NOTE *Clinical Pathology*

Serial Examinations of Anti-GFAP Autoantibodies in Cerebrospinal Fluids in Canine Necrotizing Meningoencephalitis

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ABSTRACT. Canine necrotizing meningoencephalitis (NME) is characterized by autoantibodies against glial fibrillary acidic protein (GFAP) in cerebrospinal fluids (CSFs). To clarify the time-course changes in autoantibodies, serial examinations were conducted in three dogs with NME (two Pugs and a Pomeranian) that were treated by immunosuppressive therapy. The Pugs retained high autoantibody titers throughout the observation periods (146 and 813 days) and died with neurological signs. On the other hand, the Pomeranian switched from being positive for autoantibody to negative after day 580, and its NME seemed to be in clinical remission until death on day 1238. Therefore, the anti-GFAP autoantibodies can be detected over time in canine NME even during immunosuppressive therapies. However, the autoantibodies can also disappear within a certain period after onset.

KEY WORDS: autoantibody, canine, necrotizing meningoencephalitis.

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Canine necrotizing meningoencephalitis (NME) is an idiopathic disease that affects specific breeds such as the Pug and Maltese [1, 6]. While there is little information about the pathogenesis of NME, we have previously reported that almost all NME cases possess autoantibodies against glial fibrillary acidic protein (GFAP) in their cerebrospinal fluids (CSFs) [4, 5, 8]. Our data suggests that the CSF autoantibodies can be used as a diagnostic marker of NME; however, the time-course changes in autoantibody titers remain unclear. There also remains a question of whether the antibody can be detected both in the acute and chronic stages of NME, especially under certain immunosuppressive therapies. In the present report, serial examinations of anti-GFAP autoantibodies were conducted in three dogs with NME.

Case 1 (an 8-month-old female Pug) was referred to the University of Tokyo Veterinary Medical Center with a day history of tonic-clonic seizures, lethargy and ataxia. Brain magnetic resonance images (MRI) at admission revealed diffuse inflammation in the cerebrum. A diagnosis of NME (acute stage) was tentatively made, and the dog was treated with prednisolone (1–2 mg/kg/day) and phenobarbital (4–8 mg/kg/day). While seizures were partially controlled over the following months, the dog's mental status was not improved. The dog died on day 146, and the diagnosis of NME was histopathologically confirmed. Anti-GFAP autoantibodies were tested on days 1 and 146 by the indirect fluorescent antibody (IFA) test as previously described [4]. Briefly, five-micrometer frozen sections of the normal canine brain cortex were dried, fixed with cold acetone, blocked with normal goat serum (1:50) and incubated with CSFs at dilutions of 1:1, 1:10 and 1:100 at room temperature for 60 min. After washing, FITC-conjugated goat anti-canine IgG (1:400, Bethyl, Montogomery, TX, U.S.A.) was added for 60 min. The slides were then washed, mounted with Vectashield (Vector Labs, Burlingame, CA, U.S.A.) and analyzed using a fluorescent microscope. In Case 1, both CSFs revealed high titers (1:100).

Case 2 (a 6-year-old female Pug) was referred to us with a three-day history of tonic-clonic seizures and ataxia. A brain MRI revealed diffuse inflammation and multifocal malacic lesions in the brain cortex. A tentative diagnosis of NME (acute to subacute) was made, and the dog was treated with prednisolone (1–2 mg/kg/day), cyclosporine (5 mg/kg/day) and zonisamide (6–10 mg/kg/day). For over 2 years, neurological abnormalities including generalized seizures were well improved in the dog. However, intractable cluster seizures occurred on day 812, and the dog died on day 818. A postmortem examination confirmed the diagnosis of NME. CSF examinations were conducted on days 1, 171 and 813. All CSFs had high titers (1:100) for anti-GFAP autoantibodies.

Case 3 (a 5-year-old male Pomeranian) was referred to us with a 46-day history of tonic-clonic seizures and blindness. A brain MRI revealed malacic lesions in the cortex. A tentative diagnosis of NME (chronic stage) was made, and the dog was treated with prednisolone (1–2 mg/kg/day), phenobarbital (5–8 mg/kg/day) and potassium bromide (35 mg/kg/day). For the first year, at least 1 mg/kg/day of prednisolone was needed to maintain the neurological status of the dog. The dog's autoantibody titers were 1:10 on days 1 and 265. However, around day 500, the dog exhibited improvement in relation to seizures, and the steroid therapy was tapered off and stopped without obvious recurrence of seizures. The dog eventually died on day 1238 of acute pulmonary edema from mitral valve regurgitation. A postmortem

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Table 1. Three dogs with necrotizing meningoencephalitis subjected to serial examinations of anti-GFAP autoantibodies in cerebrospinal fluids

Case	Profiles	Major symptoms	Treatment	CSF examination	Anti-GFAP autoantibody
No. 1	0Y8M, female Pug	Seizures, lethargy, ataxia	Prednisolone, phenobarbital	Day 1	1:100
				Day 146	1:100
No. 2	6Y, female Pug	Seizures, ataxia	Prednisolone, cyclosporine, zomisamide	Day 1	1:100
				Day 171	1:100
				Day 813	1:100
No. 3	5Y, male Pomeranian	Seizures, blindness	Prednisolone, phenobarbital, KBr	Day 1	1:10
				Day 265	1:10
				Day 580	ND
				Day 1238	ND

ND: Not detected.

examination confirmed the diagnosis of NME. The CSF autoantibody was not detected on days 580 and 1238.

In the present study, we were only able to include three NME cases in which CSF anti-GFAP autoantibodies were examined two or more times. Unfortunately, since many dogs with NME survive for less than a year, longitudinal observations in those dogs are difficult to carry out. Case 1, the young Pug that died within five months of onset, might be a typical case of NME in Pugs. On the other hand, Case 2 was a comparatively long-surviving Pug. In both of these Pugs, the titers of anti-GFAP autoantibody remained high throughout the observation periods, even during/after aggressive therapies. The cause of persistence of the autoantibodies remains unknown. As described in our previous report [8], Pugs with NME may have some fragility in their brain astrocytes. We hypothesize that the continuous leakage of GFAP from astrocytes is the cause of the persistent autoimmunity and inflammation in the brain. This may result in anti-GFAP autoantibodies in blood sera having limited sensitivity and specificity for canine NME [2]. However, further study is needed to confirm our hypothesis.

In addition to the Pug, several other breeds of dog develop NME [3, 6, 7]. In our previous study [4], we found that both Pugs and non-Pug dogs with NME possess anti-GFAP autoantibodies in the CSF. However, the titers were lower in non-Pug dogs than in Pugs. In the present study, although only a Pomeranian could be included, the dog presented some unique features, including positive autoantibody on admission, disappearance of the autoantibody during treatment and clinical remission of NME. While there is very little information about the pathogenesis and clinical features of NME in non-Pug dogs, there may be some differences among dog breeds. Additionally, the anti-GFAP autoantibodies may reflect the activity of NME.

In conclusion, CSF anti-GFAP autoantibodies can be detected both in the acute and chronic stages of canine NME. Thus, these autoantibodies may be used as a diagnostic marker of canine NME even if the dogs are undergoing immunosuppressive therapy. However, the autoantibodies can also disappear within certain periods after onset.

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