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Lymphocytic infiltration in the spinal cord of patients with amyotrophic lateral sclerosis

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Abstract. Amyotrophic lateral sclerosis (ALS) is a devastating systemic atrophy affecting the upper and lower motor neurons. The etiology is unknown, but one theory of pathogenesis supposes that the motor system is affected by abnormal immune responses. We have studied the prevalence and extent of lymphocytic infiltration, previously reported as a rare finding in the ALS spinal cord.

Application of monoclonal antibodies against macrophages, T- and B-cells to spinal cords from 48 ALS patients disclosed a cellular mononuclear infiltrate in 38 specimens (79%), intense enough to be revealed by routine neuropathological techniques in 6 of them (12.5%); the remaining 10 cords (21%) exhibited no infiltrates. Since duration and clinical signs of the preceeding illness were the same in cases with and without infiltrates, we consider it unlikely that such infiltrates are entirely secondary to atrophy of the cord. As Wallerian degeneration is not accompanied by infiltrates of lymphocytes, their presence in the cord tracts of our material throws doubt on the conventional view that tract degeneration in ALS is exclusively Wallerian.

Key words: amyotrophic lateral sclerosis – leukocyte common antigen – monoclonal antibodies to lymphocytes – motor neuron disease – neuronal degeneration – spinal cord

Introduction

The etiology of amyotrophic lateral sclerosis (ALS) is not known, and, to date, we cannot even decide whether the disease is an entity rather than a syndrome [Appel et al. 1986, Tandan and Bradley 1985]. Various etiological factors have been considered, including slow virus infection [Gibbs and Gajdusek 1982], intoxication with heavy metals [Patten 1984] and neurotoxins [Tandan and Bradley 1985, Calne et al. 1986] whereas the seed of the neurotoxic plant Cycas circinalis L. has recently been linked to the occurence of Guam type ALS [Spencer et al. 1987].

Histopathological differences between cases of ALS are generally explained as different stages of one disease [Bertrand and van Bogaert 1925, Brownell et al. 1970, Bonduelle 1972, Castaigne et al. 1972, Engel 1977, Hudson 1981, Lawyer and Netsky 1953, Malamud 1968, Oppenheimer 1984]. The presence of mononuclear cells in the spinal cord is one of those inconsistent histopathological features that has not yet been studied in detail in a large group of ALS patients. The presence of lymphocytic infiltrates in the spinal cord of ALS patients has been both asserted and denied [Behan and Behan 1984, Hawkes et al. 1984]. This discrepancy can be explained both by the relatively small numbers of patients that have been studied and by the difficulty in detecting lymphocytes in the parenchyma of the CNS. This difficulty is due to the close similarity between lymphocytes and oligodendrocytes in brain and spinal cord parenchyma. Since monoclonal antibodies directed against lymphocytes bypass the problem of identifying infiltrations, we have applied a combination of routine and immunocytochemical techniques to the spinal cords of 48 ALS patients. Our results will demonstrate that the presence or absence of mononuclear cell infiltrates divides our material into at least two groups.

