Acta Neuropathol (1992) 83: 579 – 583

Acta Neuropathologica

Basophilic inclusions in sporadic juvenile amyotrophic lateral sclerosis: an immunocytochemical and ultrastructural study*

S. Matsumoto[1,2], H. Kusaka[2], N. Murakami[3], Y. Hashizume[1], H. Okazaki[5], and A. Hirano[1]

[1] Bluestone Laboratory, Division of Neuropathology, Department of Pathology, Montefiore Medical Center, Bronx, NY 10467, USA
[2] Department of Neurology, Kitano Hospital and Neurological Center, Osaka, Japan
[3] Department of Neurology, Higashi Nagoya National Hospital, Nagoya, Japan
[4] First Department of Pathology, Nagoya University School of Medicine, Nagoya, Japan
[5] Department of Pathology, Mayo Medical School, Rochester, MN, USA

Received April 9, 1991/Revised, accepted December 2, 1991

Summary. This report concerns immunocytochemical and ultrastructural studies on the basophilic inclusions in two cases of sporadic juvenile amyotrophic lateral sclerosis (ALS). The inclusion had a globular, irregular-shaped, or sometimes fragmented appearance. Ultrastructurally, the inclusions consisted mainly of thick filamentous structures associated with granules. Focal neurofilamentous accumulations were occasionally observed among the granulofilamentous structures. The basophilic inclusions occasionally showed granular reaction product deposits with an antibody to ubiquitin. The inclusions did not react with antibodies to phosphorylated neurofilament and to tau protein.

Key words: Juvenile amyotrophic lateral sclerosis – Basophilic inclusion – Ubiquitin

Several pathologically documented cases of motor neuron disease (MND) with an onset before the age of 20 have been reported as sporadic juvenile ALS [2, 6, 8, 29–31, 37–39]. Cytoplasmic basophilic inclusions were observed as characteristic features in four cases [2, 29, 31, 39]. Ultrastructurally, these inclusions have been shown to consist of randomly interwoven tubules with granular endoplasmic reticulum [31]. Since no information is available regarding the immunocytochemical characteristics of the basophilic inclusions in juvenile ALS, we examined two cases with this disorder.

Materials and methods

The spinal cord and medulla oblongata were obtained postmortem from two patients with sporadic juvenile ALS. The first patient (case 1) was a 22-year-old woman with a 32-month interval between disease onset and death. Muscle weakness and atrophy were first noted in the hypothenar muscles, and progressed to involve all limbs. A moderate degree of dementia, eye movement dysfunction and autonomic symptoms developed later. During the final few months she was artifically ventilated. The second patient (case 2) was a 23-year-old woman with a 6-month history, who first noticed weakness of her left arm. She had a history of delayed development but had no autonomic or extraocular muscle disorders.

Neuropathological examination of both cases showed degeneration of anterior horn cells and pyramidal tracts with characteristic basophilic intracytoplasmic inclusions in the anterior horn cells. In case 1, these inclusions were observed in other neurons of various locations within the central nervous system. Tissues were fixed in formalin and embedded in paraffin. Sections stained with hematoxylin and eosin (H&E), modified Bielschowsky stain, Klüver-Barrera and methyl green pyronin were examined.

Immunohistochemical studies

The following antibodies were used in this study: a rabbit antibody to ubiquitin (donated by Drs. S.-H. Yen and H. Ksiezak-Reding) [19], a mouse monoclonal antibody to medium- and high-molecular weight subunit of phosphorylated neurofilament (pNF) (SMI 31, Sternberger-Meyer, Inc.) and a mouse monoclonal antibody to tau protein (Tau-2, Sigma Chemical Co.) [3, 32].

Six-micrometer sections were mounted onto poly-L-lysine-coated slides. Sections were deparaffinized, hydrated and immunostained. The antibody to ubiquitin and Tau-2 were diluted 1:2000 with phosphate buffer saline (PBS) containing 3% bovine serum albumin and SMI 31 was diluted 1:10000. Sections were incubated with primary antibodies overnight at 4°C. Bound antibodies were detected by using the appropriate avidin-biotin-peroxidase complex (ABC) Kit (Vector Laboratories) [11]. Incubation times with biotinylated secondary antibody and ABC reagent were 50 min each at room temperature. Diaminobenzidine tetrahydrochloride was used as chromogen. The sections were lightly counterstained with hematoxylin. To obtain a comparison between H&E staining and immunostaining of the same section, several sections were first stained with H&E, photographed, decolorized with ethanol, and then stained for pNF or ubiquitin.

