Pathological Features of Ganglioradiculitis (Sensory Neuropathy) in Two Dogs

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ABSTRACT. Canine ganglioradiculitis (sensory neuropathy) was examined pathologically in two dogs (dog Nos. 1 and 2). The affected dogs had 1 and 2 years clinical courses from the onset, respectively. As common clinical signs, both cases showed progressive ataxia, difficulty in prehending food, visual deficit, and several sensory abnormalities. Gross observation after tissue fixation revealed whitish discoloration in the dorsal column of the spinal cords. The histological lesions were mainly distributed in the spinal dorsal roots, ganglia, and dorsal columns. In the spinal dorsal roots and ganglia, there were striking myelin loss, mild infiltration of mononuclear cells, and proliferation of small spindle cells. In the dorsal funiculus, there were moderate to severe diffuse myelin-loss and axonal degeneration. Immunohistochemistry for substance P (SP) revealed marked reduction of SP-immunopositive granules in the spinal sub substantia gelatinosa of affected dogs. By immunohistochemistry, CD3-positive cells were observed in the dorsal roots of dog No. 2, while CD3-positive cells were rare in those of dog No. 1. In the spinal ganglion of dog No. 1 there were many CD3- and MHC class II-positive cells. By indirect immunofluorescence assay using sera from affected dogs, no autoantibodies against canine nerve tissues were detected. CD3-positive cells were rare in those of dog No. 1. In the spinal ganglion of dog No. 1 there were many CD3- and MHC class II-positive cells. By indirect immunofluorescence assay using sera from affected dogs, no autoantibodies against canine nerve tissues were detected.

Canine ganglioradiculitis is a rare idiopathic disease characterized by various degree of inflammation within the peripheral ganglia and spinal nerve roots in acute phase and reactive proliferations of satellite cells, Schwann cells and/or fibroblasts in chronic phase. The sporadic diseases have described usually to affect adult dogs with no breed and gender predilections [2, 5, 16, 17], while some familial forms are recognized [6, 20]. Since the lesions are restricted within the sensory nerve tracts, canine ganglioradiculitis is also called as “sensory neuropathy (SN)” [16, 17]. Clinical signs in canine SN are abrupt in onset and slowly progressive, some which may be asymmetric. Those include sensory ataxia, hypermetria, basewide stance, depression or fibroblasts in chronic phase. The sporadic diseases have described usually to affect adult dogs with no breed and gender predilections [2, 5, 16, 17], while some familial forms are recognized [6, 20]. Since the lesions are restricted within the sensory nerve tracts, canine ganglioradiculitis is also called as “sensory neuropathy (SN)” [16, 17]. Clinical signs in canine SN are abrupt in onset and slowly progressive, some which may be asymmetric. Those include sensory ataxia, hypermetria, basewide stance, depression or loss of spinal reflexes, reduced postural reactions, facial hypalgesia, dysphagia, masticatory muscle wasting, and megaesophagus [2, 16, 17]. Histopathologically, extensive Wallerian degeneration in the spinal cords and diffuse demyelination with mononuclear cell infiltration in both the spinal ganglia and autonomic ganglia, are characteristic [2, 5, 16, 17, 20]. Although several case reports have been published in foreign countries, there have been no reports of canine SN in Japan.

Besides SN, similar canine neurological disorder, called as idiopathic polyradiculitis (coonhound paralysis; CP), has been well known [4, 5, 11, 15]. Unlike SN, the pathologic changes are usually concentrated in the ventral roots that also called radix motoria, in which motor deficits such as tetraplegia are more conspicuous than sensory disturbances [5]. Although the etiology of these diseases has not been well elucidated, the pathogenesis of CP has been supposed to mimic that of human Guillan-Barré syndrome (GBS) [4, 11, 15]. Koski et al. [14] suggested that an autoimmune response mediated by some autoantibody in GBS patients that could be triggered by multiple infectious agents binds a surface determinant of a Fossman-like lipid of human peripheral nerve myelin. Several reports referred to the antigenic similarity between the components of Campylobacter spp. and peripheral myelin, causing immune-mediated demyelination [12, 21, 22].

