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EXPRESSION OF DYSTROPHIN-ASSOCIATED GLYCOPROTEINS DURING HUMAN FETAL MUSCLE DEVELOPMENT: A PRELIMINARY IMMUNOCYTOCHEMICAL STUDY

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Abstract-An immunocytochemical study was performed on quadriceps muscle from .eight fetuses ranging from 12 weeks of gestation to term, using antibodies against the dystrophin-associated proteins, in order to evaluate the developmental expression of these proteins. For comparison, antibodies against dystrophin and utrophin were also used. The expression of the 59 kDa dystrophin-associated protein was simultaneous with that of dystrophin. which is also a subsarcolemmal protein. The extracellular glycoprotein of 156 kDa (rY-dystroglycan) and the transmembrane glycoprotein of 43 kDa (pdystroglycan) appeared to be expressed later. The transmembrane glycoproteins of 50 kDa (adhalin) and 35 kDa were fully expressed at an even later stage of fetal muscle development. This study suggests that the subsarcolemmal proteins may have an essential role in the assembly of the transmembrane and extracellular components of the dystrophin-glycoprotein complex during fetal muscle development. The knowledge obtained from observing the developmental expression of these proteins may contribute to the understanding of the molecular mechanism of their different involvement in muscle disorders.

Human fetal muscle, development, dystrophin, utrophin, dystrophin-glycoprotein complex, dystrophin-associated proteins, dystroglycan, adhalin.

INTRODUCTION

The discovery of a large oligomeric complex of glycoproteins associated with dystrophin gave a new insight to the understanding of the molecular mechanism of Duchenne and Becker tnuscular dystrophies [l-6]. This complex spans the sarcolemma providing a link between the subsarcolemmal cytoskeleton and laminin, a major component of the extracellular matrix [a-9]. The complex is composed of four glycoproteins (156, 50, 43 and 35 kDa) and two proteins (59 and 25 kDa). The I56 and 43 kDa glycoproteins (named a-dystroglycan and pdystroglycan, respectively) are encoded by a single gene mapped to chromosome 3q21[9]. The a-dystroglycan is an extracellular glycoprotein which binds to laminin and is linked to pdystroglycan, a transmembrane protein [IO]. The 50 and 35 kDa glycoproteins and the 25 kDa protein are also transmembrane while the 59 kDa protein is cytoplasmic and is linked to dystrophi". In Duchenne muscular dystrophy (DMD), the absence of dystrophin leads to a great reduction in dystrophin-associated proteins, thus disrupting the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix. This is presumed to render muscle fibers susceptible to necrosis. In Becker muscular dystrophy (BMD) it was recently shown that the partial deficiency of dystrophin correlates both in intensity and distribution with the reduction of all ofthe dystrophin-associated proteins [I 1, 121.

The 50 kDa dystrophin-associated glycoprotein (adhalin), is a novel transmembrane glycoprotein [I31 expressed almost exclusively in skeletal and cardiac muscles [l3, 141. Adhalin is deficient in severe childhood autosomal recessive muscular dystrophy with DMD-like phenotype

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(SCARMD) [I 51, a disease first reported in Tunisia [I61 and prevalent in North African countries, which has been linked to chromosome 13ql2 [17, 181. Adhalin deficiency was subsequently found in European patients [I91 and it was recently demonstrated that SCARMD is genetically heterogeneous, as some Brazilian and French families with adhalin deficiency do not map to chromosome 13 [20, 211.

The modifications of the dystrophin-glycoprotein complex and its role in the pathogenesis of muscular dystrophies have recently been discussed in several publications [IO, 22-241. Here is presented a preliminary study of the distribution and localization of the proteins of the dystrophin-glycoprotein complex during the development of human fetal muscle.