Ultrastructural studies

These were done on deparaffinized blocks (case 1) and formalin-fixed tissues (case 2). Specimens were postfixed in 1% osmium

* Supported in part by the Amyotrophic lateral Sclerosis Society

Offprint requests to: A. Hirano (address see above)

tetroxide and embedded in epoxy resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate following standard procedures.

Results

The basophilic cytoplasmic inclusions had various shapes, sometimes they exhibited a round, elliptical, globular or irregular appearance, and some of them were foliated and fragmented. They had no core or halo, and some inclusions had a vesicular appearance (Fig. 1A). The inclusions stained intensely with cresyl violet and lightly purple with methyl green pyronin. None was argyrophilic when subjected to silver impregnation.

Immunohistochemistry

When stained with anti-ubiquitin antibody the reaction products appeared as granular dot-like structures were observed in the gray and white matters [7]. In many instances the staining intensity of the basophilic inclusions did not exceed the background level. However,

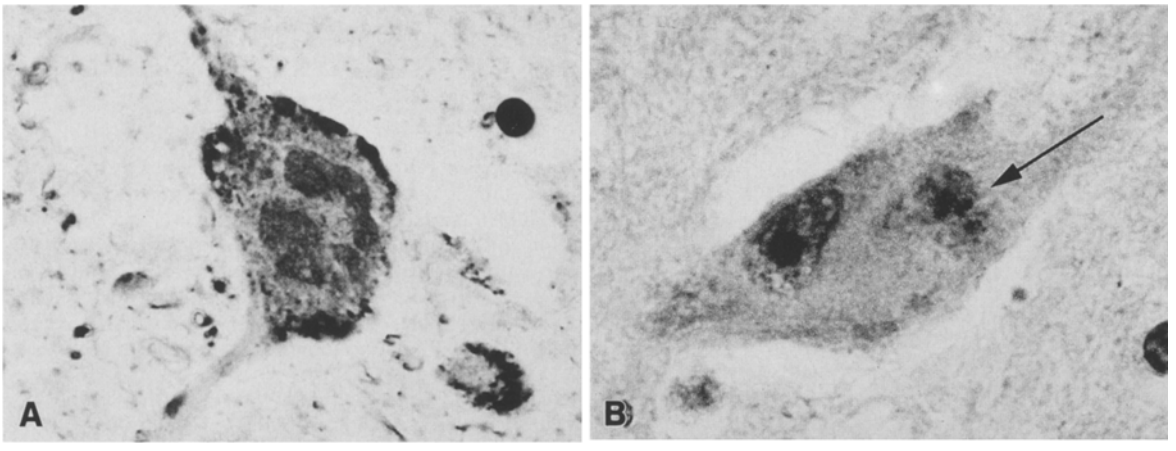

Fig. 1. **A** Basophilic inclusion in the medulla. Case 1, Klüver-Barera stain. **B** Granules in a basophilic inclusion in a lumbar anterior horn cell showing positive reaction with anti-ubiquitin antibody (*arrow*). Case 2, hematoxylin counterstain. **A** × 1,350; **B** × 1,220

