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Microtubule-Associated Protein Tau Epitopes Are Present in Fiber Lesions in Diverse Muscle Disorders

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The microtubule-associated protein tau is a major cytoskeletal protein involved in the neurofibrillary tangles of Alzheimer's disease. Although tau is predominantly a neuronal protein, it has been demonstrated in glia and other nonneuronal cells. We describe the presence of microtubule-associated protein tau epitopes in various muscle fiber lesions in oculopharyngeal and Becker muscular dystrophy, dermatomyositis, central core disease, neurogenic atrophy, and in the recovery phase of an attack of malignant hyperthermia. Western blot demonstrated a 100- to 110-kd tau-immunoreactive protein probably corresponding to 'big tau' as described in peripheral nerves. Tau immunoreactivity in muscle fiber lesions usually co-localized with tubulin, although electron microscopy failed to show an increase in microtubules. Tau and tubulin reactivity also correlated with the presence of desmin and vimentin epitopes. Possible explanations for the presence of tau are briefly discussed. (Am J Pathol 1994, 145:175–188)

The microtubule-associated protein tau is a component of the neuronal cytoskeleton that promotes the assembly and stability of microtubules. Tau also mediates interactions between microtubules and organelles and other parts of the cytoskeleton.[1] The neurofibrillary tangles of Alzheimer's disease are mainly comprised of an abnormally phosphorylated form of tau protein. It has been suggested that the subunit of the paired helical filaments (PHF) forming these neurofibrillary tangles is an antiparallel dimer of the microtubule binding region of tau.[2,3] It was first demonstrated in studies of Alzheimer's disease that tau was not limited to neurons, but could also be expressed in astrocytes.[4] Since then, the presence of tau has been described in avian erythrocytes[5] and in spermatids in mammalian testis.[6] The function of tau in nonneuronal cells is not known but may be similar to that in neurons.

Recently, we have reported that tau immunoreactivity is found in the rimmed vacuoles of oculopharyngeal muscular dystrophy (OPMD).[7] Askanas et al[8] also showed Alz-50 immunoreactivity associated with PHF-like structures in the rimmed vacuoles of inclusion body myositis (IBM). In our previous study, we used a panel of tau antibodies directed to normal and abnormally phosphorylated forms but we could not demonstrate abnormally phosphorylated tau.[7] Because Alz-50 is directed against a nonphosphorylated epitope and recognizes normal and abnormally phosphorylated tau in Alzheimer's disease,[9] we suspected that the tau in rimmed vacuoles would be normal tau. In this study, we demonstrate tau epitopes in various muscle fiber lesions in at least seven different myogenic and neurogenic muscle disorders. The tau found in muscle fibers appears to be the recently described 100- to 110-kd big tau.[10,11] In these lesions, tau expression correlates with the presence of tubulin. Furthermore, desmin and vimentin immunoreactivity is observed in these lesions.

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Table 1. *Clinical Data*

Case	Age	Sex	Muscle	Diagnosis	Pathology
1	62	F	M. Delt. L	OPMD	Rare necrosis, rimmed vacuoles, mottled fibers, most atrophic fibers of type 1
2	67	F	M. Delt. L	OPMD	Rare necrosis, numerous rimmed vacuoles, endomysial fibrosis
3	60	F	M. Delt. L	OPMD	Rare necrosis, rimmed vacuoles, mottled fibers; most atrophic fibers of type 1
4	47	F	M. Delt. L	Myopathy	Increased variation of fiber size, fiber splitting, numerous centralized nuclei, hypertrophic fibers are of type 2
5	29	M	M. Delt. L	Myopathy	Essentially normal muscle biopsy, rare centralized nuclei
6	28	F	M. Vast. Lat. R	Myopathy with cardiac involvement	Increased variation in fiber size, centralized nuclei, necrosis, myophagia, large fibers with disturbed intermyofibrillar pattern, type 2 predominance
7	4 week neonate	F	M. Quadr. L	LCHAD	Essentially normal
8	6	M	M. Vast. Lat. R	DMD	Increased variation in fiber size, atrophy, necrosis, myophagia, basophilic fibers, endomysial fibrosis, numerous type 2C fibers
9	7	M	M. Vast. Lat.	DMD	Numerous necrotic fibers, endomysial fibrosis, basophilic fibers, atrophic and hypertrophic fibers
10	31	M	Unrecorded	BMD	Centralized nuclei, necrosis, myophagia, basophilic fibers, endomysial fibrosis, disturbance of intermyofibrillar pattern in some fibers
11*	6	M	M. Vast. Lat. R	MH	Increased variation of fiber size, centralized nuclei, basophilic fibers, splitting, fiber type grouping, atrophy of type 1 fibers, some type 2C fibers
12	33	M	M. Vast. Lat. R	MH	Essentially normal, few centralized nuclei, no other abnormalities
13	54	F	M. Vast. Lat. L	MH	Essentially normal, few atrophic fibers, mild fiber type grouping
14	68	F	M. Vast. Lat. L	MH	Essentially normal, slight increased variation in fiber size
15	66	M	M. Tib. Ant. L	Neurogenic atrophy	Large field atrophy, hypertrophy, splitting, centralized pseudomyopathic changes, small angulated fibers, many fibers with disturbed intermyofibrillar pattern
16	7	M	M. Vast. Lat. R	Neurogenic atrophy	Field atrophy, angulated fibers, hypertrophic fibers with centralized nuclei, slight inflammatory infiltrate with monocytes-macrophages, endomysial fibrosis
17	58	M	M. Vast. Lat.	Dermatomyositis	Perifascicular atrophy, perifascicular fibers often basophilic, few intrafascicular necrotic fibers, type 2 predominance, slight grouping, mottled fibers
18	75	M	M. Delt. L	IBM	Numerous rimmed vacuoles, some centralized nuclei, no inflammation
19	22	M	M. Vast. Lat. L	Dermatomyositis	Perifascicular atrophy, intrafascicular necrotic fibers, perivascular inflammatory infiltrates with lymphocytes, monocytes, endomysial edema
20	19	M	M. Delt. L	CCD	Necrotic fibers, NADH-TR stain shows numerous central cores, consisting of oval to irregular, slightly PAS-positive structures
21	37	F	M. Delt. L	Acid maltase deficiency	Membrane-bound vacuoles filled with β-glycogen particles in muscle fibers and fibroblasts
22	31	M	M. Vast. Lat. L	BMD	Rare necrotic fibers, small atrophic fibers with nuclear clumps, rare basophilic fibers, type 2 predominance, mottled fibers, some centralized nuclei, rare splitting
23	35	M	M. Vast. Lat. R	Myopathy	Numerous centralized nuclei, increased variation in fiber size, slight endomysial fibrosis
24	4	F	M. Vast. Lat. L	DMD carrier	Numerous necrotic fibers, endomysial fibrosis, basophilic fibers, atrophic and hypertrophic fibers
25	1,5	F	M. Vast. Lat.	Nemaline myopathy	Increased variation in fiber size, normal distribution of fiber types, nemaline bodies

M = male; F = female; M. = musculus; Vast. Lat. = vastus lateralis; Delt. = deltoideus; Quadr. = quadriceps; Tib. Ant. = tibialis anterior; R = right; L = left; OPMD = oculopharyngeal muscular dystrophy; LCHAD = long chain hydroxyacyl-coenzyme A dehydrogenase deficiency; DMD = Duchenne muscular dystrophy; BMD = Becker muscular dystrophy; IBM = inclusion body myositis; MH = malignant hyperthermia susceptible; CCD = central core disease.

* Biopsy taken only 5 weeks after a MH attack.

Table 2. *Antibodies Used*

Antigen	Code	Clonality	Dilution	Source	Reference
Myosin	M-7523	PAb	1/50	Sigma	
Desmin	D 33	MAb	1/200	Dakopatts	14
Vimentin	V9	MAb	1/50	Dakopatts	15
α-Tubulin	DM 1 A	MAb	1/1000	Sigma	16
Actin	JLA20	MAb	1/1000	Amersham	17
MAP-2	HSM11	MAb	1/5000	Innogenetics	18
Tau	rab 220	PAb	1/1000	Innogenetics	19
Tau*	AT8	MAb	1/20,000	Innogenetics	20, 21
Tau	AT 120	MAb	1/5000	Innogenetics	22

PAb = polyclonal antibody; MAb = monoclonal antibody.
* AT8 only recognizes Tau protein when it is phosphorylated at Ser 202 (in Goedert's[23] LMW Tau numbering, corresponding to Ser 447 in 'big' Tau[10,24]).

