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Involvement of neurofilaments in motor neuron disease

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SUMMARY

Motor neuron disease is clinically characterized by progressive muscle wasting leading to total muscle paralysis. A long history of pathological study of patients has firmly established that the primary lesion site is in spinal and cortical motor neurons. In addition to the widespread loss of these neurons, neuronal abnormalities including massive accumulation of neurofilaments in cell bodies and proximal axons have been also widely observed, particularly in the early stages of the disease. To test whether high accumulation of neurofilaments directly contributes to the pathogenic process, transgenic mice that produce high levels of neurofilaments in motor neurons have been generated. These transgenic mice show most of the hallmarks observed in motor neuron disease, including swollen perikarya with eccentrically localized nuclei, proximal axonal swellings, axonal degeneration and severe skeletal muscle atrophy. These data indicate that extensive accumulation of neurofilaments in motor neurons can trigger a neurodegenerative process and may be a key intermediate in the pathway of pathogenesis leading to neuronal loss.

Key words: membrane-cytoskeleton linkage, spectrin, ankyrin, cell adhesion molecule, Ig-super family

INTRODUCTION

Neurofilaments are 10 nm filaments in many types of neurons. Assembled from three polypeptide subunits, NF-L (68 kDa), NF-M (95 kDa) and NF-H (115 kDa), neurofilaments are the most abundant structure in large myelinated axons, such as those elaborated by spinal motor neurons. Mounting evidence has strengthened the view that neurofilaments play a critical role in the development and maintainence of axonal caliber (Friede and Samorajski, 1970; Hoffman et al., 1987; Cleveland et al., 1991; Yamasaki et al., 1992), a crucial determinant for conduction velocity of axons and perhaps also a trigger for myelination (Arbuthnott et al., 1980; Voyvodic, 1989).

In addition to a function in supporting the growth and maintenance of axonal caliber in normal neurons, neurofilaments have been suspected to play a role in the pathogenesis of several types of neurodegenerative diseases, including motor neuron disease, e.g. amyotrophic lateral sclerosis or ALS (Banker, 1986; Carpenter, 1968; Inoue and Hirano, 1979; Hirano et al., 1984a,b; Mulder, 1984, 1986) and infantile spinal muscular atrophy (Byers and Banker, 1961; Wiley et al., 1987). The common clinical symptom of motor neuron diseases is progressive loss of motor neuron function, which in turn leads to wasting of skeletal muscle, paralysis and ultimately death (for reviews, see Mulder, 1984, 1986; Gomez, 1986). For most cases, neither the primary causes nor the mechanism of pathogenesis have been elucidated. A long history of pathological examination, dating from the middle of the last century, has firmly established that the primary lesion of the disease lies predominantly in the cortical and spinal motor neurons (Betz cells and the anterior horn α-motor neurons). Various degrees of motor neuron loss in either of these two areas (or both) are seen as the major pathological hallmark (Chou, 1992). However, in those cases (e.g. infantile muscular atrophy, the adult diseases which progress relatively rapidly, and the early stage of ALS), significant numbers of surviving motor neurons are observed in post-mortem examination. Most of these remaining motor neurons display various abnormal morphologies (Fig. 1A and B), including swollen cell bodies and dispersal of the rough endoplasmic reticulum (often called Nissl substance). Further, large axonal swellings that sometimes reach the size of the cell body are found (Carpenter, 1968; Chou, 1992). These swollen structures are strongly stained with silver (Fig. 1C) suggesting that they are rich in neurofilaments (Carpenter, 1968; Hughes and Jerrome, 1971; Chou and Fakadej, 1971; Inoue and Hirano, 1979; Hirano et al., 1984a,b). Many electron microscopic studies have unequivocally established that the swollen neurons and axons contain abundant swirls of neurofilaments (Carpenter, 1968; Hughes and Jerrome, 1971; Chou and Fakadej, 1971; Hirano et al., 1984a,b).

Motor neuron disease, with symptoms resembling infantile spinal muscular atrophy, has also been described in a number of animal species. Remarkable neurofilament accumulation in the anterior horn α-motor neurons has been

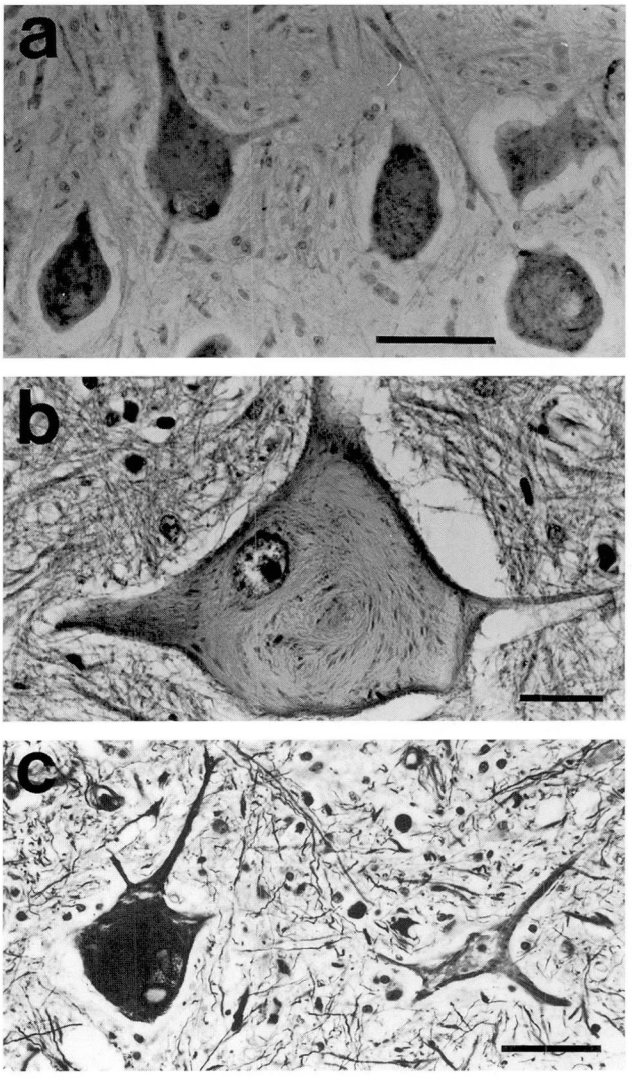

Fig. 1. Swollen neurons in the anterior horn of the spinal cord from human ALS. (a,b) Swollen neurons with neurofilament accumulation from a case of infantile spinal muscular atrophy stained with hematoxylin and eosin. Bars: (a) 150 μm; (b) 25 μm. Reproduced with permission from Wiley et al. (1987). (c) A swollen neuron from a case of familial ALS stained by Bielschowsky's silver impregnation. Bar, 50 μm. Reproduced with permission from Hirano et al. (1984).