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No	Age	Sex	Duration of disease in months	First symptoms (Nos. 1–48)/ Disease/ctrl (Nos. 49–60)	Tract degeneration subtype	Lymphocytes pyr. tract	Lymphocytes ant. horn
1	55	m	17	b	1	1	1
2	68	f	24	b	2	2	1
3	61	m	41	l	1	2	1
4	62	m	58	l	3	0	0
5	57	m	44	b	3	0	0
6	60	m	27	a	1	3	1
7	59	f	59	l	3	1	2
8	70	f	25	b	3	3	3
9	81	f	15	b	1	3	3
10	66	m	12	a	1	3	3
11	48	m	39	b	1	2	0
12	46	m	28	a	2	1	1
13	52	f	27	l	1	1	3
14	58	m	28	l	1	1	3
15	35	m	9	a	2	1	3
16	70	f	10	l	3	0	1
17	34	m	8	a	3	1	1
18	46	m	29	a	1	0	0
19	53	m	16	a	1	0	0
20	43	f	27	l	1	3	1
21	49	m	35	b	1	2	1
22	72	f	29	b	1	2	1
23	57	m	26	a	1	0	2
24	66	f	30	a	1	2	1
25	64	f	39	a	1	2	1
26	69	f	15	l	2	1	0
27	56	m	27	a	2	2	1
28	71	m	39	b	1	2	2
29	47	m	72	a	2	1	0
30	66	f	18	a	1	2	1
31	77	m	30	l	2	3	1
32	47	m	9	b	3	0	0
33	74	m	47	a	2	3	2
34	67	f	16	l	1	0	0
35	66	m	120 familial	l	2	0	1
36	63	m	57	l	1	2	0
37	68	m	58	l	1	3	2
38	61	f	21	b	1	2	2
39	67	f	44	a	1	1	1
40	51	m	19	b	1	2	1
41	67	f	25	a	1	0	0
42	69	m	20	a	3	1	1
43	50	f	26	a	2	1	0
44	55	m	36	a	2	0	0
45	72	m	27	a	1	2	3
46	77	f	216 familial	–	1	0	0
47	75	f	24	b	1	0	0
48	65	m	14	a	2	3	2
49	53	m	12	metastasis spinal cord	1	0	0
50	63	m	2	basilar thrombosis	1	1	0
51	58	f	48	CVA	1	0	0
52	83	m	192	CVA	1	0	0
53	65	f	48	CVA	1	0	0
54	67	m	36	CVA	1	0	0
55	51	f		ctrl	0	0	0
56	57	f		ctrl	0	0	0
57	65	f		ctrl	0	0	0
58	45	m		ctrl	0	0	0
59	57	m		ctrl	0	0	0
60	68	m		ctrl	0	0	0

Table 1 Clinical data histopathology.

m = male
f = female
D = duration of the disease in months
a = ALS with first symptoms in the arm
l = ALS with first symptoms in the leg
b = ALS with first symptoms in the bulbar region

Tract degeneration subtype
1. pyramidal tracts only
2. diffuse involvement of the spinal cord, with the exception of the dorsal funiculi
3. myelin pallor of the pyramidal tracts

The density of lymphocyte cells was scored in an LCA staining according to an arbitrary scale of 0–3:
0 = no positive cells detected
1 = a few positive perivascular cells
2 = some positive perivascular cells an in the parenchyma
3 = diffuse parenchymal infiltration of positive cells

Materials and methods

We obtained the spinal cords from 48 cases of ALS, 46 of the isolated and 2 of the familial type, from the Netherlands ALS tissue bank. The average age of onset of the disease was 57 years (range 34–81 years of age). The duration of the disorder varied from 8 months to 10 years. The median survival time after the first symptoms was 27 months (range 8–216 months). Pneumonia was the cause of death in 37 cases (77%). In 11 cases (23%) death was related to other non-infectious diseases. Twelve controls, i.e. three females and three males with the same age range and postmortem delay without neurological disease and six patients with Wallerian degeneration in the spinal cord after brain infarction or tumor infiltration were used. Patients and controls are listed in Table 1. Autopsy delay ranged from 6 to 16 hours, and all material was fixed in 10% formalin. In all cases a general necropsy was performed and muscle biopsies were taken. From each case, samples from area 4 and brain stem were taken; the spinal cord was sampled at C6, T4, T8 and L1. Paraffin embedded sections were stained with hematoxylin and eosin (HE), Klüver-Barrera, Nissl, Bodian and Holzer. Microglia was demonstrated by a modified rapid silver impregnation technique [Scott 1971]. For the immunohistochemical demonstration of cell surface antigens, 5-μm paraffin sections of the spinal cord were stained with a three-step indirect immunoperoxidase procedure using the following antibodies:

– DAKO-LC, reacting with the leukocyte common antigen (LCA) expressed on all leukocytes including B- and T-lymphocytes, macrophages and granulocytes [Warnke et al. 1983]

– DAKO-UCHL1, which labels resting T-cells belonging to both the CD4 and CD8 subsets, and mature activated T-cells.