MATERMLS AND METHODS

Muscle samples from quadriceps muscle were obtained under legal conditions from eight fetuses ranging from 12 weeks of gestation to term. The age of the fetuses was determined using different well established criteria [25-271. The muscle samples were collected shortly after delivery and they were frozen in isopentane cooled in liquid nitrogen and stored at – 80°C. Conventional histological and histochemical techniques were performed on IO fl transverse cryostat sections of frozen muscle specimens, as in other studies [28]. This allowed us to ascertain the state of preservation of the muscle tissue and its degree of differentiation. This study was carried out only in fetuses not presenting either autolytic or pathological changes.

For immunocytochemistry, 7 m transverse cryostat sections of muscle specimens from fetuses of different ages and from an infant of 9 months were placed on superfrost glass microscope slides and processed as previously described [5, 6, 1 I-12, 15, 291. Indirect immunofluorescence was performed using antibodies against dystrophin (directed against the C-terminal, rod and N-terminal domains), utrophi", the 59 kDa dystrophin-associated protein (59DAP), and the 156 kDa (156DAG or a-dystroglycan), 50 kDa (SODAG or adhalin), 43 kDa (43DAG or pdystroglycan) and 35 kDa (35DAG) dystrophin-associated glycoproteins, previously characterized [5, 6, 1 l-12, 15, 291. Other monoclonal anti-dystrophin antibodies (NLC DYS 2 and NLC DYS 3, Novocastra) were also used. The sections were examined under a Zeiss Axioplan fluorescence microscope and the photographs were taken under identical conditions with the same exposure time.

RES"LTS AND DISCUSSION

The antibodies against dystrophin and utrophin showed an expression of these proteins in the fetus of 12 weeks of gestational age (Fig. l), as has been reported in other studies [3&35]. The expression of these two proteins diverged during subsequent stages of fetal muscle development: dystrophin progressively increased until birth while utrophin rapidly decreased. At 12 weeks of gestation, the antibody against utrophin showed strong labelling of the periphery ofthe myotubes, but at 32 weeks the labelling of the sarcolemma of the muscle cells was already faint. At birth it was almost absent but remained prominent at the neuromuscular junctions, as was also seen in the 9.month-old child.

The first protein of the dystrophin-associated complex to be abundantly expressed was the 59DAP. In fetuses of I2 weeks of development the antibody against this protein labelled the periphery of myotubes (Fig. I). This labelling was discontinuous in most muscle cells but in older fetuses it became progressively more regular and at 32 weeks was clearly distinct around the periphery of nearly all muscle fibers. The labelling with the antibody against the 59DAP during development was concomitant with that obtained with the anti-dystrophin antibodies (Fig. I).

The 156DAG and 43DAG (œ and pdystroglycan, respectively) were little expressed in the younger fetuses (Fig. 2). Their expression became more apparent in fetuses of 16 weeks of development and in older fetuses it progressively increased in intensity. At 32 weeks most fibers were already labelled by the antibodies against these glycoproteins.

The expression of adhalin and 35DAG was delayed in relation to that of a- and pdystroglycan (Fig. 3). Until the age of 22 weeks very few muscle cells were labelled by the antibodies against these two glycoproteins, particularly with the antibody against adhalin. At 32 weeks the labelling with the antibodies against adhalin and 35DAG was still irregular and less intense than that seen at term and in the 9-month-old infant.

The neuromuscular junctions were labelled with the antibodies against all proteins of the dystrophin-glycoprotein complex. The labelling of neuromuscular junctions is illustrated in

