Deficiency of the 50K dystrophin-associated glycoprotein in severe childhood autosomal recessive muscular dystrophy

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X-LINKED recessive Duchenne muscular dystrophy (DMD) is caused by the absence of dystrophin, a membrane cytoskeletal protein. Dystrophin is associated with a large oligomeric complex of sarcolemmal glycoproteins. The dystrophin–glycoprotein complex has been proposed to span the sarcolemma to provide a link between the subsarcolemmal cytoskeleton and the extracellular matrix component, laminin. In DMD, the absence of dystrophin leads to a large reduction in all of the dystrophin-associated proteins. We have investigated the possibility that a deficiency of a dystrophin-associated protein could be the cause of severe childhood autosomal recessive muscular dystrophy (SCARMD) with a DMD-like phenotype. Here we report the specific deficiency of the 50K dystrophin-associated glycoprotein (M, 50,000) in sarcolemna of SCARMD patients. Therefore, the loss of this glycoprotein is a common denominator of the pathological process leading to muscle cell necrosis in two forms of muscular dystrophy, DMD and SCARMD.

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Figure 1 shows the immunohistochemical analysis of the muscle biopsy specimens using a monoclonal antibody against dystrophin and affinity-purified sheep polyclonal antibodies against 156K dystrophin-associated glycoprotein (156DAG), 59K dystrophin-associated protein (59DAP), 50K dystrophin-associated glycoprotein (50DAG), 43K dystrophin-associated glycoprotein (43DAG) and 35K dystrophin-associated glycoprotein (35DAG). In normal skeletal muscle, antibodies against dystrophin and dystrophin-associated proteins (DAPs) stain the sarcolemma. In addition, we have found no abnormality of these proteins in the following neuromuscular diseases: limb-girdle muscular dystrophy, myotonic dystrophy, oculopharyngeal muscular dystrophy, facioscapulohumeral muscular dystrophy, non-Fukuyama type congenital muscular dystrophy, congenital fibre type disproportion, spinal muscular atrophy and amyotrophic lateral sclerosis (not shown). In DMD patients, dystrophin was absent and the immunostaining for all of the DAPs was greatly reduced in the sarcolemma (Fig. 1), except at the neuromuscular junction and in the sarcolemma of intrrafusal muscle fibres (data not shown). In contrast, in four
FIG. 1 Immunohistochemical analysis of dystrophin (DYS) and dystrophin-associated proteins in biopsied skeletal muscle. Shown are (from left to right): 16-year-old male with no pathological changes in the skeletal muscle (CONTR); 5-year-old male with SCARMID 10-year-old female with SCARMID 11-year-old male with SCARMID 11-year-old female with SCARMID and 8-year-old male with DMD (magnification, ×28). 

METHODS. Serial transverse cryosections (7 μm) were immunostained with VIA4, a monoclonal antibody against dystrophin, and affinity-purified sheep polyclonal antibodies against 156 DAG, 50 DAG, 90 DAG, 43 DAG and 35 DAG as described previously. The diagnosis of SCARMID was made on the basis of the following: (1) DMD-like phenotype affecting both males and females; (2) mode of inheritance compatible with an autosomal recessive disease; (3) North African patients; (4) elevated serum creatine kinase level; and (5) normal expression of dystrophin in the biopsied skeletal muscle analysed both by immunohistochemistry and by immunoblotting (Fig. 3).

FIG. 3 Immunoblot analysis of the dystrophin (DYS)-glycoprotein complex in the SDS-extracts of the biopsied skeletal muscle. Shown are immunoblots stained with a polyclonal antibody against the last 10 amino acids of dystrophin (Anti-DYS), a monoclonal antibody against 156 DAG (I130) and a cocktail of affinity-purified sheep polyclonal antibodies against 90 DAG, 50 DAG and 43 DAG (Anti-DAPs), respectively. The affinity-purified sheep polyclonal antibody against 35 DAG was not strong enough to stain the 35 DAG in crude muscle extracts. Lanes 1 to 6 are samples from: 5-year-old male with SCARMID; 5-year-old female with facioscapulohumeral muscular dystrophy; 10-year-old female with SCARMID; 11-year-old male with SCARMID; 16-year-old male with no pathological changes in the skeletal muscle; and 11-year-old female with SCARMID (sister of patient in lane 1), respectively. Molecular weight standards (×10⁻³) are shown on the left.

