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Galectin-1 Is a Component of Neurofilamentous Lesions in Sporadic and Familial Amyotrophic Lateral Sclerosis

Takeo Kato,*[,1] Keiji Kurita,* Tomomi Seino,* Toshihiko Kadoya,† Hidenori Horie,‡ Manabu Wada,* Toru Kawanami,* Makoto Daimon,* and Asao Hirano§

**Third Department of Internal Medicine, Yamagata University School of Medicine, Yamagata, Japan; †Pharmaceutical Research Laboratory, Kirin Brewery Company, Ltd., Takasaki, Japan; ‡Department of Physiology, School of Medicine, Yokohama City University, Yokohama, Japan; and §Division of Neuropathology, Montefiore Medical Center, New York, New York*

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In amyotrophic lateral sclerosis (ALS), abnormal accumulation of neurofilaments induces pathological changes such as axonal spheroids, cord-like neurite swellings, and perikaryal conglomerate inclusions in degenerating motor neurons of the spinal cord, and the accumulation seems to cause motor neuron degeneration in this disease. Such ALS lesions were intensely labeled with HepSS-1, a monoclonal antibody to heparan sulfate. Since the identification of HepSS-1-immunoreactive substance seems to be an important step for understanding the molecular pathology of ALS, we purified the substance from human spinal cord tissue to homogeneity. Amino acid sequence of the protein was consistent with that of galectin-1. Immunohistochemistry using antibodies against recombinant human galectin-1 showed that galectin-1 was accumulated in these lesions in ALS. Although HepSS-1 was believed to be specific for heparan sulfate, it reacted with recombinant human galectin-1 which has no heparan sulfate moiety. The results show that galectin-1 is a component of the neurofilamentous lesions in ALS. Since galectin-1 has axonal regeneration-enhancing activity, the abnormal accumulation of galectin-1 to the lesions seems to be related to the pathological process of ALS.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving motor neurons of the spinal cord and brain. Five to ten percent of all ALS patients show a family history, and ~20% of them (1–2% of all ALS) have a causative mutation of the Cu/Zn superoxide dismutase (SOD1) gene (1). However, the cause of the majority of ALS is still unknown.

A strategy for elucidating the molecular basis of the pathogenesis and etiology of neurodegenerative diseases is to identify the molecule(s) constituting the neuropathological changes. In the case of Alzheimer's disease (AD), the β/A4 peptide was discovered in the amyloid vessels (2) and the amyloid core of senile plaques (3), and phosphorylated tau protein was identified as a major component of the neurofibrillary tangles in the AD brain (4–6). In prion diseases, amyloid plaques consist of prion proteins (7–9). In ALS, several neuropathological hallmarks such as Bunina bodies (10, 11), Lewy body-like hyaline inclusions (12–14), and skein-like inclusions (14, 15) have been reported in degenerating motor neurons of the spinal cord. Abnormal accumulation of neurofilaments in the axons and perikarya of motor neurons (16, 17) seems to be especially important for the pathogenesis of ALS, because it is an early pathological change of motor neurons in ALS (12, 13, 16, 18, 19). Indeed, transgenic mice overexpressing neurofilament proteins develop an abnormal accumulation of neurofilaments in motor neurons with ensuing motor neuron death, similar to human ALS (20, 21). The abnormal accumulation of neurofilaments produces such morphological changes as axonal spheroids, "cord-like neurite swellings" and conglomerate inclusions (excess accumulation of neurofilaments in the perikaryal area) in ALS.

In 1993, we reported that a monoclonal antibody HepSS-1, which is believed to be specific for heparan sulfate (22), immunostained areas of neurofilament accumulation in the spinal cord of ALS, e.g., axonal spheroids and conglomerate inclusions (23). The present experiments were carried out to isolate and identify HepSS-1-immunoreactive substance in the spinal cord. The results indicate that the substance is galectin-1,

Abbreviations used: ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; HepSS-1, monoclonal antibody to heparan sulfate; SOD, superoxide dismutase; TBS, Tris-buffered saline.

[1] To whom correspondence should be addressed at Third Department of Internal Medicine, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan. Fax: +81-23-628-5318. E-mail: tkato@med.id.yamagata-u.ac.jp.

