Peripheral Neuropathy Associated with Sjögren's Syndrome: Pathologic and Immunologic Study of Two Patients

Atsushi INOUE, Chang-Sung KOH, Naoyuki TSUKADA
and Nobuo YANAGISAWA

Two cases of Sjögren's syndrome accompanied by peripheral neuropathy are reported. The level of anti-endothelial cell antibody was increased in both patients. Immunofluorescent deposits of immunoglobulin and C3 component were detected in the vasa nervorum of both cases. The pathological findings showed damage to the endothelial cells in the same vessels. The findings suggest that injury from immune complex and anti-endothelial cell antibody may be the immunological factor in the induction of peripheral neuropathy.

Key words: Immune complex, Anti-endothelial cell antibody

Sjögren's syndrome is a chronic autoimmune disease characterized primarily by xerophthalmia and xerostomia, accompanied by destructive infiltration of inflammatory cells in the lacrimal and salivary glands. Patients with the syndrome may show extrasalivary manifestations, such as polyarthropathy, lymphadenopathy, splenomegaly, pneumonitis, nephritis, and myositis (1).

Peripheral neuropathy associated with Sjögren's syndrome has been previously reported (2-7), but pathogenetic studies are rare. We encountered two cases of Sjögren's syndrome complicated by peripheral neuropathy: one had mild sensory peripheral neuropathy, and the other mononeuritis multiplex with severe weakness and sensory disturbance. The peripheral nerves of these cases were examined morphologically using histochemical techniques and electron microscopy.

CASE REPORT

Case 1

A 33-year-old woman was admitted to our hospital because of Raynaud's phenomenon in October 1983. She first experienced the phenomenon

in March 1983. In April she developed the sicca complex, with xerophthalmia and xerostomia, and in October the Raynaud's phenomenon worsened.

There was no previous history of weakness of sensory disturbance. Blood pressure was 100/50 mmHg while lying down, the pulse was 72/min and regular. Body temperature was 36.4°C. There was no anemia or jaundice. Oral cavity and pharynx were rather dry. There were no skin lesions, edema, myalgia or abnormal findings in the joints. On neurological examination, she had a normal mental state and there were no cranial nerve abnormalities. But she had decreased pain sensation in the lateral right lower leg. She had no weakness and her deep tendon reflexes were normal. Co-ordination was normal. There was no dysfunction of the autonomic nervous system. Motor nerve conduction velocities of ulnar and tibial nerves were normal. Sensory conduction velocity in the right tibial nerve was 26.7 m/s ($N > 45$). Schirmer's test showed a marked reduction of lacrimation in both eyes (rt 2 mm, lt 3 mm moistened after 5 min). Rose bengal test showed conjunctival erosions.

Erythrocyte sedimentation rate was 28 mm/h.

From The Department of Medicine (Neurology), Shinshu University School of Medicine, Matsumoto

Received for publication April 27, 1990; Accepted for publication January 14, 1991

Reprint requests should be addressed to Atsushi Inoue, MD, PhD, Department of Medicine (Neurology), Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390, Japan

Serum electrolyte, BUN, creatinine, creatine phosphokinase and urinalysis values were all normal. CRP and RA test were negative. Serum IgG was 3,007 mg/dl (N 800–1,600), IgM 119 mg/dl and IgA 197 mg/dl. The titer of anti-nuclear antibody was $\times 5,120$ (speckled pattern) and anti-SSA antibody was more than $\times 256$. Anti-RNP antibody was $\times 8$. But anti-DNA antibody, anti-SSB antibody, anti-Sm antibody and anti-phospholipid antibody were all negative. Serum C3 was 59 mg/dl (N 60–130). Blood viscosity was normal. The cerebrospinal fluid (CSF) was clear and colorless. The cell count was $2/\text{mm}^3$ with a glucose value of 60 mg/dl and a protein value of 62 mg/dl. The CSF IgG level was 16 mg/dl.