METHODS. Cryosections (20 μm) from skeletal muscle biopsy specimens were homogenized in 50 vols of SDS-extraction buffer (80 mM Tris-HCl, pH 6.8, 10% SDS, 0.115 M sucrose, 1% β-mercaptoethanol, 1 mM PMSF, 1 mM benzamidine and 1 mM EDTA) and incubated at 50 °C for 10 min. After centrifugation, 10-μl samples were separated on 3-12% SDS-PAGE. The gel was stained with Coomassie blue and the density of the myosin heavy chain band was measured using a computing laser densitometer (model 300S; Molecular Dynamics, Sunnyvale, CA). On the basis of this result, samples were run on 3-12% SDS-PAGE so that the amount of myosin heavy chain was equal for all specimens. Transfer to nitrocellulose membrane and immunostaining with antibodies were done as described.

patients with severe childhood autosomal recessive muscular dystrophy (SCARMID), including two siblings, immunostaining for the 50 DAG was drastically diminished in the sarcolemma of all muscle fibres, including the neuromuscular junction and the sarcolemma of intrafusal muscle fibres, whereas immunostaining for dystrophin, 156 DAG, 50 DAG and 43 DAG was preserved. Loss of 50 DAG in SCARMID patients was more severe than in DMD patients. Immunostaining for 35 DAG in SCARMID was reduced compared with normal control but was not as severely reduced as in DMD patients. Loss of 50 DAG in the sarcolemma of SCARMID patients was confirmed using three other specific antibodies against the 50 DAG, a monoclonal antibody (Fig. 2), a sheep polyclonal antibody affinity-purified against a 50 DAG peptide (Fig. 2) and an affinity-purified guinea-pig polyclonal antibody (data not shown).

To confirm the deficiency of the 50 DAG in SCARMID, skeletal muscle biopsy extracts were analysed by immunoblotting. Although dystrophin, 156 DAG, 50 DAG and 43 DAG were detected, 50 DAG was undetectable in all four SCARMID patients (Fig. 3). The affinity-purified antibody against the 35 DAG was not strong enough to stain the 35 DAG in crude muscle extracts. The deficiency of the 50 DAG in SCARMID muscle extracts was confirmed using two other antibodies against the 50 DAG (data not shown).

SCARMID (MIM number 253700) is a progressive muscular dystrophy prevalent in North Africa. This disease shares several clinical features with DMD: mode of onset, rapid progression, hypertrophy of calves and extremely high serum creatine kinase levels in the initial stages of the disease. Dystrophin and dystrophin-related protein, an autosomal homologue of dystrophin, are expressed normally in skeletal muscle in this disease. The structure and function of the dystrophin-glycoprotein complex (DGC) as a transsarcomembral linker between the subsarcolemmal cytoskeleton and the extracellular component, laminin, suggest that a deficiency of a DAP could be the cause of an autosomal recessive muscular dystrophy with a DMD-like phenotype. Here we have demonstrated the specific deficiency of the 50 DAG in the
SCARMID sarcolemma. In the DMD sarcolemma, in contrast, the absence of dystrophin leads to a large reduction in all of the DAPs, including the 50DAG (Fig. 1, DMD panel). Our results indicate that the loss of the 50DAG in the sarcolemma is a common denominator of the pathological process that leads eventually to muscle cell necrosis in two forms of muscular dystrophy, DMD and SCARMID.

On the basis of the function of the DGC mentioned above, we propose a hypothesis on the molecular mechanism of muscle cell necrosis in these two diseases where the disruption/dysfunction of the DGC plays a key role in the cascade of events leading to muscle cell necrosis (Fig. 4). In DMD, the absence of dystrophin leads to a loss of all DAPs, causing the disruption of the link between the subsarcolemmal cytoskeleton and the extracellular matrix that leads to sarcolemmal instability. In the case of SCARMID, in contrast, the deficiency of the 50DAG may severely disturb the function of the DGC and/or destabilize the DGC, also leading to sarcolemmal instability, which eventually causes muscle cell necrosis (Fig. 4).

The moderate reduction of the 35DAG in SCARMID could be secondary to the loss of the 50DAG. The 50DAG and 35DAG may form a tight subcomplex in the DGC and the loss of the 50DAG may cause a secondary reduction of the 35DAG in SCARMID. Dystrophin and 150DAG appeared reduced in the immunoblot analysis of the muscle specimen from the SCARMID patient with the most severe phenotype (lane 3 in Fig. 3). This suggests that the other components of the DGC could also be affected in the advanced stages of the disease.

Our results demonstrate the specific deficiency of the 50DAG in the SCARMID sarcolemma, leading to the reasonable hypothesis that this deficiency is causative for the disease. At present, the primary defect causing this deficiency is not known. It could be caused by a primary defect in the structure or expression of the gene for the 50DAG or could be due to a secondary effect of an unknown primary defect. Molecular biological and linkage analysis will be needed for the elucidation of the primary cause of SCARMID. But our results demonstrate that the diagnosis of SCARMID is now already possible by the immunohistochemical analysis of the 50DAG in muscle biopsy specimens. This will greatly aid the differential diagnosis between DMD and SCARMID.

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