Axonal and perikaryal involvement in chronic inflammatory demyelinating polyneuropathy

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Abstract

**Objectives**—To assess the extent of loss of myelinated nerve fibres and spinal motor neuron loss in chronic inflammatory demyelinating polyneuropathy (CIDP), a clinicopathological study was conducted on biopsied sural nerves and necropsied spinal cords from patients with CIDP.

**Methods**—The myelinated fibre pathology of 71 biopsied sural nerves and motor neuron pathology of nine necropsied spinal cords at L4 levels in patients with CIDP were quantitatively and immunohistochemically assessed.

**Results**—Myelinated nerve fibre density was significantly diminished to 65.4% of the control values (p < 0.0001), correlating inversely with the extent of segmental demyelination and remyelination (r = −0.43, p < 0.0005) and duration of illness (r = −0.31, p < 0.01). Numbers of large spinal motor neurons in CIDP were variably but significantly diminished (range from 46.0 to 97.6% of the age matched control value (p < 0.005)), and reactive astrogliosis was evident in the ventral horn in CIDP. The frequency of ventral horn neurons exhibiting central chromatolysis and the accumulation of phosphorylated high molecular weight neurofilament protein was significantly higher in CIDP than in controls (p<0.01 and p<0.05).

**Conclusions**—The loss of nerve axons and spinal motor neurons is common in CIDP, and extensive in some cases. These neuronal and axonal losses may influence the functional prognosis in CIDP.

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Keywords: chronic inflammatory demyelinating polyneuropathy; axon loss; spinal motor neuron

The pathological hallmark of chronic inflammatory demyelinating polyneuropathy (CIDP) is segmental demyelination and mononuclear inflammatory cell infiltration in the peripheral nerves, accompanied by varying degrees of axonal degeneration, myelinated fibre loss, and endoneurial oedema.1 Regarding the pathology of the CNS in CIDP, central chromatolysis in spinal motor neurons has often been reported,2 and sporadic cases with a slight loss of spinal motor neurons have occasionally been documented.3–6 Spinal motor neuron loss as well as nerve myelinated fibre loss could be important factors influencing functional recovery. In this study, we assessed the degree of involvement of spinal motor neurons and peripheral nerve axons in CIDP.

**Methods**

**SPECIMENS**

After informed consent was given, sural nerve biopsy specimens from 71 patients with CIDP (50 males and 21 females) were obtained at the Nagoya University School of Medicine and its affiliated hospitals over 11 years. Age at biopsy ranged from 2 to 81 years; mean (SD) age 48.5 (21.9) years. The duration of illness before biopsy ranged from 2 months to 28 years; mean (SD) 2.9 (5.8) years. The spinal cords were obtained at necropsy from nine patients with CIDP. Three of these patients were necropsied at the Nagoya University Hospital and affiliated hospitals, and others were necropsied in hospitals located throughout Japan during the past 11 years. These patients consisted of six men and three women, aged 49 to 73 years; mean (SD) 62.4 (8.9) years. Their duration of illness ranged from 4 months to 8 years. Clinical profiles of necropsy cases are summarised in table 1.10

The diagnosis of CIDP in our study was assessed using the criteria of Barohn et al11 or the ad hoc committee of the American Academy of Neurology.12

