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INCREASE OF B-50/GAP-43 IMMUNOREACTIVITY IN UNINJURED MUSCLE NERVES OF *MDX* MICE

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Abstract—Lack of dystrophin in *mdx* mice leads to muscle fibre degeneration followed by the formation of new myofibres. This degeneration–regeneration event occurs in clusters. It is accompanied by inflammation and remodelling of the intramuscular terminal nerve fibres. Since the growth-associated protein B-50/GAP-43 has been shown to be involved in axonal outgrowth and synaptic remodelling following neuronal injury, we have investigated the presence of B-50 in gastrocnemius and quadriceps muscles of *mdx* mice. Using immunocytochemistry we demonstrate increased presence of B-50 in terminal nerve branches at motor endplates of *mdx* mice, particularly in the clusters of de- and regenerating myofibres. In comparison, the control mice displayed no B-50 immunoreactivity in nerve fibres contacting motor endplates. Our findings indicate that during axonal remodelling and collateral sprouting the B-50 level in the terminal axon arbours is increased although there is no direct injury to the motoneurons.

We suggest that the degenerating target and/or the inflammatory reaction induces the increased B-50 level in the motoaxons. The increased B-50 may be important for sprouting of the nerve fibres and re-establishment of synaptic contacts, and in addition, for maturation and survival of the newly formed myofibres.

Key words: sprouting, motoneuron, axonal remodelling, dystrophin, Duchenne muscular dystrophy.

Axonal growth is of great importance for the wiring of the central and peripheral nervous systems both during development and following injury. The mechanisms which control such growth are largely unknown. Axonal elongation is often characterized by the expression of a group of proteins called growth associated proteins. The best studied of this group is B-50, also called GAP-43 (see Ref. 20 for review). During development this protein is expressed in large amounts decreasing substantially after synaptogenesis, although to a different degree in different structures (see Ref. 35 for review). In the peripheral nervous system of the adult, B-50 decreases to a very low level in sensory and motor neurons, but not in the postganglionic autonomic neurons.[25,38] In the CNS B-50 is present in several types of adult neurons which are assumed to be involved in synaptic plasticity.[2,3,18,31] It has been proposed that the neuronal target plays an important role in controlling B-50 expression through the release of a factor which could control the expression of the gene for B-50.[35]

In the adult, particularly in the peripheral nervous system, the growth of an axon with its terminal arborization may occur following axotomy. In this case, lack of the target would be responsible for inducing B-50 up-regulation, although an effect of the direct injury is also possible. An axonal elongation may also occur in the absence of any direct injury to the neuron. In this case it is the manipulation of the target which is able to trigger the axonal growth under two conditions: (i) under a form of collateral sprouting when the neurone maintains its normal target, but a new denervated target becomes available; (ii) as a simple remodelling of the terminal arborization when it is the normal target which is affected or manipulated.

While there is a wide agreement on the fact that during regeneration of injured peripheral nerves there is a consistent up-regulation of B-50 until reinnervation is achieved,[4,10,34,42,44] it is less clear what happens in relation to collateral sprouting and axonal remodelling. Mehta *et al.*[29] have found that following partial muscle denervation B-50 immunoreactivity was increased in the terminals of collaterals from axons of motoneurons, as well as in Schwann cells covering denervated endplates. However, it has been shown that there is no change in the level of B-50 mRNA during sprouting of the motoneurons induced both by a partial muscle denervation[6] and by application of botulinum toxin to the muscle.[5] In these experiments, the level of B-50 mRNA expression was

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Abbreviations: DMD, Duchenne muscular dystrophy; TBS, Tris-buffered saline.

assessed only at about one week. Caroni and Becker[8] found that the developmental down-regulation of B-50 mRNA and protein in the motoneurons and at motor endplates was prevented when the muscle was paralysed. In addition, Caroni and Grandes[9] induced a sprouting of motoneurons by intramuscular application of insulin-like growth factors and they found a B-50 up-regulation in the axonal terminals of motoneurons. B-50 expression in motoneuron terminals at several weeks following application of botulinum toxin to the muscle, but not in Schwann cells, is also reported by Hassan *et al.*[21]

