Detection of Serum Antibody against Arrestin from Patients with Acute Disseminated Encephalomyelitis

YOKO IKEDA, AKIRA SUDOH,¹ SUSUMU CHIBA,² HIROYUKI MATSUMOTO,² TAKASHI NAKAGAWA and HIROSHI OHHGURO

Departments of Ophthalmology and ²Neurology, Sapporo Medical University School of Medicine, Sapporo 060-8543, and ¹Department of Pediatrics, Hokkaido University School of Medicine, Sapporo 001-0014

IKEDA, Y., SUDOH, A., CHIBA, S., MATSUMOTO, H., NAKAGAWA, T. and OHHGURO, H. Detection of Serum Antibody against Arrestin from Patients with Acute Disseminated Encephalomyelitis. Tohoku J. Exp. Med., 1999, 187 (1), 65-70 ——— In our previous study, we found the presence of serum autoantibody against arrestin in patients with multiple sclerosis (MS), while such serum autoantibody was not detected from patients with other neurological diseases and control subjects. We suggested that serum arrestin antibody titers may be useful for the diagnosis and evaluation of the disease’s course. In the present study we examined sera from 7 patients, who were initially diagnosed as having acute disseminated encephalomyelitis (ADEM), for the presence of serum antibody against arrestin, in order to study the specificity of the serum antibody among demyelinated diseases. High titers were detected from 2 patients out of 7. One of the patients, a 4 year-old girl, presented with an additional neurological attack during the 6 months after the initial attack, resulting in change of diagnosis to MS. During her disease course the serum titers against arrestin fluctuated in correspondence with the disease’s activity. These observations suggest that the presence of serum autoantibody against arrestin may be specific to MS and be helpful for differential diagnosis of ADEM and MS. ——— multiple sclerosis; acute disseminated encephalomyelitis; demyelination; arrestin; autoantibody © 1999 Tohoku University Medical Press

Acute disseminated encephalomyelitis (ADEM) is, similarly to multiple sclerosis (MS), an inflammatory demyelinated disease that affects the central nervous system. MS is a multiphasic disease that frequently results in stepwise or steadily progressive deterioration in neurologic function. Conversely ADEM is
usually a monophasic illness that usually occurs 7-21 days after a viral infection or vaccination (Prineas and McDonald 1997). At the initial neurological attack of MS, its clinical course sometimes closely resembles with ADEM, and there are no definitive clinical signs to differentiate them. Therefore, we sometimes change the diagnosis of ADEM to MS during the clinical course.

Our group recently found the presence of autoantibodies against arrestins, 45-48 kDa proteins widely distributed within retina and brain, in sera of 8 of 14 MS patients. Such autoantibody was not detected from 50 serum samples from healthy subjects and those with other neurological diseases, including autoimmune based neurological disorders such as Guillain-Barré syndrome and Miller Fischer syndrome. In two patients with MS, the serum titers during relapse were higher than found during remission (Ohguro et al. 1993). Based upon the above observations, we suggested that the presence of circulating antibodies reactive with arrestin are possibly specific to MS, and related to the course of MS progression. These observations allowed us to speculate that the presence of the serum arrestin antibody might be useful for the differential diagnosis of MS and ADEM.

In this study, to prove our hypothesis, we performed serological examination related to the arrestin antibody using 7 patients who were initially diagnosed as ADEM. We found that serum autoantibodies against arrestin were detected from 2 out 7 patients who were initially diagnosed as ADEM. One of the patients turned out to be MS because she showed an additional neurological attack during the following 6 months, and the serum antibody levels correlated with the severity of the disease.

Patients and Methods

Patients and sera preparation

Seven patients initially diagnosed as ADEM were recruited from Sapporo Medical University or Goryoukaku Hospital. The initial diagnosis of ADEM was based on the guideline described in Merritt’s textbook of Neulology (Jubert and Miller 1995). Patients’ ages were distributed from 4 to 47 years. Demyelinated plaques were confirmed from all patient by MR imaging examination. Peripheral venous bloods obtained from 7 patients and 10 age-matched healthy subjects were immediately subjected to serum separation and stored at –80°C.

ELISA

ELISA was performed as described previously (Ohguro et al. 1993). Briefly, freshly purified bovine retinal arrestin (Buczyłko and Palczewski 1991) was subjected to a microtiter plate. Patients’ serum at dilution of 10 to 640 and a horse radish peroxidase (HRP) conjugated anti-human IgG goat sera (Dako, Kyoto) were used as primary and secondary antibodies, respectively. The specific antigen-antibody binding was visualized with o-phenylenediamine as a substrate. The optical density (OD) was measured at 495 nm.
Western blots

The electrophoretic transfer of retinal soluble proteins which were obtained by homogenization and centrifugation of fresh bovine retinas onto a polyvinylidene difluoride membrane was performed by using an electrotransfer instrument (Biocraft Co., Tokyo). The immunoblot staining was performed using patient sera (1:200 dilutions) and horse radish peroxidase conjugated goat anti-human IgG (Dako, Kyoto) as 1st and 2nd antibodies as described previously (Ohguro et al. 1993).