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and suggest that association of galectin-1 with neurofilament may play an important role in the pathogenesis of ALS.

MATERIALS AND METHODS

Extraction of spinal cord. Spinal cords were obtained from three autopsied patients (age: 64–72 years) with no neurological diseases. The interval between death and autopsy was 6–11 h. The spinal cord tissue was stored at −80°C until use. After removal of blood vessels and pia mater, 36 g of spinal cord tissue was homogenized with 72 ml of Tris–buffered saline (TBS: 50 mM Tris–HCl and 150 mM NaCl, pH 7.6) containing 50,000 U aprotinin (Bayer), 5 mM EDTA (Wako), and 1 mg/ml leupeptin (Sigma). The homogenate was centrifuged at 100,000*g* for 1 h at 4°C, and the resultant supernatant was designated as TBS-soluble fraction. The pellet was homogenized with 18 ml of 8 M urea (Pharmacia) in TBS and centrifuged as above. The supernatant was designated as urea-soluble fraction. Both TBS- and urea-soluble fractions were kept at −80°C until use.

Antibodies. HepSS-1 is a monoclonal mouse IgM antibody obtained by immunization with methylcholanthrene-induced fibrosarcoma cells. It was reported that this antibody recognizes an epitope present in heparan sulfate glycosaminoglycan (22). HepSS-1 employed in the present experiments was obtained from Seikagaku Co (No. 270426, Tokyo). The antibodies to human galectin-1 were raised in rabbits by immunization with recombinant human galectin-1 included in complete Freund's adjuvant (24, 25). The isolation of human galectin-1 cDNA from human liver cDNA library, the construction of the expression vector, and the purification of *E. coli*-derived recombinant human galectin-1 have been described previously (24, 25). The recombinant human galectin-1 contains entire amino acid sequence of human galectin-1 and represents the oxidized form having no affinity for galactose (24, 25). The IgG fraction of the anti-galectin-1 antiserum was obtained by affinity chromatography on protein-A Sepharose (Amersham). The antibodies recognize recombinant human galectin-1, but failed to bind recombinant human galectin-3 (24, 25).The antibody specific for neurofilament proteins is a monoclonal mouse IgG antibody that has been shown to recognize 200 and 160 kDa components of neurofilament triplets (26). The monoclonal antibody was supplied from Dr. I Toyoshima (Akita University, Japan).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting. SDS–PAGE was set up according to the method of Laemmli (27). Protein samples were analyzed by SDS–PAGE using 15% polyacrylamide gels, and electroblotted onto a nitrocellulose membrane. After incubation with 5% skim milk in TBS for 1 h, the membrane was treated with HepSS-1 (1.0 μg/ml) overnight at 4°C; or the IgG fraction of anti-galectin-1 antibodies (0.6 μg/ml) for 2 h at room temperature, followed by incubation with biotinylated anti-mouse IgM or anti-rabbit IgG antibody (1:2000 dilution, Vector). After treatment with streptavidin-labeled alkaline phosphatase (1:3000 dilution, Amersham), color was developed with nitro-blue tetrazolium (Sigma) and 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

Ion-exchange column chromatography. The buffer of the urea-soluble fraction of spinal cord tissue was replaced with 8 M urea in 20 mM Tris–HCl, pH 8.3, using a HiTrap Desalting column (Pharmacia). Two milliliters of the sample were applied to a Mono Q HR 5/5 column (Pharmacia) and proteins were eluted with a linear gradient of NaCl (0 to 1 M) in 20 mM Tris–HCl and 8 M urea, pH 8.3 for 96 min at a flow rate of 0.25 ml/min using the FPLC system (Pharmacia). Fractions of the eluate were collected at 4-min intervals.

Two-dimensional gel electrophoresis. After concentration of a sample using Vivapore Concentrator (VivaScience), two-dimensional gel electrophoresis was performed by the method of O'Farrell (28). In brief, a concentrated sample mixed with an equal volume of a lysis buffer consisting of 8 M urea (Pharmacia), 2% Nonidet P-40 (Nacalai), 2% Bio-Lyte 5/8 (Bio-Rad) and 5% β-mercaptoethanol was subjected to isoelectric focusing between pH 5 and pH 8 (Bio-Lyte 5/8; Bio-Rad) at a constant voltage of 400 V to a total of 8800 Vh. The second dimension was done by SDS–PAGE in 15% polyacrylamide gel as described above, and proteins in the gel were electroblotted onto a nitrocellulose membrane.