An axonal remodelling without a direct damage to motoneurons occurs in Duchenne muscular dystrophy (DMD), an X-linked recessive disorder which is characterized by the lack of dystrophin expression. This protein is present in the membrane of muscle fibres and of many cells in the CNS[22,26,27] (see Ref. 33 for review). The lack of dystrophin in the muscle leads to a progressive degeneration accompanied by fibrotic proliferation. Although in physiological conditions muscle fibres do not degenerate, they are able to regenerate after necrosis. Muscle precursor cells become mitotically active, fuse together and differentiate to form new muscle fibres. This repairing process is present in DMD, but it is not sufficient to compensate for the necrotic process and the fibrosis, with a consequent fatal exit of the patient usually before the age of 20 years.[1,28]

A well studied animal model of DMD is the *mdx* mutant mouse which is also characterized by a similar lack of dystrophin and by the same type of biochemical and histopathological muscle alterations. In fact, the muscle presents extensive areas of myofibre necrosis accompanied by regeneration and often by active cellular infiltration.[7,12,39,40] The *mdx* mouse differs from DMD patients in that it exhibits a greater degree of compensatory muscle regeneration and a scarce fibrotic replacement.[11] Indeed, a vigorous muscle regeneration is present in *mdx* mice during the first year of life, which declines up to 15 months of age and becomes negligible thereafter.[32] Such milder histopathologic modifications are reflected by a slower progression of the disease.

The presence of a degeneration–regeneration pattern in DMD patients and *mdx* mice implies the necessity of a continuous remodelling of the nerve fibres which are innervating the muscle. In addition, the inflammatory type of reaction which is present in the affected areas of the muscle is likely to be accompanied by a remodelling of the vessels and their innervation.

Here we report that during the inflammatory reaction in the muscle of *mdx* mice the level of B-50 immunoreactivity in the remodelling axon terminals is increased in comparison to that in muscles of control mice. This response occurs, though the peripheral nerve is not directly injured, but the primary deficit is in the target muscle.

EXPERIMENTAL PROCEDURES

The animals were provided by Harlan CBP, Austerlitz (The Netherlands). We used gastrocnemius and quadriceps muscles of 10 C57/BL10 *mdx/mdx* and of 5 C57/10Sc/Sn mice. All the animals were one to 12 months old.

Immunocytochemistry and histological procedures

The animals were terminally anaesthetized using a mixture of ketamine (Ketalar, Bayer, 100 mg/kg) and xylazine (Rompum, Bayer, 5 mg/kg i.p.) and transcardially perfused with a rinsing solution (0.8% sucrose, 0.8% NaCl, 0.4% glucose in 0.12 M phosphate buffer, pH 7.3) followed by 300 ml of 4% paraformaldehyde in 0.12 M phosphate buffer. The gastrocnemius and quadriceps muscles were removed, pinned out on a silicon rubber, kept in the same fixative overnight at 4°C and then cryoprotected in 30% sucrose in 0.12 M phosphate buffer at 4°C for 48 h.

Serial sections (50-μm thick) were cut with a cryostat and immediately mounted on chrome-alum gelatinized slides. Endogenous peroxidase was quenched by 0.3% H_2O_2 in Tris-buffered saline (TBS, 0.9% NaCl in 0.05 M Tris–HCl, pH 7.4) for 30 min. The sections were incubated overnight at 4°C in a humid box with the primary antiserum, affinity purified polyclonal rabbit anti-B-50, diluted 1:300 in TBS with 0.25% Triton X-100.[41] A few sections were incubated with a non-immune serum and always yielded negative reactions. In addition, in some cases part of the sections were incubated with a polyclonal anti-S-100 antibody (1:400, Dakopatts) to stain Schwann cells. The ensuing detections were performed according to the avidin-biotin-peroxidase method (Vectastain, Vector) using diaminobenzidine as a chromogen. The reacted sections were usually air dried, rapidly dehydrated in graded ethanol concentrations, cleared in xylene and coverslipped in Entellan (Merck). In some instances, however, the sections were also counterstained with haematoxylin–eosin in order to visualize muscle fibres.