**ASSESSMENT OF SURAL NERVE BIOPSY**

Sural nerve biopsy specimens were fixed in glutaraldehyde in 0.025 M cacodylate buffer (pH 7.4) and embedded in epoxy resin. Semithin sections were stained with toluidine blue, and the density of myelinated fibres was analysed quantitatively using a computer assisted imaging system (Luzex FS, Nireco, Tokyo, Japan). The extent of subperineurial oedema was assessed as an increase in the subperineurial space by comparing the subperineurial area to the total endoneurial area using the same imaging system. A part of the nerve specimen was processed for teased fibre analysis, and the condition of each fibre was assessed according to our previously indicated criteria.13 A portion of the nerve specimen was fixed in 10% buffered formalin and embedded in paraffin, and then processed for immunohistochemical study. Mouse monoclonal antibodies (mAb) to human leucocyte common antigen (LCA, DAKO, Denmark; dilution, 1:50), helper/inducer T cells (CD4, Novocastra, UK; dilution, 1:10), cytotoxic/suppressor T cells (CD8, DAKO; dilution, 1:25), and macrophages (CD68, DAKO; dilution, 1:10) were used. The avidin-biotin-peroxidase complex
Modified Rankin score: 0=asymptomatic; 1=non-disabling symptoms that do not interfere with lifestyle; 2=minor disability symptoms that lead to some restriction of lifestyle but do not interfere with the patients’ capacity to look after themselves; 3=moderate disability symptoms that significantly interfere with lifestyle or prevent totally independent existence; 4=moderately severe disability symptoms that clearly prevent independent existence, although patient does not need constant attendance day and night; and 5=severely disabled, totally dependent requiring constant attention day and night.10 Modified Rankin scores of some patients were assessed on the clinical records. Therapeutic response was evaluated as positive if the modified Rankin score improved one grade or more. Muscle atrophy and weakness were respectively assessed as 1, 2, and 3 denoting mild, moderate, and severe degrees. NA=not available. The grading was made within 3 months before death.

### Table 1 Clinical features of necropsied patients with CIDP

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at death (y)</th>
<th>Sex</th>
<th>Onset to death (s)</th>
<th>Motor/Sensory symptoms</th>
<th>Modified Rankin score (pretreatment)</th>
<th>Corticosteroid</th>
<th>Plasmaphoresis</th>
<th>IV Ig</th>
<th>Therapeutic response</th>
<th>Muscle</th>
<th>Modified Rankin score (before death)</th>
<th>CSF protein (mg/dl)</th>
<th>Cause of death</th>
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<td>1</td>
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<td>M</td>
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<td>4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>105</td>
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<td>55</td>
<td>F</td>
<td>0.8</td>
<td>M&gt;S</td>
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<td>+</td>
<td></td>
<td>+</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>800</td>
<td>Cardiovascular disease</td>
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<tr>
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<td>49</td>
<td>M</td>
<td>8</td>
<td>M&gt;S</td>
<td>3</td>
<td>-</td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>99</td>
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<tr>
<td>4</td>
<td>68</td>
<td>M</td>
<td>3</td>
<td>M&gt;S</td>
<td>3</td>
<td>+</td>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>218</td>
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<tr>
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<td>54</td>
<td>F</td>
<td>0.3</td>
<td>M=S</td>
<td>5</td>
<td>+</td>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>5</td>
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<td>57</td>
<td>M</td>
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<td>+</td>
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<td>1</td>
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<td>110</td>
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<td>1</td>
<td>M&gt;S</td>
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<td>8</td>
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<td>+</td>
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<td>1</td>
<td>2</td>
<td>NA</td>
<td>Bronchitis/Meningitis</td>
</tr>
</tbody>
</table>

Modified Rankin scores of some patients were assessed on the clinical records. Therapeutic response was evaluated as positive if the modified Rankin score improved one grade or more. Muscle atrophy and weakness were respectively assessed as 1, 2, and 3 denoting mild, moderate, and severe degrees. NA=not available. The grading was made within 3 months before death.
cells (CD20, DAKO; dilution, 1:50), and a broad range of T cells (UCHL1, DAKO; dilution, 1:125). CD68, CD4, CD8, and LCA antibodies were used in the same manner as the sural nerves. To assess the occurrence of motor neurons expressing pNFH in neuronal perikarya, Ta-51 positive ventral horn neurons with obvious nucleoli were counted, and a ratio to the total neuronal population was expressed as described previously. Gliosis in the ventral horn was assessed using Holzer stain and immunohistochemistry for GFAP. Motor neurons satisfying the criteria for central chromatolysis by Campbell and Novick were designated as undergoing active central chromatolysis, and their occurrence among total neurons was estimated on Klüver-Barrera stained sections as described before.