Table 3. *Immunocytochemistry Results*

Case	Diagnosis	Tau (rab220)	Tau (AT120)	Tau (AT8)	Vimentin	Desmin	Tubulin
1	OPMD	+	+	–	+	+	+
2	OPMD	+	+	–	+	+	+
3	OPMD	+	+	ND	+	+	+
4	Myopathy	+	+	–	+	+	+
5	Myopathy	–	–	–	+‡	–	+‡
6	Myopathy with cardiac involvement	++	++	–	++	++	++
7	LCHAD	–	–	–	–	–	–
8	DMD	–	–	–	ND	ND	ND
9	DMD	–	–	–	+++	+	++
10	BMD	–	–	–	ND	ND	ND
11†	MH	+	+	ND	++	+	+
12	MH	–	–	–	–	–	–
13	MH	–	–	–	–	–	–
14	MH	–	–	–	–	–	–
15	Neurogenic atrophy	++	+	–	++	++	+
16	Neurogenic atrophy	–	–	–	–	–	–
17	Dermatomyositis	+	+	–	+	+	+
18	IBM	–	–	–	–	–	–
19	Dermatomyositis	+++	+++	–	+++	+++	+++
20	CCD	+++	++	–	–	++	+
21	Acid maltase deficiency	–	–	–	–	–	–
22	BMD	+	+	–	+	+	+
23	Myopathy	+‡	+‡	–	+‡	+‡	+‡
24	DMD carrier	–	–	ND	+++	++	+++
25	Nemaline myopathy	–	–	ND	–	–	–

OPMD = oculopharyngeal muscular dystrophy; LCHAD = long chain hydroxyacyl-coenzyme A deficiency; DMD = Duchenne muscular dystrophy; BMD = Becker muscular dystrophy; MH = malignant hyperthermia susceptible; IBM = inclusion body myositis; CCD = central core disease. +, Rare positive lesions (less than 1 lesion/field); ++, frequent positive lesions (1 to 10 lesions/field); +++, abundant positive lesions (more than 10 lesions/field); ‡, only a single positive fiber was observed.

Materials and Methods

Tissue

Muscle biopsies were obtained from 25 patients with a variety of muscle disorders. Clinical data are summarized in Table 1. One (patient 11) of four patients susceptible to malignant hyperthermia (MH), as demonstrated by the standardized contracture assay,[12] was biopsied during the recovery phase of an attack. The diagnosis of three patients with myopathy and of one patient with cardiomyopathy could not be further specified on the basis of the muscle biopsy alone. The neurogenic muscle changes in the two patients with neurogenic atrophy were examined on the occasion of a nerve biopsy.

Before immunohistochemical study, muscle biopsies were examined with a standard series of staining techniques including hematoxylin and eosin, trichrome Masson, periodic acid-Schiff (PAS), PAS after amylase treatment, Oil Red O, and enzyme histochemical techniques for succinate dehydrogenase, NADH tetrazolium reductase, menadione α-glycerophosphate dehydrogenase, phosphorylase, and ATPase.[13]

Immunocytochemistry

Muscle biopsies were snap-frozen in liquid nitrogen-cooled isopentane. Cryostat sections (8 μ) were cut and fixed for 10 minutes in acetone or left unfixed and

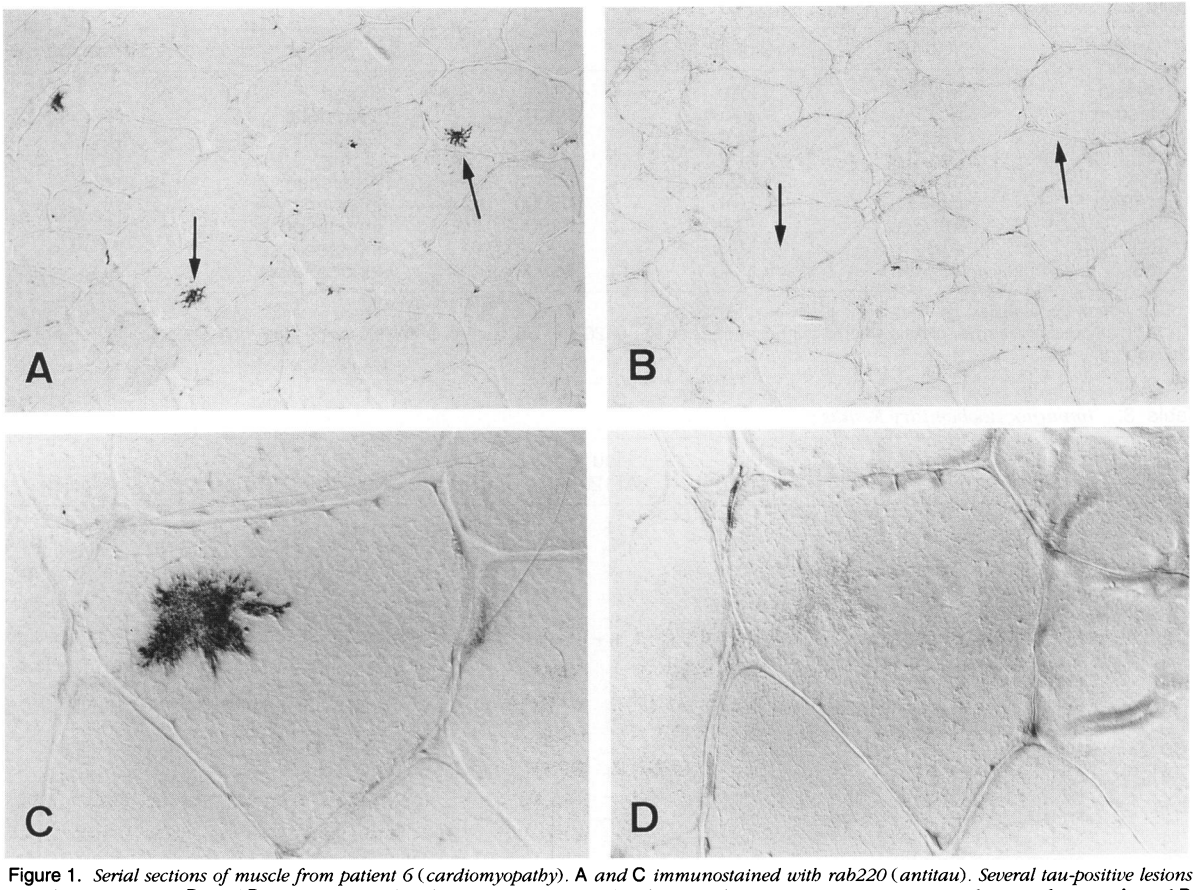


Figure 1. *Serial sections of muscle from patient 6 (cardiomyopathy).* **A** *and* **C** *immunostained with rab220 (antitau). Several tau-positive lesions are shown (arrows).* **B** *and* **D** *immunostained with HSM11 (anti-MAP-2). The same lesions are not immunostained. Magnification:* **A** *and* **B** *×108;* **C** *and* **D** *×436.*

incubated with antibodies against cytoskeletal proteins. The antibodies used are listed in Table 2. The AT8 antibody recognizes a phosphorylation-dependent epitope found only in Alzheimer's disease and in fetal tau.[20,21,25] The AT120 antibody is a subclone of the AT12 antibody previously described.[20,22] Like AT12, it is not phosphatase sensitive and recognizes all forms of tau indiscriminately. Sections from a few biopsies were subjected to phosphatase treatment according to Sternberger.[20,26] For control experiments the immunostaining of the tau antibody (AT 120) was absorbed by prior incubation with purified human tau prepared as described.[19]

Sections were incubated overnight at room temperature with primary antibodies. Tissues were immunostained with the peroxidase-antiperoxidase (PAP) technique[27] for the polyclonal antibodies and with the avidin-biotin-complex (ABC) technique[28] for the monoclonal antibodies using Dakopatts (Glostrup, Denmark) and Amersham (Amersham, UK) reagents, respectively. Color was developed with 3,3′-diaminobenzidine (DAB) tetrahydrochloride (Janssen Chimica, Geel, Belgium). Sections were counterstained with Harris' hematoxylin (Sigma, St Louis, MO), dehydrated, coverslipped with Eukitt (O. Kindler, Freiburg, Germany), and viewed in a light microscope.

Electron Microscopy

For electron microscopic examination, muscle tissue from patients 6, 16, and 18 to 25 was fixed in 4% glutaraldehyde and postfixed in 2% osmium tetroxide, dehydrated in graded alcohols, and embedded in Araldite (Fluka, Buchs, Switzerland). Ultrathin sections were stained with 2% uranylacetate and lead citrate and observed in a Philips CM 10 electron microscope at 60 kV.