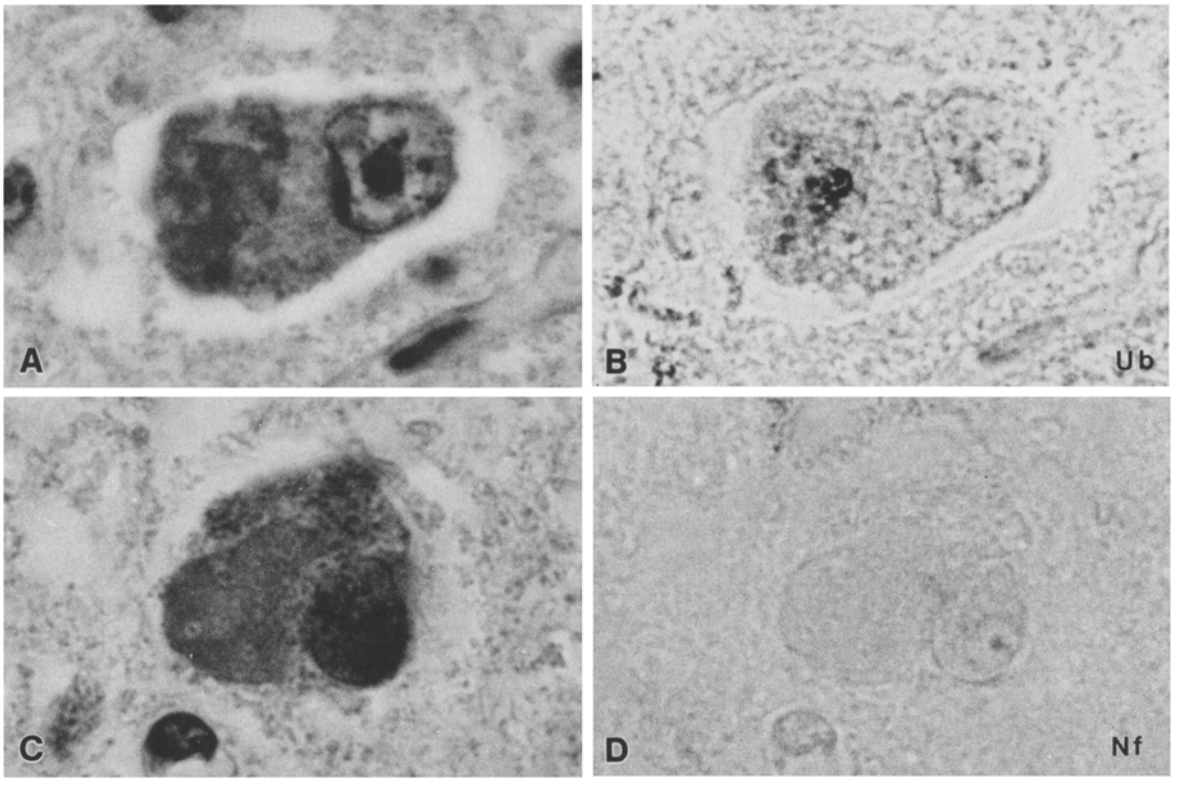

Fig. 2A–D. Basophilic inclusions of case 1. **B** Ubiquitinated granules; **A** same section stained with H&E. **D** Absence of positive reaction with anti-pNF antibody; **C** section stained with H&E. *Ub:* ubiquitin; *Nf:* neurofilament; **A, B** × 1,780; **C, D** × 1,740

