

Vaccine-associated acute polyneuropathy resembling Guillain-Barré syndrome in a dog

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S U M M A R Y

A 3.5 year-old male Rottweiler developed generalized acute progressive weakness 15 days after receiving an inactivated rabies vaccine. At clinical examination there was symmetrical quadriparesis with reduced spinal reflexes, cranial nerve function was normal and neither ataxia nor abnormal postural reactions were detected. Muscle biopsies from the semitendinosus muscle showed clear signs of denervation atrophy. Immunohistochemistry revealed the presence of antibodies against the myelin sheaths of peripheral nerves. Three months later clinical signs relapsed after receiving an inactivated tetravalent vaccine not including rabies virus. The pathogenesis, clinical and pathological signs of this case report resemble the acute forms of human Guillain-Barré syndrome. The presence of anti-myelin antibodies and the association between clinical signs and previous vaccination with two different vaccines free of myelin, strongly suggests a polyclonal immune response induced by the vaccine immunoadjuvants or by the viral antigens. Although this side effect can be considered exceptional it may encourage the use of non-adjuvated vaccines.

Key words: polyneuritis, Guillain-Barré, adjuvants, vaccination, dog

INTRODUCTION

Peripheral neuropathies are common entities in domestic animals, particularly dogs. Apart from those due to trauma, the pathophysiology of most neuropathies is poorly understood. These peripheral neuropathies result in lower motor neuron signs, which include flaccid muscle weakness or paralysis, significant muscular atrophy, reduced or absent muscle tone (hypotonia, atonia) and reflexes (hypo reflexia) [4].

The Guillain-Barré syndrome (GBS) or acute inflammatory demyelinating polyneuropathy is a condition characterized by transient neurological signs associated with an inflammatory

demyelination of peripheral nerves in which myelin is the target of immune attack [10]. The typical course of the syndrome shows ascending motorparesis, peaking within 4 weeks, diminished or absent muscle stretch reflexes, sensory clinical signs with minimal objective sensory loss and electrophysiological evidence of a demyelinating neuropathy [16]. In the dog, three early reports described the electrophysiological changes in individuals with idiopathic acute progressive quadriparesis compatible with a GBS-like polyneuropathy [7,12,13]. However, none of the aetiologies previously reported in human GBS could be established in these dogs and only some of them had been exposed to

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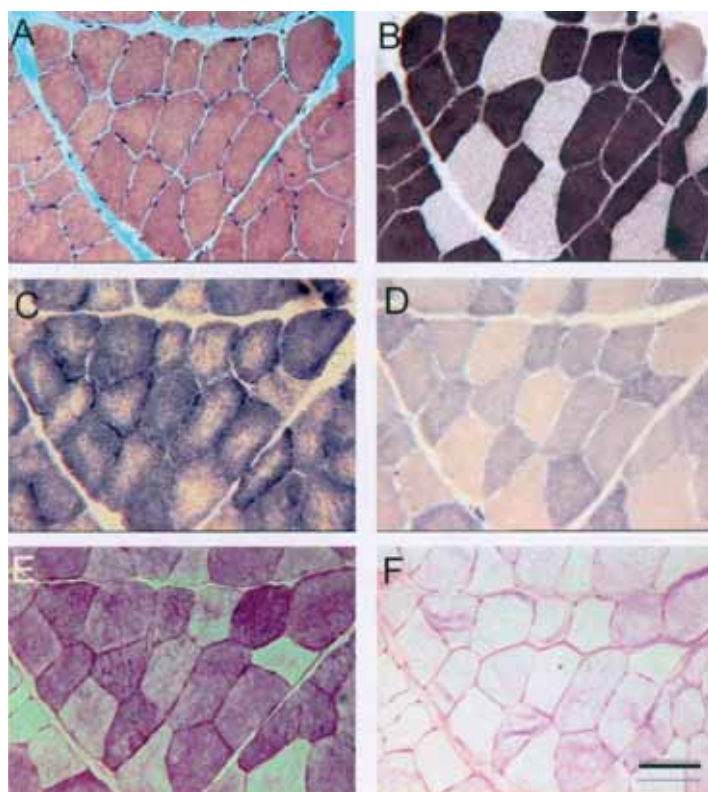
raccoon bites. On the other hand, several neurological abnormalities have been reported in Rottweilers dogs, including spinal muscle atrophy of puppies, neuroaxonal dystrophy in immature and mature dogs, leuko encephalopathy in mature dogs [6,18] and an isolated case of chronic polyneuritis in a mature dog [2]. In this report we describe the clinical signs and pathological lesions of a Rottweiler dog which suffered two separated episodes of acute polyneuropathy after receiving two different vaccines and where the presence of antibodies against peripheral nerve myelin was demonstrated.