found in all such cases (Delahunta and Shively, 1974; Vandevelde et al., 1976; Higgins et al., 1977, 1983; Shields and Vandevelde, 1978; Cork et al., 1982). These studies have collectively fueled the speculation that neurofilament accumulation may represent a common initial pathology of the motor neuron disease process.

Additional experimental support for this view has emerged from various toxin-induced neuropathies. Intoxication with aluminum, β,β′-iminodiproionitrile (IDPN) or 3,4-dimethyl-2,5-hexanedione (DMHD) causes prominent axonal swelling as well as (in the case of aluminum intoxication) swollen neuronal soma. In each case the swellings are accompanied by massive accumulation of neurofilaments in the anterior horn of the spinal cord (Troncoso et al., 1982; Chou and Hartman, 1965; Anthony et al., 1983). Impairment of slow axonal transport of neurofilaments has been demonstrated in both the IDPN- and aluminum-treated animals (Griffin et al., 1978; Bizzi et al., 1984; Troncoso et al., 1985).

Despite these widely observed examples of neurofilament misaccumulation in α-motor neurons in humans and animals with motor neuron disease, a central unsolved question is whether the aberrant accumulation is merely a harmless by-product of the pathogenic process or a central element in the pathogenic pathway that leads to neuronal dysfunction and ultimately cell death. To distinguish between these these two possibilities, transgenic technology has now been used to demonstrate that forcing neurons to increase expression of neurofilaments is sufficient to yield morphologic features of motor neuron disease, including excessive accumulation of neurofilaments in perikarya and proximal axons and increased axonal degeneration (Xu et al., 1993; Cote et al., 1993). In addition, these neuronal changes result in neuronal dysfunction, as indicated by severe skeletal muscle atrophy. These results demonstrate that misaccumulation of neurofilaments can be a integral part of the pathogenic pathway in motor neuron degeneration.

RESULTS

Doubly transgenic lines accumulate high levels of NF-L in their nervous tissue

To examine the consequence of forcing increased expression of wild-type murine NF-L, several lines of transgenic mice were produced that accumulated amounts up to twice the normal level of wild-type NF-L in peripheral nerves. No overt phenotypic change was observed in any of these transgenic mice (Monteiro et al., 1990). To increase further the number of neurofilaments in the nervous tissue, we mated two independent, highly expressing NF-L lines (MSV-NF-L58 and MSV-NF-L103) and screened for progeny that carried copies of both transgenes. Since transgenes of both transgenic lines are incorporated in a tandem, repeated fashion but have integrated at different chromosomal loci, we could distinguish the presence of each transgene by using genomic DNA blotting to detect the unique sized fragments located at the 5′ junction of the incorporation sites. Using this approach, we readily detected animals that carry transgenes from both founder lines (to be referred to as doubly NF-L transgenic mice).

To determine the levels of accumulated NF-L in doubly transgenic animals, we used protein immunoblotting of extracts of sciatic nerves from 3-week-old control and transgenic animals (Fig. 2). We analyzed known amounts of purified neurofilament proteins in parallel, to provide accurate quantitation standards. The level of NF-L in either parental transgenic line was increased approximately 2-fold (Fig. 2, lanes 2 and 3) more than wild-type controls (Fig. 2, lane 1) and 4-fold in the doubly transgenic animals (Fig. 2, lane 4), reaching about 2% of the total sciatic nerve protein. Immunohistochemistry of spinal cord and sciatic nerves revealed that accumulated NF-L, both endogenous

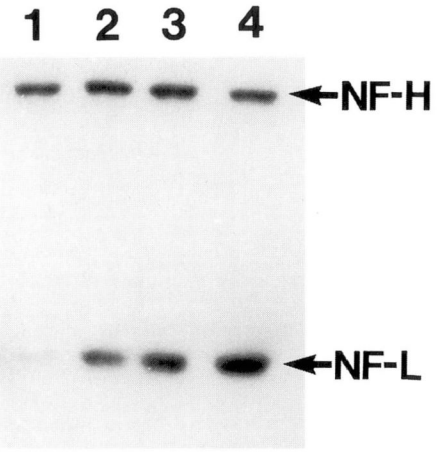

Fig. 2. Accumulation of excess NF-L in sciatic nerves of singly and doubly transgenic mice determined by immunoblot. Total proteins extracted from the sciatic nerve of wild-type (lane 1), line MSV-NF-L103 (lane 2), line MSV-NF-L58 (lane 3) and a doubly transgenic mouse (lane 4) were taken from 20-day-old animals, separated by SDS-PAGE and immunoblotted with a mixture of anti-NF-L and phosphorylation-independent NF-H monoclonal antibodies.

and transgene-encoded, was present only in the neurons, with none detectable in the surrounding Schwann cells or oligodendrocytes (see also Monteiro et al., 1990).

Accumulation of high levels of NF-L results in morphologic characteristics of motor neuron disease

Inspection of litters during the first 21 postnatal days revealed that some mice from matings of the two transgenic lines were significantly smaller in size than their littermates (around 1/3-2/3 of the normal weight) and all displayed progressive reduction in kinetic activity, cultimating in eventual death during the third postnatal week. With careful feeding, two of these doubly transgenic animals survived past 3 weeks of age. Genomic DNA blotting revealed that 8 of 8 animals showing this slow growth phenotype were doubly transgenic mice, while all other littermates were wild type or singly transgenic.