Granulocytes and monocytes are also labelled by DAKO-UCHL1 [Smith et al. 1986].

– DAKO-L26, reacting with a major 33 kD and a minor 30 kD polypeptide present on the majority of B-cells [Cartun et al. 1987].

For the immunohistochemical demonstration of glial fibrillary acid protein (GFAP) a three-step unlabelled peroxidase-anti-peroxidase (PAP) method using polyclonal rabbit anti-GFAP, swine antirabbit immunoglobulin and rabbit PAP complexes was used (all reagents obtained from Dakopatts a/s).

The density of the lymphocytic infiltration was arbitrarily scored from zero to 3 : 0 = no positive cells detected; 1 = a few positive perivascular cells; 2 = some positive perivascular and parenchymal cells; 3 = diffuse parenchymal infiltration with positive cells. From each spinal level at least five slides were stained with LCA, UCHL1 and L26.

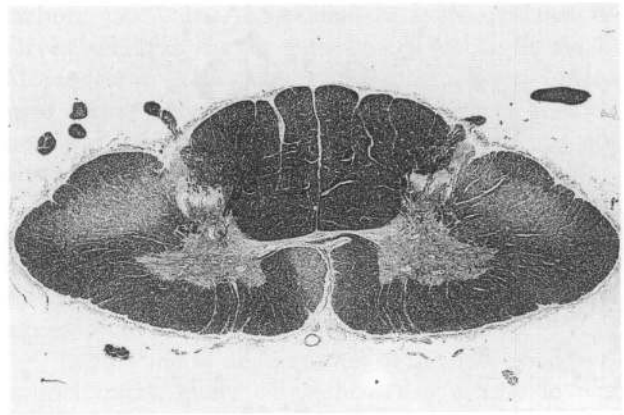

Fig. 1 Degeneration restricted to the pyramidal tracts in ALS patient 30 (Klüver-Barrera, × 20).

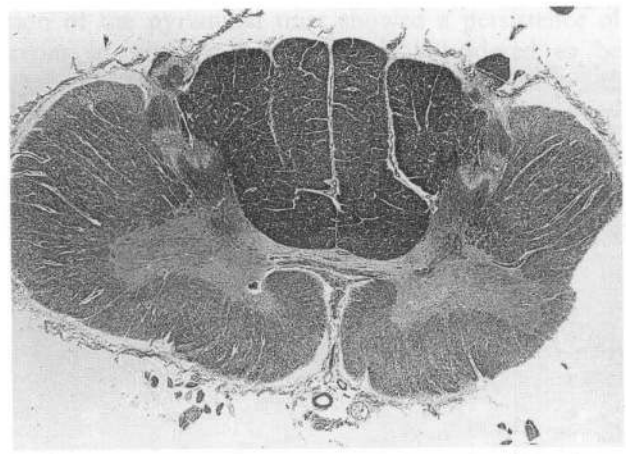

Fig. 2 Diffuse degeneration of lateral and anterior funiculi in ALS patient 33 (Klüver-Barrera, × 20).

Results

Anterior horns

The major histopathological lesion in the spinal cord consisted of a variable loss of motor neurons in the anterior horn. Cell loss was almost complete at the thoracic level and less severe at the cervical and lumbar levels. The remaining neurons showed hyaline change, lipoidosis, swelling, vacuolation and pyknosis. In addition, spheroids, argyrophilic "neuritic" remnants, argyrophilic motor neurons and ghost cells were present. Neurofibrillary changes were not observed. Involvement of Clarke's column was observed in 41 cases (85%).