In-gel digestion and protein sequencing. To obtain an exact topographical correlation between HepSS-1-positive spots on nitrocellulose membrane and Coomassie brilliant blue (CBB)-stained spots obtained in the two-dimensional gel electrophoresis, many places of the membrane and gel were stuck through with a needle at the end of electroblotting before peeling off the membrane from the gel. A CBB-stained spot in gel corresponding to a HepSS-1-positive spot on the membrane was excised and subjected to in-gel digestion with trypsin (Sigma; 250 μg/ml in 200 mM ammonium carbonate, pH 8.9) overnight at 35°C by the method of Rosenfeld *et al.* (29). The digested peptide fragments were separated by reverse-phase HPLC on a TSK gel ODS-80Ts QA column (2.0 × 250 mm, Tosoh) with a linear gradient of 0–90% acetonitrile containing 0.1% trifluoroacetic acid for 87 min at a flow rate of 0.2 ml/min. Fractions were collected at 1-min intervals. Amino acid sequence analysis was performed in a Shimadzu PQS-1 gas-phase sequenator, and phenylthiohydantoin derivatives were identified with an on-line HPLC system equipped with a TSK gel PTH pak (2 × 250 mm, Tosoh).

Immunohistochemistry. The spinal cords were obtained from eleven autopsied cases, including 8 cases of sporadic ALS (23), 4 cases of familial ALS with the A4V mutation of the SOD1 gene (30), and 3 control cases. Clinical and pathological findings of the ALS cases have been reported previously (23, 30). Formalin-fixed, paraffin-embedded tissue sections of 4–6 μm thickness were deparaffinized and incubated with 3% hydrogen peroxide (Wako) in methanol for 15 min for blocking endogenous peroxidase. After incubation with 20% normal goat serum (Vector) in TBS for 1 h, the sections were reacted with HepSS-1 (1.0 μg/ml), anti-neurofilament antibody (1.0 μg/ml), or IgG fraction of anti-galectin-1 antiserum (0.6–3.0 μg/ml) overnight at 4°C, followed by incubation with a corresponding biotinylated secondary antibody (1:200 dilution, Vector). After reaction with an avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector), labeling was visualized with 0.05% diaminobenzidine (Aldrich) and 0.024% hydrogen peroxide.

Immunoelectron microscopy. Fixed tissue of the spinal cord from four cases of ALS was cut into 50 μm-thick sections with a DSK microslicer. These sections were incubated with HepSS-1 (1.0 μg/ml), followed by 10-nm gold-labeled goat anti-mouse IgM (BioCell). After washing, the sections were fixed with 1.25% glutaraldehyde in 0.1 M cacodylate-buffered saline (CBS) for 1 h, post-fixed with 1% osmium tetroxide in 0.1 M CBS, and embedded in epon. Ultra thin sections were cut and stained with uranyl acetate and lead citrate.

RESULTS

Previous experiments demonstrated the most intense immunostaining with HepSS-1 in axonal spheroids and conglomerate inclusions in ALS spinal cords (23). However, less intense and subtle HepSS-1 immunoreactivity was also encountered in blood vessel walls and some axons of normal spinal cords. Thus, we employed normal spinal cords for purification of HepSS-1-immunoreactive substance. Immunoblotting using HepSS-1 showed a single band of ~14 kDa in the urea-soluble fraction but not in the TBS-soluble fraction (Fig. 1A). Thus, the urea-soluble fraction was fractionated by ion-exchange chromatography (Mono Q,