Acetylcholinesterase histochemistry and microscopic evaluation

In some cases B-50 immunostained sections were subsequently treated according to the method of Koelle[24] to visualize acetylcholinesterase activity. Briefly, the sections were first incubated in a 0.1 mM solution of tetra-isopropyl-pirophosphoramide (Sigma) in distilled water for 30 min at 37°C, to inhibit aspecific cholinesterase activity. After a 10 min rinse in distilled water, the sections were incubated with the acetylcholinesterase substrate acetylthiocholine iodide (Sigma, 6 mg/5 ml of 0.05 M acetate buffer, pH 5.6–5.9) for 30 min at 37°C. Finally the reaction product was revealed by incubation in 5% ammonium sulfide (Merck) in distilled water for a few minutes at room temperature. In these cases the sections were mounted with an aqueous mounting medium for microscopy (Aquatex, Merck).

The histological preparations were evaluated by bright field and Nomarski interference contrast on a Zeiss Axiophot light microscope. In order to determine the distribution of B-50 immunolabelled fibres in relation to the degenerative–regenerative processes of the *mdx* muscle, we analysed one 50-μm-thick section from a *mdx* quadriceps muscle immunolabelled for B-50 and counterstained with haematoxylin–eosin. The section was projected through a magnifying lens in order to reproduce its outline and that of the areas of muscle degeneration–regeneration. Thereafter, all the immunolabelled fibres present on this section were reproduced by means of a camera lucida coupled to a Leitz Dialux light microscope at 400× magnification and their position with respect to the normal or affected muscle areas was determined. Each labelled profile not directly connected to any other immunostained element was considered as an individual entity and counted as one B-50 immunopositive

fibre. The drawings were digitized by means of a magnetic graphic tablet and filed in a 80486 PC. Two-dimensional morphometric measurements (i.e. total surface area of the section, extent of the affected and normal areas, length of the labelled fibres) were carried out by means of the Sigma Scan Scientific Measurement System (Jandel).

RESULTS

The typical histopathologic modifications of *mdx* mice muscles were confirmed in our experimental material.[12,32,40] The mutant mouse muscles displayed circumscribed areas of myofibre degeneration–regeneration, which contained both eosinophilic fibres in the process of degeneration and basophilic elements of smaller diameter representing regenerating muscle cells. The affected areas were also characterized by an intense cellular infiltration. This picture, although variable in its intensity and distribution, was consistently present in all the *mdx* mice examined in our experiments.

In our control mice the analysis of muscle sections treated with the anti-B-50 antibody revealed the presence of very few, scattered, thin nerve fibres which were clearly positive. Some of them were present in nerve bundles running through the adipose tissue along vascular peduncles of the muscle (Fig. 1A), while others were placed along the vessels (Fig. 1B, arrowheads). Some of them had the aspect of a thin terminal plexus across the wall of the vessels (Fig. 1C). In control muscles B-50 immunostained fibres never contacted acetylcholinesterase-stained endplates (Fig. 1B, arrows). Our findings are in agreement with previously reported observations describing that in the muscle, only autonomic nerve fibres innervating arteries are displaying B-50 immunoreactivity.[21,25,38]

The picture was strikingly different for the sections of the *mdx* mouse muscles which displayed a large number of B-50 immunoreactive nerve fibres in different patterns. Figure 1D shows a longitudinal section of a muscle with a nerve bundle running almost parallel to the myofibres. The number of B-50 immunolabelled profiles is quite high in comparison to that seen in control mice where immunoreactive nerve fibres were rarely present in nerve bundles. In addition, large numbers of strongly immunoreactive nerve fibres were abundant along the course of blood vessels (Fig. 1E) and also as terminal plexuses innervating the depth of their walls (Fig. 1F).

In the *mdx* mice numerous B-50 immunoreactive nerve fibres were also consistently present in small nerve bundles spreading towards the muscle endplates (Fig. 2A). These fibres terminate inside the endplates identified by acetylcholinesterase staining. Poor B-50 immunorcactivity is also present in the muscle regions characterized by the absence of degenerating events, where the myofibres identified by central nuclei have already regenerated. Since centrally nucleated muscle elements represent new myofibres, this means that B-50 immunoreactivity is a transitory event timely correlated with the degeneration–regeneration process.