MATERIAL AND METHODS

Clinical examination

A 3.5 years-old male Rottweiler weighting 42 kg was presented with a history of generalized acute progressive weakness. After the initial evaluation, a complete neurological examination was carried out and the following diagnostic tests performed: a complete haemogram, biochemistry profile, CSF analysis, spinal radiography, serological tests for ehrlichiosis, leishmaniasis and canine distemper virus and total T4 concentrations.

Fig. 1. Transverse serial sections of the long head of the triceps brachii muscle stained with haematoxylin and eosin (A), myofibrillar adenosine triphosphatase after preincubation at pH 10.2 (B), succinic dehydrogenase (C), a-glycerophosphate dehydrogenase (D), periodic acid Schiff (E) and a-amylase periodic acid Schiff (F). Bar, 50 µm.



Skeletal muscle biopsies

Muscle biopsies were obtained under general anaesthesia from the long head of the triceps brachii (distal third) and semitendinosus (middle third) muscles using Braund's technique [5]. Multiple biopsy specimens (approximately 0.2 cm x 0.2 cm x 0.5 cm) were collected from each muscle, frozen by immersion in isopentane precooled in liquid nitrogen, and stored at -70 °C until analyzed. Samples from both muscles were identically processed. Serial sections were cut at 10 µm and stained with hematoxylin and eosin (HE), myofibrillar adenosine triphosphatase (mATPase) for fibre type differentiation after alkaline (pH 10.2) and acid (pH 4.3) preincubations [11] succinic dehydrogenase [3] and glycerol-3-phosphate dehydrogenase (SDH and GPDH) to study metabolic characteristics [11], periodic acid Schiff (PAS) to assess glycogen content, and a-amylase PAS for visualizing capillaries [1]. Control of non specific reactivity and analytical precision of this histological and histochemical methods are described elsewhere [15]. Serial sections were analyzed with a Leica DMLS microscope (Leica Microsistemas, Barcelona, Spain) and image-analysing software (Visiolog 5, Noemi, Microptic, Barcelona, Spain).

Immunohistochemistry

The presence of antibodies against myelin was demonstrated using the avidin-biotin-peroxidase complex (ABC) immunohistochemical technique. Tissue sections of subcutaneous connective tissue containing peripheral nerves and sections of sciatic nerve of normal dogs were used. Serum samples of the affected dog obtained during the two episodes of acute weakness, and serum from a normal dog were used as primary antibodies. Endogenous peroxidase activity was inactivated by incubation with 3% hydrogen peroxide in methanol for 30 min at room temperature (RT, 20-25°C). Normal rabbit serum (Vector Laboratories, Burlingame, CA, USA) diluted 1:10 was applied for 30 min at RT. Then the primary serum diluted 1:10 in PBS was applied for 18 h at 4°C. After three 10 min washes in PBS, a biotinylated rabbit anti-dog immunoglobulin (Sigma Chemical, St. Louis, MO, USA) diluted 1:100 was applied for 30 min at RT. After three 10 min washes in PBS, an avidin-biotin complex (Vector) diluted 1:50 was applied for 1 hour at RT as third reagent. Then, 3-3'-diaminobenzidine tetrahydrochloride (Sigma Chemical) diluted 0.035% in tris buffered saline (pH 7.6) containing 0.01% hydrogen peroxide was used as chromogen for one minute. The primary test antibodies were replaced by rabbit non-immune serum in negative control tissue sections.