Western Blot

Cryosections (20 μ) from muscle biopsies 6, 13, 17, 19, and 20 were homogenized in sodium dodecyl sulfate (SDS) extraction buffer, placed in a boiling water bath for 10 minutes, and centrifuged. Supernatants

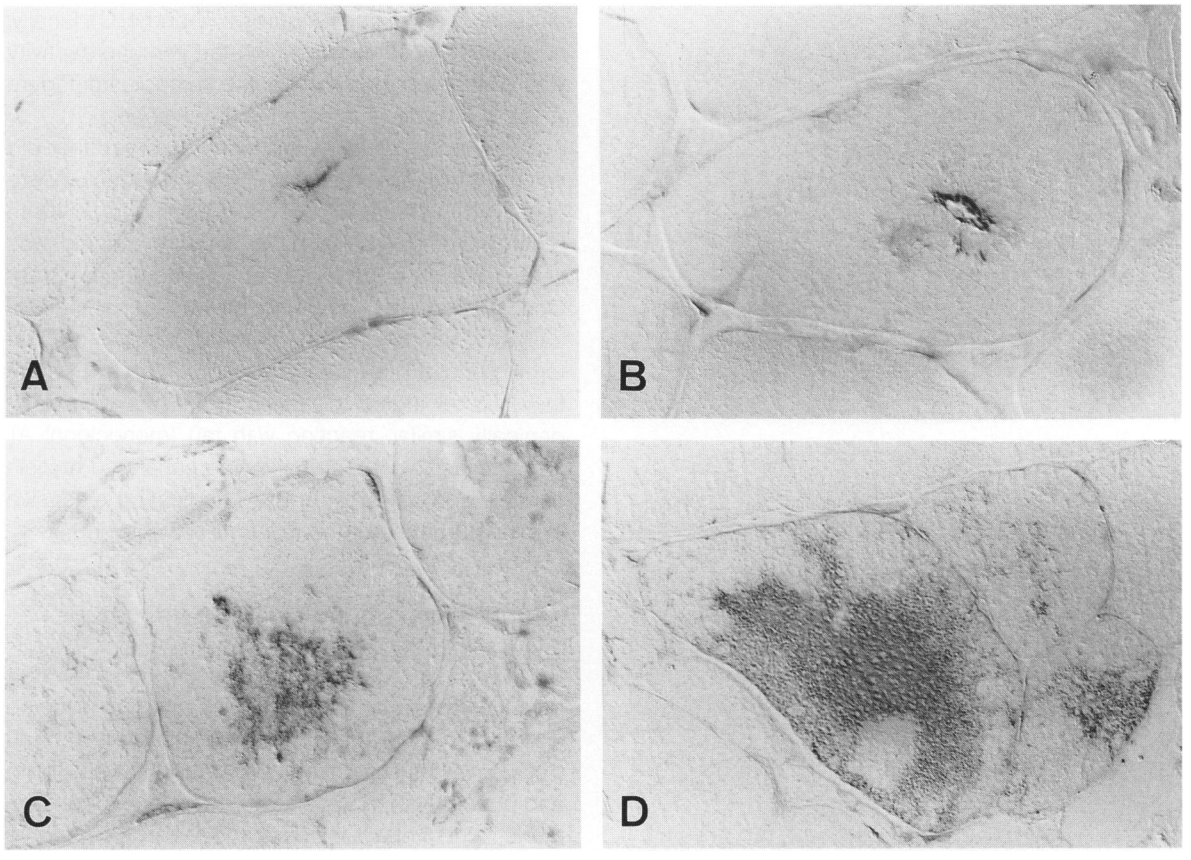


Figure 2. *Morphological diversity of tau-positive lesions.* A: *Small centrally located focal lesion (core) in patient 20 (CCD) immunostained with AT120.* B: *Focal lesion with clear center in patient 23 (myopathy) immunostained with rab220.* C: *Larger central lesion in patient 19 (dermatomyositis) immunostained with AT120.* D: *Spreading of tau immunoreactivity (AT120) over a large area of the sarcoplasm in patient 2 (oculopharyngeal muscular dystrophy). Magnification (all):* ×436.

containing 100 μg of protein were loaded on a 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE was performed according to the method of Laemmli[29] and protein was transferred to nitrocellulose (Hybond-C; Amersham). Blotted proteins were incubated with primary antibodies (rab220 1/200, AT 120 1/1000, tubulin 1/50, and actin 1/2000) overnight at 4 C and detected with the ABC technique using Biogenex (San Ramon, CA) reagents. DAB was used as chromogen for peroxidase-labeled conjugate and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT; Boehringer Mannheim, Mannheim, Germany) as chromogen for alkaline phosphatase-labeled conjugate.

Results

Tau Immunoreactivity in Muscle Fibers

As summarized in Table 3, intrafibrillar lesions immunoreactive with AT120 and rab220 antitau antibodies were observed in the following patients: 1, 2, and 3 (OPMD); 4 and 6 (myopathy); 11 (post-MH); 15 (neurogenic atrophy); 17 and 19 (dermatomyositis); 20 (central core disease, CCD); 22 (Becker muscular dystrophy); and 23 (myopathy).

The phosphorylation-dependent antibody AT8, on the other hand, did not stain any of these lesions. No significant difference in immunostaining was observed after alkaline phosphatase treatment of the sections (data not shown). Omission of the fixation step did not influence the intensity of immunostaining. Serial sections stained with the anti-MAP-2 antibody HSM11 were negative, confirming the specificity of the tau immunostaining (Figure 1).

Morphological Diversity of Lesions Showing Tau Immunoreactivity

Tau-immunoreactive structures ranged along a continuum of morphologies, from focal lesions to diffuse staining of the entire fiber (Figure 2). Focal lesions showed a similar aspect in different disorders and were often centrally located (Figure 2, A, B). These

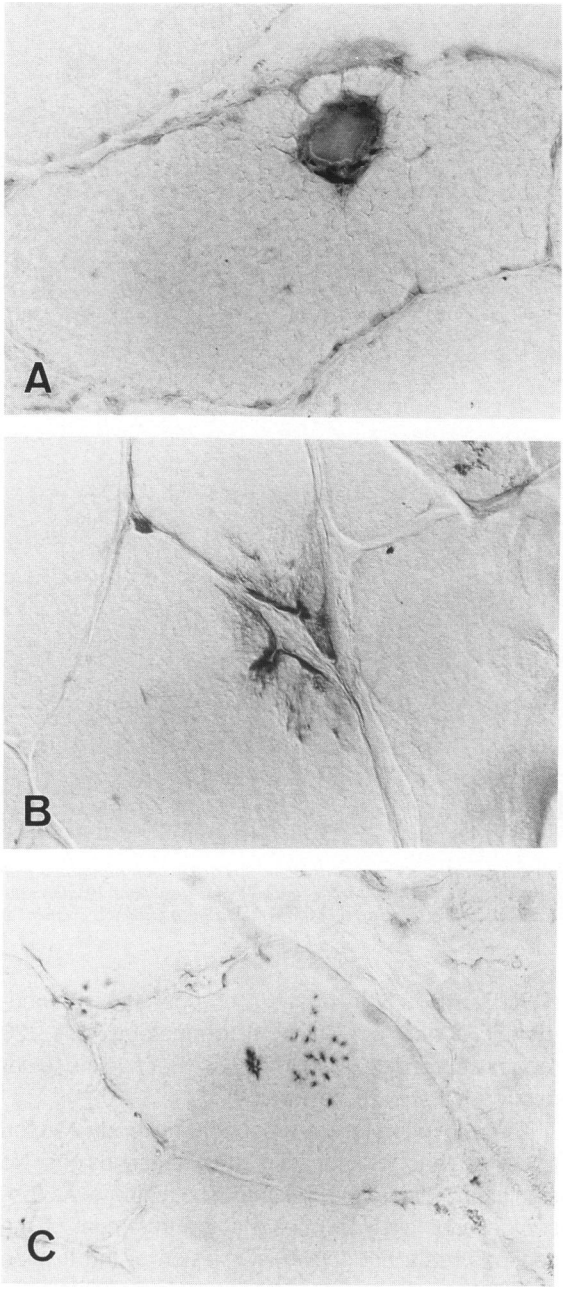


Figure 3. *Other tau-immunoreactive structures.* A: *Rimmed vacuole-like structure in patient 2 (OPMD) immunostained with AT120.* B: *Subsarcolemmal tau immunoreactivity (rab220) in two adjacent fibers, possibly a consequence of fiber splitting, in patient 22 (Becker muscular dystrophy).* C: *Punctate tau (rab220)-positive deposits and a small focal lesion in patient 15 (neurogenic atrophy). Magnification (all): ×436.*

lesions seemed to correspond to cores in CCD (Figure 2, A) and target or targetoid fibers in neurogenic atrophy (data not shown)[13] but were also seen in other disorders (Figures 1, C and 2, B). They often showed a clear central area (Figure 2, B). Other lesions, though still somewhat centrally located, showed a more diffuse or heterogenous immunostaining over a larger area of the fiber (Figures 2, C and 4, C). Finally, fibers were observed in which tau immunoreactivity was distributed over most of the sarcoplasm (Figure 2, D), giving these fibers a 'mottled' aspect.[13]