Pyramidal tracts

In all cases, degeneration affected the pyramidal tracts, but strong variation in the extent of this process was apparent. In 17 cases (35%) tract degeneration was symmetrical and restricted to the lateral pyramidal tracts. In 5 cases (10%) the lateral and anterior pyramidal tracts were symmetrically involved. In 13 cases (27%) the lateral pyramidal tracts were symmetrically involved while the anterior pyramidal tract showed an asymmetrical degeneration. In 8 cases (17%) large parts of the lateral and anterior funiculi were involved as well. In 5 cases (10%) the pyramidal tracts showed only a pallor of myelin without clearcut destruction whereas in 4 cases (8%) a slight pallor in the median portion of the dorsal funiculi was observed.

On the basis of these differences in tract degeneration, three histopathological subtypes could arbitrarily be distinguished (Table 1): type one showed degeneration restricted to the pyramidal tracts (Figure 1); type-two patients showed a more diffuse involvement of the spinal cord including areas outside the pyramidal tracts (Figure 2), whereas type-three patients showed only a myelin pallor of the pyramidal tracts.

Macrophages

Few macrophages (lipid phagocytes or scavenger cells) were observed in the anterior horns of type-1 and type-2 patients. The pyramidal tract showed larger numbers of macrophages reactive with LCA and UCHL1 (Figure 3). No macrophages were ob-

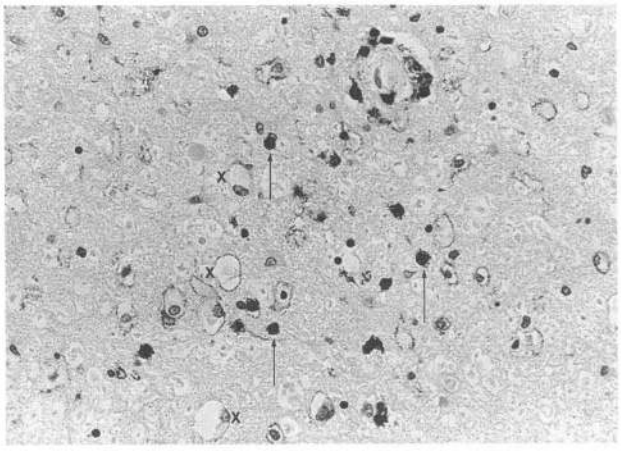

Fig. 3 Lateral pyramidal tract with lymphocytes (arrows) and macrophages (×) scattered through the parenchyma and around blood vessels in patient 11 (Leukocyte common antigen, paraffin (LCA), × 500).

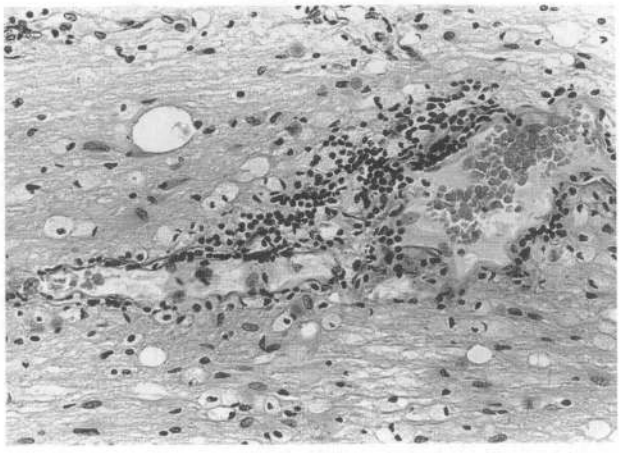

Fig. 4 Lateral pyramidal tract with lymphocytes and macrophages around blood vessels in patient 37 (HE, paraffin, × 325, longitudinal section).