Several hypotheses concerning the etiology of canine SN, such as an immune-mediated pathogenesis, some virus infections, intoxication, or inherited factors have been proposed [2, 5, 6, 16, 17, 20]. Previously, some viral infections and/or immune response affecting on ganglionic neurons were supposed as possible etiologies of canine SN [5]. Wouda et al. [20] had concluded that canine SN might be a cryptogenic disease targeting on the sensory neurons. T cell or NK cell-mediated immune responses have been supposed to play the role on the peripheral nerve injury of canine SN based on immunohistochemical findings [16, 17]. The intoxication followed by silica exposure was supposed to induce sensory tract lesions in canine SN [2]. Hereditary form of canine SN has been recognized in a few canine breeds, such as Shorthaired Pointer, Dachshund, and English Pointer [6, 20]. Cummings et al. [6] examined the immunoreactivity of substance P (SP); the undecapeptide imputed to mediate nociception at the first synapse, in the spinal cords of hereditary SN in English Pointer and reported marked reduction of SP-like immunoreactivity. They suggested that the phenomena were led by progressive
changes of sensory neurons that caused by a localized deficiency of some tropic factors, such as nerve growth factor or its receptors [6].

The present paper describes clinicopathological features of canine SN of two dogs. In addition, the purpose of this study is to examine the cell types of the inflammatory cells within the spinal ganglia and nerve roots of affected dogs by immunohistochemistry. The presence of the autoantibodies against nerve tissues is also evaluated by indirect immunofluorescence assay (IFA).

MATERIALS AND METHODS

Cases and tissue samples: Two affected dogs; dog No. 1, 9-year-old, spayed female Maltese, and dog No. 2, 6-year-old, spayed female American Cocker Spaniel, and one neurologically normal necropsied dog, 9-year-old, castrated male Labrador Retriever, were examined. The tissue samples were taken from the brain, spinal cord, nerve root, and ganglion of these three dogs. The peripheral nerves including the sciatic, tibial, ulnar, common peroneal, and left median nerves, and skeletal muscles including the tongue, semitendinosus, right tendinosus, and triceps brachii muscles were also taken from dog No. 2. Fresh samples from the spinal cords including the roots and ganglia of neurologically normal dog were taken and kept at −20°C for cryostat sections. Sera from dog Nos. 1 and 2, and a clinically normal dog, 3-year-old, female beagle, were stored at −20°C until use for autoantibody assay.

Tissue processing: All tissue samples were fixed in 10% formalin, processed routinely, and embedded in paraffin. Then, paraffin sections of 6 µm were made and stained with hematoxylin and eosin (HE). Some selected sections were also stained with Luxol-fast blue (LFB) and cresyl echt violet.

Immunohistochemistry: Immunohistochemistry was performed using Envision polymer reagent (DAKO-Japan, Kyoto, Japan). The antigen retrieval procedure was performed by heating with autoclave, at 121°C for 5 min, except for SP-immunostaining. These sections were incubated with 3% hydrogen peroxidase in methanol at room temperature for 10 min to block endogenous peroxidase activity. Sections for SP-immunostaining were incubated with normal goat serum for 30 min at 37°C to reduce non-specific reactions, and also washed with PBS. Then, the slides were incubated with primary antibodies for 30 min at 37°C. The primary antibodies were rabbit sera against human CD3 (1:50, DAKO-Japan), cow S-100 (prediluted, DAKO-Japan), myelin basic protein (MBP, prediluted, DAKO-Japan), and SP (1:25, Biogenesis, Poole England, UK), mouse monoclonal antibodies against human BLA-36 (1:25, DAKO-Japan), MAC387 (prediluted, DAKO-Japan), HLA-DR (MHC class II, 1:20, TAL.1B5, DAKO-Japan), neurofilament (NF, prediluted, DAKO-Japan), and vimentin (prediluted, DAKO-Japan), and biotin-labeled sheep antiserum against canine IgG (1:100, American Qualex, San Clemente, CA, U.S.A.). After reaction with each primary antibody, the sections were incubated with Envision polymer reagent (DAKO-Japan) for 30 min at 37°C. For canine IgG-immunostaining, avidin-biotin peroxidase complex method (PK-4000, Vector Laboratories, Burlingame, CA, U.S.A.) was used. All reaction products were visualized with 3,3′-diaminobenzidine (Sigma, St. Louis, MO, U.S.A.). Sections were counterstained with Mayer’s hematoxylin. Then, semi-quantitative analysis for inflammatory cells was performed. The mean number of positive cells in the dorsal roots and ganglion was calculated in randomly selected high power magnification fields (× 40 objective lens).