To determine the consequence of increased NF-L accumulation, four of the doubly transgenic animals were sacrificed by perfusion at day 21 or 22 and their tissues examined morphologically. In all of these animals, striking changes were observed, predominantly in the anterior horn motor neurons of the spinal cord. Compared with non-transgenic littermates, almost all of the motor neurons (at all levels of the spinal cord) displayed features of chromatolysis, including enlarged, ballooned perikarya with depleted rough endoplasmic reticulum and eccentrically positioned nucleus (Fig. 3A). These features are strikingly similar to what has been seen in many reported cases of motor neuron disease (e.g. Fig. 1).

At higher magnification, massive accumulation of filaments in all motor neuron compartments (cytoplasm, dendrites and axons) was confirmed (Fig. 3B; see also Xu et al., 1993). Accompanying this increased accumulation of filaments were two obvious axonal abnormalities. First, as in ALS, on the edge of the anterior horn of a 2-month-old doubly transgenic mouse, numerous axonal swellings were present (Fig. 4A). These swellings were filled with neurofilaments (Fig. 4B). Second, in the ventral roots containing the corresponding motor axons from doubly transgenic animals of various ages, degenerating axons were present (Fig. 4C). While the proportion of degenerating axons was less than 0.2%, this does represent a marked increase in the frequency of degeneration from that seen in control littermates. From the four ventral roots examined from doubly transgenic animals, three degenerating axons were found, whereas an exhaustive search of three ventral roots from control animals revealed no degenerating axons.

The two doubly NF-L transgenic mice that survived past 3 weeks of age gradually recovered, and by 2 months of age were four-fifths of the weight of littermate con-

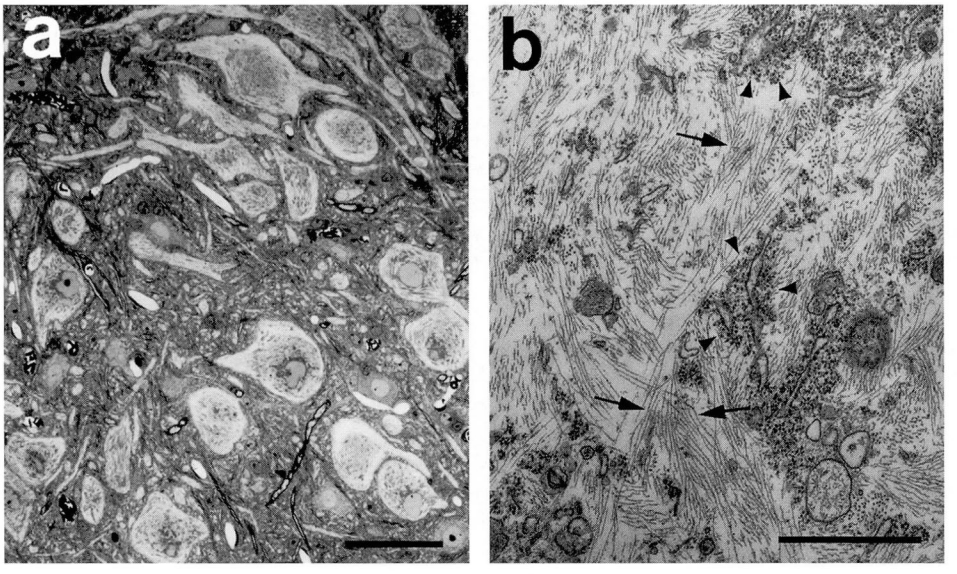

Fig. 3. Morphology of spinal anterior horn motor neurons expressing high levels of NF-L. (a) The anterior horn of a spinal cord from a 21-day-old MSV-NF-L58/MSV-NF-L103 doubly transgenic animal. Sections (1 μm) stained with toliudine blue. Bar, 60 μm. (b) Electron micrograph of the cytoplasm from doubly transgenic anterior horn motor neurons showing massive filament accumulation. Arrows point to bundles of filaments; arrowheads point to clusters of RER. Bar, 1 μm.

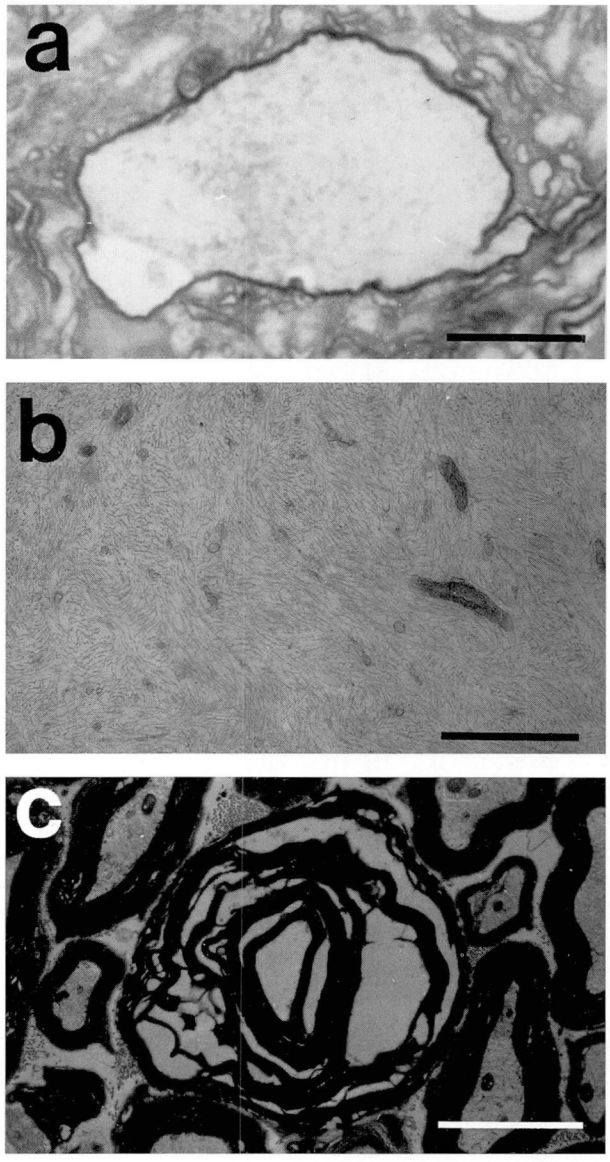

Fig. 4. Axonal abnormalities in the doubly transgenic NF-L mice. (a) Light microscopic view of an axonal swelling in the proximal axon of an anterior horn motor neuron from a doubly transgenic animal. Bar, 10 μm. (b) Higher magnification view of an area inside the axonal swelling. Bar, 1.5 μm. (c) Degenerated axon from a ventral root of a doubly transgenic NF-L mouse. Bar, 3 μm.

