Altered membrane proteins and permeability correlate with cardiac dysfunction in cardiomyopathic hamsters

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Altered membrane proteins and permeability correlate with cardiac dysfunction in cardiomyopathic hamsters. Am J Physiol Heart Circ Physiol 278: H1362–H1370, 2000.—A mutation in the δ-sarcoglycan (SG) gene with absence of δ-SG protein in the heart has been identified in the BIO14.6 cardiomyopathic (CM) hamster, but how the defective gene leads to myocardial degeneration and dysfuncion is unknown. We correlated increased membrane permeability with increased sarcolemmal membrane permeability and investigated the LV distribution of the dystrophin-dystroglycan complex in BIO14.6 CM hamsters. On echocardiography at 5 wk of age, the CM hamsters showed a mildly enlarged diastolic dimension (LVDD) with decreased LV percent fractional shortening (%FS), and at 9 wk further enlargement of LVDD with reduction of %FS was observed. The percent area of myocardium exhibiting increased membrane permeability or membrane rupture, assessed by Evans blue dye (EBD) staining and wheat germ agglutinin, was greater at 9 than at 5 wk. In areas not stained by EBD, immunostaining of dystrophin was detected in CM hearts compared with control hearts. As previously described, abnormally expressed at the intercalated discs; in addition, the expression of β-dystroglycan was significantly reduced compared with control hearts. As previously described, α-SG was completely deficient in CM hearts compared with control hearts. In myocardial areas showing increased sarcolemmal permeability, neither dystrophin nor β-dystroglycan could be identified by immunoblotting. Thus, together with the known loss of δ-SG and other SGs, abnormal distribution of dystrophin and reduction of β-dystroglycan are associated with increased sarcolemmal permeability followed by cell rupture, which correlates with early progressive cardiac dysfunction in the BIO14.6 CM hamster.

δ-sarcoglycan; dystrophin-dystroglycan complex; heart failure; Evans blue dye

Numerous studies on muscular dystrophy have focused on dystrophin with its associated membrane-glycoprotein complex and their pathophysiological role (see Ref. 3 for review). The transmembrane dystrophin-glycoprotein complex (DGC) serves as a link between the intracellular cytoskeleton and the extracellular matrix, connecting intracellular F-actin, dystrophin, the transmembrane sarcoglycans, and the dystroglycans (α-, β-subunits) with extracellular laminin-α2. The sarcoglycan complex of dystrophin-associated glycoproteins is composed of four subunits (α, β, γ, δ), sarcospan, the dystrobrevins, and the syntrophins (3, 24). Although the precise function of these components has not yet been elucidated, the main current hypothesis regarding the primary role of the DGC holds that it has a mechanical function to strengthen the plasma membrane during muscle contraction (3). Also, several lines of data support a role for dystrophin in regulating certain signal transduction pathways, along with the syntrophins and/or calmodulin (24). The function of the sarcoglycan complex is not known, although the sarcoglycan-sarcospan complex is assumed to serve as a molecular stabilizer of the DGC because mutations in any sarcoglycan subunit lead to a concomitant loss or reduction of all four sarcoglycans and sarcospan, manifested in various types of muscular dystrophy or cardiomyopathy (24). In addition, the molecular function of each subunit can differ (4); for example, deficiencies of γ- and δ-sarcoglycan are associated with a cardiomyopathy, whereas α-sarcoglycan deficiency is not (5).

The BIO14.6 cardiomyopathic (CM) hamster, an autosomal recessive strain, recently has been found to have a mutation in the δ-sarcoglycan gene (19). Sakamoto et al. (22) subsequently reported that two other strains of cardiomyopathic hamsters (UMX7.1 and TO-2) also possess a mutation in the δ-sarcoglycan gene. In addition, δ-sarcoglycan was reported to be involved in type F limb girdle muscular dystrophy (18). Deficiency of δ-sarcoglycan leads to a concomitant loss of the other sarcoglycan subunits (α, β, γ), sarcospan, and δ-dystroglycan (25). Holt et al. (9) have recently shown that transfer of the normal human δ-sarcoglycan gene can restore membrane structure and function in skeletal muscle of the BIO14.6 hamster.