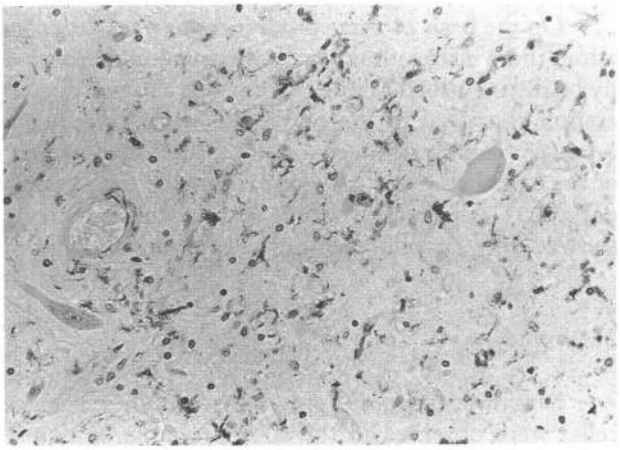

Fig. 5 Anterior horn at the level of T8. LCA-positive microglia is diffusely scattered in between large motor neurones (LCA, × 200) (patient 11).

served in the cases showing myelin pallor only (type-3 patients) and in the control cases. Although exact quantative estimations were not performed, it was apparent that the numbers of macrophages in long- and short-lived cases were similar. A significant relation between the number of macrophages and the presence of lymphocytic infiltrates was lacking. One case (patient 34) showed a closely packed infiltrate of macrophages in the pyramidal tract which failed to react with LCA and UCHL1.

Lymphocytes

Using routine staining procedures, lymphocytic infiltration was observed in 21 ALS patients (44%). The infiltrates were localized as small cuffs around the blood vessels (Figure 4) LCA and UCHL1 labelled similar numbers of lymphocytes detected in 38 (79%) of the ALS patients. B-cells immunoreactive to L26 were not observed in any of the ALS cases.

Except for the small "dendrites of reactive microglial cells", no staining of cellular elements of the central nervous system was observed (Figure 5). The density of the cellular infiltrate as indicated in Table 1 was estimated at the cervical level. The infiltrate was generally denser in the pyramidal tract than in the anterior horns and no obvious relationship between the presence of infiltrates and the extension of pyramidal tract degeneration, neuronal loss, age of the patients or survival time was noted.

Only minor differences were noted in the density of the cellular infiltrate at different levels of the spinal cord. Cases lacking lymphocytic infiltration in the spinal cord also failed to show lymphocyte infiltration in the brain stem and cortical areas. On the other hand, in cases showing lymphocytic infiltration, the infiltrate extended cranially up to the level of the mesencephalic pedunculi, in some patients even to the level of the subcortical area of the precentral gyrus. This phenomenon will be discussed extensively elsewhere.

Lymphocytic infiltration, as observed in routinely stained sections, was not restricted to the damaged parts of the pyramidal tracts. In eight cases (16%) lymphocytic infiltration was observed in histologically normal areas of the brain stem, particularly in the pyramidal tract.

Ten patients (21%) showed no lymphocytes at all in the spinal cord; their myelin degeneration ranged from mild (pallor only) to severe.

Controls

The normal controls showed no lymphocyte infiltration. In the six patients with Wallerian degenera-

tion, lymphocytic infiltration was found in only one case. In this case the infiltrate was composed of a mixture of B- and T-lymphocytes and plasma cells.

Discussion

The histopathological changes observed in the anterior horn and motor neurons of the spinal cord in ALS were similar to those that have been documented before using conventional staining methods [Bertrand and van Bogaert 1925, Brownell et al. 1970, Bonduelle 1972, Castaigne et al. 1972, Engel 1977, Hudson 1981, Lawyer and Netsky 1953, Malamud 1968, Oppenheimer 1984]. Motor neuron loss was more severe in the cervical and thoracic regions than in the lumbar region. Clarke's column was generally involved in the disease process, which is in agreement with the findings of Averback [Averback and Crocker 1982]. In many ALS patients the smaller axons of the pyramidal tract are relatively spared as has been described before and confirmed in the present study [Colmant 1957].

