Fig 2). The α2M levels in the CSF differed markedly among patients with ADEM, regardless of α2M in their serum. There was almost no correlation between α2M levels in the CSF samples and those in the serum samples ($R = 0.264$, $P = 0.668$) from patients with ADEM. On the contrary, in patients with AE, α2M levels in the CSF were low.

Receiver operating characteristics curve

To distinguish ADEM from the other diseases (AE, FSE, and FS) based on the ratio of α2M levels in the CSF and in serum, receiver operating characteristics curve analysis was performed (Fig 3). The α2M cutoff level, according to Youden's index (sensitivity + specificity − 1), was 0.42 μg/mg. The sensitivity and specificity were 1.00 and 0.79, respectively. Furthermore, to analyze α2M levels in the CSF, the cutoff level was set as 2.5 μg/mL. The sensitivity was 1.00 and specificity was 0.80, which were almost the same as those for the ratio of the α2M level in the CSF and that in the serum.

Changes in α2M band levels in the CSF of patients with ADEM after treatment

We examined the temporal changes in α2M band levels in the CSF (Fig 4). Compared with CSF samples obtained at disease onset, α2M band levels decreased after steroid pulse therapy (no. 1 versus no. 2 and no. 3 versus no. 4). On the contrary, in samples obtained from a patient before being diagnosed with ADEM who did not receive therapeutic intervention, no significant decrease was found in the band levels even 10 days after disease onset (no. 5 versus no 6).

α2M immunoreactive cells in herpes viral encephalitis

To examine α2M production in the CNS, the tissue sections of patients with herpes simplex encephalitis were subjected to immunohistochemistry. As shown in Fig 5A, astrocyte-like cells (arrows) and microglia-like cells (circle) were immunoreactive against anti-α2M antibodies. Vascular endothelial cells were also immunoreactive (asterisk). Many macrophage-like cells were also immunoreactive in the perivascular Virchow-Robin spaces of blood vessels (data not shown). No immunoreactive signal was observed without the first antibody (Fig 5B).

Discussion

α2M is a glycoprotein with a molecular weight of 725.000, making it one of the most polymeric substances among the plasma proteins, and it exhibits a mass of approximately 180 kDa by electrophoresis.[14] α2M is produced in the Kupffer cells of the liver

TABLE 3.
α2M Levels in CSF, Serum, and Ratio in CSF to Serum

Classification of Disease	ADEM	AE	FSE	FS
CSF (μg/mL)	4.7* (3.8-8.4)	2.1 (1.1-2.3)	1.1 (0.9-6.4)	1.0 (0.8-1.1)
Serum (mg/dL)	363.9 (352.3-380.8)	353.8 (328.7-429.7)	356.4 (335.1-392.3)	263.6 (174.7-284.9)
CSF/Serum (μg/mg)	1.46† (0.75-2.28)	0.28 (0.27-0.34)	0.3 (0.27-0.50)	0.17 (—)

Abbreviations:
ADEM = acute disseminated encephalomyelitis
AE = infection-related acute encephalopathy
CSF = cerebrospinal fluid
FS = febrile seizure simplex type
FSE = febrile status epilepticus
The number of CSF samples: ADEM (n = 5), AE (n = 7), FSE (n = 12), and FS (n = 11). The number of serum samples: ADEM (n = 8), AE (n = 6), FSE (n = 13), and FS (n = 3). The ratio in CSF to serum (CSF/serum): ADEM (n = 5), AE (n = 5), FSE (n = 8), and FS (n = 1).
All concentrations and ratio are expressed as median level (interquartile range).
* *P* values (<0.05) for CSF cells are detected versus AE and FS.
† *P* values (<0.05) for CSF cells are detected versus AE and FSE.

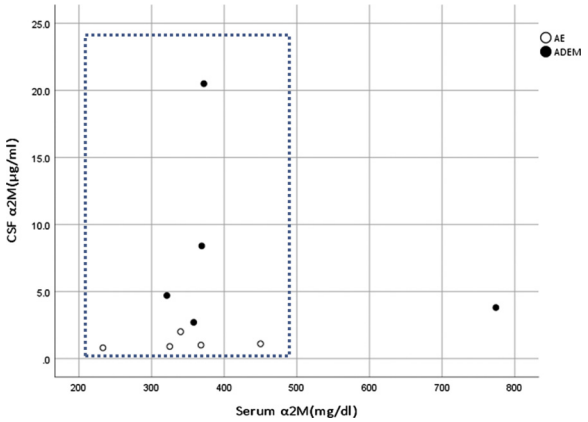

FIGURE 2. Scatter diagram of α2M levels in the CSF and serum. α2M levels in the CSF and serum are plotted along the vertical and horizontal axes, respectively. Closed circles represent α2M levels in patients with ADEM and open circles represent those in patients with AE. The boxed area in the figure is the reference range of α2M levels in the serum. α2M, alpha 2-macroglobulin; ADEM, acute disseminated encephalomyelitis; AE, acute encephalopathy; CSF, cerebrospinal fluid. The color version of this figure is available in the online edition.