Detection of immune complexes: Immune complexes in the serum and cerebrospinal fluid was measured by the modified Raji cell assay (8) using an enzyme-linked immunosorbent assay (9) (ELISA). The amount of immune complex was expressed as microgram of aggregated human gamma globulin (AHG) equivalent per milliliter. The levels of immune complex were increased to 240 $\mu\text{gAHG}/\text{ml}$ (N < 50) in the cerebrospinal fluid and 1,200 $\mu\text{gAHG}/\text{ml}$ (N < 100) in the serum.

Measurement of anti-endothelial cell antibody: Endothelial cells were derived from the isolated capillaries by trypsin-collagenase digestion using established method (10, 11) and grown in tissue culture as monolayers. The level of anti-endothelial cell antibody was quantitated using an ELISA method. After 7 days in culture, endothelial cell monolayers of 1.5×10^6 cells were incubated with 100 μl of test serum diluted in Hank's balanced salt solution (HBSS) (Gibco, Long Island, NY) for 60 min at 37°C , washed 3 times in HBSS, and then incubated with 1:20 diluted alkaline-phosphatase-labelled goat anti-human IgG serum (Miles Laboratories, Indianapolis, IN). The cell pellet was washed with physiological saline containing 0.05% Tween 20, and 1 ml of a 1 mg/ml solution of p-nitrophenyl phosphate was added. After 30 min of incubation at 37°C , NaOH (sodium hydroxide) was added. The optical density (O.D.) of each sample was measured in a microplate 2 wavelength photometer (Corona MTP 12) (12, 13).

The titer of anti-endothelial cell antibody in the serum was increased to 0.150 O.D. (N < 0.10). RI

Fig. 1. Minute granular IgG deposits in the vasa nervorum. Immunofluorescent staining of the longitudinal section. $\times 300$

sialography showed a decreased RI uptake and secretion in bilateral submandibular glands. Labial salivary gland biopsy showed lymphocyte aggregates. Sural nerve biopsy showed slight lymphocyte infiltration around the endoneural small vessels, which were somewhat thickened. Minute granular IgG and C3 deposits were demonstrated surrounding the wall of the same vessels under fluorescence microscopy (Fig. 1).

Density of myelinated fibers: Sural nerve specimens were fixed with 3% glutaraldehyde in a 0.1 M cacodylate buffer at pH 7.2, and then washed, postfixed in 0.1% osmium tetroxide in the same buffer, dehydrated, and embedded in epon-araldite. Transverse section of 1 μm thickness were cut, stained with 1% toluidine blue and studied under a light microscope. Photographic enlargement ($\times 1,000$) of transverse nerve sections were used to measure the number of myelinated fibers. Then the diameter frequency histogram of myelinated fibers was made (14).

The mean density of myelinated fibers was 7,658/ mm^2 (control 9,563 $\pm$ 896) and a predominant loss of large myelinated fibers (diameter $\geq 6 \mu\text{m}$) was evident (Fig. 2). She was treated with prednisolone 1 mg/kg daily, and gradually improved over the next two months.

Case 2

A 62-year-old woman was admitted to our hospital because of difficulty in walking in November 1985. Three years before admission she

Fig. 2. A predominant loss of large myelinated fibers in case 1.

frequently experienced Raynaud's phenomenon. In 1984, she developed purpura on her torso and lower extremities, xerophthalmia and xerostomia. These symptoms remitted. In August 1985, she noticed motor weakness and dysesthesia in both legs, especially in the left. Within a month she found it hard to walk and developed a high fever.

She was admitted to another hospital, where she was treated with prednisolone and her symptoms improved. After the dose was tapered, however, muscle weakness and atrophy in all limbs increased and she became unable to walk. General examination on admission was normal except for xerostomia and xerophthalmia. Blood pressure was 136/90 mmHg while lying down. The pulse was 88/min and regular. Body temperature was 36.2°C. There was no anemia or jaundice. There were no skin lesions, abnormal findings in the joints, edema and myalgia.

Neurologic examination showed a normal mental state. She had mild bilateral facial weakness and mild weakness of neck flexion and extension. Manual muscle strength of the proximal muscle was 4 (good) in the arms and 3 (fair) in the legs, and that of the distal muscle was 3 in the arms and 1 (trace) in the legs (15). The muscle weakness was more severe in the right leg. She had severe atrophy in all

limbs, especially the distal portions. Deep tendon reflexes were absent and plantar response was flexor bilaterally. Sensory examination revealed moderately decreased pinprick, temperature and light touch sensations and position sense in the distal area of all extremities. The sensory disturbance was more prominent in the left arm and leg. Co-ordination was normal. There was no dysfunction of the autonomic nervous system.