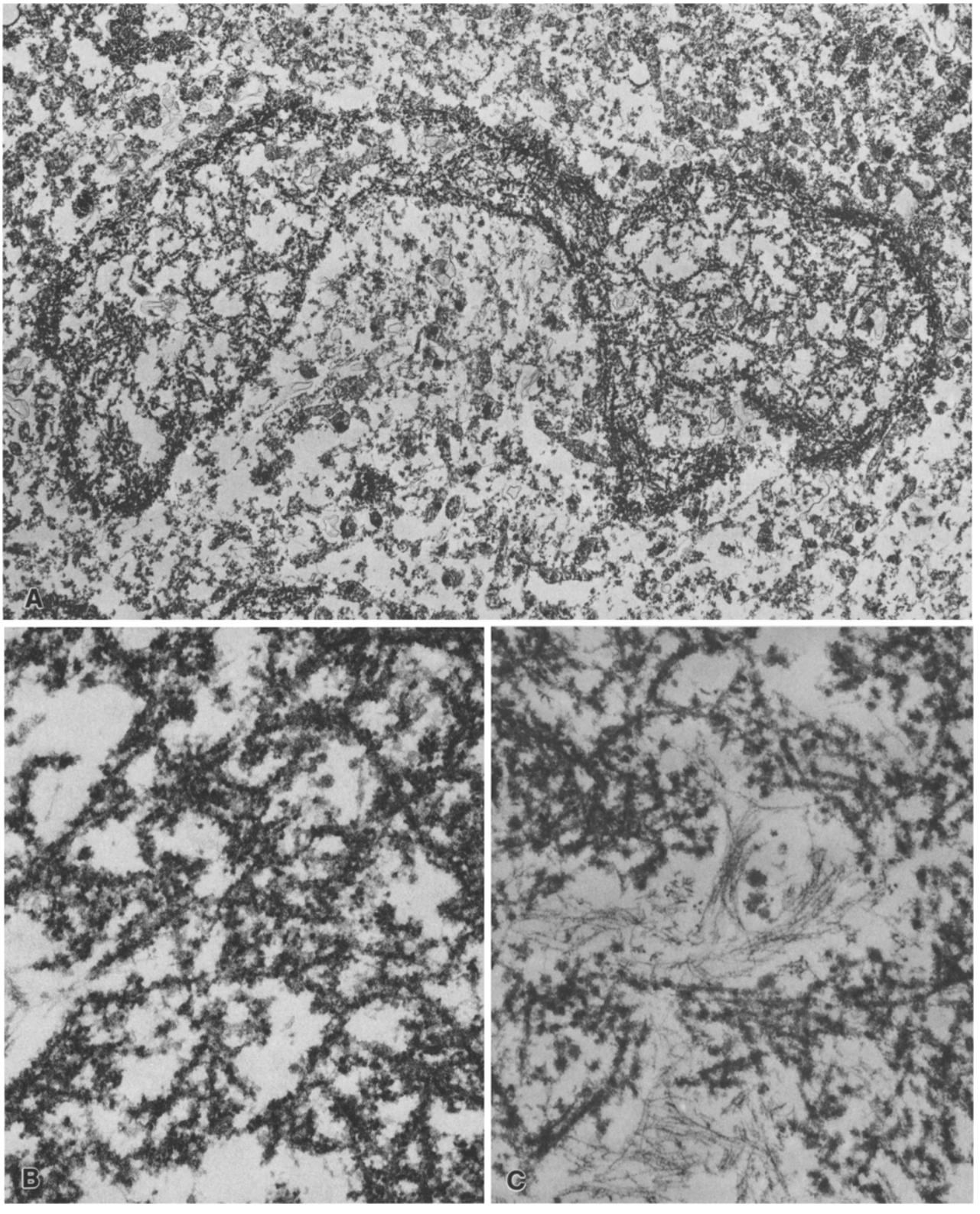

Fig. 3A–C. The inclusion consists of a meshwork of thick filamentous structures associated with granules (**A, C** case 2 of sporadic juvenile ALS; **B** case 1). Focal neurofilamentous accumulation can be seen among the granulofilamentous structures (**C**). **A** × 7,300; **B** × 44,000; **C** × 34,000

both cases had some basophilic inclusions which showed granular immunoreactivity (Figs. 1B, 2B). The reaction products were irregularly distributed in the inclusions. No ubiquitin-positive filamentous inclusions were found in the soma of anterior horn cells. The antibody to pNF labelled axons and globules, but did not stain the basophilic inclusions (Fig. 2D). The inclusions also failed to react with Tau-2.

Ultrastructure

The ultrastructural appearances were similar in case 1 and 2, consisting of a meshwork of thick filamentous structures associated with granules (Fig. 3A–C). The inclusions had no limiting membranes and the granulofilamentous profiles varied from being compactly arranged to a more loosely packed arrangement. Focal neurofilamentous accumulations were occasionally observed among the granulofilamentous structures in case 2 (Fig. 3C). Mitochondria and vesicles were also enclosed in the inclusions. At higher magnification, the filaments appeared to be straight or curved with a diameter ranging from about 13 to 17 nm. The granules measured approximately 20 to 30 nm in diameter.

Discussion

Basophilic cytoplasmic inclusions were observed in four cases of pathologically documented sporadic juvenile ALS [2, 29, 31, 39], and it has been suggested that this represents an entity distinct from sporadic adult ALS [29]. In several systematic surveys of adult-onset ALS cases, no basophilic inclusions have been observed [4, 9], although Chou reported that such inclusions were frequently associated with Bunina bodies [5]. Recently, we reported an adult-onset case of sporadic MND with basophilic inclusions [17]. It has been shown that the basophilic inclusions are widely distributed in the central nervous system as well as somatic motor neurons [17, 29, 31], and that they contain RNA-protein compounds [29, 31].