trols. The non-progressive course of transgene-mediated pathology in these animals was a surprise, initially. However, quantitation of protein blots of sciatic nerve extracts from both doubly and singly NF-L transgenic mice revealed that although NF-L accumulation initially rises significantly above littermate controls, after 3 weeks of age it gradually falls back to about the same as in wild-type animals (Table I). Concomitant with this decrease in excessive NF-L, morphological examination of one doubly transgenic animal revealed restoration of a nearly wild-type appearance at 9 months of age. Similarly, neurofilament density in axons also declined with age. Since

Table 1. Comparison of NF-L levels in the sciatic nerves of MSVNF-L transgenic and wild-type mice at different ages

Transgenic lines	NF-L in transgenic mice* / NF-L in wild-type mice*		
	20-day-old	120-day-old	300-day-old
58	2-3	2	1
103	~2	1	1
58-103	>4	n.d.	1-2

*The quantification was carried out by immunoblotting using the total proteins extracted from sciatic nerve as described in the legend to Fig. 2.

the only difference between the transgene and the wild-type gene lies in their promoters, the most reasonable explanation for the decline in transgene-encoded NF-L is an age-dependent reduction in activity of the MSV promoter used to drive transgene transcription. In any event, loss of both phenotypic and morphological abnormalities in neurons, coincident with the age-dependent reduction in NF-L content, combined with the absence of abnormalities in either singly transgenic mouse line, strongly support the view that only those increases in neurofilament economy above a threshold level result in obvious neurological abnormalities.

Spinal motor neurons with high neurofilament accumulation contain phosphorylated NF-H, a marker of motor neuron pathology

Aberrant accumulation of phosphorylated NF-H in motor neuron soma has been described in a number of motor neuron disease cases, in both human and animal species (Cork et al., 1988; Munoz et al., 1988; Manetto et al., 1988; Sobue et al., 1990). Although this phenomenon is not specific for motor neuron disease, it is an indication that the neuron is undergoing a pathological process. In spite of the fact that more than 20 phosphates are added to NF-H (Julien and Mushynski, 1982; Jones and Williams Jr, 1982; Wong et al., 1984), most of the phosphates are normally added only in the axon so that antibodies specific to the phosphorylated NF-H do not detect reactivity in the cell bodies (Sternberger and Sternberger, 1983). To test whether increased expression of wild-type NF-L and the corresponding accumulation of large numbers of filaments in motor neuron cell bodies was associated with phosphorylation of NF-H, the spinal cords from wild-type and doubly transgenic NF-L mice were embedded in paraffin, sectioned and stained for phosphorylated NF-H. In doubly transgenic animals (Fig. 5A), hematoxylin- and eosin-stained sections revealed strikingly similar neuronal morphologies to motor neurons in human motor neuron disease (compare Fig. 5A to Fig. 1). These include swollen perikarya, depleted Nissl substance and eccentric nuclei in the motor neurons of the anterior horn. Using a well characterized monoclonal antibody that only detects phosphorylated NF-H (Sternberger and Sternberger, 1983), strong staining was found in the transgenic soma (Fig. 5C), while no staining was detectable in a parallel analysis of normal motor neurons (Fig. 5D).

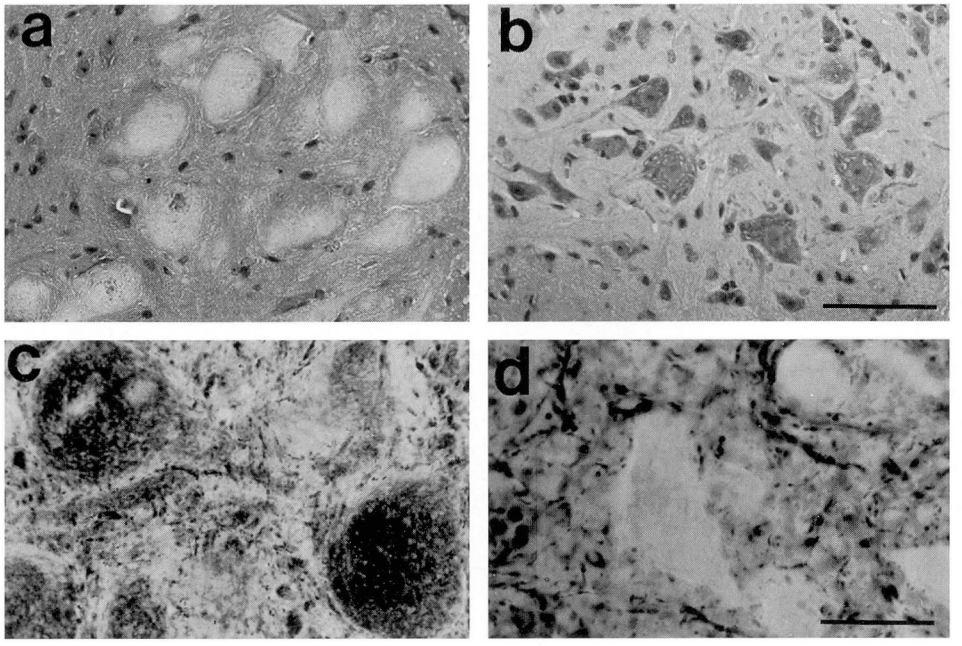

Fig. 5. Abnormal masses of filaments in the neuronal cell bodies of doubly transgenic mice contain phosphorylated NF-H. (a,b) Hematoxylin- and eosin-stained paraffin sections of the anterior horn of the spinal cord from a 21-day-old doubly transgenic (a) and a control mouse (b). Bar, 80 μm. (c,d) Anterior horn motor neurons from a doubly transgenic (c) and a control mouse (d) stained with an anti-phosphorylated NF-H antibody (Ab3-44). Bar, 25 μm.