during the early stage of inflammation, and it appears in the blood. Regarding its function in the blood *in vivo*, α2M is known to nonspecifically capture proteases released from bacteria and act as a protease inhibitor, resulting in the inhibition of cellular immunity.[15] α2M is characterized by its rapid response, which is more rapid than antigen-antibody reactions. After capturing proteases, α2M-protease complexes are taken up by α2M receptors on the surfaces of hepatocytes, macrophages, fibroblasts, and mast cells. Therefore the complexes disappear from the blood on the resolution of the inflammation.[16,17]

In this study, α2M levels in the CSF of patients with ADEM were higher than those in patients with AE, FSE, and FS (Fig 1). α2M levels in the CSF and serum from patients with bacterial meningitis showed a significantly strong correlation ($r = 0.74$, $P < 0.0001$).[3] In

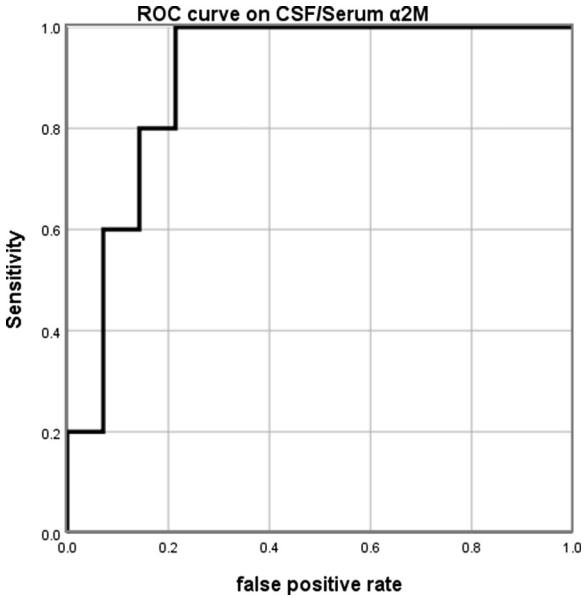

FIGURE 3. Receiver-operator characteristic (ROC) curve for the ratio of the α2M level in the CSF to that in the serum. At a cutoff level of 0.42 μg/mg, the sensitivity and specificity for ADEM were 1.00 and 0.79, respectively. α2M, alpha 2-macroglobulin; ADEM, acute disseminated encephalomyelitis; CSF, cerebrospinal fluid.

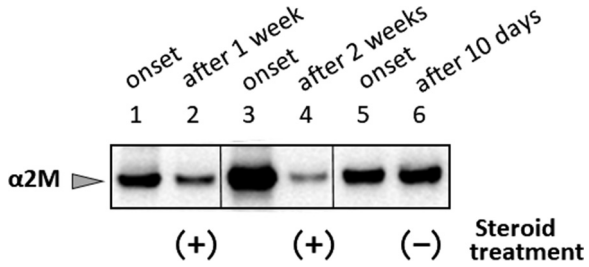

FIGURE 4. Changes in α2M band levels in the CSF after treatment for ADEM. Paired samples of the CSF (on disease onset and after treatment) were obtained from patients with ADEM. Western blotting was performed in the same manner as in Fig 1. Nos. 1 and 3 show α2M bands at onset, and nos. 2 and 4 show their α2M bands after steroid pulse therapy. Both α2M levels decreased after treatment. On the contrary, there was no change in α2M bands between nos. 5 and 6, obtained at a 10-day interval from a patient before being diagnosed with ADEM. α2M, alpha 2-macroglobulin; ADEM, acute disseminated encephalomyelitis; CSF, cerebrospinal fluid.

other words, it appears that α2M in the case of bacterial meningitis is produced by the liver and that α2M levels in the CSF increase as a result of the concentration gradient of α2M in the serum. Kanoh and Ohtani concluded that α2M levels in the CSF of patients with bacterial meningitis increase as a result of the permeation of α2M from the serum to the CSF. α2M has a high molecular weight, so this permeation depends on the degree of inflammation-induced BBB damage.[10] However, no studies on α2M levels in the CSF of patients with ADEM have been reported. A significant difference was found in α2M levels in the CSF between patients with ADEM and those with AE, whereas no significant differences were observed in serum α2M levels of patients with these diseases (Table 3).