Electromyographic examination showed an increase in the portion of typical polyphasic motor unit potentials and a decreased interference pattern, changes compatible with a peripheral neuropathy. Motor conduction velocity of the right ulnar nerve was 41.9 m/s ($N > 50$) and sensory conduction velocity of the same nerve was 41.1 m/s ($N > 45$). No sensory or motor nerve action potentials were detectable when recording from the right tibial nerve. Schirmer's test showed decreased lacrimation (rt 1 mm, lt 3 mm moistened after 5 min). Slit-lamp examination after fluorescein staining and rose bengal test confirmed keratoconjunctivitis sicca. A labial salivary gland biopsy showed focal infiltrates of lymphocytes consistent with the typical lesion of Sjögren's syndrome. Laboratory findings included hemoglobin 10.4 g/dl and white blood cell count 11,000/mm³. Erythrocyte sedimentation rate was 37 mm/h. RA test was positive (+). CRP was 6+. Serum electrolyte, GOT, GPT, BUN, creatinine, creatine phosphokinase and urinalysis values were all normal. Serum IgG was 1,050 mg/dl, IgA 210 mg/dl and IgM 105 mg/dl. Other laboratory data were as follows: Anti-nuclear antibody $\times 160$ (speckled and peripheral pattern), serum C3 28 mg/dl, LE test negative, anti-DNA antibody negative, anti-RNP antibody negative, anti-Sm antibody negative, anti-SSA antibody positive ($\times 4$), anti-SSB antibody negative, serum cryoglobulin positive, the level of immune complex in the serum 70 μ g AHG eq/ml, the titer of anti-endothelial cell antibody 1.018 O.D. ($N < 0.10$), tuberculin reaction negative. The blood viscosity was normal and anti-phospholipid antibody was negative. The CSF was clear and colorless, the cell count was 4/mm³, with a glucose level of 66 mg/dl and a protein value of 4 mg/dl. The CSF IgG level was 5 mg/dl. Salivary gland scintigraphy revealed severely decreased function of the bilateral parotid glands and no function

Fig. 3. Mononuclear cell infiltrates around the epineural small blood vessels in the sural nerve of case 2. H-E. $\times 300$.

Fig. 4. Marked reduction of myelinated fibers in case 2. Toluidine blue. $\times 200$.

Fig. 5. A total loss of myelinated fibers in case 2.

Fig. 6. Proliferation of endothelial cells and thickness of basement membrane in the vasa nervorum. $\times 3000$.

at all in the bilateral submandibular glands.

Sural nerve biopsy showed mononuclear cell infiltrates in and around the small vessels of the epineurium (Fig. 3). The number of myelinated fibers was markedly decreased (Fig. 4), with a mean density of $845/\text{mm}^2$ (control $9,563 \pm 896$) (Fig. 5). Electron microscopic study showed proliferation of endothelial cells and thickening of the basement membrane in the vasa nervorum (Fig. 6). The patient was treated with prednisolone 1 mg/kg daily, and her neurological symptoms improved over the next 3 months.

DISCUSSION

Two cases of Sjögren's syndrome accompanied by peripheral neuropathy are reported. In case 1, the only neurological symptom was decreased pain sensation in the lateral area of the right lower leg. In case 2, the neurological symptoms were severe motor weakness and sensory disturbance, and the patient had a chronic relapsing course. Although peripheral neuropathy in the course of Sjögren's syndrome has been described (4, 16) precise examination of such cases is rare (17). Kaltreider and Talal (4) reported that this association occurred in 10 of 109 patients with Sjögren's syndrome. The peripheral neuropathy in Sjögren's syndrome is mainly sensory neuropathy, and sensory trigeminal neuropathy may occur (4, 16–21). Other cranial nerve palsies however, have been reported only rarely (22).