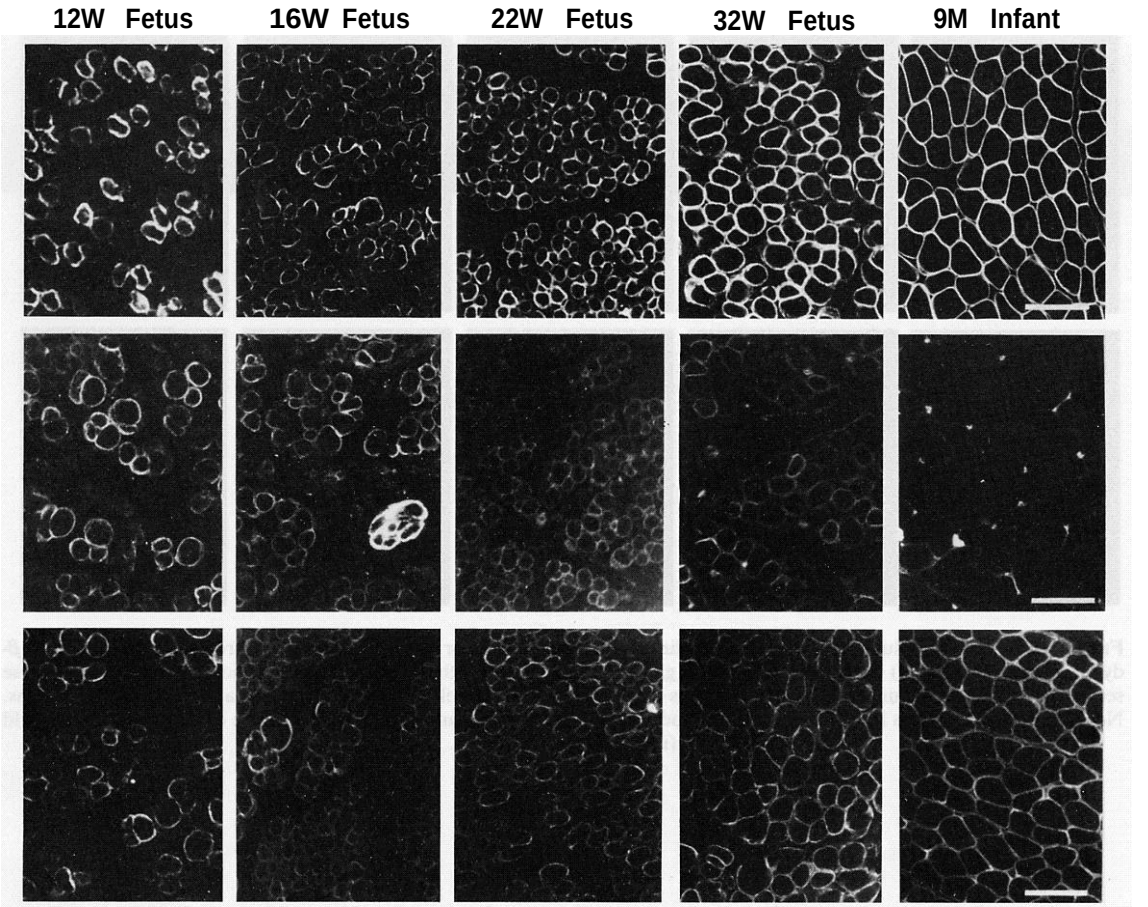

FIG. 1. Indirect immunofluorescence with antibodies against the C-terminal domain of dystrophin (upper row), utrophin (middle row) and the 59 kDa dystrophin-associated protein (lower row) showing the expression of these proteins in cryostat transverse sections of quadriceps muscle from human fetuses of 12, 16, 22 and 32 weeks of development and a child aged nine months. Bar 20 μm.

the 9-month-old child with antibodies against α–dystroglycan (Fig. 2) and adhalin (Fig. 3). No labelling with any of the antibodies used was seen in cells at the myoblast stage, lying between the myotubes in the younger fetuses.