Our electron microscopic study revealed that basophilic inclusions are composed mainly of thick filaments and associated granules. The ultrastructural features of the inclusions described by Oda et al. [31] in a case of sporadic juvenile ALS are almost identical to those found in our two cases. Focal neurofilamentous accumulation was occasionally found in case 2 but few in case 1 and the case reported by Oda et al. [31]. It should be noted that in the basophilic inclusions of an adult-onset case of MND, similar granule-associated filaments were seen [17].

The Lewy body-like hyaline inclusions in familial ALS with posterior column and spinocerebellar tract involvement have been reported to contain somewhat similar ill-defined coarse linear structures with a meshwork of neurofilaments [10, 35]. Recent immunocytochemical studies have demonstrated the presence of epitopes of pNF [23, 24, 26, 27] and ubiquitin [24, 27], but not of tau protein [27]. In an immunoelectron microscopic study of familial ALS it was demonstrated that the granule-associated filaments were ubiquitin positive [27]. The Lewy body-like hyaline inclusions in sporadic ALS [15, 16, 28] and sporadic MND [33] have similar granule-associated filaments. However, there may exist differences between sporadic and familial ALS with respect to the abundance of neurofilaments [16, 33]. These inclusions were also intensely ubiquitinated [13, 28, 33]. It is not still clear whether ubiquitin is present in entire inclusions [13], the core [27], or halo [24] of the Lewy body-like hyaline inclusions. Moreover, Murayama et al. [28] have postulated that ubiquitin-positive granule-associated filaments are commonly related to the degeneration of lower motor neurons in both sporadic and familial ALS.

In neurodegenerative disorders other than motor neuron disease, intracytoplasmic inclusions with a similar ultrastructure have been reported. These include cortical Lewy bodies [14], inclusions in generalized variants of Pick's disease [25] and argyrophilic inclusions in olivopontocerebellar atrophy (OPCA) [12]. The cortical Lewy bodies contain criss-crossing filaments with fuzzy deposits [14, 36] that react with antibodies to ubiquitin [1, 18] and pNF [1], but not with the antibody to tau [1]. Cytoplasmic inclusions in the generalized variants of Pick's disease are composed of straight filaments coated with granular material along most of their length [25] and stained poorly with antibodies to neurofilament [25]. Argyrophilic inclusions in OPCA are also composed of fibrils coated with granules and the entire inclusion is stained by the antibody to ubiquitin. By contrast, the basophilic inclusions in sporadic juvenile ALS only occasionally had focal granular reaction products when stained for ubiquitin, and they did not react with antibodies to pNF and tau. Thus, the basophilic inclusions of sporadic juvenile ALS show some unique characteristics which differentiate them from the Lewy body-like hyaline inclusions and other intracytoplasmic inclusions.

The presence of ubiquitin-immunoreactive filamentous inclusions in anterior horn cells has been demonstrated only in sporadic ALS [20–22, 28, 34], familial ALS with Lewy body-like hyaline inclusions [27] and Guamanian ALS [22]. It has been suggested that they reflect degenerative alterations of neurons and may be characteristic of these diseases. In this study, we detected no ubiquitin-immunoreactive filamentous inclusions in the perikarya of the anterior horn cells in the two cases studied. In juvenile ALS, atypical clinical features such as extraocular muscle disorders [38], autonomic signs [31] and mental disorder [6, 29] have been occasionally observed. The widespread presence of basophilic inclusions and absence of ubiquitin-immunoreactive filamentous inclusions in anterior horn cells may indicate that sporadic juvenile ALS represents a subgroup of MND different from adult-onset ALS. Further studies on more cases are required to clarify this point.

Acknowledgements. The authors wish to thank Drs. S.-H. Yen and H. Ksiezak-Reding for generously providing the antibody to ubiquitin and to Dr. F. Herz for reviewing the manuscript.