In a study of alterations of B-50 immunoreactivity after partial muscle denervation, Mehta *et al.*[29] have described axon-like and Schwann-like B-50 labelling patterns of nerve fibres. In Fig. 2B–D, the nerve fibres appear to be very thin and the pattern corresponds to the axon-like type.[29] In Fig. 2E it is shown that in a muscle section, which has been immunostained with an anti-S-100 antibody, a marker of Schwann cells, the terminal fibres contacting the motor endplate appear to have a different morphology and a thicker diameter than those of Fig. 2B–D. This difference suggests that the B-50 immunoreactive fibres of Fig. 2B–D originate from motoneurons, rather than Schwann cells. In preparations labelled by anti-B-50 antibodies and acetylcholinesterase histochemistry, some endplates have a round-shaped and uniform aspect and are innervated by a single fibre (Fig. 2C), similar to those observed in control mice. Other ones have a less regular profile and are entered by several motoneuronal branches (Fig. 2B). Finally, in some instances the endplates are clearly broken up into small islands and distinct B-50 immunoreactive nerve fibres innervate different islands of the same endplate (Fig. 2D). This abnormal morphology has been previously described in *mdx* mice and it has been attributed to newly formed endplates in which different islands are innervated by distinct nerve fibres.[40]

Because of the widespread presence of nerve fibres containing B-50 immunoreactivity in the muscle of the *mdx* mice, we investigated whether there was any correlation between the areas of muscle necrosis and the presence of B-50 immunoreactive nerve fibres. Figure 3A shows one complete section of an *mdx* quadriceps muscle. The shaded areas represent regions of muscle degeneration–regeneration. Morphometric evaluations on camera lucida drawings of the B-50 immunopositive fibres present in this section showed that such axons are primarily associated with affected muscle regions (Fig. 3A–D). Among the 382 fibres observed in this section, 230 (60%) were localized in areas of muscle degeneration–regeneration, which represented only 19.4%, of the total surface area of the section. Accordingly, the total length of labelled fibres was 1501.8 μm/mm^2 in the affected areas, whereas it was only 201 μm/mm^2 in the normal regions. Such large difference in fibre length can also be attributed to the different morphology displayed by the labelled elements in the different muscle areas. In fact, in the unaffected areas immunopositive profiles were short and scarcely ramified axon segments which, for the most, run perpendicularly to the myofibre longitudinal axis (Fig. 3C). By contrast, in the areas of degeneration–regeneration they frequently appeared as long, highly branched processes, without any preferential orientation in space (Fig. 3D).

DISCUSSION

B-50 immunoreactive fibres are increased in number

Our experiments confirm that in normal mice little B-50 immunoreactivity is detectable in intramuscular nerve fibres. There were nerve fibres with strong B-50 immunoreactivity, but these were mainly around blood vessels or as terminal plexuses inside their walls. These nerve fibres have been identified as autonomic fibres usually innervating the arterioles.