ADEM is defined as the first episode of inflammatory demyelination, with polyfocal neurological deficits implicating the involvement of multiple sites of the CNS.[18] There have been no reports on vascular permeability in patients with ADEM. Furthermore, the mechanism relating to the extent of BBB damage and the concentration gradient of serum α2M is yet to be elucidated. In patients with ADEM, α2M levels in the CSF are not correlated with those in the serum, suggesting that α2M is produced in the CNS (Fig 2). Serum α2M plays an important role as a carrier protein for IL-6 and activates IL-6 produced at local inflammatory sites.[19] Our present results indicate that astrocyte-like cells may produce α2M in brain tissues (Fig 5). This observation is supported by neuroinflammation relating to a microglia-astrocyte-mast cell network.[20] In addition to the findings from patients with CNS infections, such as herpes simplex encephalitis, those from patients with neurodegenerative diseases, such as Alzheimer disease, multiple sclerosis, and Parkinson disease, also demonstrates neuroinflammation induced by glial cells of the brain.[21] To date, there have been no reports on α2M production in the brain. The results of our study suggest that α2M is, at least partly, produced in the CNS under inflammatory conditions.

In ADEM, it is unknown whether the increase in α2M is because of brain production or vascular permeability. The mechanism of neuroinflammation is related to microglia and mast cells, which act as macrophages in the brain and react with cytokines, chemokines, and neurotransmitters. When lesions form in CNS, microglia are activated to produce inflammatory cytokines and mediate inflammatory responses to disease or injury. One of these responses may be α2M production.

We investigated whether α2M levels in the CSF could be used clinically. α2M levels in the CSF and the ratio of the α2M level in the CSF to that in the serum were significantly increased in the acute phase of ADEM. On the contrary, a comparison of the α2M bands in the CSF samples obtained at disease onset and after treatment

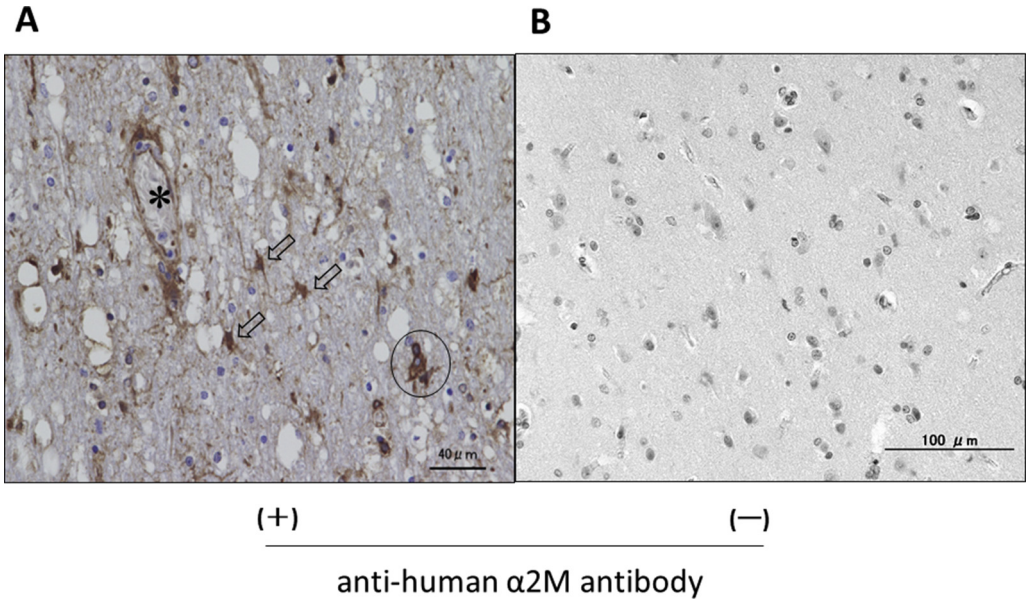

FIGURE 5. α2M immunoreactive cells in herpes simplex encephalitis. On encephalitis tissue, astrocyte-like cells (arrows) and microglia-like cells (circle) were immunoreactive against anti-α2M antibodies (A). Vascular endothelial cells were also immunoreactive (asterisk). No immunoreactive signal was observed without the first antibody and only signals of counter staining with cresyl violet were observed (B). α2M, alpha 2-macroglobulin.

showed that the bands were weaker after steroid pulse treatment. However, in a patient not receiving treatment, the α2M bands remained strong over time (Fig 4). Changes in α2M levels in the CSF may have reflected the effect of intervention. These data suggest that the ratio of the α2M level in the CSF to that in the serum reflects the neuroinflammatory status of patients with ADEM and indicates the expression of early neuroinflammation in ADEM.

Limitations

In this study, pathogens are not clearly known in any AEE cases. In addition, as our study uses only a small number of samples, further accumulation of data is warranted. Finally, the presence of α2M in the CSF does not indicate disease specificity; therefore the results should be interpreted quantitatively, considering the clinical course.

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