Abnormal morphological changes in motor neurons are accompanied by severe muscle atrophy

The low body weight phenotype of the doubly transgenic mice was accompanied by a progressive loss in kinetic activity of the doubly transgenic animals. Postmortem examination of 21-day-old animals revealed widespread skeletal muscle atrophy. For example, Fig. 6A,B displays cross sections of the anterior tibial muscle from a doubly transgenic and an age-matched control animal. Individual muscle fibers in the transgenic sample are <20% of the cross sectional area of the wild type, a phenotype consistent with denervation-induced muscle atrophy. However, as noted previously for the singly transgenic lines (Monteiro et al., 1990), transgene-encoded NF-L is not expressed exclusively in neurons but also accumulates in skeletal muscles. To distinguish whether atrophy was a consequence of nerve dysfunction or a direct effect of NF-L accumulation in muscle, we evaluated the level of NF-L in muscles from animals of different ages (Fig. 7). In contrast to the decline of transgene expression in neurons as the animals age beyond 21 days (Table 1), NF-L accumulation in muscle continues to increase up to 2 months of age (Fig. 7B, lane 2) and remains higher than the level seen in a 2.5-week-old animal for at least 11 months thereafter (compare lanes 3 and 1 in Fig. 7B). Since the muscle was most severely

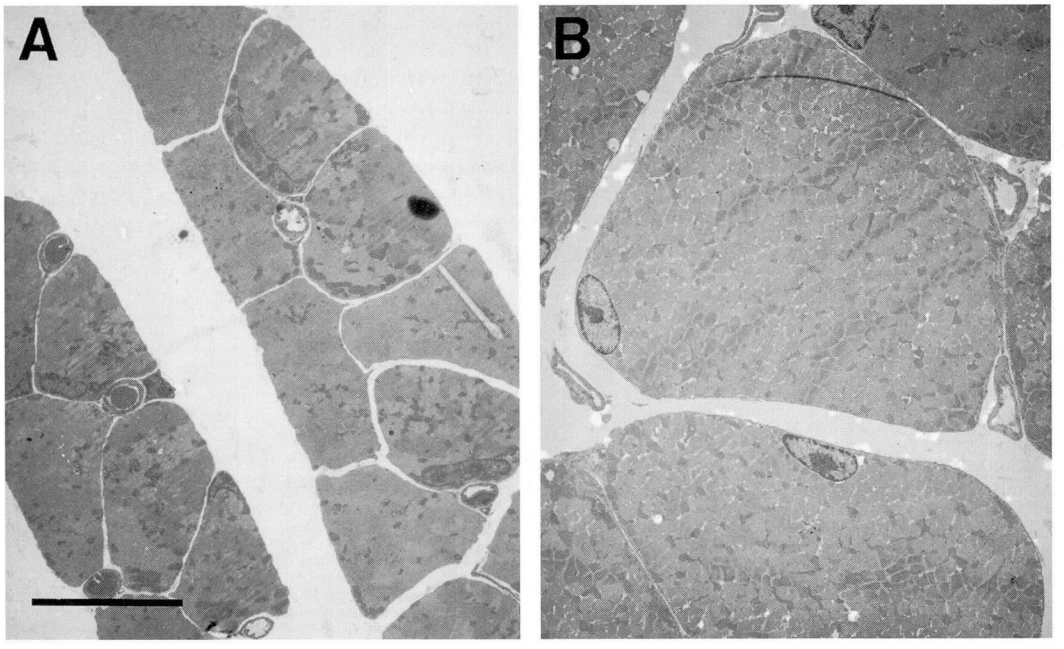

Fig. 6. Severe muscle atrophy in NF-L doubly transgenic mice. (A,B) Electron micrographs of cross sections of muscle fibers from anterior tibial muscle of a 21-day-old doubly transgenic mouse (A) or a non-transgenic littermate (B). Bar, 10 μm. Reproduced with permission from Xu et al. (1993).

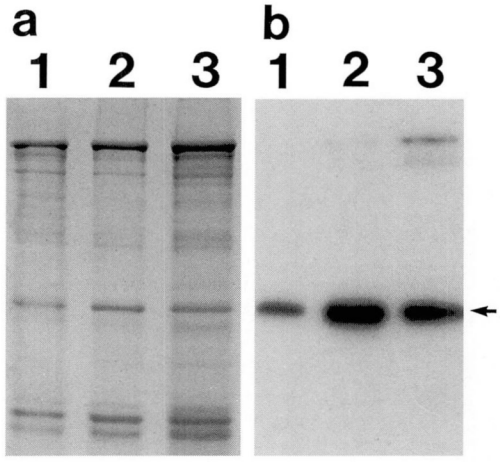

Fig. 7. The level of NF-L accumulation in skeletal muscle does not correlate with the severity of muscle atrophy. (a) Total proteins (10 μg) extracted from skeletal muscle (biceps) from 18-day-old (lane 1), 2.5-month-old (lane 2) and 11-month-old (lane 3) transgenic animals of line MSV-NF-L58 were separated by SDS-PAGE and stained with Coomassie blue. (b) A duplicate gel was immunoblotted with anti-NF-L monoclonal antibodies. An arrow points to the NF-L band.

atrophic between 2 and 3 weeks of age but recovered to nearly normal size by 2 months despite the increasing burden of transgene encoded NF-L, we conclude that there is no correlation between the level of NF-L accumulation in muscle and muscle atrophy. In contrast, the muscle atrophy correlates well with the peak accumulation of NF-L in neurons. These data strongly support the view that the predominant cause of muscle atrophy is the dysfunction of motor neurons resulting from the excessive accumulation of neurofilaments.