Spinal cords of seven subjects who died of non-neurological disorders, (aged 47 to 81 years; mean (SD) 66.6 (12.7) years), were examined in the same manner as age matched controls.

**STATISTICAL ANALYSIS**

Statistical analyses were by Student’s *t* test, Mann-Whitney *U* test, and Pearson’s correlation coefficient; *p*<0.05 was taken as significant.

**Results**

**SURAL NERVES**

The myelinated fibre density of the sural nerves was diminished in varying degrees (mean (SD) 5679 (2370) /mm²; significantly less than control values, 8679 (1336) /mm²; *p*<0.0001; fig 1A). The incidence of abnormalities in the teased fibre preparation was also increased to various degrees (fig 1 B): 0 to 52% for segmental demyelination (mean (SD) 7.2 (10.6)%); significantly greater than controls 0 (0)%; *p*<0.0005; 0 to 100% for segmental demyelination and remyelination (mean (SD) 23.0 (16.6)%); significantly increased compared with controls, 9.5 (8.8)%; *p*<0.05. The extent of active axonal degeneration in CIDP varied widely, ranging from 0 to 51%, (mean (SD) 5.5 (11.1)%), and 23% of the nerves showed values exceeding the mean +2 SD control level (controls, mean (SD) 1.7 (1.4)%). The extent of subperineurial oedema in CIDP (mean (SD), 11.0 (3.1)%) was significantly greater than in controls (mean (SD), 4.6 (1.0)%; *p*<0.0001; fig 1C). The density of myelinated fibres was significantly inversely correlated with the extent of segmental demyelination and remyelination (fig 2A), and with the extent of segmental demyelination and remyelination plus axonal degeneration (fig 2C). Patients with a marked increase in axonal degeneration were also accompanied by a considerable myelinated fibre loss (fig 2B). Myelinated fibre loss was directly correlated with the duration of illness (fig 3), and severe fibre loss (<2000/mm² in remaining fibres) was found in some patients with a duration of illness exceeding 5 years before the nerve biopsy. Myelinated fibre loss was not significantly correlated with the extent of mononuclear cellular infiltrates as assessed by immunohistochemistry. Infiltrates of LCA positive cells were found using immunohistochemistry in the endoneurium or epineurium in 72% of patients with CIDP. Infiltrates of CD4 positive cells were found in 37%, CD8 positive cells in 35%, and CD68 positive cells in 67% of the nerves from patients with CIDP.

**SPINAL CORDS**

The mean number of large motor neurons in the unilateral L4 ventral horn in CIDP (mean (SD) 466 (99)/50 sections) was significantly diminished compared with that of age matched
controls (mean (SD) 632 (55)/50 sections, p <0.005; fig 4 and 5 A). The extent of loss of large motor neurons in CIDP was highly variable, the average value being 73.7% of the controls, and the minimum value only 46% of the mean control value (fig 4 and 5A). The small neurons were also significantly depopulated in CIDP, although to a lesser extent (fig 5C). The number of medium sized neurons was not significantly decreased (fig 5B). The frequency of ventral horn neurons exhibiting central chromatolysis and accumulations of pNFH was significantly higher in CIDP than in controls (p<0.01 and p<0.05, respectively; fig 6 and table 2). Reactive astrogliosis in the ventral horn was also more prominent in CIDP (table 2). Neuronophagia was occasionally demonstrated by immunohistochemistry using CD68, but lymphocytes positive for CD4, CD8, or UCHL1 were not found in the ventral horns of CIDP. Varying degrees of myelinated fibre loss in the dorsal columns were seen at the L4 level in five of nine patients (table 2).

Discussion

Our study showed that myelinated nerve fibre loss is common in CIDP, and that it is correlated with the extent of demyelination and remyelination on teased fibre analysis as well as with the duration of illness. These findings indicate that both the intensity of the inflammatory demyelinating process and the longevity of
the disease process are the factors influencing the severity of myelinated fibre loss.