IFA: To detect the autoantibody against canine nerve tissues, cryostat sections of the spinal cords including the roots and ganglia of a neurologically normal dog (Labrador Retriever) were prepared. The sections were incubated with the sera (1:10) from two affected dogs and a negative control (Beagle) at 37°C for 30 min. Sections then were incubated with a FITC-labeled sheep antiserum against canine IgG (1:100, American Qualex) and subsequently observed with a fluorescence microscope.

RESULTS

Clinical findings: Dog No. 1 exhibited weakness of four limbs of about 1-year duration. One-week prior to admission to a private animal hospital, because the symptoms suddenly progressed, the dog showed astasia and difficulty in prehending food due to insufficiency of the tongue. Neurological examinations revealed quadriaparesis, right optical insufficiency, and dysfunctions of the bilateral oculomotor, right trigeminal, right facial, and hypoglossal nerves. The dog was euthanized by owner’s request.

Dog No. 2 had initially coughing, vomiting, and dysphagia of about 2-year duration. The dog was treated with metoclopramide and prednisolone, while significant remission was not confirmed. When the dog was presented to the Nihon University Animal Medical Center, corticosteroid treatment was once suspended because of corneal ulceration and scleritis. Because of dry-eyes after keratoplasty, the dog was administered again by corticosteroid. Then, the increased dosage of corticosteroid was given. Three weeks prior to the admission, the dog suddenly exhibited left forelimb raising and knuckling, and the left hindlimb was diffusely alopecic due to continuous self-biting. When the dog was admitted to Nihon University Animal Medical Center for precise examinations, the dog showed quadriaparesis and neurological examinations revealed poor postural reactions on the left forelimb and hindlimb, absence of patellar reflex of bilateral hindlimbs, flexion reflex of the left forelimb, and superficial and deep hypalgesia of the left fore and hind limbs. The cranial nerve tests revealed the absence of corneal reflex and sensory nerve ataxia on the upper and lower jaws. These signs continuously progressed, and the dog finally developed dysstasia 1 week later. Then, steroid treatment had been done everyday in the other private hospital, but the dog showed dysphagia and died by aspiration.
pneumonia.

Gross findings: At necropsy, the right temporal muscle of dog No. 1 exhibited severe atrophy. The skeletal muscles of dog No. 2 showed severe generalized atrophy throughout the body (Fig. 1). The other significant gross changes were found in the spinal cords of both cases. A whitish discoloration in the dorsal column of the spinal cord was observed in the lumbar vertebrae of dog No. 1. More prominent gross lesions characterized by discolored V-shaped zones were detected in the spinal cords of dog No. 2 (Fig. 2). In the visceral organs other than the nervous and muscular systems, the lungs were partly (dog No. 1) or generally (dog No. 2) collapsed due to aspiration pneumonia.

Histopathology: Both examined dogs had similar pathological features and the major lesions were distributed in the spinal dorsal roots, ganglions, and dorsal columns. In the dorsal roots of both dogs, there were mild to moderate infiltration of mononuclear cells and proliferation of small spindle cells (Fig. 3). In dog No. 2, vacuolar changes of the nerve sheath were also diffusely observed (Fig. 4). In contrast to severe lesions of the dorsal roots, the ventral roots of both dogs were well preserved. The inflammatory reactions consisting of diffuse infiltration or perivascular accumulation of mononuclear cells (Fig. 5) were widely distributed in the dorsal roots in both cases. The inflammatory lesions were prominent in the lumbar and sacral spinal cords in both cases. In the spinal ganglions, moderate to severe neuronal cell-loss, marked increase of the sounding satellite cells sometimes forming "nageotte's nodules" (Fig. 6), diffuse proliferation of small spindle-shaped cells, and mild to moderate inflammatory reactions in the series of the dorsal roots. The inflammatory changes appeared as diffuse infiltration and occasional nodular accumulation of mononuclear cells. In the spinal cords of both dogs, there were severe to moderate vacuolar changes characterized by diffuse myelin-loss and axonal degeneration in the dorsal...
funiculus (Fig. 7). In the lesions, vacuolated myelin sheaths occasionally contained swollen axons forming spheroids and foamy macrophages, while inflammatory reactions by other mononuclear cells were absent. The ventral and lateral funiculi were well preserved. In the central nervous tissues other than the spinal cords, there were no significant lesions.