DISCUSSION

Neurofilamentous accumulations in perikarya, dendrites and axons occur in a variety of degenerative, toxic and heritable diseases. Particularly striking examples have been reported in different types of motor neuron diseases, including infantile spinal muscular atrophy (Byers and Banker, 1961; Chou and Fakadej, 1971; Wiley et al., 1987), familial ALS (Hughes and Jerrome, 1971; Takahashi et al., 1972; Hirano et al., 1984b) and sporadic ALS (Carpenter, 1968; Schochet, Jr et al., 1969; Hirano et al., 1984a). In various animal species including dog (Delahunta and Shively, 1974; Cork et al., 1982), zebra (Higgins et al., 1977), rabbit (Shields and Vandevelde, 1978), cat (Vandevelde et al., 1976), pig (Higgins et al., 1983) and cattle (Rousseaux et al., 1985), spontanous motor neuron disease with symptoms resembling those of human infantile spinal muscular atrophy have been reported. Invariably, in each of these cases, severe neurofilament accumulation in the anterior horn α-motor neurons has been found, although in none of these diseases is the pathogenesis fully understood. Even in animal models where similar neurofilament accumulations in the α-motor neurons were induced by administration of neurotoxins (e.g. Chou and Hartman, 1965; Troncoso et al., 1982; Anthony et al., 1983), the precise mechanisms of injury are only partially understood (Griffin et al., 1978; Bizzi et al., 1984; Troncoso et al., 1985), since the agents may have multiple effects on neurons. The present results, in conjunction with similar findings using transgenic technology to force excessive accumulation of NF-H (Cote et al., 1993) provide an unambiguous demonstration that primary alterations in neurofilament economy can (1) lead to structural changes of the type seen in these neurodegenerative disorders and (2) ultimately lead to axonal breakdown and loss.

The morphological effects of over-producing neurofilaments in motor neurons bears most striking resemblance to those observed in rapidly progressing infantile spinal muscular atrophy (Byers and Banker, 1961; Chou and Fakadej, 1971; Wiley et al., 1987) and the early stages of ALS (Inoue and Hirano, 1979; Hirano et al., 1984a,b). This raises an interesting speculation that marked neurofilament accumulation in perikarya and proximal axons may be an early pathological change that preceeds the widespread neuronal loss. Consistent with this is the observation that in virtually all the reported cases of spinal muscular atrophy from various animal species, large numbers of swollen neurons with high neurofilament accumulation are a prominent feature. Further, in dogs with rapidly progressing spinal muscular atrophy, severe neurofilamentous accumulations are accompanied by only a minor motor neuron loss. However, in the cases in which the disease progresses relatively slowly, more prominent motor neuron loss is observed (Cork et al., 1982). In this context, in many human examples a relatively low frequency of swollen perikarya and a higher proportion of degenerating axons may simply reflect the slow progression of disease which allows compromised neurons to initiate subsequent degeneration. From a slightly

Fig. 8. Schematic model for involvement of neurofilaments in motor neuron disease. Reproduced with permssion from Xu et al. (1993).

different perspective, the remarkable extent of neurofilament accumulation in both naturally occurring motor neuron disease and the transgenic mice further indicates a great degree of tolerance of the neuron for the substantial increases in total 'neurofilament burden', suggesting that filament-induced degeneration is a slow process. This is consistent with the gradual progression of many of the disorders with neurofilament accumulation.

Although direct measurements of motor neuron function have not been carried out, the presence of widespread muscle atrophy in the doubly transgenic mice suggests that severe misaccumulation of neurofilaments can cause an impairment of motor neuron function. Consistent with this are the human cases where muscle atrophy becomes prominent before the widespread loss of motor neurons (Inoue and Hirano, 1979; Wiley et al., 1987). In dogs with spinal muscular atrophy, a disproportionately greater muscle weakness relative to neuronal loss has also been reported (Cork et al., 1982).

If it is true, as our data imply, that NF accumulation may be an active participant in the pathogenesis of motor neuron disease, then what mechanisms can lead to the accumulation of neurofilaments in the perikarya and proximal axons? The various possibilities (shown in Fig. 8) include increased synthesis, decreased degradation, and defective transport of neurofilaments. Slowed degradation seems unlikely in the pathogenesis of motor neuron disease because increased neurofilament stability would be expected to lead to more extreme distal accumulations of neurofilaments, which has not been observed. While a concomitant increase in neurofilament synthesis is a possibility, the most plausible mechanism (and one which appears to be consistent with the neuropathological findings) is an alteration in slow axonal transport of neurofilaments. This could be derived from defects either in the machinery that moves the filaments or in the filaments themselves. The former may be a realistic possibility for cases in which not only neurofilaments but also other organelles accumulate in perikarya and proximal axons (Chou and Fakadej, 1971; Cork et al., 1982; Hirano et al., 1984b). However, the latter alternative is also attractive, particularly in cases where abnormal filament structure and organization are found, including paracrystalline arrays, beaded filaments or various types of focal accumulation of neurofilaments (Schochet et al., 1969; Hirano et al., 1984a; Banker, 1986).