The results of this study are schematically illustated in Fig. 4. The strong expression of utrophin at an early age of fetal development has been reported previously [33-351. Dystrophin and 59DAP are also expressed early but at a lower rate. These three proteins are subsarcolemmal and their expression appears to precede the expression of the transmembrane and extracellular glycoproteins of the dystrophin-glycoprotein complex. This suggests that they may play an essential role in the assembly of the glycoproteins of the dystrophin-glycoprotein complex. The concomitant expression of both the α– and β–dystroglycan correlates with the fact that these two glycoproteins are closely related and have a common messenger RNA [6, 9]. The concomitant expression of adhalin and 35DAG may also suggest a link between the two glycoproteins. This may explain why in SCARMD, a condition characterized by a deficiency in adhalin, there is a slight reduction in 35DAG [15, 19]. It is interesting to note that the drastic decrease in utrophin occurs in fetuses of about 22-24 weeks of gestation and this is concomitant with the installation of monoaxonal innervation [36] and muscle fiber type cyto-enzymological differentiation [37, 38]. Utrophin persists at the neuromuscular junction in Duchenne muscular dystrophy and ***mdx*** mice [39-41], and may compensate for dystrophin deficiency [42, 43]. It appears to be nerve dependent, as it is overexpressed after experimental denervation in ***mdx*** mice [44, 45].

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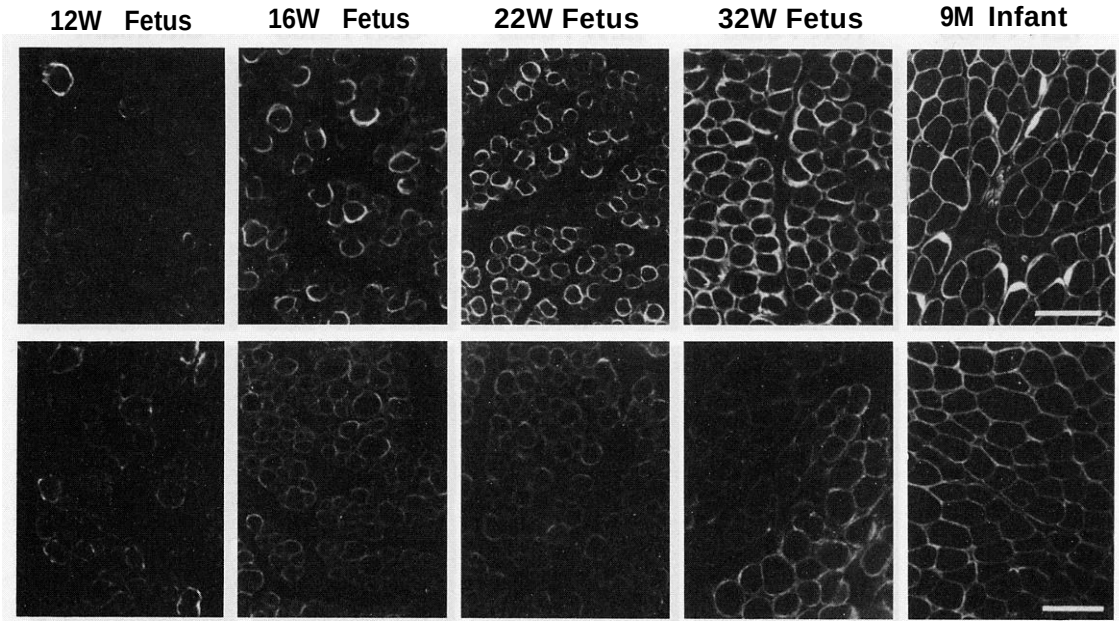
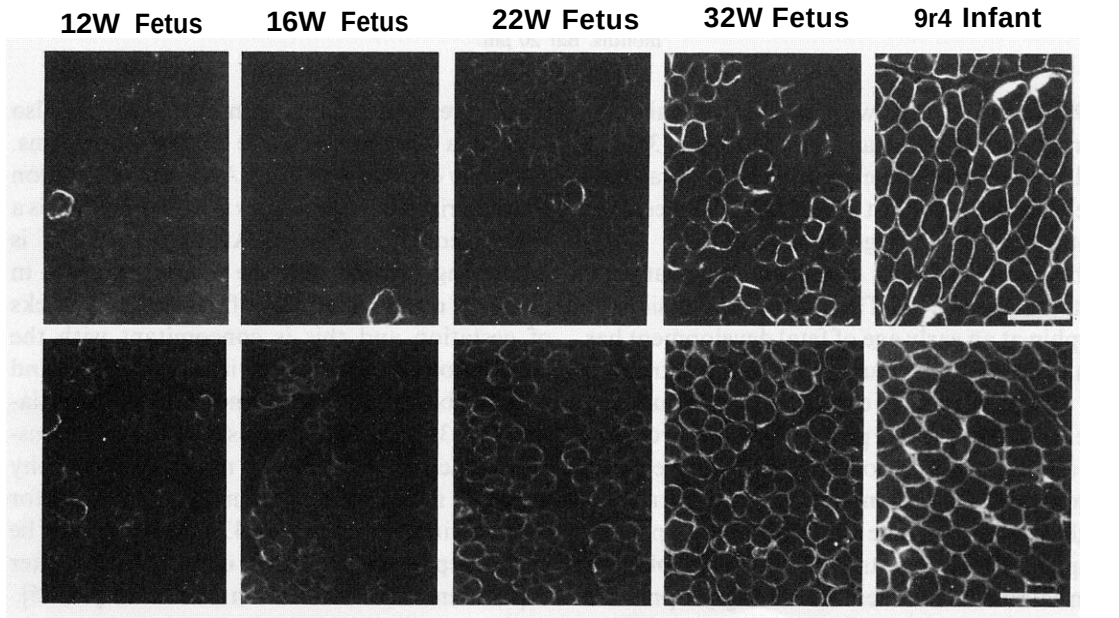