Case Report (Patient 1)

A 4-year-old girl noticed a sudden onset of neurological symptoms including paralysis in her right upper extremity, tremor in bilateral upper extremities, dysuria, and dysarthria, 2 weeks after fever and headache. Then she was treated with corticosteroid therapy for a diagnosis of ADEM. Her symptoms were diminished during the next one and a half months. However five months later, she presented with additional neurological attacks including optic neuritis, abnormal eye movements, and sudoresis, and was treated again with steroid therapy. Based on her clinical course, she was diagnosed as having MS not ADEM. Laboratory examinations including complete blood cell count, blood chemistry, and liquid pressure and cell count were within normal ranges except for slight serum hyperleukocytosis. Tests for serum anti-virus antibody titers, serum antinuclear titers and liquid oligoclonal IgG were negative. ELISA test revealed that her serum anti-arrestin titers were 40 times, 5 times and 15 times higher than normal at initial attack, after remission and in relapse phase, respectively.

Results and Discussion

Several lines of evidences have suggested that an autoimmune basis should be involved in the pathogenesis of MS and other demyelinating diseases. However, it is still controversial with regard to the precise pathophysiology and autoantigen (Lisak 1980; Martin et al. 1992). Recently, we found the presence of serum autoantibodies against arrestins, 45-48 kDa proteins widely distributed within retina and brain, in 8 out of 14 MS patients. However, such antibodies were not found in sera of patients with other neurological diseases and control subjects. In two MS patients, the serum titers during relapse were higher than in remission (Ohguro et al. 1993). Therefore we suggested that the presence of circulating antibodies reactive with arrestin may be related to the course of MS progression, and that monitoring of the serum arrestin antibody titers may be useful to predict additional neurological attack of MS. If our speculation is true, the serum arrestin antibody titers would be helpful for differential diagnosis of ADEM and initial attack of MS, which are sometimes very difficult to distinguish. Here, to test our hypothesis, we examined seven patients initially diagnosed as ADEM and
found that serum arrestin-antibody titer levels were significantly high in 2 patients (cases 1 and 2, \( p < 0.01 \)) and relatively raised in one patient (case 4) as compared with controls (mean 0.007, s.d. = 0.01), but cases 3 and 5-7 were considered to be within normal range (absorbance at 492 nm < 0.037 ± 0.007 (mean) + 3 s.d., Fig. 1). Western blot analysis using retinal homogenate showed that a prominent immunoreactivity toward arrestin was also detected in the two patients' sera (Fig. 2). However, one of the patients, a 4 year-old girl (case 1), presented with additional neurological attack during the 6 months after the initial attack. Her serum titers against arrestin fluctuated (high in relapse and low in remission phases) as shown in Fig. 3. In addition, a 47 year-old female patient (case 4) also presented with an additional neurological relapse one year after the onset. From their clinical episodes, they were diagnosed as MS not ADEM. While, although such neurological relapses have not observed so far in the other patient, an 18-year old male (case 2), we are afraid that this will occur at some time in the future.

Arrestin is a family of proteins which is functionally implicated in inactivating the active state of G-protein coupled receptors (Palczewski 1994). Retinal arrestin is also known to be a highly pathogenic molecule inducing autoimmune uveitis to susceptible strains of animals (Wacker et al. 1977; Nessenblatt et al. 1980). In our previous study, we found the major epitope for the anti-arrestin

![Graph](image)

**Fig. 1.** Comparative absorption at 495 nm by HRP enzyme-linked immunosorbent assay reactions of control and ADEM serum samples. All serum samples were assayed at 1 to 100 dilution on microplate coated with purified arrestin as describe in Method section. Serum samples tested were withdrawn from patients with ADEM at the initial attack phase, and age-matched healthy control subjects (\( n = 10 \)). Experiments were performed in duplicate. Serum arrestin-antibodies titer levels were significantly high in 2 patients (\( p < 0.01 \)) and relatively raised in one patient (Case 4) as compared with controls (Mean 0.007, s.d. = 0.01), while Cases 3 and 5-7 were considered to be within normal range (absorbance at 492 nm < 0.037 ± 0.007 (mean) + 3 s.d.)
Serum Arrestin Antibody in Patient with ADEM

Fig. 2. Binding of patient’s serum to a blot of bovine retinal soluble fractions. Blots of bovine retinal soluble fractions were incubated with sera from patient 1 and 2, and immunoreactive band were visualized as described in Materials and Methods. A prominent band at 45 kDa labeled with the serum is identical with that labeled with anti-arrestin sera (data not shown). The data of control subject is identical with that shown in Fig. 1.

Fig. 3. Comparative absorption at 495 nm by ELISA of control and patient 1 serum samples. All serum samples were assayed at 1 to 100 dilution on microplate coated with purified arrestin as describe in Method section. Control sera (upper first column), patient 1 serum (initial attack; second column, after recovery; third column, second attack; fourth column). Experiments were performed in duplicate.

Antibody in MS patients located between residues 290–320 (Ohguro et al. 1993) which is similar to amino acids sequences of several virus including Theiler murine encephalomyelitis virus which cause demyelination in animal, hepatitis B virus and other viruses. This suggests to us that molecular mimicry is an
underlying mechanism of specific antibody production. If this is true, we can speculate that the infection which precedes ADEM, in which the virus has arrestin homologous sequence, might be involved in the pathogenesis of additional neurological attack in ADEM. Although this possibility is still speculative at present, autoimmune response against arrestin was detected in some cases of MS and ADEM. Therefore, we will study further to clarify the relationship between arrestin and demyelinating diseases.

Acknowledgments

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References