If neurofilamentous accumulations are important intermediates in the neurodegenerative process, how can this be reconciled with the recent discovery that half of the familial cases of ALS (≈5% of total ALS cases) result from mutations in the enzyme superoxide dismutase (SOD) (Rosen et al., 1993)? How too can we explain the puzzle that SOD mutations lead to the selective death of motor neurons even though SOD is expressed in most (possibly all) cells? Since SOD acts to block oxidative damage by converting oxygen radicals into peroxide (Fridovich, 1986), and if the sites of primary damage are proteins, we suggest that it is reasonable that the most affected proteins will be those that have long turnover times, since proteins with short half-lives are quickly replaced. In this context, neurofilaments are slowly transported proteins with transit times from synthesis to arrival near a nerve terminus as long as three years (calculated for a meter-long axon and a rate of transport at 1 mm/day; see Oblinger and Lasek, 1985). It is conceivable, therefore, that damaged proteins of this class of slowly transported proteins gradually accumulate to poison the transport machinary. Such a mechanism can explain the particular vulnerability of motor neurons in motor neuron disease: whatever the cause, if damage to neurofilaments or their transport ultimately results in neuronal degeneration, then cells that normally have the highest neurofilament burden will be the ones first and most severely affected. Consistent with this prediction, both in our mice and in human motor neuron diseases, the neurons that are most severely affected by accumulation of neurofilaments are among the largest neurons with the longest axons that normally contain abundant amounts of neurofilaments.

In any event, our evidence has established one point of pathological significance for human motor neuron disease: primary changes in the cytoskeleton, and specifically in neurofilaments, are sufficient to produce most of the pathological changes encountered in neurodegenerative diseases such as ALS. Our data further promote the suggestion (shown in Fig. 8) of a common pathogenetic sequence that includes neurofilament accumulation as a central pathological intermediary, leading to subsequent axonal swelling and degeneration. Even in disorders where the neurofilamentous abnormalities are secondary to other types of neuronal injury, neurofilaments may contribute to the ultimate loss of the neuron or its axon. Indeed, cytoskeletal abnormalities may increase the susceptibility of the neuron to other insults (e.g. excitotoxicity), so that multiple factors could culminate in production of disease.

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REFERENCES

Anthony, D. C., Giangaspero, F. and Graham, D. G. (1983). The spatiotemporal pattern of the axonopathy associated wiht the neurotoxicity of 3, 4-dimethyl-2, 5-hexanedione in the rat. *J. Neuropath. Exp. Neurol.* **42**, 548-560.

Arbuthnott, E. R., Boyd, I. A. and Kalu, K. U. (1980). Ultrastructural dimensions of myelinated peripheral nerve fibres in the cat and their relation to conduction velocity. *J. Physiol.* **308**, 125-157.

Banker, B. Q. (1986). The pathology of the motor neuron disorders. In *Myology* (ed. A. G. Engel and B. Q. Banker), pp. 2031-2066. McGraw-Hill, New York.

Bizzi, A., Crane, R. C., Autilio-Cambetti, L. and Gambetti, P. (1984). Aluminum effect on slow axonal transport: a novel impairment of neurofilament transport. *J. Neurosci.* **4**, 722-731.

Byers, R. K. and Banker, B. Q. (1961). Infantile spinal muscular atrophy. *Arch. Neurol.* **5**, 140-164.

Carpenter, S. (1968). Proximal axonal enlargement in motor neuron disease. *Neurology* **18**, 841-851.

Chou, S. M. (1992). Pathology - light microscopy of amyotrophic lateral sclerosis. In *Handbook of Amyotrophic Lateral Sclerosis* (ed. R. A. Smith), pp. 133-181. Marcel Dekker, Inc.

Chou, S. M. and Fakadej, A. V. (1971). Ultrastructure of chromatolytic motor neurons and anterior spinal roots in a case of Werdnig-Hoffmann Disease. *J. Neuropathol. Exp. Neurol.* **30**, 368-379.

Chou, S. M. and Hartmann H. A. (1965). Electron microscopy of focal neuroaxonal lesions produced by β-β′-iminodipropionitrile (IDPN) in rats. *Acta Neuropathol.* **4**, 590-603.

Cleveland, D. W., Monteiro, M. J., Wong, P. C., Gill, S. R., Gearhart, J. D. and Hoffman, P. N. (1991). Involvement of neurofilaments in the radial growth of axons. *J. Cell Sci.* **15**, 85-95.

Cork, L. C., Troncoso, J. C., Klavano, G. G., Johnson, E. S., Sternberger, L. A., Sternberger, N. H. and Price, D. L. (1988). Neurofilamentous abnormalities in motor neurons in spontaneously occurring animal disorders. *J. Neuropathol. Exp. Neurol.* **47**, 420-431.

Cork, L. C., Griffin, J. W., Choy, C., Padula, C. A. and Price, D. L. (1982). Pathology of motor neurons in accelerated hereditary canine spinal muscular atrophy. *Lab. Invest.* **46**, 89-99.

Cote, F., Collard, J.-F. and Julien, J-P. (1993). Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell* **73**, 35-46.

Delahunta, A. and Shively J. N. (1974). Neurofibrillary accumulation in a puppy. *Cornell Vet.* **65**, 240-247.

Fridovich, I. (1986). Superoxide dismutase. *Advan. Enzymol.* **58**, 61-97.

Friede, R. L. and Samorajski, T. (1970). Axon caliber related to neurofilaments and microtubules in sciatic nerve fibers of rats and mice. *Anat. Rec.* **167**, 379-388.

Gomez, M. R. (1986). Motor neuron diseases in children. In *Myology* (ed. A. G. Engel and B. Q. Banker), pp 1993-2012. McGraw-Hill, New York.

Griffin, J. W., Hoffman, P. N., Clark, A. W., Carroll, P. T. and Price, D. L. (1978). Slow axonal transport of neurofilament proteins: impairment by β, β′-iminodipropionitrile administration. *Science* **202**, 633-636.

Higgins, R. J., Rings, D. M., Fenner, W. R. and Stevenson, S. (1983). Spontaneous lower motor neuron disease with neurofibrillary accumulation in young pigs. *Acta Neuropathol. (Gerl)* **59**, 288-294.

Higgins, R. J., Vandevelde, M., Hoff, E. J., Jagar, J. E., Cork, L. C. and Selbermann M. S. (1977). Neurofibrillary accumulation in the zebra (Equus burchelli). *Acta Neuropathol. (Gerl)* **37**, 1-5.

Hirano, A., Donnenfeld, H., Shoichi, S. and Nakano, I. (1984a). Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* **43**, 461-470.