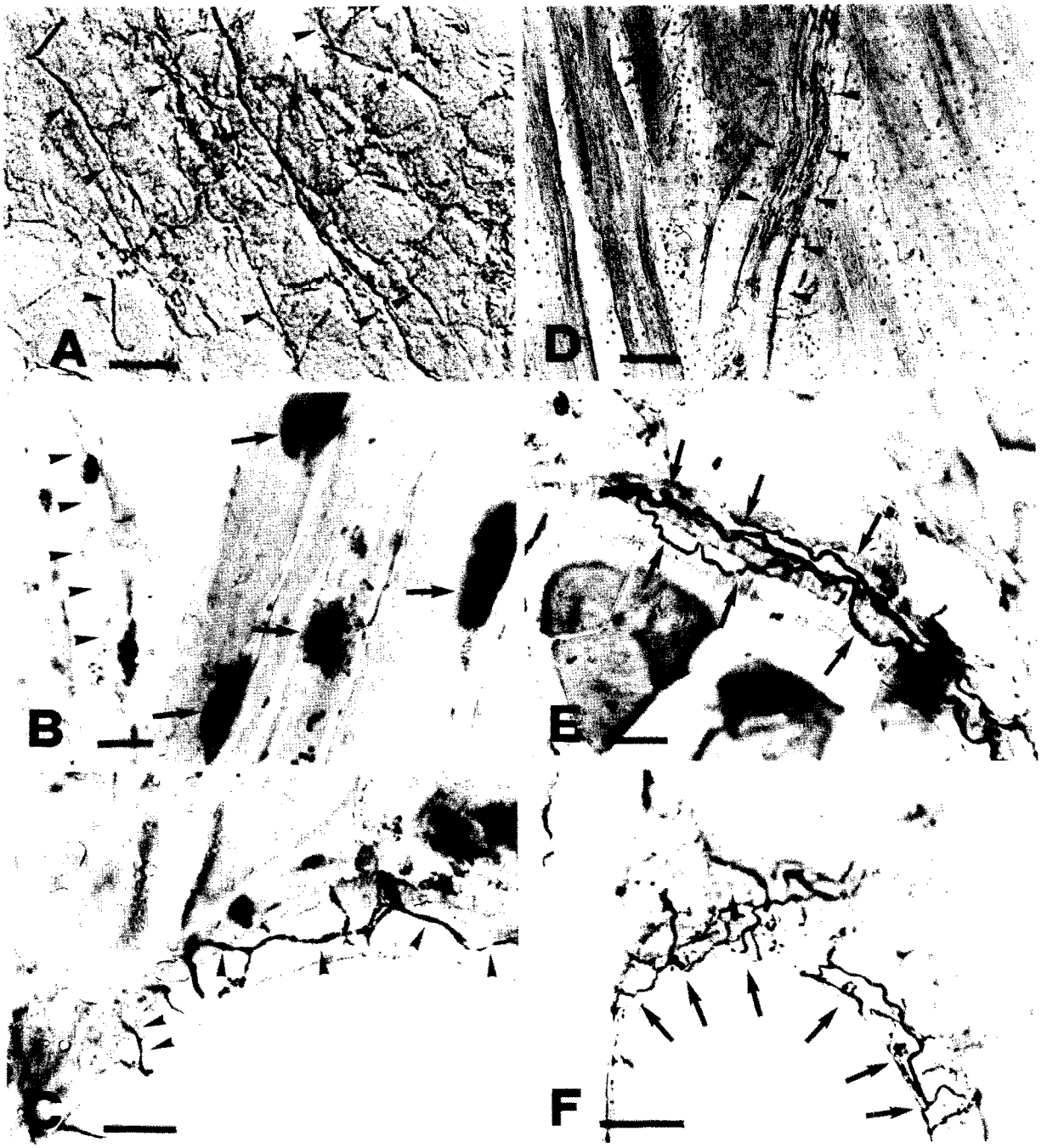
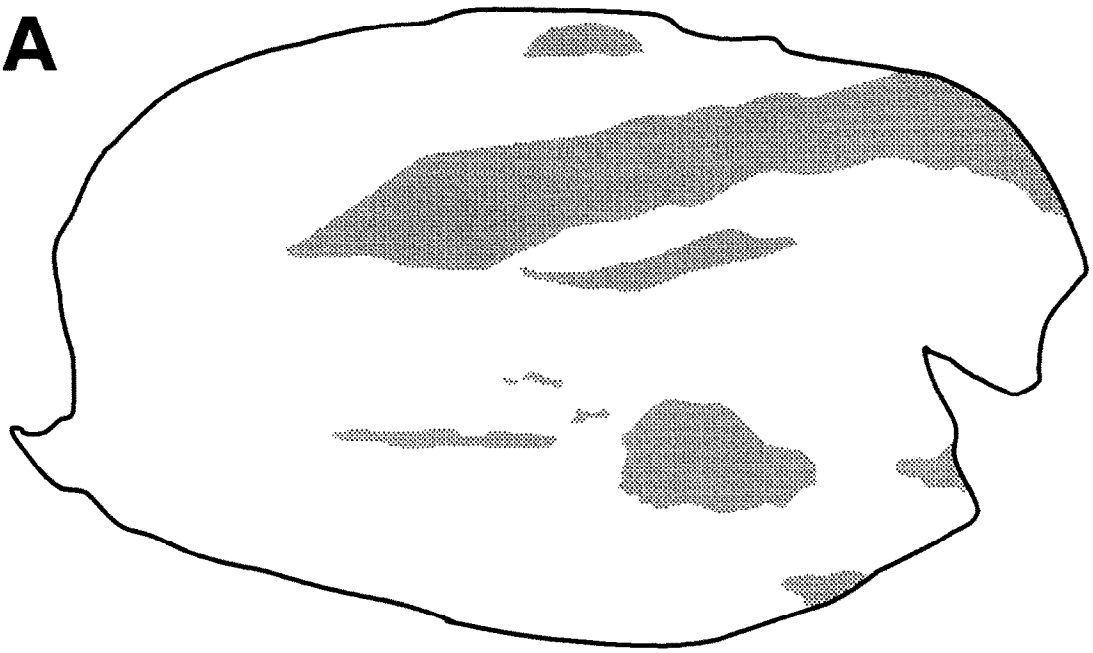