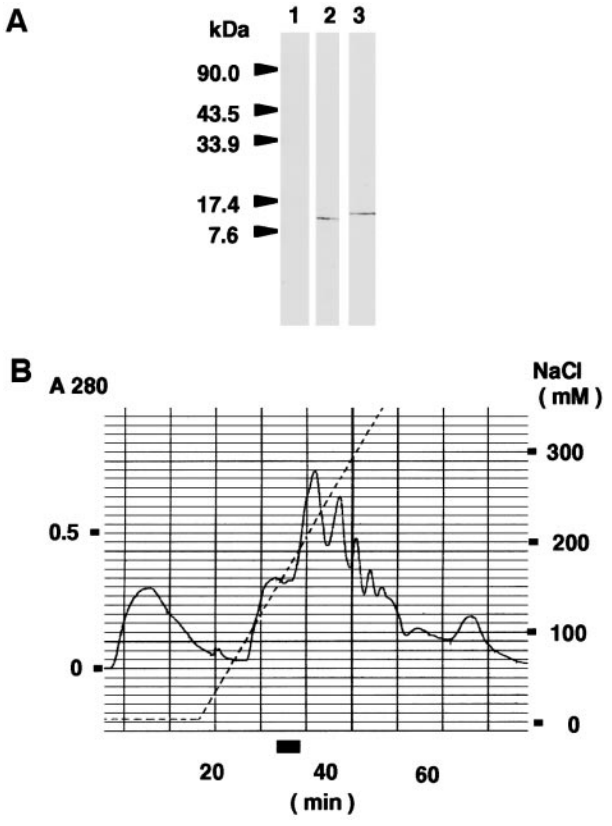

FIG. 1. (A) Immunoblotting of the extract of human spinal cord using HepSS-1. Human spinal cord tissues were extracted with TBS, followed by 8 M urea, and the TBS-soluble and urea-soluble fractions were analyzed by SDS–PAGE on 15% polyacrylamide gel. After transfer onto a nitrocellulose membrane, immunoblotting was performed using HepSS-1. A single, HepSS-1-positive band of ~14 kDa is seen in the urea-soluble fraction (lane 2), but not in the TBS-soluble fraction (lane 1). Extraction of the spinal cord with TBS in the presence of 5% β-mercaptoethanol, and analysis of the extract by the same method gave a HepSS-1-positive band (lane 3). (B) Ion-exchange column chromatography. Two ml of urea-soluble fraction were applied to a Mono Q HR 5/5 column (Pharmacia). A linear gradient of NaCl is shown by a dotted line. HepSS-1-immunoreactive protein was eluted in the fraction (—) indicated at the bottom of the figure.

Pharmacia) using NaCl gradient. The HepSS-1-positive protein was recovered from the column at 125–167 mM NaCl (Fig. 1B), as determined by immunoblotting. The fraction was concentrated, and the concentrated sample was analyzed by two-dimensional (2-D) gel electrophoresis. Immunoblotting of nitrocellulose membrane showed that HepSS-1 bound at least three spots with slightly different isoelectric points (Fig. 2A). The corresponding spots were excised from the 2-D gel stained with Coomassie brilliant blue, and the N-terminal amino acid sequence of the protein was determined by a protein sequencer. Since the N-terminus was found to be blocked, we performed in-gel digestion of the protein with trypsin by the method of Rosenfeld *et al.* (29). The digested peptide fragments were separated by reverse-phase HPLC to obtain three fragments, and each fragment was analyzed by the protein sequencer. The amino acid sequences of the three fragments were: Asp-Ser-Asn-Asn-Leu-Cys-Leu-His-Phe-Asn-Pro; Glu-Ala-Val-Phe-Pro-Phe-Gln-Pro-Gly-Ser; and Leu-Pro-Asp-Gly-Tyr-Glu-Phe-Lys, respectively. These sequences are consistent with the internal amino acid sequences of human galectin-1 (Fig. 2B) (31–33). The N-terminus of galectin-1 has been reported to be blocked by acetyl residue (31–33). Since galectin-1 can be solubilized by β-mercaptoethanol without urea (34–36), spinal cord tissue was homogenized with TBS containing 5% β-mercaptoethanol. Immunoblotting of the extract using HepSS-1 showed a single band with a slightly higher molecular weight than that detected in the urea-soluble fraction (Fig. 1A, lane 3).