The precise mechanism of nerve fibre loss in CIDP is unknown. In Guillain-Barré syndrome, axonal degeneration has been found in addition to macrophage mediated segmental demyelination in severe nerve lesions. Immuno-histochemical study showed antibody and complement mediated attacks on the axolemma of nerve fibres in a Chinese series of axonal Guillain-Barré syndrome, suggesting that axonal damage can occur as a primary process. However, because antibody and complement deposits in nerves from patients with CIDP are rare, axonal injury and subsequent fibre loss may be caused by a different mechanism from that suggested in the Chinese series of axonal Guillain-Barré syndrome.

In experimental allergic neuritis (EAN) and chronic EAN, models of acute and chronic inflammatory demyelinating neuropathy, axonal degeneration has also been found. Hahn et al and Madrid and Wisniewski reported that the extent of axonal degeneration in nerve roots corresponded to the degree of inflammation and demyelination in their models of EAN. Moreover, Said et al also documented axonal degeneration distal to the demyelinated nerve segments by the intraneural injection of antiserum from EAN rabbits.

Soluble factors such as proteases, phospholipases, lymphotoxins or tumour necrosis factor-α (TNF-α) from infiltrating mononuclear cells are supposed to be responsible for axonal degeneration in EAN. Meanwhile, in biopsied nerves from patients with CIDP, macrophages express TNF-α when attached to myelinated fibres. Some of the infiltrating macrophages in the endoneurium as seen in the present study might contribute to axonal damage by releasing soluble factors such as TNF-α.

In our study, the duration of illness is another factor influencing the extent of myelinated fibre loss. Severe myelinated fibre loss in the sural nerves was found in patients with CIDP associated with marked onion bulb formation, suggesting that the longstanding, repeated inflammatory demyelinating process is related to myelinated fibre loss. On the other hand, myelin regulates axonal properties such as the focal number, spacing, and phosphorylation level of neurofilaments, axonal calibre, and slow axonal transport. Therefore, long-standing, repetitive, or persistent demyelination itself might play some part in the process of axonal damage by influencing axonal properties. Actually, hereditary demyelinating neuropathies with abnormal myelin protein genes such as Charcot–Marie–Tooth disease type 1A and 1B, and Déjérine-Sottas disease are often accompanied by a considerable degree of myelinated fibre loss during a lengthy process.

The most striking finding in our study was the spinal motor neuron loss in CIDP, which was extensive in some patients. The average loss of one fourth of the large spinal motor neurons, corresponding to α-motor neurons, with a maximum loss of one half in our necropsied series, is more severe than so far inferred. The loss of spinal motor neurons was also evidenced by astrogliosis occurring in the lateral and medial nuclei of the ventral horn, particularly when the loss of these neurons was extensive. Muscle weakness and atrophy were pronounced in patients with extensive spinal motor neuron loss (table 1). This finding indicates that the residual disability in patients with CIDP, especially muscle wasting, is in some way related to spinal motor neuron loss.

Loss of large motor neurons has been reported a long time after limb amputation in humans and cats, suggesting that long-standing axonal damage may secondarily induce spinal motor neuron loss. Nerve cells are also supposed to be lost when axonal damage occurs close to the cell body. Loss of large motor neurons in the spine has been found in necropsied patients with Guillain-Barré syndrome accompanying marked proximal axonal involvements. In our series of CIDP, the absence of inflammatory lymphocytic infiltrates in the ventral horn suggests that the ventral horn is not the primary site of inflammation, and that longstanding or proximal motor axonal damage might cause spinal motor neuron loss. An increased rate of central chromatolysis in the spinal motor neurons also suggests the axonal involvement of motor nerves. In addition, an increased accumulation of pNFH in the perikarya of spinal motor neurons is related to myelinated fibre loss.
neurons may also support this view. pNPH accumulation in the spinal motor neurons has been shown in a wide variety of pathological conditions, including amyotrophic lateral sclerosis,14 17 toxic neuropathies, and experimental nerve crush.40 In most cases, a certain degree of impairment of axonal transport is speculated to underlie pNPH accumulation.