RESULTS

Clinical examination

The progression of clinical signs was rapid and after 9 days the dog could only walk a few metres before collapsing. There was no known exposure to toxic substances or trauma. The dog had received an inactivated rabies vaccine (Rabdomun, Pfizer) 15 days before clinical signs were first noted. At clinical examination the dog was alert and in good body condition. The most prominent clinical sign was a severe weakness that

made the dog walk with exaggerated head movements and a waddling gait. Neurologically, cranial nerve function was normal and neither ataxia nor abnormal postural reactions or other sensory deficits were detected. There was symmetrical quadriparesis with reduced spinal reflexes. Results of haemogram, routine biochemistry profile, serum creatine kinase and lactate dehydrogenase determinations, spinal radiographies and CSF analysis were all normal. Immunological tests for ehrlichiosis, leishmaniasis and canine distemper were negative. Test for serum total T4 (competitive RIA) yielded a concentration of 35.2 microg/dl which suggested the presence of antibodies against T4.

Administration of piridostigmine bromide (1.5 mg/kg/12h) to rule out myasthenia gravis induced vomiting with no improvement on the neuromuscular signs. Weakness with reduced segmental reflexes, normal sensory function, lack of cranial nerve involvement, normal CFS results and failure to respond to piridostigmine suggested a predominantly motor polyneuropathy. Prednisolone was started at a dose of 3 mg/kg/12h and clinical improvement was evident after 2 days of treatment. The same dose was continued over 2 weeks and tapered progressively for another 2 weeks. By this time the dog was fully recovered and treatment could be discontinued. Three months later the dog was presented with the same clinical signs. History revealed that again the dog had received 12 days before, an inactivated tetravalent that did not include rabies virus antigen virus (Tetradog, Merial). A similar treatment resulted in a complete clinical recovery.

Skeletal muscle biopsies

In the long head of the triceps brachii muscle, morphological features were largely normal (Fig 1). Sections stained with HE showed polygonal myofibres tightly packed together and separated only by a thin layer of endomysial connective tissue (Fig.1A) and capillaries (Fig.1F). The two main fibre types were distributed in a normal mosaic pattern (Fig.1B). Histochemical SDH revealed a relatively high reaction of all fibre types (Fig.1C). Type 1 fibres showed a lower a GPD reaction than type II fibres, but a spectrum in this reaction was observed within fast - twitch fibres (Fig.1D). In general type I fibres had lower glycogen content than II fibres and had also an unusual low PAS staining (Fig.1E).

In the semitendinosus muscle, by contrast, clear signs of denervation atrophy were evident (Fig.2). Small, sharply angulated muscle fibres with small peripheral nuclei were interposed between fibres of normal or hypertrophied size (Fig.2A). A selective hypertrophy of some rounded type I fibres was also observed (Fig.2B). A tendency for certain fibre type grouping was also evident (Fig.2B). Scattered fibres with target-like structures in their centres were observed (Figs.2C and 2D). Type II fibres were intensely stained with a-GPD reaction (Fig.2D). Myofibres showed, in general, a weak PAS staining (Fig.2E). The atrophic and hypertrophic muscle fibres were separated by an increased amount of endomysial fibrous tissue and by the presence of numerous capillaries (Fig.2F).

Immunohistochemistry

Tissue sections stained with the serum from the affected dog showed granular immunolabelling in the myelin sheaths of