Massey (5) emphasized mononeuritis multiplex,

however motor dominant mononeuritis multiplex, as in case 2 which took a chronic relapsing course, is uncommon. It has been proposed that the peripheral neuropathy accompanying Sjögren's syndrome usually results from vasculitic damage to the vasa nervorum (4, 17). This is supported by the abundant evidence of vasculitis and perivasculitis in both the muscle and nerve (4). Direct infiltration of nerve sheaths by chronic inflammatory cells has been reported (23). However, some authors claim that there is no systemic or peripheral nerve vasculitis though the disease shows immunological abnormalities (20, 21, 24, 25).

Mainlow et al (19) reported that the thoracic dorsal root ganglion biopsy from a patient with primary Sjögren's syndrome who developed subacute sensory neuronopathy showed lymphocytic infiltration and degeneration of ganglion cells. In the present study, sural nerve biopsy of case 1 showed mild lymphocytic infiltration around the endoneural vessels, which were slightly thickened. Minute granular IgG and C3 deposits were demonstrated around the blood vessel wall of the same vessels. These findings suggest immune complex deposition around the blood vessel. The level of circulating immune complex was high and the level of the anti-endothelial cell antibody was also slightly increased.

Although the level of circulating immune complex was not increased in case 2, the level of anti-endothelial cell antibody was significantly increased. Sural nerve biopsy in this case showed vasculitis in the small vessels of the epineurium with proliferation of endothelial cells and thickening of the basement membrane in the vasa nervorum.

The pathogenesis of peripheral neuropathy is uncertain. Association with anti-SSA antibody and systemic vasculitis has been proposed (18). Alexander et al (18) examined 16 patients with Sjögren's syndrome unassociated with another connective tissue disease. In their study, the peripheral nerve system involvement occurred in 10 patients (63%), and peripheral vasculitis occurred in 12 patients (70%), 83% of whom had anti-SSA antibody. They stressed the significance of anti-SSA antibody in the patients with Sjögren's syndrome. They pointed out that this antibody appeared in a subset of Sjögren's syndrome patients with a

predilection for vasculitic complications. They also showed that the frequency of anti-SSA antibody was 86% among 28 Sjögren's syndrome patients with vasculitis and 19% among 47 Sjögren's syndrome patients without vasculitis. They claimed that an immune vasculopathy might play a role in the pathogenesis of the central nervous system and peripheral nervous system disease of Sjögren's syndrome. But the precise mechanism associating anti-SSA antibody and neurological involvement is unclear.

In the present two cases, anti-SSA antibody was positive. So it might be a pathogenic factor in the production of peripheral nerve involvement in these two cases. Furthermore we feel that immune complex and anti-endothelial cell antibody are also of significance. The immunologic and pathologic findings in the present cases may indicate injury to endothelial cells in the vasa nervorum. Nerve ischemia due to vascular change of the vasa nervorum may play a role in the pathogenesis of peripheral neuropathy accompanying Sjögren's syndrome. Although the relation between the peripheral neuropathy and anti-endothelial cell antibody is unclear, it could be suggested that immune complex and anti-endothelial cell antibody may be immunological factors in the production of the peripheral neuropathy of Sjögren's syndrome.

REFERENCES

- 1) Bloch KJ, Buchanan WW, Wohl MJ, Bunim JJ. Sjögren's syndrome. A clinical, pathological and serological study of sixty-two cases. *Medicine* **44**: 187, 1965.
- 2) Spillane JD, Wells CEC. Isolated trigeminal neuropathy. A report of sixteen cases. *Brain* **82**: 391, 1959.
- 3) Attwood W, Poser C. Neurologic complication of Sjögren's syndrome. *Neurology* **11**: 1034, 1961.
- 4) Kaltreider HB, Talal N. Neuropathy of Sjögren's syndrome. Trigeminal nerve involvement. *Ann Intern Med* **70**: 751, 1969.
- 5) Massey EW. Sjögren's syndrome and mononeuritis multiplex. *Ann Intern Med* (letter) **63**: 87, 1980.
- 6) Alexander GE, Provost TT, Stevens MB, Alexander EL. Sjögren's syndrome: Central nervous system manifestations. *Neurology* **31**: 1391, 1981.
- 7) Molina R, Provost TT, Alexander EL. Peripheral inflammatory vascular disease in Sjögren's syndrome: Association with nervous system complications. *Arthritis Rheum* **28**: 1341, 1985.