References

1. Bancher C, Lassmann H, Budka H, Jellinger K, Grundke-Iqbal I, Iqbal K, Wiche G, Seitelberger F, Wisniewski HM (1989) An antigenic profile of Lewy bodies: immunocytochemical indication for protein phosphorylation and ubiquitination. J Neuropathol Exp Neurol 48:81–93
2. Berry RG, Chambers RA, Ducktt S, Terrero R (1969) Clinicopathological study of juvenile amyotrophic lateral sclerosis (abstract). Neurol 19:312

3. Binder LI, Frankfurter A, Rebhun LI (1985) The distribution of tau in the mammalian central nervous system. J Cell Biol 101:1371–1378
4. Brownell B, Oppenheimer DR, Hughes JT (1970) The central nervous system in motor neuron disease. J Neurol Neurosurg Psychiatry 33:338–357
5. Chou SM (1978) Pathognomy of intraneuronal inclusions in ALS. In: Tsubaki T, Toyokura Y (eds) Amyotrophic lateral sclerosis. University of Tokyo Press, Tokyo, pp 135–176
6. Cognazzo A, Martin L (1970) A sporadic case of juvenile amyotrophic lateral sclerosis. Semi-quantitative and histoenzymatical study of the denervated muscles. Eur Neurol 3:211–230
7. Dickson DW, Wertkin A, Kress Y, Ksiezak-Reding H, Yen S-H (1990) Ubiquitin immunoreactive structures in normal human brains. Distribution and developmental aspects. Lab Invest 63:87–99
8. Grunnet ML, Donaldson JO (1985) Juvenile multisystem degeneration with motor neuron involvement and eosinophilic intracytoplasmic inclusions. Arch Neurol 42:1114–1116
9. Hirano A, Iwata M (1979) Pathology of motor neurons with special references to amyotrophic lateral sclerosis and related diseases. In: Tsubaki T, Toyokura Y (eds) Amyotrophic lateral sclerosis. University of Tokyo Press, Tokyo, pp 107–133
10. Hirano A, Nakano I, Kurland LT, Mulder DW, Holley PW, Saccomanno G (1984) Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 43:471–480
11. Hsu S, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580
12. Kato S, Nakamura H (1990) Cytoplasmic argyrophilic inclusions in neurons of pontine nuclei in patients with olivopontocerebellar atrophy: immunocytochemical and ultrastructural studies. Acta Neuropathol 79:584–594
13. Kato T, Katagiri T, Hirano A, Kawanami T, Sasaki H (1989) Lewy body-like hyaline inclusions in sporadic motor neuron disease are ubiquitinated. Acta Neuropathol 77:391–396
14. Kosaka K (1978) Lewy bodies in cerebral cortex. Report of three cases. Acta Neuropathol (Berl) 42:127–134
15. Kuroda S, Kuyama K, Morioka E, Ohtsuki S, Nanba R (1986) Sporadic amyotrophic lateral sclerosis with intracytoplasmic eosinophilic inclusions. A case closely akin to familial ALS. Neurol Med Chir (Tokyo) 24:31–37
16. Kusaka H, Imai T, Hashimoto S, Yamamoto T, Maya K, Yamasaki M (1988) Ultrastructural study of chromatolytic neurons in an adult-onset sporadic case of amyotrophic lateral sclerosis. Acta Neuropathol (Berl) 75:523–528
17. Kusaka H, Matsumoto S, Imai T (1990) An adult-onset case of sporadic motor neuron disease with basophilic inclusions. A case report. Acta Neuropathol 80:660–665
18. Kuzuhara S, Mori H, Izumiyama N, Yoshimura M, Ihara Y (1988) Lewy bodies are ubiquitinated. A light and electron microscopic immunocytochemical study. Acta Neuropathol (Berl) 75:345–353
19. Lee S, Park YD, Yen S-HC, Ksiezak-Reding H, Goldman JE, Dickson DW (1989) A study of infantile motor neuron disease with neurofilament and ubiquitin immunocytochemistry. Neuropediatrics 20:107–111
20. Leigh PN, Anderton BH, Dodson A, Gallo J-M, Swash M, Power DM (1988) Ubiquitin deposits in anterior horn cells in motor neuron disease. Neurosci Let 93:197–203
21. Lowe J, Lennox G, Jefferson D, Morrell K, McQuire D, Gray T, Landon M, Doherty FJ, Mayer RJ (1988) A filamentous inclusion body within anterior horn neurons in motor neuron disease difined by immunocytochemical localization of ubiquitin. Neurosci Lett 94:203–210