Other tau-positive lesions were observed that did not easily fit in this continuum. Rimmed vacuoles were observed in all patients with OPMD (Figure 3, A). In some patients, local subsarcolemmal staining was seen, possibly a result of fiber splitting (Figure 3, B). Occasionally, tau immunoreactivity was neither focally nor diffusely distributed but consisted of clustered punctate tau deposits (Figure 3, C). Despite their morphological diversity, all lesions were antigenically similar, reacting with tau (monoclonal AT 120 and polyclonal) and showing enhanced myosin immunoreactivity (Figure 5, A), indicating extensive local disturbance of the myofibrillary network.

Tau Immunoreactivity Correlates with Tubulin

Immunostaining by the antitubulin antibody revealed lesions very similar to those demonstrated with tau (Figure 4). Lesions observed included focal, diffuse, and punctate morphologies. Furthermore, tubulin immunostaining of whole fibers was also noted (Figures 4, B and 7, A). The latter were usually small and often more basophilic than adjacent tubulin-negative fibers. Serial sections showed that tau-immunoreactive lesions almost invariably contained tubulin, confirming the correlation between tau and tubulin (Figure 5, B to D). Electron microscopic observations showed only sporadic single microtubules between the myofibrils, as previously described in normal skeletal muscle by Kano et al (data not shown).[30] One highly disturbed fiber in patient 6 contained focal aggregations of microtubules (Figure 6).

Tau and Tubulin Immunoreactivity Correlate with Desmin and Vimentin

Antivimentin antibodies intensely immunostained occasional small basophilic fibers (Figure 7, B). Antidesmin antibodies diffusely stained normal muscle fibers, owing to their normal reactivity with the intermyofibrillar network, but some small basophilic fibers were stained far more strongly (data not shown). Moreover, focal and diffuse lesions similar to those stained by antitubulin and antitau were also immunoreactive with antidesmin and antivimentin. Serial sections established a correlation in the same lesions between tau and tubulin immunoreactivity on the one hand, and desmin and vimentin immunoreactivity on

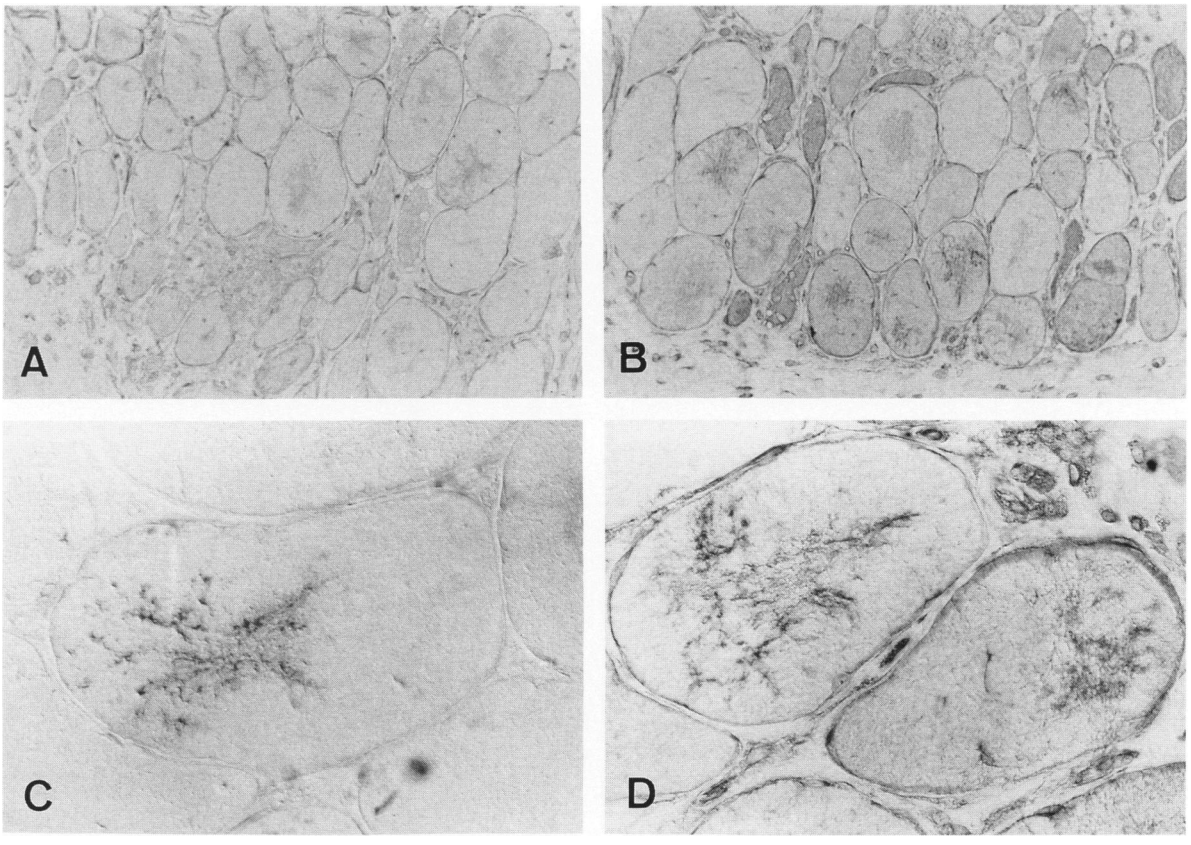


Figure 4. *Comparison of tau and tubulin immunoreactivity.* **A:** *Muscle biopsy of patient 19 (dermatomyositis) showing tau (rab220) immunoreactivity in several fibers.* **B:** *Another section (not serial) from the same biopsy showing tubulin immunoreactivity. Note the similarity of positive structures.* **C:** *Tau (AT120)-immunoreactive diffuse lesion from section, (A).* **D:** *Similar tubulin-immunoreactive lesions from section (B). Magnification:* **A** *and* **B** *×117;* **C** *and* **D** *×436.*

the other (Figure 5). A good correlation was also found between tubulin and desmin/vimentin in whole fibers, though these were not always tau immunoreactive (Figure 7).

Tau Detection on Western Blot Demonstrates a 100- to 110-kd protein

Western blot on homogenates of several biopsies demonstrated the presence of actin (42 kd; Figure 8, lane 2), tubulin (50 kd; Figure 8, lane 1), and tau bands (Figure 8, lanes 3 to 8). Some bands of lower molecular weight, probably degradation products, were also stained. The antiactin antibody appeared to cross-react with tubulin, but this may be an aspecific reaction because the antibody in question is an IgM.[17] The tau bands were located in the molecular weight range 100 to 110 kd, corresponding to the big tau described in the peripheral nervous system.[10,11] Tau bands were clearly seen in all five patients examined, including a patient without observable histological abnormalities. The tau bands were more intensely stained in a patient showing numerous positive lesions by immunocytochemistry.

Discussion

Studies on the microtubule-associated protein tau have hitherto always been focused on the nervous system, where microtubules and their associated proteins play a crucial role in maintaining the integrity of the neurons with their long and vulnerable neurites. Indeed, tau has been specifically implicated in the pathology of Alzheimer's disease, which is marked by extensive modification and disruption of the neuronal cytoskeleton.