Small neurons in the intermediate zone of the ventral horn, corresponding to interneurons, were mildly but significantly decreased in CIDP. The background mechanism of this loss of small neurons is uncertain. Suzuki et al43 have reported a decrease in small neurons in the cervical intermediate zone as well as in large neurons on the opposite side in necropsy case studies years after a proximal amputation of one arm, suggesting that contralateral interneurons may be damaged by transneuronal degeneration after loss of large neurons. Small neurons in the intermediate zone in our patients with CIDP might have been lost by a similar mechanism. However, as the degree of loss of small neurons in patients with CIDP was mild, this finding should be confirmed by studying additional cases of CIDP.

In summary, we showed that loss of spinal motor neurons is common in CIDP, and that substantial neuronal loss may occur in some cases. Myelinated fibre loss also occurred and was correlated with the extent of segmental demyelination and remyelination, and the duration of illness. These findings are important regarding the long term functional prognosis of CIDP. Ultimately, prevention of these neuronal and axonal losses would be another therapeutic goal in CIDP.

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Stjepan Betlheim (1898–1970)

The Croatian neuropsychiatrist Stjepan Betlheim was born in Zagreb in 1898. He began his medical studies in Graz and graduated at the University of Vienna in 1922. He specialised in neuropsychiatry in Vienna, Berlin, Zürich, and Paris. In Vienna he worked in the neuropsychiatric clinic headed by Professor J Wagner-Jauregg. During his specialization, he published six articles in distinguished Austrian and German neurological or neuropsychiatric journals. The article he wrote in collaboration with Heinz Hartmann, Über Fehlreaktionen bei der Korsakoffschen Psychose (On paraphraxes in the Korsakoff psychosis), published in Arch f Psychiat u Nervenkrank (1925;72:275–86) is known best. As a medical student, Betlheim showed interest in psychoanalysis and attended Freud’s lectures. His educators in psychoanalysis were Paul Schilder, Helen Deutsch, Sandor Rado, and Karen Horney. He returned to Zagreb in 1928, and began work as neuropsychiatrist. In the same year he founded the station for mental-hygiene. He continued working as a psychoanalyst in his private practice. He gave many public lectures, and wrote popular articles on psychoanalysis. In 1941, after the German and Italian armies occupied Yugoslavia, the pro-fascist quisling Ante Pavelić became the president of Croatia. Dr I Petrić, the Minister of health (16 April 1941 to 10 October 1942) sent the group of mostly Jewish physicians and their families, to Bosnia, in the region with endemic syphilis, aimed at protecting them from prosecution and deportation to camps. Betlheim very soon voluntarily joined Marshal Tito’s army—the antifascist movement—with his little daughter Ruth and wife Marie Luise, néé Morgenroth. After the second world war he returned to Zagreb and became Professor of Neuropsychiatry in 1959. He wrote the first Croatian psychiatric textbook (1959), the book Neuroze i njihovo liječenje (The treatment of neuroses, 1963), the articles for Medicinska enciklopedija (Medical Encyclopedia, 1957), and also wrote for numerous Croatian and foreign medical journals. He was a propagator of psychoanalysis, psychoanalytic psychotherapy, and group analysis not only in Croatia but also in other republics of the former Yugoslavia. Betlheim thought that neurology and psychiatry were two branches of medicine which had many connections and there was a need for neuropsychiatry. He was a member of the Vienna Psychoanalytic Society from 1928 to 1938, and of the International Psychoanalytical Association since 1952, the New York Academy of Sciences, The Royal Society of Medicine (London), Group-Analytic Society (London), and French Psychosomatic Society. For many years he was the WHO Consultant for Mental Health. In 1998, Croatia commemorated the 100th anniversary of his birth with a stamp.
Stjepan Betlheim (1898–1970)

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