Hirano, A., Nakano, I., Kurland, L. T., Mulder, D. W., Holley, P. W. and Sccomanno, G. (1984b). Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* **43**, 471-480.

Hoffman, P. N., Cleveland, D. W., Griffin, J. W., Landes, P. W., Cowan, N. J. and Price, D. L. (1987). Neurofilament gene expression: a major determinant of axonal caliber. *Proc. Nat. Acad. Sci. USA* **84**, 3272-3476.

Hughes, J. T. and Jerrome, D. (1971). Ultrastucture of anterior horn motor neurons in the Hirano-Kurland-Sayre type of combined neurological system degeneration. *J. Neurol. Sci.* **13**, 389-399.

Inoue, K. and Hirano, A. (1979). Early pathological changes of amyotrophic lateral sclerosis--autopsy findings of a case of 10 months' duration. *Neurol. Med. (Tokyo)* **11**, 448-455.

Jones, S. M. and Williams, R. C., Jr (1982). Phosphate content of mammalian neurofilaments. *J. Biol. Chem.* **257**, 9902-9905.

Julien, J.-P. and Mushynski, W. E. (1982). Multiple phosphorylation sites in mammalian neurofilament polypeptides. *J. Biol. Chem.* **257**, 10467-10470.

Manetto, V., Sternberger, N. H. and Gambetti, P. (1988). Phosphorylation of neurofilaments is altered in amyotrophic lateral sclerosis. *J. Neurol. Sci.* **47**, 642-653.

Monteiro, M. J., Hoffman, P. N., Gearhart, J. D. and Cleveland, D. W. (1990). Expression of NF-L in both neuronal and nonneuronal cells of transgenic mice: increased neurofilament density in axons without affecting caliber. *J. Cell Biol.* **111**, 1543-1557.

Mulder, D. W. (1984). Motor neuron disease. In *Peripheral Neuropathy* (ed. P. J. Dyck, P. K. Thomas, E. H. Lambert and R. Bunge), pp. 1525-1536. Saunders, Philadelphia.

Mulder, D. W. (1986). Motor neuron disease in adults. In *Myology* (ed. A. G. Engel and B. Q. Banker), pp 2013-2029. McGraw-Hill, New York.

Munoz, D. G., Greene, C., Perl, D. P. and Selkoe, D. J. (1988). Accumulation of phosphorylated neurofilaments in anterior horn motoneuron of amyotrophic lateral sclerosis patients. *J. Neuropath. Exp. Neurol.* **47**, 9-18.

Oblinger, M. M. and Lasek, R. J. (1985). Selective regulation of two axonal cytoskeletal networks in dorsal root ganglion cells. In *Neurobiology: Molecular Biological Approaches to Understanding Neuronal Function and Development* (ed. P. O'Lagre), pp The Shering Corp.: UCLA Symposium on Molecular and Cellular Biology, vol. 24. New York: Liss.

Rosen, D. R., Siddique, T., Patterson, D. et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59-62.

Rousseaux, C. G., Klavano, G. G., Johnson, E. S., Shnitka, T. K., Harries, W. N. and Snyder, F. F. (1985). 'Shaker' calf syndrome: A newly recognized inherited neurodegenerative disorder of horned Hereford calves. *Vet. Pathol.* **22**, 104-111.

Schochet, S. S. Jr, Hardman, J. M., Ladewig, P. P. and Earle, K. M. (1969). Intraneuronal conglomerates in sporadic motor neuron disease. *Arch. Neurol.* **20**, 548-553.

Shields, R. P. and Vandevelde, M. (1978). Spontaneous lower motor neuron disease in rabbits (*Oryctolagus cuniculus*). *Acta Neuropathol. (Berl)* **44**, 85-90.

Sobue, G., Hshizume, Y., Yasuda, T., Mukai, E., Kumagai, T., Mitsuma, T. and Trojanowski, J. Q. (1990). Phosphorylated high molecular weight neurofilament protein in lower motor neurons in amyotrophic lateral sclerosis and other neurodegenerative diseases involving ventral horn cells. *Acta Neuropathol.* **79**, 402-408.

Sternberger, L. A. and Sternberger, N. H. (1983). Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments *in situ*. *Proc. Nat. Acad. Sci. USA* **80**, 6126-6130.

Takahashi, K., Nakamura, H. and Okada, E. (1972). Hereditary amyotrophic lateral sclerosis. *Arch. Neurol.* **27**, 292-299.

Troncoso, J. C., Hoffman, P. N., Griffin, J. W., Hess-Kozlow, K. M. and Price, D. L. (1985). Aluminum intoxication: a disorder of neurofilament transport in motor neurons. *Brain Res.* **342**, 172-175.

Troncoso, J. C., Price, D. L., Griffin, J. W. and Parhad, I. M. (1982). Neurofibrillary axonal pathology in aluminum intoxication. *Ann. Neurol.* **12**, 278-283.

Vandevelde, M., Greene, C. E. and Hoff, E. J. (1976). Lower motor neuron disease with accumulation of neurofilaments in a cat. *Vet. Pathol.* **13**, 428-435.

Voyvodic, J. T. (1989). Target size regulates caliber and myelination of sympathetic axons. *Nature* **342**, 430-432.

Wiley, C. A., Love, S., Skoglund, R. R. and Lampert, P. W. (1987). Infantile neurodegenerative disease with neuronal accumulation of phosphorylated neurofilaments. *Acta Neuropathol.* **72**, 369-376.

Wong, J., Hutchison, S. B. and Liem, R. K. H. (1984). An isoelectric variant of the 150,000-dalton neurofilament polypeptide. *J. Biol. Chem.* **259**, 4019-4025.

Xu, Z-S., Cork, L. C., Griffin, J. W. and Cleveland, D. W. (1993). Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* **73**, 23-33.

Yamasaki, H., Bennett, G. S., Itakura, C. and Mizutani, M. (1992). Defective expression of neurofilament protein subunits in hereditary hypotrophic axonopathy of quail. *Lab. Invest.* **66**, 734-743.