Fig. 1. B-50 immunoreactivity in nerve fibres of muscle and blood vessels of control (A–C) and *mdx* (D–F) mice. The picture in A shows the loose meshwork of B-50 immunostained axons (arrowheads) in the adipose tissue along a vascular peduncle of the control gastrocnemius muscle. Terminal immunopositive fibres running in the wall of small intramuscular vessels are indicated by arrowheads in B and C (quadriceps muscles). Note that no immunolabelled fibres are present in the vicinity of acetylcholinesterase-stained endplates (arrows) displayed in B. In the micrograph in D is shown a bundle of B-50 immunoreactive axons (arrowheads) in an intramuscular nerve branch of a *mdx* mouse (gastrocnemius muscle). The arrows in E, F point to the terminal network of B-50 immunostained axons running in the vessel walls of the *mdx* quadriceps muscle. Scale bars = 30 μm in A–D, 100 μm in E, 50 μm in F.

Fig. 2. B-50 immunoreactivity in terminal axonal branches at motor endplates of the *mdx* mouse (A–D) and S-100 immunostaining of Schwann cell processes (E) at a motor endplate of a control mouse. The arrows in A indicate a bundle of B-50 immunolabelled axons in the gastrocnemius muscle. Single fibres (arrowheads) emanate from this bundle and end on acetylcholinesterase-labelled endplates. The high magnification micrographs in B and C show two examples of single B-50 immunoreactive axons (arrows) ending in endplates with typical morphology. By contrast, the endplate shown in D appears to be broken up in several small islands. Note that two distinct immunoreactive axons (arrows) terminate in two different islands of this endplate. The comparison between the micrograph E, showing the morphology of Schwann cell processes, and B and C, revealing B-50 immunoreactive fibres, suggests that in *mdx* mice B-50 is detected in terminal axon branches of motoneurons. Scale bars = 50 μm in A, 25 μm in B–D, 20 μm in E.

A



B

	Affected	Normal
Area (mm^2)	11.6	48.1
Number of fibres	230	152
Number of fibres/mm^2	19.8	3.16
Total fibre length (μm)	17421	9693
Fibre length/mm^2 (μm)	1501.8	201.5