The occurrence of tau outside nervous tissues has only rarely been reported.[5,6] An isolated study[31] reported the presence of MAP-2, a protein partially homologous to tau,[32] in several nonneuronal tissues, including heart muscle. It would be premature, however, to extrapolate these results to tau because, even in the nervous system, the distributions of tau and MAP-2 are significantly different.[33,34] Our own

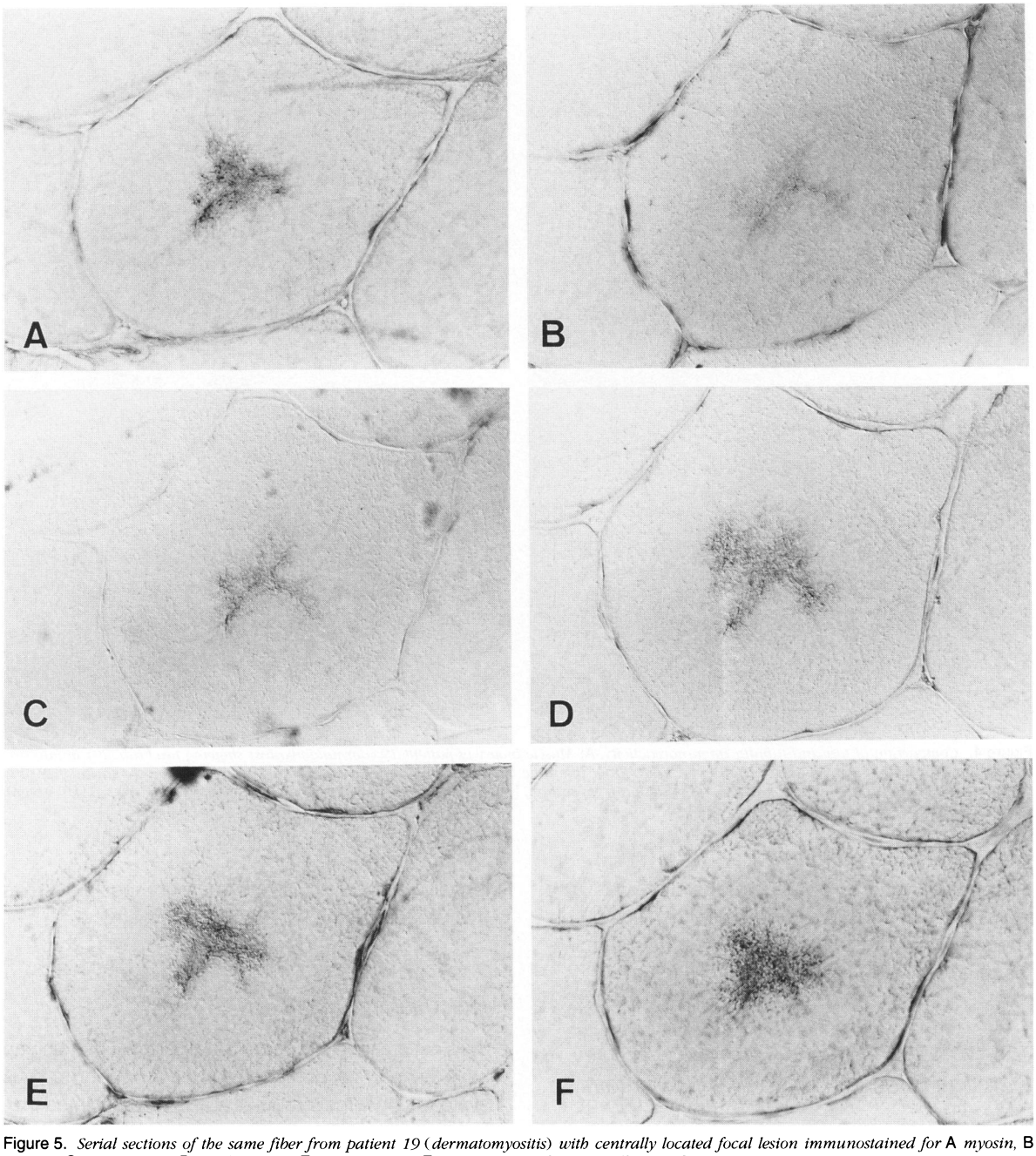


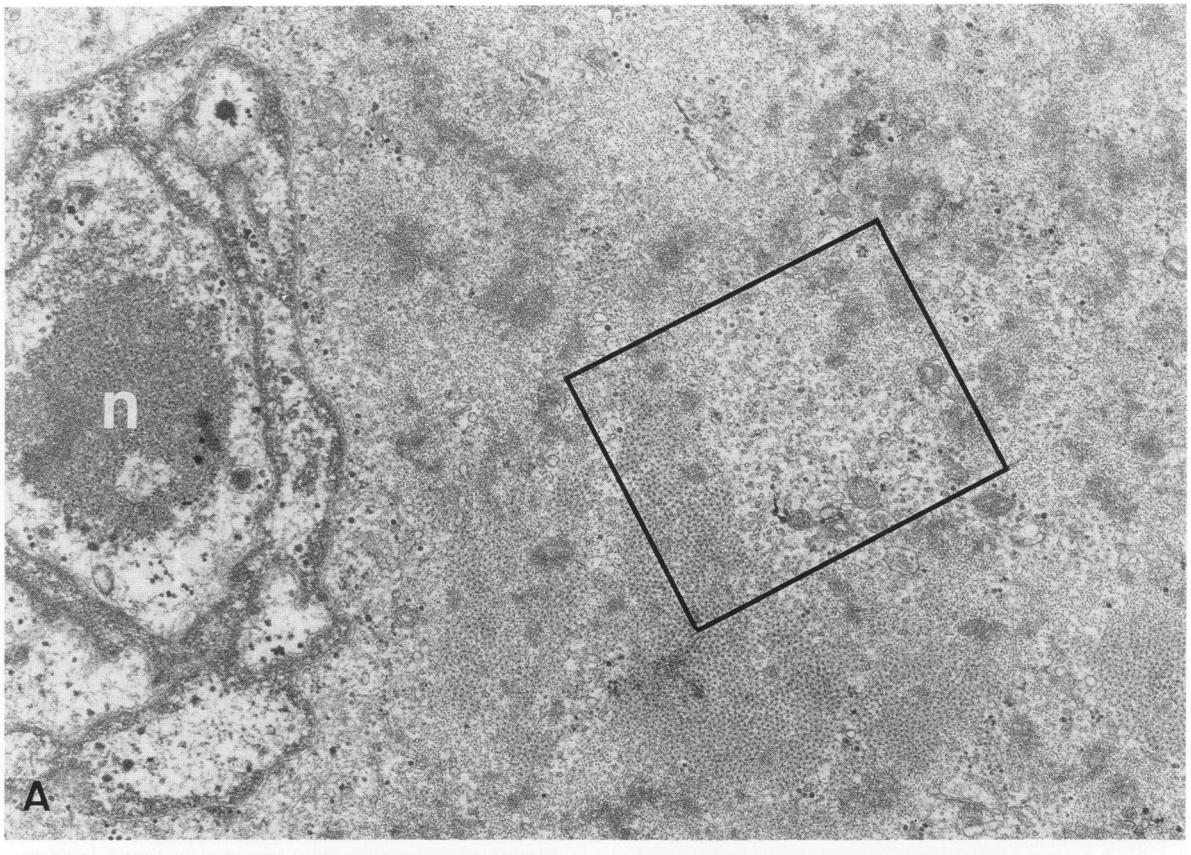
Figure 5. *Serial sections of the same fiber from patient 19 (dermatomyositis) with centrally located focal lesion immunostained for* A *myosin,* B *tubulin,* C *tau (AT120),* D *tau (rab220),* E *vimentin, and* F *desmin. Magnification (all): ×436.*

antibody HSM11[18] failed to detect MAP-2 in normal or diseased skeletal muscle.

We demonstrated the presence of tau epitopes immunohistochemically in diverse lesions in several unrelated muscle disorders. The diversity of the lesions and diseases in which this reaction was observed suggests that tau is quite common in damaged muscle fibers and, contrary to previous suggestions,[7,8] is not limited to vacuolated muscle fibers.