To confirm that the HepSS-1-reactive protein in the urea-soluble fraction of the spinal cord actually contains galectin-1, the presence of galectin-1 in the fraction was tested by SDS–PAGE and immunoblotting using anti-galectin-1 antibodies. As shown in Fig. 3, the fraction contained a 14–15 kDa protein which bound anti-galectin-1. Similar to the HepSS-1-reactive protein, the mobility of the galectin-1-containing protein in SDS–PAGE became lower under reducing conditions. Nevertheless, the molecular size of the galectin-1-containing protein in the urea-soluble fraction was significantly larger than that of human recom-

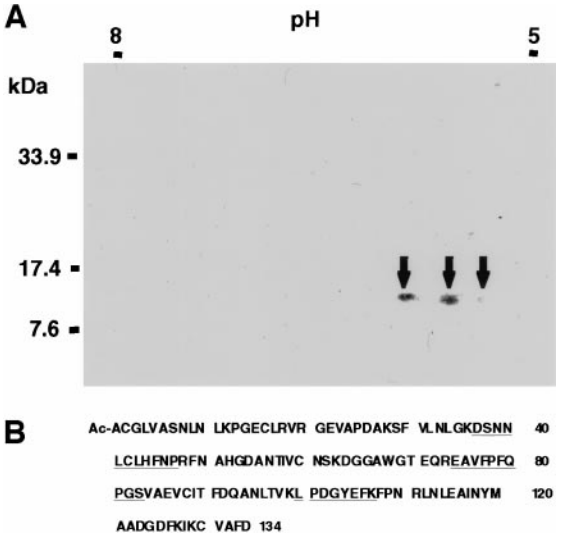

FIG. 2. (A) Two-dimensional gel electrophoresis and immunoblotting. HepSS-1-positive fraction from ion-exchange chromatography (cf. Fig. 2) was subjected to two-dimensional gel electrophoresis. Isoelectric focusing was performed between pH 5–8. After transfer onto a nitrocellulose membrane, immunoblotting was performed using HepSS-1. At least three spots are labeled with HepSS-1 (arrows). (B) Amino acid sequence of human galectin-1 and those of the proteolytic fragments of a HepSS-1-immunoreactive protein in two-dimensional gel. The amino acid sequences of these peptides (underlining) are consistent with that of human galectin-1.

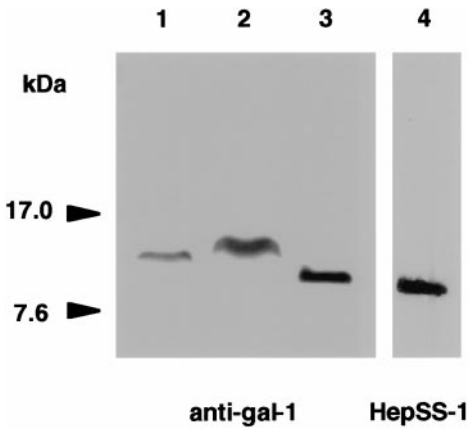

FIG. 3. Immunoblotting of urea-soluble fraction of human spinal cord tissue in the absence (lane 1) or in the presence of β-mercaptoethanol (lane 2), and recombinant human galectin-1 (0.1 μg/lane, lanes 3 and 4) using anti-galectin-1 antibodies (lanes 1, 2, and 3) or HepSS-1 (lane 4). Recombinant human galectin-1 is recognized not only by anti-galectin-1 antibodies (lane 3) but also by HepSS-1 (lane 4).

binant galectin-1. Since the findings shown in Figs. 1A and 3 strongly suggested that the same molecules bound both HepSS-1 and anti-galectin-1 antibodies in immunoblotting, we tested as to whether HepSS-1 might bind recombinant human galectin-1. Surprisingly, the monoclonal antibody bound to the recombinant protein (Fig. 3, lane 4). We wondered that the recombinant galectin-1 may have nonspecifically bound the monoclonal IgM antibody through galactose residues in the antibody molecules. However, the binding of HepSS-1 to the recombinant galectin-1 in Western blotting was not affected by the presence of 10 mM lactose in the diluent of the monoclonal antibody applied. The results clearly indicate that the HepSS-1-reactive protein in the extract of spinal cord actually contains galectin-1.