In several peripheral nervous tissues from dog No. 2, there were mild diffuse vacuolation of myelin sheaths and proliferation of spindle cells (Fig. 8). The mononuclear cell infiltration was less prominent compared to those in the spinal dorsal roots and ganglions. The lesions in the peripheral nerves other than spinal roots and ganglions were more apparent in the left sides of this case. In almost all skeletal muscles examined in both dogs, extensive diffuse atrophy with moderate fibrosis and occasional calcium deposits was observed (Fig. 9). In the ganglions around the adrenal gland of dog No. 2 there were mild infiltrations of mononuclear cells.

**Immunohistochemistry:** Immunohistochemistry for MBP and NF revealed that the affected dorsal roots in both cases contained few MBP- and NF-positive structures, suggesting severe loss of both myelin and axons in the lesions. In addition, the proliferated spindle cells in the affected dorsal roots in both cases were negative for S-100 and positive for vimentin. Immunohistochemistry for SP revealed that the number of SP-immunopositive materials (or neurites) was reduced in the spinal substantia gelatinosa of affected dogs as compared to that of normal control (Figs. 10a-c). Among affected cases, the reduction of SP-immunopositive granules (or neurites) was more severe in dog No. 2 (Fig. 10c).

To confirm the population of the inflammatory cells
within the lesions in both dorsal roots and ganglia, the results of immunohistochemistry for CD3, BLA.36, and MHC class II were analyzed semi-quantitatively. In the dorsal roots of both cases, there were many MHC class II-immunopositive cells, while there were only few MAC387-positive cells in the lesions (Fig. 11). The results might indicate that these MHC class II-positive cells were histiocytic cells as antigen-presenting cells. The number of BLA.36-positive cells was almost equal to that of IgG-positive cells in the dorsal roots in dog No. 1 (Fig. 11). The BLA.36- and IgG-positive cells might represent B or plasma cells. Many CD3-positive cells were detected in the dorsal roots of dog No. 2, while CD3-positive cells were rare in those of dog No. 1 (Fig. 11). However, there were relatively many CD3- and MHC class II-positive cells in the spinal ganglion of dog No. 1 (Fig. 12). Semi-quantitative analysis for inflammatory cells in the ganglion from dog No. 2 was unable due to the lack of specimen.

Autoantibody analysis: By IFA using cryostat sections of the canine spinal cords including the nerve roots and ganglia from neurologically normal dogs, any significant positive signals were not detected following the reaction of sera from dogs Nos. 1 and 2.

DISCUSSION

The clinical and pathological features of two cases examined in this study were similar to each other and were almost consistent with those in previous reports of canine SN [2, 5, 16, 17, 20]. However, several minor differences were present in their clinical and pathological findings such as the degree and distribution of the inflammatory changes. Unlike in dog No. 1, dog No. 2 showed coughing and self-biting. Previously, a Welsh Corgi dog with SN exhibited coughing over the long term [5]. Furthermore, two English Pointer dogs that affected with hereditary SN had been described to lick and bite their digits during the clinical courses [6]. Wouda et al. [20] also reported that a Dorberman Pinscher with SN was biting her left hindlimb. These clinical findings of the previous cases of SN seem not to conflict with those recorded in dog No. 2. On the other
hands, in the early clinical course in dog No. 2 corneal ulceration and scleritis were recognized, suggesting caused by some corneal reflex deficits. A Whippet dog with SN had also ulcerative keratitis [20]. In the case, the branches and descending tract of the trigeminal nerve were affected with similar pathological lesions to the dorsal roots including the fragmentation of the axons and myelin sheaths [20]. Unfortunately, the trigeminal nerves was failed to examine in this study, while the clinical symptoms in dog No. 2 might suggest the presence of similar trigeminal nerve lesions.