- 8) Theofilopoulos AN, Wilson CB, Bokisch VA, Dixon FJ. Binding of soluble immune complexes in animal and human sera. *J Exp Med* **140**: 1230, 1974.
- 9) Cunningham-Rundles C, Brandies WE, Zacharczuk T, Good RA, Day N. Quantitation of circulating immune complexes in serum by Raji cells using an enzyme-linked immunosorbent assay. *Clin Exp Immunol* **40**: 411, 1980.
- 10) Mrsulja BB, Mrsulja BJ, Fuimoto T, Klatzo I, Spatz M. Isolation of brain capillaries: a simplified technique. *Brain Res* **110**: 361, 1976.
- 11) Spatz M, Bembry J, Dodson RF, Hervonen H, Murray MR. Endothelial cell cultures derived from isolated cerebral microvessels. *Brain Res* **191**: 577, 1980.
- 12) Tsukada N, Inoue A, Yanagisawa N, Behan WMH, Behan PO. Anti-endothelial cell antibody and immune complexes in the sera of animals with acute experimental allergic encephalomyelitis and chronic relapsing experimental allergic encephalomyelitis. *J Neuroimmunol* **12**: 89, 1986.
- 13) Tanaka Y, Tsukada N, Koh C-S, Yanagisawa N. Anti-endothelial cell antibodies and circulating immune complexes in the sera of patients with multiple sclerosis. *J Neuroimmunol* **17**: 49, 1987.
- 14) Hanyu N, Oguchi K, Yanagisawa N, Tsukagoshi H. Degeneration and regeneration of ventral root motor fibers in amyotrophic lateral sclerosis. Morphometric studies of cervical ventral roots. *J Neurol Sci* **55**: 99, 1982.
- 15) Daniel L, Williams M, Worthingham C. Muscle testing in: *Techniques of Manual Examination*, 2nd ed. W.B. Saunders, Philadelphia, 1956, p.2.
- 16) Kennett RP, Harding AE. Peripheral neuropathy associated with the sicca syndrome. *J Neurol Neurosurg Psychiatry* **49**: 90, 1986.
- 17) Pittsley RA, Talal N. Neuromuscular complications of Sjögren's syndrome. in: *Handbook of Clinical Neurology*, Vinken PJ, Bruyn GW, Eds. Vol. 39. North Holland Publishing Co., Amsterdam, 1980, p.419.
- 18) Alexander EL, Provost TT, Stevens MB, Alexander GE. Neurologic complications of primary Sjögren's syndrome. *Medicine* **61**: 247, 1982.
- 19) Mainlow K, Yannakakis GD, Glusman SM, et al. Subacute sensory neuronopathy secondary to dorsal root ganglionitis in primary Sjögren's syndrome. *Ann Neurol* **20**: 535, 1986.
- 20) Hankey GJ, Gubbay SS. Peripheral neuropathy associated with sicca syndrome. *J Neurol Neurosurg Psychiatry* **50**: 1085, 1987.
- 21) Graus F, Pou A, Kanterewicz E, Anderson NE. Sensory neuropathy and Sjögren's syndrome: Clinical and immunologic study of two patients. *Neurology* **38**: 1637, 1988.
- 22) Vincent D, Laron P, Awada A, Gautier JC. Paralysis Multiples et Recidivantes des Nerfs Craniens. Syndrome de Gougerot-Sjögren. *Rev Neurol (Paris)* **141**: 318, 1985.
- 23) Marbini A, Gemignani F, Manganelli P, Govoni E, Bragaglia MM, Ambanelli U. Hypertrophic neuropathy in Sjögren's syndrome. *Acta Neuropathol (Berl)* **57**: 309, 1982.
- 24) Szanto L, Farkas K, Gyulai E. On Sjögren's disease. *Rheumatism* **13**: 67, 1957.
- 25) Gross M. Chronic relapsing inflammatory polyneuropathy complicating sicca syndrome. *J Neurol Neurosurg Psychiatry* **50**: 939, 1987.