As might be expected from a microtubule-associated protein, tau reactivity correlated well with the presence of tubulin. However, electron microscopy did not show a significant increase of microtubules, except for a single, possibly atypical fiber. This

Figure 6. *Electron micrograph of lesioned fiber in patient 6 (cardiomyopathy).* A: *General view showing disturbed sarcoplasm with groups of myofibrils interspersed with clusters of microtubules. n = nucleus.* B: *Higher magnification of bracketed area in* A *showing clustered microtubules. Magnification:* A *25 × 840;* B *80 × 500.*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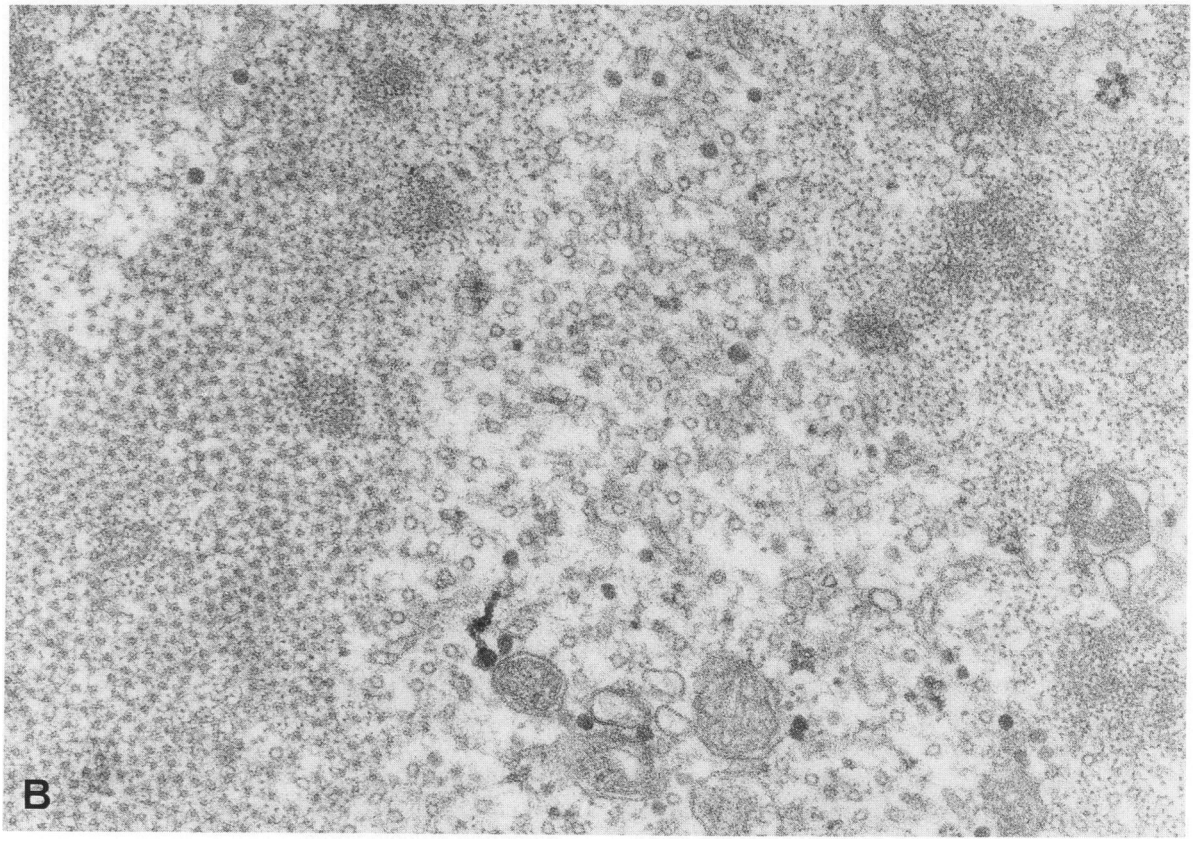
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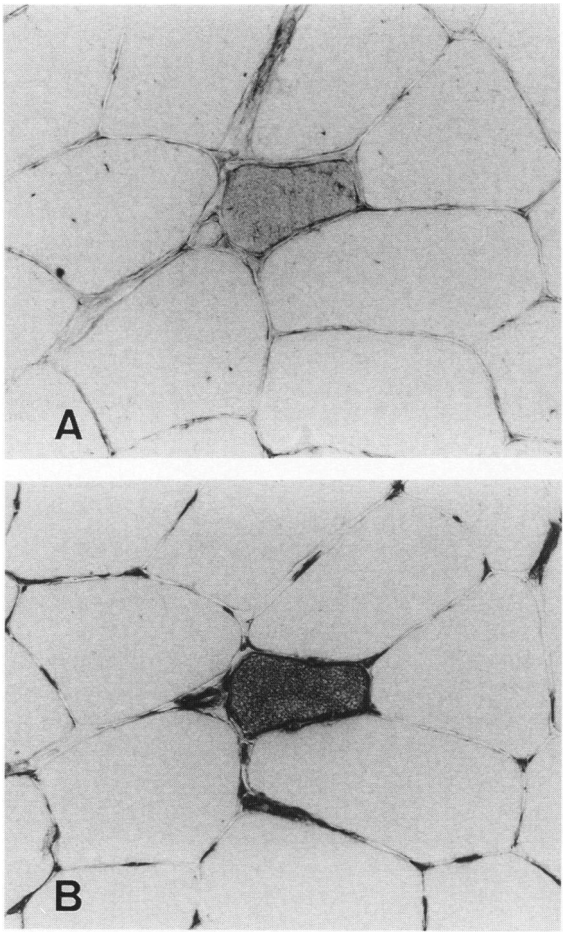

Figure 7. *Serial sections from patient 22 (Becker muscular dystrophy) showing whole fiber staining for* A *tubulin* B *vimentin. Magnification (all):* ×*182.*

lack of microtubules may indicate that the tubulin detected by immunocytochemistry is present mainly in free monomeric or dimeric form in the sarcoplasm. Alternatively, any microtubules present may be so ephemeral as to elude detection. Microtubules can be very unstable[35,36] and although tau enhances microtubule assembly, it does not prevent dissociation of microtubules.[37]

The presence of arrays of microtubules in diseased muscle fibers has been described before, although anecdotally.[38] It is rare for microtubules to be observed in normal adult skeletal muscle where the myofibrils and their associated proteins nearly completely fill the sarcoplasm and microtubules are restricted to a subsarcolemmal lattice work, with no more than the occasional single microtubule penetrating deeper into the sarcoplasm.[30,39] However, extensive networks of microtubules have been observed transiently in muscle tissues during development[40,41] and during regeneration after amputation[42] or drug-induced destruction of the sarcoplasmic cytoskeleton.[43,44] It has been hypothesized that these microtubules serve as a temporary scaffolding during myogenesis and regeneration, acting as a template for the spatial organization of the developing myofibrils.[45] Although we have not observed such a microtubule network, the possible presence of short-lived microtubules in the lesions could explain the presence of tau. Interestingly, one of the few instances where tau has been described outside the nervous system involves spermatids in mammalian testis[6] where tau is associated with the manchette, a transient microtubule network possibly involved in the extensive reshaping that takes place in developing spermatids.

The presence of tau and tubulin in muscle fiber lesions also correlated with desmin and vimentin immunoreactivity. Both desmin[38,46] and vimentin[46,47] are often considered to be indicative of muscle fiber regeneration. Notably, besides small basophilic regenerating fibers, Misra et al[47] also described focal lesions similar to ours, which they named perinuclear in a patient with myotubular myopathy. However, our focal lesions did not appear associated with nuclei, although they sometimes showed a clear central area. These observations suggest that the presence of tau and tubulin could be associated with a regenerative or developmental process in these lesions.

The presence of tau could be solely due to its association with tubulin. Although tau has not been described in normal muscle, it could be present in amounts too minute to be detected immunocytochemically by our antibodies. Tau could be demonstrated by Western blot even in a malignant hyperthermia-susceptible but histologically normal patient. In some instances tau was seen in a subsarcolemmal distribution, which would probably reflect the distribution of tubulin.[30,39] Sometimes, the subsarcolemmal staining that was observed could also be ascribed to fiber splitting.

On the other hand, tau might also play an active role in these lesions. There is some evidence that tau mediates interactions between microtubules and other cytoskeletal structures, organelles, and membranes. Tau or tau-related proteins have been reported to bind to actin[48–50] and neurofilament proteins,[51,52] to calcium binding proteins such as calmodulin[53,54] and S100,[55,56] to mitochondrial membranes[57,58] and other membranous organelles,[59,60] possibly through other proteins such as spectrin,[61] and possibly even to nucleic acids in ribosomes[62] and nuclei.[63,64] Many of these putative functions for tau are of potential significance in either normal or damaged muscle fibers.