FIG. 2. Indirect immunoAuorescence with antibodies against the 156 kDa or edystroglycan (upper row) and the 43 kDa or p dystroglycan (lower row) dystrophin-associated glycoproteins, showing their progressive expresion in cryostat transverse sections of quadriceps muscle from human fetuses of I2. 16.22 and 32 weeks ofdevelopment and a child aged nine months. Neuromuscular junctions labelled with the antibody against a-dystroglycan are seen in the muscle of the %month-old child (upper row). Bar 20 μm.



FE 3. Indirect immunofluorescence with antibodies against the 50 kDa (adhalin) (upper TOW) and 35 kDa dystrophin-associated glycoproteins (lower row), sbowmg then expression in cryostat transverse sections of quadriceps muscle from humanfetusecof!2,16,22and32 weeksofdevelopment andachildaEedninemonths.Neuromuscularjunctionslabelledwith the antibody against adhalin are seen in the muscle aCthe 9.month-old child (upper row). Bar 20 p.

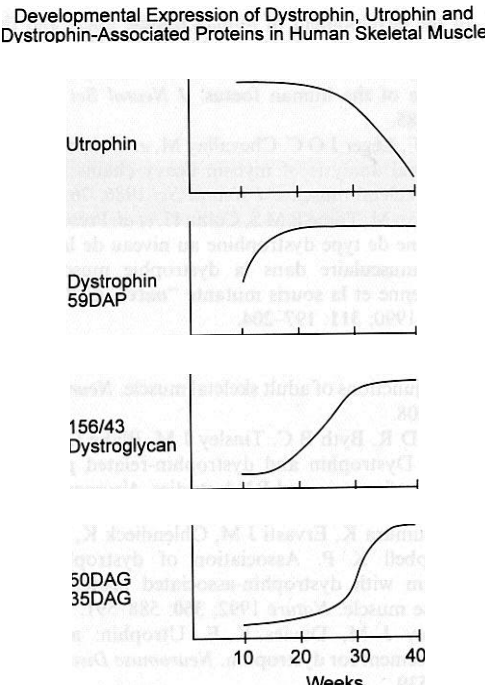


FIG. 4. Schematic representation afthe sequential expression of utraphin, dystrophin and dystrophin-associated proteins during human fetal muscle development.

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