The pathological findings in both cases obviously restricted within the sensory tracts including the dorsal roots, spinal ganglions, and dorsal columns as previous cases of canine SN [2, 5, 16, 17, 20]. Although the lesions of the dorsal funiculus were considered as diffuse Wallerian degeneration, the dorsal roots and its ganglions were more severely affected by myelin-loss, axonal degeneration, spindle cell proliferation, and inflammatory changes. Nerve fibers that connect the dorsal root to the dorsal funiculus by way of the ganglion ascend without changing the nerve cells. Therefore, damages to the dorsal roots and its ganglions cause Wallerian degeneration of the nerve fibers in the dorsal funiculus. Since nerve fibers connecting to the dorsal horn change the cell and ascend the same side of dorsal spinocerebellar tract, the nerve cells in dorsal horn were well preserved in our dogs. In this study, reduced SP immunoreactivity of the spinal substantia gelatinosa was also confirmed as well as the observations of previous hereditary SN in English Pointer dogs [6]. Although the reduction of SP immunoreactivity is one of the dramatic changes in two dogs with SN, the event might reflect secondary events that led by destruction of the dorsal roots and its ganglions.

The proliferated spindle cells within the dorsal roots and its ganglions were completely negative for S-100 and intensely positive for vimentin. These spindle cells have been considered as Schwann cells for remyelination [5, 16, 17, 20]. However, present immunohistochemical findings indicated that these spindle cells had the nature of fibroblasts. The proliferation of these spindle cells might represent the irreversible fibrosis processes.

The distal peripheral nerves other than spinal roots had the same, but milder, histopathological changes as the dorsal roots. Similar histological findings were also described in some previous reports [2, 5, 16, 20]. As the inflammatory damage was often most severe in the ganglions [5, 16], the neurons in the cranio spinal sensory ganglia had been supposed as the primary target for the immune response in canine SN [2, 5, 16, 17, 20]. Keane et al. [13] indicated that peripheral nervous system neurons are more susceptible to T cell-mediated immune response than central nervous system. Their hypothesis may also explain the lack of central nerve lesions in canine SN. Regardless of whether the distal peripheral nerve lesions are primary or secondary changes, the lesions have clinical utility for the diagnosis using biopsy of the distal peripheral sensory nerves. In fact, biopsy specimens of the sural nerve mostly consisting of sensory nerves have been used for pathological evaluations of acute SN in humans [8, 10].

In both affected dogs, the persisting inflammatory changes were present, while the degree and distribution pattern of cellular infiltrations were somewhat varied in each case. The inflammatory changes in the dorsal roots of dog No. 1 were inconspicuous, and there was moderate diffuse fibrosis, representing chronic status of the disease. However, many MHC class II-positive cells were detected in the dorsal roots, and CD3-positive cells were distributed focally within the adjacent ganglions. Moreover, many CD3-positive cells infiltrated in the dorsal roots of dog No. 2 in spite of long-term corticosteroid treatment. The presence of inflammatory cells in the dorsal roots and ganglions of both affected dogs, regardless of the chronic status and/or corticosteroid treatment, indicates that the inflammatory reactions might be closely associated to the progress of canine SN.

By IFA, no autoantibodies against canine ganglion tissues were detected in sera from both affected dogs. The participation of both cellular and humoral immunity has been demonstrated in several immune-mediated diseases [3, 16, 19]. In human idiopathic SN, both T cell-mediated immune response and autoantibody against spinal ganglion neurons were involved on its pathogenesis [1, 9, 16, 18]. However, a previous report of human acute SN [10] described that CD8 positive-T cell-mediated immunity against ganglion neurons might mostly contribute the pathologic events and humoral immunity had only a limited role. Some forms of human SN have been associated with lung or breast cancers called as “paraneoplastic syndromes” [7, 9]. In the conditions, the anti-Hu antibody against neurons in both serum and cerebrospinal fluid caused the syndrome together with cytopathic effect mediated by CD8-positive T cells.

Although the presence of any autoantibodies has not been confirmed in canine SN, further investigations using more sensitive procedures than IFA such as radioimmuno assay will be need.

In conclusion, the present paper described clinicopathological features and immunohistochemical features of canine SN. The typical clinical symptoms in both affected dogs were slowly progressive ataxia and more striking paresthesis than motorial dysfunction. The prescription by common dose of corticosteroid seems to be difficult to obtain complete remission, suggesting other adequate medications would be needed. As an early diagnostic procedure, biopsy of the peripheral sensory nerves may be possible utility. Several etiologies other than the immune response, such as some hereditary factors [6], viral infections [5], and intoxications [2], had been proposed for canine SN, while there were few evidences concerning these causative factors in our cases. Thus, the etiology of the unique canine disorder remains unclear. To clarify the pathogenesis, further clinical data and some specialized investigations focusing these etiologies are necessary.
REFERENCES