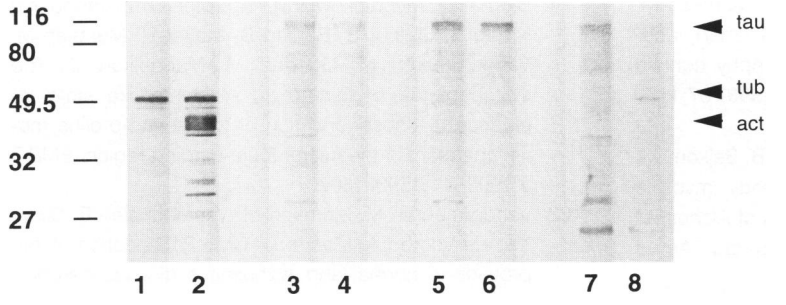

Figure 8. *Immunoblot of muscle tissue homogenates from three biopsies. Lanes 1, 2, 5, and 6: patient 19 (dermatomyositis); lanes 3 and 4: patient 13 (malignant hyperthermia susceptible but histologically normal); lanes 7 and 8: patient 20 (CCD) immunostained with antitubulin (lane 1), antiactin (lane 2), antitau (AT120) (lanes 3, 5, and 7), antitau (rab220) (lanes 4, 6, and 8). Arrowheads at right designated HMW tau (tau), tubulin (tub), and actin (act) bands. Molecular weight markers (in kd) are at left.*

It may also be significant that tau protein has been implicated in nervous system development.[65,66] At least some of this development-related tau protein appears to be modified by phosphorylation in the same way as in Alzheimer's disease.[25,67] The tau we have detected in muscle, however, does not appear to be phosphorylated in this way, as demonstrated by the lack of AT8 immunoreactivity. The AT8 antibody recognizes a specific site (Ser 202 in LMW tau,[23] which is equivalent to Ser 447 in big tau[10,24]) only when it is phosphorylated, ie, in Alzheimer's disease and fetal tissue, but not in normal brain.[21,25] In this respect at least, the phosphorylation state of tau in these muscle disorders differs from that of PHF tau in Alzheimer's disease. Moreover, phosphatase treatment did not influence the immunoreactivity of these lesions. We therefore conclude that, contrary to the findings of Askanas et al,[8] phosphorylation state of tau in these muscle fibers is different from that in PHF and fetal tau. Nevertheless, the presence of tau might indicate a reactivation of developmental mechanisms over a broad range of pathologies.

Recent observations on β/A4 amyloid precursor protein (βAPP) provide an interesting parallel to this study. The involvement of βAPP was described in regenerative processes in a number of different muscle diseases.[68] The close spatial association of βAPP with tau proteins has been demonstrated both in Alzheimer's disease[69,70] and OPMD/IBM.[7,71] Thus, these findings may provide independent evidence for the involvement of tau in regenerative and developmental processes in muscle disorders.

In conclusion, we have demonstrated the presence of tau epitopes in a number of different muscle fiber lesions. The presence of tubulin, which has been implicated in myogenesis and regeneration of muscle fibers, and the correlation with desmin and vimentin immunoreactivity all suggest a regenerative or developmental process possibly involving the formation of a short-lived microtubule network in which tau protein could play a role. More generally, because a considerable number of different interactions between microtubules and other structures may be mediated by tau protein, many different potential roles for tau protein in normal and diseased muscle could be imagined. Further studies are in progress to determine the full significance of these observations.

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References

1. Goedert M, Crowther RA, Garner CC: Molecular characterization of microtubule-associated proteins tau and MAP2. Trends Neurosci 1991, 14:193–199
2. Ksiezak-Reding H, Yen SH: Structural stability of paired helical filaments requires microtubule-binding domains of tau: a model for self-association. Neuron 1991, 6:717–728
3. Wille H, Drewes G, Biernat J, Mandelkow EM, Mandelkow E: Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau in vitro. J Cell Biol 1992, 118: 573–584
4. Papasozomenos SC: Tau protein immunoreactivity in dementia of the Alzheimer type I: morphology, evolution, distribution and pathogenetic implications. Lab Invest 1989, 60:123–137
5. Murphy DB, Wallis KT: Erythrocyte microtubule assembly in vitro: determination of the effects of erythrocyte tau, tubulin isoforms, and tubulin oligomers on erythrocyte tubulin assembly and comparison with brain microtubule assembly. J Biol Chem 1985, 260:12293–12301
6. Ashman JB, Hall ES, Eveleth J, Boekelheide K: Tau, the neuronal heat-stable microtubule-associated protein, is also present in the cross-linked microtubule network of the testicular spermatid manchette. Biol Reprod 1992, 46:120–129

7. Villanova M, Lübke U, Vandermeeren M, Mercken M, Guazzi GC, Martin JJ, Perry G, Cras P: Rimmed vacuoles in oculopharyngeal muscular dystrophy contain amyloid precursor protein. Adv Biosci 1993, 87:157–158
8. Askanas V, Engel WK, Bilak M, Alvarez RB, Selkoe DJ: Twisted tubulofilaments of inclusion body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. Am J Pathol 1994, 144:177–187
9. Ksiezak-Reding H, Davies P, Yen SH: Alz-50, a monoclonal antibody to Alzheimer's disease antigen cross-reacts with tau proteins from bovine and normal human brain. J Biol Chem 1988, 263:7943–7947
10. Goedert M, Spillantini MG, Crowther RA: Cloning of a big tau microtubule-associated protein characteristic of the peripheral nervous system. Proc Natl Acad Sci USA 1992, 89:1983–1987
11. Georgieff I, Liem RKH, Mellado W, Nunez J, Shelanski ML: High molecular weight tau: preferential localization in the peripheral nervous system. J Cell Sci 1991, 100:55–60
12. European MH Group: A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. Br J Anaesth 1984, 56:1267–1269
13. Dubowitz V, Sewry CA, Fitzsimons RB: Muscle biopsy: a practical approach, ed 2. London, Baillière Tindall, 1985
14. Van Muijen GNP, Ruiter DJ, Warnaar SO: Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 1987, 57:359–369
15. Osborn M, Debus E, Weber K: Monoclonal antibodies specific for vimentin. Eur J Cell Biol 1984, 34:137–143
16. Blose SH, Meltzer DI, Feramisco JR: 10-nm filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. J Cell Biol 1984, 98:847–858
17. Lin JJC: Mapping structural proteins of cultured cells by monoclonal antibodies. Cold Spring Harbor Symp Quant Biol 1982, 46:769–783
18. Six J, Lübke U, Mercken M, Vandermeeren M, Ceuterick C, Van de Voorde A, Boons J, Gheuens J: Specific monoclonal antibodies against normal microtubule-associated protein-2 (MAP2) epitopes present in Alzheimer pathological structures do not recognize paired helical filaments. Acta Neuropathol 1992, 83:179–189
19. Mercken M, Vandermeeren M, Lübke U, Six J, Boons J, Vanmechelen E, Van de Voorde A, Gheuens J: Affinity purification of human tau proteins and the construction of a sensitive sandwich enzyme-linked immunosorbent assay for human tau detection. J Neurochem 1992, 58: 548–553
20. Mercken M, Vandermeeren M, Lübke U, Six J, Boons J, Van de Voorde A, Martin JJ, Gheuens J: Monoclonal antibodies with selective specificity for Alzheimer tau are directed against phosphatase-sensitive epitopes. Acta Neuropathol 1992, 84:265–272
21. Biernat J, Mandelkow EM, Schröter C, Lichtenberg-Kraag B, Steiner B, Berling B, Meyer H, Mercken M, Vandermeeren M, Goedert M, Mandelkow E: The switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the microtubule-binding region. EMBO J 1992, 11:1593–1597
22. Vandermeeren M, Mercken M, Vanmechelen E, Six J, Van de Voorde A, Martin JJ, Cras P: Detection of Tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme linked immunosorbent assay. J Neurochem 1993, 61:1828–1834
23. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA: Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron 1989, 3:519–526
24. Georgieff IS, Liem RKH, Couchie D, Mavilia C, Nunez J, Shelanski ML: Expression of high molecular weight tau in the central and peripheral nervous systems. J Cell Sci 1993, 105:729–737
25. Goedert M, Jakes R, Crowther RA, Six J, Lübke U, Vandermeeren M, Cras P, Trojanowski JQ, Lee VMY: The abnormal phosphorylation of tau protein at Ser 202 in Alzheimer's disease recapitulates phosphorylation during development. Proc Natl Acad Sci USA 1993, 90:5066–5070
26. Sternberger LA, Sternberger NH: Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. Proc Natl Acad Sci USA 1983, 80:6126–6130
27. Sternberger LA, Hardy PHjr, Cuculis JJ, Meyer HG: The unlabeled antibody enzyme method of immunohistochemistry: preparations and properties of soluble antigen-antibody complexes (horseradish peroxidase-anti peroxidase) and its use in the identification of spirochetes. J Histochem Cytochem 1970, 18:315–333
28. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
29. Laemmli UK: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970, 227:680–685
30. Kano Y, Fujimaki N, Ishikawa H: The distribution and arrangement of microtubules in mammalian skeletal muscle fibers. Cell Struct Funct 1991, 16:251–261
31. Wiche G, Briones E, Koszka C, Artlieb U, Krepler R: Widespread occurrence of polypeptides related to neurotubule-associated proteins MAP1 and MAP2 in non-neuronal cells and tissues. EMBO J 1984, 3:991–998
32. Lewis SA, Wang D, Cowan NJ: Microtubule-associated protein MAP2 shares a microtubule-binding motif with tau protein. Science 1988, 242:936–939