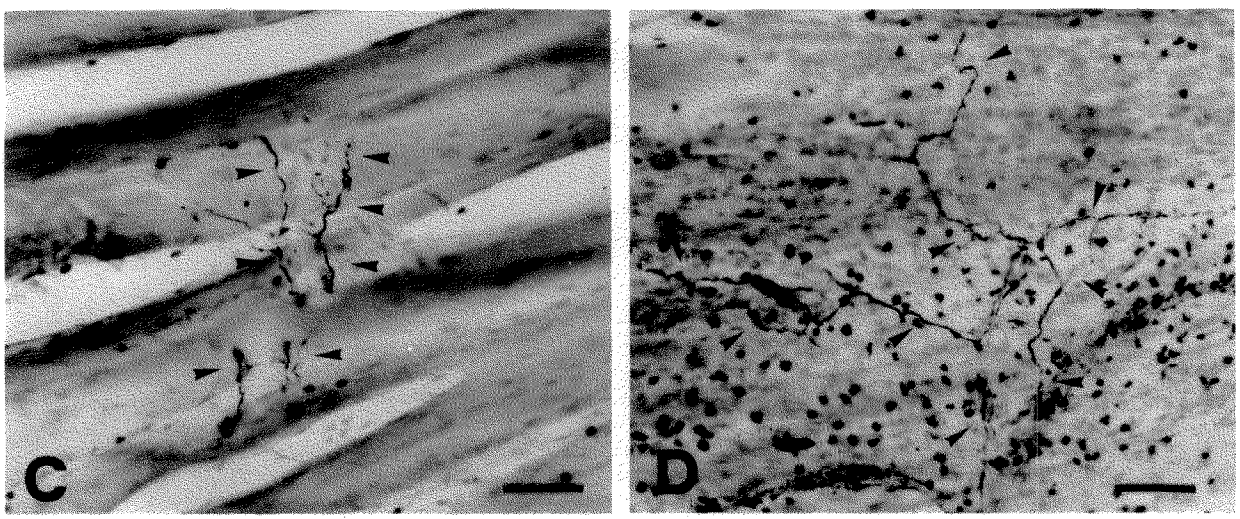


Fig. 3. Distribution of B-50 immunopositive fibres in relation to areas of muscle degeneration–regeneration. The drawing in A shows a complete section of a *mdx* quadriceps muscle in which regions of myofibre degeneration–regeneration are indicated by shaded areas. The table in B displays the results of a morphometric analysis carried out on the B-50 immunoreactive fibres present in this section. Note that the affected areas, which only represent 19.4% of the total area of the section, contain the majority of the labelled axons. Accordingly, the density and total length of labelled fibres is also larger in the affected regions than in normal ones. The micrographs C and D show the different morphological features of the labelled axons in the different muscle areas. In non-affected areas (C), the labelled axons (arrowheads) appear as short axon segments with scarce ramifications, which run perpendicularly to the longitudinal axis of muscle fibres. By contrast, in the areas of degeneration–regeneration B-50 immunoreactive fibres (arrowheads) often appear as long, highly branched processes without any preferential orientation in space. Scale bars = 50 μm in C and D.

All the other fibres in the normal muscle were not B-50 immunoreactive.[21,25,38] Ulenkate *et al.*[41] have observed a weak B-50 immunoreactivity at the level of the endplates. This means that under normal conditions the B-50 signal is very weak or absent compared to that found in autonomic nerve fibres.

In contrast, in the *mdx* muscles there was a conspicuous number of B-50 immunoreactive elements which appear to be localized, even at a first inspection, to those areas characterized by clusters of degenerating–regenerating myofibres. They were present in high amounts not only in the nerve bundles and around the vessels, where they are normally present, but also in other regions of the muscular structure. Numerous B-50 immunoreactive nerve fibres occurred in the small muscle nerves close to the areas which are rich in axon terminals of motor nerves (Fig. 2A).

B-50 immunoreactive fibres are more abundant in necrotic areas

The increased presence of B-50 in nerve fibres of the *mdx* mice muscle, in comparison to that of the control, is likely to be the consequence of the necrotic process typical of this disease which is due to the lack of dystrophin in the muscle fibres. However, since dystrophin is also lacking in several regions of the CNS,[22,26,27] one may argue that the increase of B-50 immunoreactivity is due to some abnormal feature of central neurons independent of their targets. That this is unlikely is shown by the fact that there is a correlation between the number of B-50 immunoreactive fibres and the location of the muscular necrotic process (Fig. 4A–D). In addition, dystrophin is almost absent in the spinal cord.[26]

The location of these areas changes during the course of the disease, as there is a cumulative increase in the number of newly regenerated muscle fibres identified by having central nuclei.[12] Our experiments have shown that B-50 immunoreactive axons are mostly associated with necrotic areas characterized by intense cellular infiltration. This fact suggests that the B-50 increased immunopositivity is a transitory event which mainly occurs during the degeneration–regeneration process of muscle fibres. In addition, together with the remodelling of the target for motoneurons, the inflammatory response associated to the necrotic phenomena induces a vascular proliferation and the remodelling of the autonomic nerve fibres which innervate the vessels.

Localization of B-50 immunoreactivity in motoaxons

With respect to the identification of the B-50 immunolabelled elements in the *mdx* mice, the question is whether the B-50 immunoreactivity has to be attributed to motoneurons and/or to glia. In fact, it is now known that B-50 is also located in developing glia and Schwann cells[14–17,43,45] and an up-regulation has been shown to be present in Schwann cells in the adult following neuronal injury.[13,15,29,41,45]

In studying the morphological appearance of B-50 immunolabelled elements in relation to sprouting of motoneurons, Mehta *et al.*[29] have distinguished the morphology of the B-50 immunolabelling of process outgrowth by an axon-like and a Schwann-like pattern of immunoreactivity. The former is characterized by thin structures ending in a lace- or basket-like pattern at the motor endplate that was similar to the morphology of fibres stained by antibodies to PGP 9.5, a protein marker for axons. The latter one is instead characterized by a thicker diameter and a more irregular appearance. In our *mdx* mice we observed clear axon-like patterns of B-50 immunolabelling suggesting that this protein is increased in motoneurons.