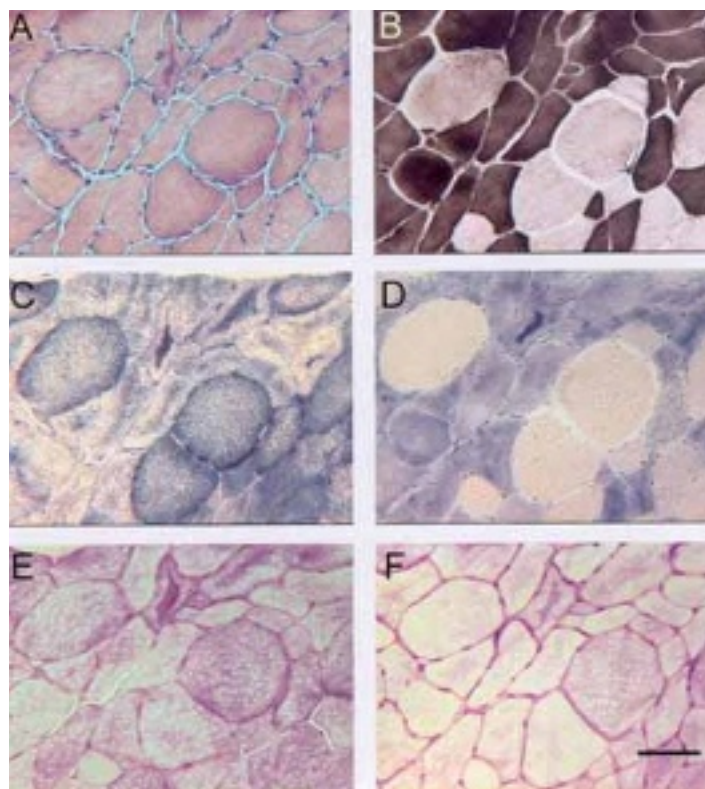


Fig. 2. Transverse serial sections of semitendinosus muscle stained with haematoxylin and eosin (A), myofibrillar adenosine triphosphatase after preincubation at pH 10.2 (B), succinic dehydrogenase (C), a-glycerophosphate dehydrogenase (D), periodic acid Schiff (E) and a-amyase periodic acid Schiff (F). Bar, 50 μ m.

peripheral nerves (Fig.3), whereas tissue sections stained with the serum of normal dogs, and with rabbit serum used as negative controls did not show reactivity in the myelin sheaths of peripheral nerves. The immuno-staining was clearly observed both in transversal and longitudinal sections of the nerves (Fig.3).

DISCUSSION

In the dog, acute polyneuropathies have aroused interest because of its resemblance with GBS in humans and its potential as an elucidating model [4,9]. The term coonhound paralysis has been used to describe this condition in North America but the exact pathogenesis of this syndrome still remains unknown worldwide although it may have an immunological basis. As in coonhound paralysis and other acute polyneuropathies, clinical signs in this case report appeared around 10 to 14 days after contact with the possible causative antigen (vaccination), ventral roots were more severely affected with pronounced motor impairment, and bladder and rectal paralysis were not observed [18] Although the distribution of weakness was compatible with

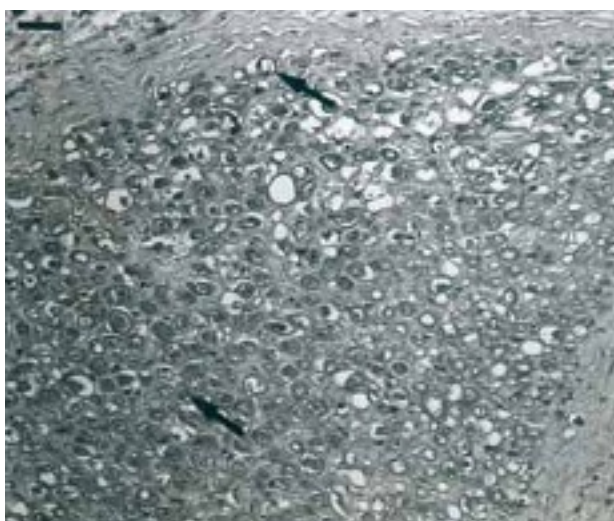


Fig. 3. Transverse sections of a sciatic nerve from a normal dog labelled with serum of the affected dog. Note the positive immunolabelling in the majority of myelin sheaths (arrows). ABC method, Mayers' haematoxylin counterstain. Bar = 60 μ m.