33. Trojanowski JQ, Schuck T, Schmidt ML, Lee VMY: Distribution of phosphate-independent MAP2 epitopes revealed with monoclonal antibodies in microwave-denatured human nervous system tissues. J Neurosci Methods 1989, 29:171–180
34. Trojanowski JQ, Schuck T, Schmidt ML, Lee VMY: Distribution of tau proteins in the normal human central and peripheral nervous system. J Histochem Cytochem 1989, 37:209–215
35. Salmon ED, McKeel M, Hays T: Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. J Cell Biol 1984, 99:1066–1075
36. Sammak PJ, Borisy GG: Direct observation of microtubule dynamics in living cells. Nature 1988, 332:724–726
37. Pryer NK, Walker RA, Skeen VP, Bourns BD, Soboeiro MF, Salmon ED: Brain microtubule-associated proteins modulate dynamic instability in vitro: real-time observations using video microscopy. J Cell Sci 1992, 103: 965–976
38. Thornell LE, Edström L, Eriksson A, Henriksson KG, Ängqvist KA: The distribution of intermediate filament protein (skeletin) in normal and diseased human skeletal muscle: an immunohistochemical and electron microscopic study. J Neurol Sci 1980, 47:153–170
39. Boudriau S, Vincent M, Côté CH, Rogers PA: Cytoskeletal structure of skeletal muscle: identification of an intricate exosarcomeric microtubule lattice in slow- and fast-twitch muscle fibers. J Histochem Cytochem 1993:1013–1021
40. Auber J: La myofibrillogenèse du muscle strié I. Insectes. J Microsc 1969, 8:197–232
41. Gundersen GG, Khawaja S, Bulinski JC: Generation of a stable posttranslationally modified microtubule array is an early event in myogenic differentiation. J Cell Biol 1989, 109:2275–2288
42. Warren RH: Microtubular organization in elongating myogenic cells. J Cell Biol 1974, 63:550–566
43. Antin PB, Forry-Schaudies S, Friedman TM, Tapscott SJ, Holtzer H: Taxol induces postmitotic myoblasts to assemble interdigitating microtubule-myosin arrays that exclude actin filaments. J Cell Biol 1981, 90:300–308
44. Toyama Y, Forry-Schaudies S, Hoffman B, Holtzer H: Effects of taxol and colcemid on myofibrillogenesis. Proc Natl Acad Sci USA 1982, 79:6556–6560
45. Holtzer H, Forry-Schaudies S, Dlugosz A, Antin P, Dubyak G: Interactions between intermediate filaments, microtubules and myofibrils in fibrogenic and myogenic cells. Ann NY Acad Sci 1985, 455:106–125
46. Gallanti A, Prelle A, Moggio M, Ciscato P, Checcarelli N, Sciacco M, Comini A, Scarlato G: Desmin and vimentin as markers of regeneration in muscle diseases. Acta Neuropathol 1992, 85:88–92
47. Misra AK, Menon NK, Mishra SK: Abnormal distribution of desmin and vimentin in myofibers in adult onset myotubular myopathy. Muscle Nerve 1992, 15:1246–1252
48. Griffith LM, Pollard TD: Evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins. J Cell Biol 1978, 78:958–965
49. Correas I, Padilla R, Avila J: The tubulin-binding sequence of brain microtubule-associated proteins tau and MAP2 is also involved in actin binding. Biochem J 1990, 269:61–64
50. Yamauchi PS, Purich DL: Microtubule-associated protein interactions with actin filaments: evidence for differential behavior of neuronal MAP2 and tau in the presence of phosphatidyl-inositol. Biochem Biophys Res Commun 1993, 190:710–715
51. Miyata Y, Hoshi M, Nishida E, Minami Y, Sakai H: Binding of microtubule-associated protein 2 and tau to the intermediate filament reassembled from neurofilament 70 kDa subunit protein and its regulation by calmodulin. J Biol Chem 1986, 261:13026–13030
52. Flynn G, Joly JC, Purich DL: The 28 000 Mr microtubule-binding domain of microtubule-associated protein 2 also contains a neurofilament-binding site. Biochem Biophys Res Commun 1987, 148:1453–1459
53. Lee YC, Wolff J: Calmodulin binds to both microtubule-associated protein 2 and tau proteins. J Biol Chem 1984, 259:1226–1230
54. Padilla R, Maccioni RB, Avila J: Calmodulin binds to a tubulin binding site of the microtubule-associated protein tau. Mol Cell Biochem 1990, 97:35–41
55. Baudier J, Cole RD: Interactions between the microtubule-associated tau proteins and S100b regulate tau phosphorylation by the Ca^{2+}/calmodulin-dependent protein kinase II. J Biol Chem 1988, 263: 5876–5883
56. Donato R, Giambanco I, Aisa MC: Molecular interaction of S100 proteins with microtubule proteins in vitro. J Neurochem 1989, 53:566–571
57. Linden M, Nelson BD, Leterrier JF: The specific binding of the microtubule-associated protein 2 (MAP2) to the outer membrane of rat brain mitochondria. Biochem J 1989, 261:167–173
58. Jancsik V, Filliol D, Felter S, Rendon A: Binding of microtubule-associated proteins (MAPs) to rat brain mitochondria: a comparative study of the binding of MAP2, its microtubule-binding and projection domains, and tau proteins. Cell Motil Cytoskeleton 1989, 14:372–381
59. Pfeffer SR, Drubin DG, Kelly RB: Identification of three coated vesicle components as alpha- and beta-tubulin linked to a phosphorylated 50000-dalton polypeptide. J Cell Biol 1983, 97:40–47
60. Allan VJ, Kreis TE: A microtubule-binding protein associated with membranes of the Golgi apparatus. J Cell Biol 1986, 103:2229–2239
61. Carlier MF, Simon C, Cassoly R, Pradel LA: Interaction between microtubule-associated protein tau and spectrin. Biochimie 1984, 66:305–311

62. Papasozomenos SC, Binder LI: Phosphorylation determines two distinct species of tau in the central nervous system. Cell Motil Cytoskeleton 1987, 8:210–226
63. Marx KA: High affinity DNA/microtubule-associated protein interaction. Mol Cell Biochem 1992, 113:55–61
64. Wang Y, Loomis PA, Zinkowski RP, Binder LI: A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau. J Cell Biol 1993, 121:257–267
65. Valverde F, Lopez-Mascaraque L, De Carlos JA: Distribution and morphology of Alz-50 immunoreactive cells in the developing visual cortex of kittens. J Neurocytol 1990, 19:662–671
66. Miller MW, Al Ghoul WM, Murtaugh M: Expression of Alz-50 immunoreactivity in the developing principal sensory nucleus of the trigeminal nerve: effect of transecting the infraorbital nerve. Brain Res 1991, 560:132–138
67. Kanemaru K, Takio K, Miura R, Titani K, Ihara Y: Fetal-type phosphorylation of the tau in paired helical filaments. J Neurochem 1992, 58:1667–1675
68. Sarkozi E, Askanas V, McFerrin J, Johnson SA, Engel WK: Human muscle fibers regenerating in vivo and in vitro express strong β-amyloid precursor protein mRNA while normal adult fibers do not. Soc Neurosci Abstr 1993, 19:1314
69. Grundke-Iqbal I, Iqbal K, George L, Tung YC, Kim KS, Wisniewski HM: Amyloid protein and neurofibrillary tangles coexist in the same neuron in Alzheimer's disease. Proc Natl Acad Sci USA 1989, 86:2853–2857
70. Spillantini MG, Goedert M, Jakes R, Klug A: Topographical relationship between β-amyloid and tau protein epitopes in tangle-bearing cells in Alzheimer's disease. Proc Natl Acad Sci USA 1990, 87:3952–3956
71. Askanas V, Alvarez RB, Engel WK: β-amyloid precursor epitopes in muscle fibers of inclusion body myositis. Ann Neurol 1993, 34:551–560