Immunohistochemical examination was carried out to confirm that galectin-1 is actually a component of axonal spheroids, cord-like neurite swellings, and perikaryal conglomerate inclusions of the spinal cord in sporadic and familial ALS. Axonal spheroids and conglomerate inclusions in the motor neurons of the spinal cords, which were all labeled with the antibody to neurofilament proteins (Fig. 4A) as well as HepSS-1 (Fig. 4B), were also intensely immunostained with the anti-galectin-1 antibodies in all eight cases of sporadic ALS examined (Fig. 4C). Cord-like neurite swellings observed in the spinal cords of the familial ALS cases were intensely immunostained with the anti-galectin-1 antibodies as well (Figs. 4D and 4E). No intense immunoreactivity of galectin-1 to other abnormal structures including Bunina body, hyaline inclusion, and

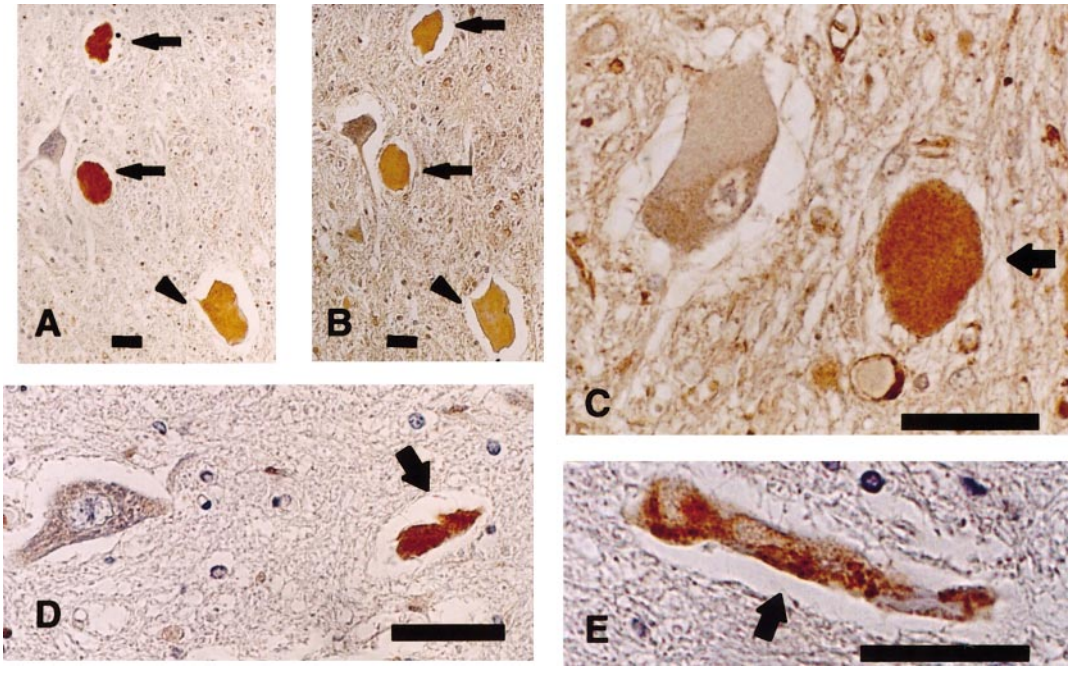

FIG. 4. Immunohistochemistry of axonal spheroids, cord-like neurite swellings, and perikaryal conglomerate inclusions in motor neurons of the spinal cord in sporadic (A–C) and familial (D and E) ALS. Antibodies used are: (A) a monoclonal antibody specific for neurofilament proteins; (B) HepSS-1; (C, D, and E) polyclonal antibodies to recombinant human galectin-1. The sections were counterstained with hematoxylin. A bar in each micrograph represents 50 μm. Axonal spheroids (arrows in A and B) and perikaryal conglomerate inclusions (arrowheads in A and B) are labeled with both anti-neurofilament antibody (A) and HepSS-1 (B). Axonal spheroid (arrow in C) and cord-like neurite swellings (arrows in D and E) are intensely immunostained with anti-galectin-1 antibodies.