Significance of B-50 expression in terminal remodelling in the absence of direct neuron injury

The most significant finding of our research is the demonstration that an increased B-50 immunoreactivity may occur in motoneurons in the absence of any direct injury to them. This issue is controversial. Brown *et al.*[6] and Bisby *et al.*[5] reported that B-50 mRNA was not up-regulated during collateral sprouting, concluding thus that injury to the neuron was an important factor in eliciting an increase in B-50 expression. However, as mentioned above, in the latter experiments the expression of B-50 mRNA in uninjured motoneurons was assessed only at about one week after partial denervation or botulinum toxin application, a time interval that may be too short to detect changes in the B-50 expression in the absence of a direct injury. In contrast, Mehta *et al.*[29] have provided evidence for B-50 expression in the collateral sprouting of uninjured motoneurons. Also when sprouting was induced by muscle inactivity[21] or by administration of insulin-like growth factor,[8,9] B-50 expression was increased in the motoneurons.

Our experiments show that B-50 immunoreactivity is present in the motoneurons in the absence of any direct injury, during the remodelling of their terminal arborization which occurs when their target degenerates and newly formed myoblasts become available. It should be mentioned that in mucosal nerves in the intestines of rats affected by an inflammatory reaction to a nematode infection B-50 is up-regulated.[36] Also in chronic pancreatitis nerve fibres up-regulate B-50.[19] It has been proposed[35] that B-50 expression is normally high during development when a nerve fibre is growing towards its target cell. Following synaptogenesis, a stop signal from the target would repress B-50 gene expression. In the *mdx* mice, motoneurons which display B-50 immunoreactivity in their terminal axon branches, are not directly damaged, as shown by Torres and Duchen,[40] and the target degeneration is likely responsible for the increased levels of protein content. When the new innervation is established, the B-50 expression returns to normal. Since an inflammatory response is also present in the affected areas of the muscle of the *mdx* mice, it is not

possible to exclude that the phenomena accompanying such an inflammatory process may also contribute to increase B-50 immunoreactivity.

A second important finding of our experiments is the demonstration of the capacity of the neurons of the *mdx* mice to increase B-50 immunoreactiyity. Although the primary deficit of the Duchenne muscular dystrophy and of the *mdx* mice responsible for the muscle degeneration is the lack of dystrophin in the muscle, the evolution of the disease is likely to be dependent on other factors. Among them the strength of the regenerative capacity of the motoneurons may still be an important factor by facilitating muscle reinnervation. In addition, because of the undoubted trophic action exerted by the motoneurons on the muscle fibres, a higher capacity for nerve regeneration may be of substantial importance for the control of the degeneration–regeneration pattern of the muscle fibres. Although muscle mechanical work is a detrimental factor in the evolution of the disease[30,37] and muscle denervation or chordotomy prevents myonecrosis,[23] poor reinnervation of the new muscle fibres by the motoneurons may contribute to the evolution of the disease by decreasing the probability of myofibre regeneration and the survival of the new muscle fibres. It is interesting that muscular dystrophy is very severe in human and dogs (see Ref. 33 for review), whereas in mice and cats the muscle regenerative capacity is strong and the disease has a slower progression. It is tempting to speculate that the milder form of mice and cats depends, at least in part, on a higher capacity of the motoneurons to express growth associated proteins. Such a higher expression would be accompanied by a stronger reinnervation which would sustain a better survival of the regenerated muscle fibres. Once the new muscle fibre becomes reinnervated, a different phenotype of the same fibre is likely responsible for the necrosis resistance.

CONCLUSIONS

The present results show that B-50 immunoreactivity is increased in uninjured axons of the *mdx* mouse muscle, including both motor axons and nerve fibres associated with blood vessels. The majority of the labelled axons are located within areas of muscle degeneration–regeneration. This fact indicates that the expression of B-50 is related to axonal remodelling phenomena which occur in response to the degenerative and regenerative processes of muscular tissue and/or to the associated inflammatory reaction. It is proposed that the plastic properties of *mdx* neurons may improve the regeneration of muscle fibre and contribute to the mild progression of the disease observed in these mice.

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