myopathy, muscle tenderness was not a feature, creatine phosphokinase determinations were normal and muscle biopsy basically showed changes of neurogenic origin. Thus, the histological and cytological features of muscle biopsies examined were consistent with a denervating myopathy [17], a diagnosis supported by the atrophy of both type I and type II fibres seen in the semitendinosus muscle. Swelling and hypertrophy of scattered type I fibres indicate a compensatory response in non denervated motor units, whereas target fibres and fibre type grouping are indicators of reinnervation [19]. Clinically, both forelimbs showed marked signs of paresis. In contrast with this finding, the morphometric data obtained in the long head of the triceps brachii muscle were within the range previously published for this muscle in other breeds of dogs [5]. This discrepancy between muscle biopsy results from fore and hindlimbs was not surprising since in cases of acute canine polyneuropathy, demyelination was more severe in the sciatic/tibial nerve than in the thoracic nerves [7].

In this dog, although electromyographic testing was not available, the acute course and the rapid recovery after corticosteroid administration suggests that demyelination of intact axons rather than axonal changes was the more likely mechanism involved [12].

The immunolabelling in myelin sheaths of peripheral nerves obtained with the serum of the affected dog, would indicate an immune-mediated process directed against the myelin of peripheral nerves affecting more severely the ventral nerve roots as supported by the lack of sensory deficits.

Complement fixing antibodies that react to normal peripheral nerve myelin are consistently present in acute-phase GBS serum. The pathogenesis of the acute GBS forms is related to antibody responses against a myelin glycolipid with antigenic cross-reactivity to Forssman hapten. This anti peripheral nerve myelin antibody activity [10] may be triggered by cross-reacting bacterial antigens, especially *Campylobacter jejuni*, but other

antigens such as viral vaccine antigens may be involved. The data regarding the relation between vaccination and autoimmune disease is conflicting but the role of vaccines as triggering events has been demonstrated in the case of rabies vaccination with extracts contaminated by myelin [17]. In susceptible individuals, reactivation of the immune-mediated neuropathy can occur subsequently to other antigenic stimuli as has been demonstrated with tetanus toxoid [14]. In the present dog, there was an apparent cause-effect relationship between vaccination and onset of clinical signs associated with the presence of antibodies against myelin. According to the manufacturers' information, the vaccines used were obtained by cultures in renal cells and did not contain nervous tissue antigens. Thus, viral or other vaccine antigens, or the adjuvants included in the vaccines might have triggered the formation of anti-myelin antibodies by over stimulation of the immune system of this particular dog. The fact that two different vaccines from two different manufacturers were involved, suggests a polyclonal activation induced by the vaccine adjuvants without the participation of myelin as the more probable pathogenesis.

In a controlled experimental study to test the effects of vaccination on the immune system, dogs that were immunized with commercially available rabies and canine distemper vaccines developed a significant increase in the titre of IgG antibodies reactive with 10 autoantigens. There was no increase in the non-vaccinated dogs [8].

Although several mechanisms, such as cross-reactivity or a 'bystander activation' of self reactive lymphocytes, could explain this response, the variety of auto-antigens found suggests a polyclonal activation or adjuvant reaction. This adjuvant effect, associated with the development of a wide range of autoantibodies has been observed more often with those vaccines with higher adjuvant contents [17]. Information about the content and type of adjuvants used in commercially available canine vaccines is not usually provided by the manufacturer, and currently, vaccine-induced autoimmunity can be regarded as rare. However, the study of Hogenesch[8] clearly shows that immunized dogs have significant titres of autoantibodies and it is likely that, a genetically-predisposed dog, will also developed autoimmune disease.

Recovery seemed to be induced by steroids due to the rapid response in both clinical episodes. However, recovery could have been spontaneous as in acute GBS where steroids are not considered useful [9]. In conclusion, the pathogenesis, clinical signs and neurogenic muscle changes of this case report support a diagnosis of polyneuropathy resembling the acute forms of GBS. The presence of anti-myelin antibodies and the association between clinical signs and previous vaccination with two different vaccines free of myelin, strongly suggests a polyclonal immune response induced by the vaccine immunoadjuvants or by the viral antigens. Although this side effect can be considered exceptional it may encourage the use of non-adjuvated vaccines.

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