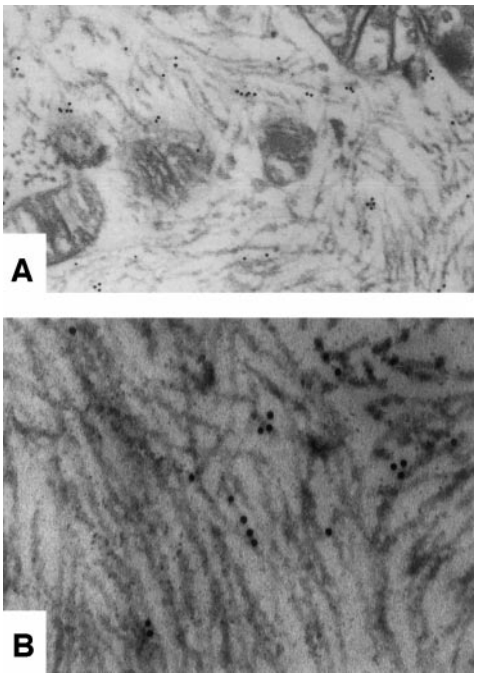

FIG. 5. Immunoelectron microscopy of an axonal spheroid in an ALS spinal cord, showing aggregates of neurofilaments with interspersed mitochondria (A). Ten-nm gold particles are associated with neurofilaments (A and B). No labeling is observed on mitochondria (A). The diameter of gold particle is 10 nm.

skein-like inclusion was observed. Their staining intensity was almost the same as that of the background in the sporadic ALS cases. Further experiments showed that among various structures in the brain and spinal cord, the abnormal neurofilamentous lesions in ALS were most intensely labeled with the anti-galectin-1 antibodies. Immunostaining of normal-looking axons, neuronal and glial cells, and blood vessel walls were much less intense in both ALS and control spinal cords. By electron microscopy, axonal spheroids appeared to consist of aggregates of neurofilaments with interspersed organelles such as mitochondria (Fig. 5A). Immunoelectron microscopy revealed that HepSS-1-coupled gold particles were associated with neurofilaments (Figs. 5A and 5B), and that no gold particle was observed in mitochondria (Fig. 5A).

DISCUSSION

The present study demonstrated that HepSS-1-immunoreactive substance accumulating in neurofilamentous lesions in ALS is galectin-1 (Figs. 2–4), and that HepSS-1 recognizes human galectin-1 (Fig. 3, lane 4). The monoclonal antibody HepSS-1 was believed to be specific for heparan sulfate moieties in heparan sulfate proteoglycan (22). However, the present experiments clearly showed that the antibody bound to *E. coli*-derived recombinant galectin-1 which contained no sugar moiety. One major evidence for the heparan sulfate specificity of HepSS-1 was that the HepSS-1-reactive molecules on the surface of the fibrosarcoma was susceptible to heparinase (22). However, the same experiment showed that the cell surface molecules were more susceptible to trypsin treatment of the cells (22). In the present experiment, we did not study as to whether the monoclonal antibody reacts to heparan sulfate, however, we wonder that the antibody may be specific for galectin-1.

Galectin-1 is a member of β-galactoside-binding lectins, and exists as a homodimer of the 14.5 kDa subunit (37, 38). It is present in various tissues and organs including the lung, heart, muscle, placenta, thymus, lymph nodes, brain, and spinal cord. Several different functions have been proposed for galectin-1: cell growth, cell differentiation, apoptosis, cell-cell interaction, and cell-matrix interaction (37–52). Recently, galectin-1 has been reported to have the ability to enhance axonal regeneration (24). The galectin-1 molecule has six cysteine residues and, when it is oxidized, three disulfide bonds are formed (25). Oxidized form of galectin-1 showed the axonal regeneration-enhancing activity, but lacks its property as a lectin to bind to lactose (25). On the other hand, reduced form of galectin-1 possessed lectin properties but showed no axonal regeneration-enhancing activity (25). In SDS–PAGE, mobility is different between oxidized galectin-1 and reduced galectin-1 due probably to differences in molecular conformation. Oxidized galectin-1 seems to take a compact conformation by intramolecular disulfide bonds and therefore runs faster than reduced galectin-1 in SDS–PAGE (25). In the present experiments, HepSS-1-reactive protein in the urea extract of human spinal cord homogenate ran faster than that observed under reducing conditions (Fig. 1A). The results support the concept that the protein represents galectin-1, and suggest that galectin-1 in the human spinal cord is in the oxidized form (Figs. 1A and 3).