22. Matsumoto S, Hirano A, Goto S (1990) Ubiquitin-immunoreactive filamentous inclusions in anterior horn cells of Guamanian and non-Guamanian amyotrophic lateral sclerosis. Acta Neuropathol 80:233–238
23. Mizusawa H, Matsumoto S, Yen S-H, Hirano A, Rojas-Corona RR, Donnenfeld H (1989) Focal accumulation of phosphorylated neurofilaments within anterior horn cell in familial amyotrophic lateral sclerosis. Acta Neuropathol 79:37–43
24. Mizusawa H, Hirano A, Yen S-H (1991) Anterior horn cell inclusions in familial amyotrophic lateral sclerosis contain ubiquitin and phosphorylated neurofilament epitopes. Neuropathology 11:11–20
25. Munoz-Garcia D, Ludwin SK (1984) Classic and generalized variants of Pick's disease: a clinicopathological, ultrastructural, and immunocytochemical comparative study. Ann Neurol 16:467–480
26. Munoz DG, Greene C, Perl DP, Selkoe DJ (1988) Accumulation of phosphorylated neurofilaments in anterior horn motoneurons of amyotrophic lateral sclerosis patients. J Neuropathol Exp Neurol 47:9–18
27. Murayama S, Ookawa Y, Mori H, Nakano I, Ihara Y, Kuzuhara S, Tomonaga M (1989) Immunocytochemical and ultrastructural study of Lewy body-like hyaline inclusions in familial amytrophic lateral sclerosis. Acta Neuropathol 78:143–152
28. Murayama S, Mori H, Ihara Y, Bouldin TW, Suzuki K, Tomonaga M (1990) Immunocytochemical and ultrastructural studies of lower motor neurons in amyotrophic lateral sclerosis. Ann Neurol 27:137–148
29. Nelson JS, Prensky AL (1972) Sporadic juvenile amyotrophic lateral sclerosis. A clinicopathological study of a case with neuronal cytoplasmic inclusions containing RNA. Arch Neurol 27:300–306
30. Nishigaki S, Takahashi A, Matuoka Y (1971) Ein Obduktionsfall der sporadischen juvenilen amyotrophischen Lateralsklerose. Clin Neurol (Tokyo) 11:500–506
31. Oda M, Akagawa N, Tabuchi Y, Tanabe H (1978) A Sporadic Juvenile case of the amyotrophic lateral sclerosis with neuronal intracytoplasmic inclusions. Acta Neuropathol (Berl) 44:211–216
32. Papasozomenos SC (1989) Tau protein immunoreactivity in dementia of the Alzheimer type. I. Morphology, evolution, distribution and pathogenetic implications. Lab Invest 60:123–127
33. Sasaki S, Yamane K, Sakuma H, Murayama S (1989) Sporadic motor neuron disease with Lewy body-like hyaline inclusions. Acta Neuropathol 78:555–560
34. Schiffer D, Autilio-Gambetti L, Chio A, Gambetti P, Giordana MT, Gullotta F, Migheli A, Vigliani MC (1991) Ubiquitin in motor neuron disease: study at the light and electron microscope. J Neuropathol Exp Neurol 50:463–473
35. Takahashi K, Nakamura H, Okada E (1972) Hereditary amyotrophic lateral sclerosis. Histochemical and electron microscopic study of hyaline inclusions in motor neurons. Arch Neurol 27:292–297
36. Tiller-Borcich JK, Forno LS (1988) Parkinson's disease and dementia with neuronal inclusions in the cerebral cortex: Lewy bodies or Pick bodies. J Neuropathol Exp Neurol 47:526–535
37. Tsujihata M, Taguchi H, Oku Y, Takamori M, Terao H (1978) A sporadic case of juvenile amyotrophic lateral sclerosis. Clin Neurol (Tokyo) 18:82–88
38. van Bogaert L (1925) La sclerose laterale amyotrophique et la paralysie bulbaire progressive chez l'enfent. Rev Neurol (Paris) 1:180–192
39. Wohlfart G, Swank RL (1941) Pathology of amyotrophic lateral sclerosis. Fiber analysis of the ventral roots and pyramidal tracts of the spinal cord. Arch Neurol Psychiatry 46:783–799

Addendum. After submitting this manuscript, immunohistochemical study of basophilic inclusions with two cases of juvenile ALS [32, 38] has been reported by Dr. H. Mizusawa (32th Japanese Conference of Neuropathology, Yamagata, Japan, May 1991). Basophilic inclusions were largely weakly immunostained with anti-ubiquitin antibody, and often showed focal granular immunoreactivity in the inclusion; this is similar to our results.