Tract degeneration in ALS is generally considered to be of the Wallerian type [Bertrand and Bogaert 1925, Brownell 1970, Bonduelle 1972, Castaigne et al. 1972, Engel 1977, Hudson 1981, Lawyer and Netsky 1953, Malamud 1968, Oppenheimer 1984] but the possibility of direct damage to the pyramidal tract or its myelin has never been excluded. In Wallerian degeneration lymphocytic infiltrates have not been reported [Duchen 1984, Jacob 1957, Wolman 1968] and in our material, infiltrates similar to those observed in ALS, i.e. composed of lymphocytes and macrophages, were not observed. This indicates that the infiltrate in ALS is not only secondary to tract degeneration. Using immunocytochemical techniques, we found that ten (21%) patients did not show lymphocytic infiltration. No relationship was observed between the presence or absence of lymphocytic infiltration in ALS and the degree of degeneration or survival time or the cause of death, i.e. infectious (pneumonia) or noninfectious. The absence of infiltrates in some patients with severe tract degeneration reinforces the idea that the infiltrates in the remaining 79% of the ALS patients can not simply be secondary to neuronal degeneration or demyelination. A number of other observations support this possibility. In subacute degeneration of the spinal cord and the brain due to B12 deficiency, large amounts of degeneration products are seen and the perivascular spaces contain sparse amounts of mononuclear cells, including macrophages [Roizin et al. 1982]. Similarly, in degenerative diseases such as leukodystrophies, large amounts of cell debris are present and lymphocytes and plasma cells have occasionally been observed in perivascular regions [Scholz 1957]. In ALS, extensive infiltrates are observed whereas the amount of cell debris is small. Moreover, in ALS cases where macrophages loaded with debris were observed and where active destruction was going on, lymphocytic infiltration was not always a prominent feature.

Finally, the absence of a mixed inflammatory infiltrate in 21% of the patients with similar survival time and identical symptomatology militates against the hypothesis that the inflammatory infiltrate is only a reaction to tissue destruction.

In addition to degeneration of the uncrossed pyramidal tracts, many cases showed a diffuse loss of myelin in the anterior and lateral columns of the spinal cord white matter (Table 1). Whether this is primarily due to demyelination or to degeneration of axons is not known [Oppenheimer 1984]. Patients with myelin pallor and several patients with a pronounced destruction of the pyramidal tract showed a persistence of axons in silver stains, which is considered to be characteristic for demyelinative diseases [Allen 1984]. In such diseases viral and/or immunological mechanisms are thought to be of particular importance [Ter Meulen and Stephenson 1983]. In multiple sclerosis the myelin damage is associated with CNS infiltration by IgG-secreting cells [Esiri 1980] and T-lymphocytes predominantly of the suppressor/cytotoxic subset. In ALS IgG-secreting cells are not present in the CNS and the lymphocytes that are present in ALS patients have not been phenotyped yet.

Although IgG-secreting cells are probably not present in the spinal cord of ALS patients and subsets of the lymphocyte infiltrate are not yet known, the possibility therefore exists that inflammatory infiltrates in ALS may reflect demyelination due to direct contact between a lymphocyte and intact myelin, followed by phagocytosis. However, the existence of an ALS patient population with primary demyelination, i.e. persistence of axons with loss of myelin of the pyramidal tract, remains to be proved. If the lymphocytic infiltration in some of the patients were not reactive but causal, this would imply an immunologic disease process. However, previous studies did not report consistent HLA findings in sporadic ALS [Mitsumoto et al. 1988]. The presence of an infiltrate in some ALS patients and not in others may, alternatively, reflect a difference in etiology. This might explain why, e.g., some investigators showed an increase in T-cells reactive to Ia-antigen whereas others were not able to confirm this.

In conclusion, we have shown that lymphocytic infiltration in ALS patients is a much more common phenomenon than it is generally considered to be. By means of immunocytochemical detection methods the proportion of patients with infiltrates is as high as 79%. The infiltrate does not seem to be a simple sec-

ondary reaction to tissue damage, but rather appears to be a disease process, and may shed new light on the etiology of ALS.

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