In two-dimensional gel electrophoresis, galectin-1 from human brain, lung and mouse melanoma cells has been reported to exhibit several spots with slightly different isoelectric points (34, 35, 54). Similar findings were obtained in the present study (Fig. 2). Since only one nucleotide sequence was detected in human brain galectin-1 (31), the molecular microheterogeneity may be due to posttranslational modifications such as glycosylation of an identical polypeptide. Although the vast majority of human glycoproteins are known to bind either concanavalin A or wheat germ agglutinin, or both, galectin-1 failed to bind either of these plant lectins (34). It was also reported that neither neutral carbohydrates nor hexosamines was detected in the hydrolytic products of galectin-1 from the human brain (36). From these observations, it was concluded that galectin-1 was unlikely to be glycosylated. Thus, no

explanation has yet been given for the microheterogeneity of galectin-1 seen in two-dimensional gel electrophoresis (34–36, 54). The present study showed that the molecular size of galectin-1 in the urea-soluble fraction of the human spinal cord was significantly higher than that of recombinant human galectin-1 (Fig. 3). Since the recombinant human galectin-1 employed in the present experiments contains the entire amino acid sequence of human galectin-1 (24, 25), the size difference between the natural and recombinant galectin-1 suggests that galectin-1 is posttranslationally modified.

Recombinant human galectin-1 is soluble in phosphate-buffered saline (24, 25). On the other hand, HepSS-1-reactive substance (galectin-1) was not detected in the TBS-extract of human spinal cord tissue (Fig. 1A). This suggests that galectin-1 is bound to some insoluble molecule(s) or structure(s) in the human spinal cord. The association of galectin-1 with neurofilaments in immunoelectron micrographs (Fig. 5) suggests that galectin-1 is bound to neurofilaments in the human spinal cord. This speculation is in agreement with the presence of galectin-1 in the extract with 8 M urea (Fig. 1A), which solubilizes neurofilaments. The close association of galectin-1 with neurofilaments, demonstrated in the present study, suggests a possible role of galectin-1 in the physiological function of neurofilaments in motor neurons and/or in the formation of neurofilamentous lesions in ALS.

In familial ALS with SOD1 mutation, deposit of SOD1 was observed in the intracytoplasmic inclusion bodies of the degenerating motor neurons (1, 30). The deposits are not observed in the sporadic form of ALS. The accumulation of neurofilaments in the perikarya and axonal swellings of degenerating motor neurons is a common feature in both sporadic and familial ALS. Thus, the neurofilamentous lesions seem to be closely related to motor neuron degeneration in ALS. Indeed, the overexpression of neurofilament proteins induces motor neuron degeneration in mice (20, 21). Moreover, in transgenic mice expressing mutant SOD1, neurofilamentous lesions are a prominent feature of degenerating motor neurons (55), and deletion of the neurofilament subunit-L (NF-L) or overexpression of NF-H slows the disease progression in such mice (56, 57), indicating that neurofilament is involved in the pathogenesis of motor neuron degeneration even in mutant SOD1-related ALS. The present experiments showed that galectin-1 is closely associated with neurofilaments in the neurofilamentous lesions of ALS, suggesting that the neurofilament-galectin-1 complex may play an important role in the pathological process of motor neuron degeneration in ALS.

In ALS, disturbance of axonal transport may occur initially (21, 58–62) and may subsequently develop accumulation of galectin-1 and neurofilaments in the perikarya of motor neurons and in the proximal portion of their axons. One might expect that such disturbance may cause depletion of galectin-1 in the distal portion of axons. If motor neuron axons and skeletal muscles need galectin-1 for their maintenance or survival, depletion of this protein may cause degeneration of the axons and skeletal muscles. Alternatively, considering that HepSS-1 immunoreactivity appears at an early stage of pathological changes of ALS spinal cord (23), and that oxidized galectin-1 has a potent axonal regeneration-enhancing activity (24, 25), galectin-1 may play a more active role for regeneration and survival of motor neurons against the degenerative process in ALS. If this is the case, oxidized galectin-1 could be useful for a therapy in ALS.

In conclusion, the present study reveals that galectin-1 is a component accumulating in axonal spheroids, cord-like neurite swellings, and conglomerate inclusions of degenerating motor neurons in both sporadic and familial ALS. The close association of galectin-1 with abnormally accumulated neurofilaments in ALS suggests its involvement in a pathological process of ALS. Galectin-1 has not been reported in the field of ALS study nor in any other diseases. This study may provide with target molecules for understanding the molecular basis of the pathogenesis of ALS.

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