10. Negative Immunostaining of Duchenne Muscular Dystrophy (DMD) and mdx Muscle Surface Membrane with Antibody against Synthetic Peptide Fragment predicted from DMD cDNA

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Recent studies of Kunkel and his associates1)-5) have clarified the cloned complementary DNA sequences corresponding to the Duchenne muscular dystrophy (DMD) gene. In the end of 1987, Hoffman et al.6),7) obtained specific antibodies directed to the proteins encoded by fragments of the mouse DMD gene which is very similar to the human DMD gene.

These antibodies recognized a specific protein, named “dystrophin” in normal skeletal and cardiac muscle of both humans and mice, which is absent in two DMD patients and mdx mice.

By using these antibodies, Hoffman et al.5) have shown that dystrophin is associated with intracellular membrane fraction, especially the triads, on the basis of Western blot analysis using the pellet obtained by the subcellular fractionation of the mouse and rabbit skeletal muscle.

Here we report that the antiserum raised against peptide fragment prepared as predicted from the distinctive region of DMD cDNA stained clearly the surface membranes of normal skeletal muscles of both humans and mice, but did not those of both DMD and mdx skeletal muscles, immunohistochemically.

Materials and methods. The peptide fragment was predicted from position 1526 to 1675 on the human cDNA map7) and an extra Tyr was added to the N-terminus. The protected peptide was synthesized by the solid phase method on Applied Biosystems model 430A and deprotected with HF.9) The peptide was purified by preparative reversed phase HPLC. It consists of 51 amino acids, having a molecular weight of 6,267.

New Zealand white female rabbit was immunized with intradermal injection of 2 mg of the peptide with Freund’s complete adjuvant 3 times every 2 weeks to obtain the antibody.

The 4 μm frozen sections of the biopsied muscles of 5 patients with DMD and 5 other related neuromuscular diseases and 3 normal controls and 3 mdx (C57BL/10ScSn-mdx) and normal control mice (C57BL/10ScSn) hind limb muscles were incubated with antiserum and stained with fluorescence labeled antibody against rabbit IgG and were observed under fluorescence microscope (Zeiss, Axiophot).

Results. As shown in Fig. 1B, the antiserum stained the whole surface

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membrane of all the muscle fibers of 3 normal controls homogeneously without interruption, but no other specified locus inside the fiber in the cross section. The longitudinal section showed no periodic staining corresponding to the repeat of sarcomere, only continuous fluorescence throughout the whole length of the surface membrane (data not shown).

In sharp contrast with this, the surface membranes of the biopsied muscle fibers from 5 human DMD muscles were not stained at all as shown in Fig. 1D. It should be emphasized that lack of staining of the surface membrane was observed not only in opaque or necrotic fibers but also apparently normal fibers with hematoxylin and eosin staining (Fig. 1C).

Fig. 2 shows the staining of the mdx and normal control mouse hind limb muscles with the antiserum. The antiserum stained the surface membranes of all the fibers of 3 control muscles (Fig. 2A), but not mdx muscles (Fig. 2B) as observed in the case of DMD.

The biopsied muscles of 5 related neuromuscular diseases, including one case of amyotrophic lateral sclerosis, 2 cases of polymyositis, one case of limb-girdle muscular dystrophy, and one case of facioscapulohumeral muscular dystrophy showed clear and definite staining of the surface membranes, in the same way as that in normal controls.

Discussion. The present study has clearly demonstrated that the antiserum...
raised against a synthetic peptide fragment predicted from the 5' region of DMD cDNA sequences stained only the surface membrane of the control muscle fibers in cross and longitudinal sections, suggesting that the immunoreactive protein is located on or attached to the surface membrane.

The staining with this antiserum, however, was not observed at all in all the cases of DMD so far examined. The fact that not only opaque or necrotic fibers, but also normal looking fibers are also unstained, definitely indicates the primary defect of the protein in DMD. Essentially the same result was observed in the case of mouse skeletal muscle. Biopsied muscles of 5 patients with various neuromuscular diseases other than DMD showed essentially the same positive staining of the surface membrane as the normal control. Therefore, we conclude that lack of protein is specific for DMD and mdx muscle.

The characterization of the antibody, especially whether this antibody cross reacts with 400 kD dystrophin reported by Hoffman et al. is now under way in our laboratory.

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