The BIO14.6 hamster develops severe cardiomyopathy with hypertrophic features and heart failure but manifests a comparatively mild skeletal muscle phenotype. In dystrophin-deficient mdx mice, apoptosis appears to play a role in the onset of skeletal muscle degeneration (16, 27), whereas in one strain of CM hamster with heart failure (CHF147) we (8) have found that apoptosis plays a minor role compared with necrosis in the early deterioration of left ventricular function during the first 4 wk of life. Others (20) have reported a
correlation between the degree of muscle activity and sarcosomal damage in skeletal muscle of mdx mice. However, the correlation between changes in sarcosomal permeability and left ventricular (LV) function has not been quantified. Therefore, we investigated changes in cardiac function and membrane permeability, as well as the subcellular distribution of dystrophin and β-dystroglycan, during early progression of the cardiomyopathy in the BIO14.6 CM hamster.

MATERIALS AND METHODS

Animals. Four-week-old male hamsters (F1B strain) and age-matched male BIO14.6 CM hamsters were obtained from B10 Breeders (Fitchburg, MA) and housed under controlled temperature and humidity conditions during the serial echocardiographic study. The experimental protocol was approved by the Animal Subjects Committee of the University of California San Diego, and all procedures were conducted in accordance with institutional guidelines.

Echocardiography. Ten normal and eleven BIO14.6 CM hamsters underwent serial transthoracic echocardiographic examinations at 4, 6, 8, 10, 12, and 14 wk of age to assess LV size and function. The echocardiographic methods have been described in detail in the rat (10) and mouse (26). The CM hamsters showed normal cardiac function at 4 wk of age and subsequently manifested LV dysfunction; we selected 5 and 9 wk of age to correlate changes of membrane permeability and immunostaining with echocardiographic findings. Briefly, after anesthesia with pentobarbital sodium (65 mg/kg ip for normal hamsters and 55 mg/kg ip for BIO14.6 hamsters), the anterior chest was shaved, and small needle electrodes for recording the electrocardiogram were inserted into the upper two extremities and lower left extremity. Using the left lateral position, we placed the echocardiographic probe on the surface of the chest and left ventricular end-systolic and end-diastolic dimensions (LVSD, LVDD, respectively), and the thicknesses of the LV posterior wall and interventricular septum (PWT, IVST) were measured from the LV M-mode tracing. Ejection time was estimated from the aortic Doppler flow signal at the LV outflow tract. Variables derived from the echocardiographic data, including percent fractional shortening (%FS) of the LV diameter, the percent thickening of the posterior wall (%WT), mean circumferential fiber shortening rate (VCF) corrected by the R-R interval, and the estimated LV mass index, were calculated as previously described (26).

In vivo assessment of membrane permeability. To assess changes in sarcosomal membrane permeability of cardiac myocytes, in vivo Evans blue dye (EBD) distribution was assessed. EBD is a nontoxic, low-molecular-weight dye (mol wt 960) that binds to serum albumin when infused intravenously and allows to recirculate (16). EBD cannot pass the sarcolemma of intact myocytes, but if the membrane is ruptured, it is found in the intracellular space where it binds to intracellular proteins (16). Intracellular staining by EBD was evaluated with OCT compound (Miles, Elkhart, IN) and frozen in isopentane chilled with liquid nitrogen. Consecutive sections were made from each frozen heart with the use of a cryostat (Microm, Heidelberg, Germany) with a chamber temperature of −25°C. Several pairs of cross-sectional 10-µm-thick adjacent sections were randomly selected from the consecutive sections. To visualize the sarcolemma and transverse tubules, sections were treated with 4% paraformaldehyde for 30 min and then stained with wheat germ agglutinin conjugated to fluorescein isothiocyanate (WGA-FITC) and coverslipped with anti-fading media (Gelvatol, Air Products and Chemicals, Allentown, PA). This section was observed under a fluorescent microscope (HFX-DX, Nikon, Japan) equipped with blue and green activation filters (488 and 546 nm, respectively); different barrier filters (520 and 590 nm, respectively) were used to view and photograph WGA and EBD staining separately. Adjacent sections were stained with hematoxylin-eosin (HE), and observed through bright-field optics. Because heart muscle has a strong autofluorescence in the green and red fluorescent channels, control sections were made from EBD-free hearts treated in the same fashion and compared with EBD-stained sections. With the use of ×100 magnification, 10 microscopic fields were randomly selected from each section, photomicrographs were taken, and the myocardial regions that were unstained and stained with EBD were scanned using a Hewlett-Packard PhotoSmart Photo Scanner. EBD-positive areas were estimated using SigmaScan Pro (Jandel Scientific Software, San Rafael, CA). The percent area of stained myocardium was calculated from each field and averaged.

Antibodies. Mouse monoclonal antibodies against the COOH-terminal domain of human dystrophin (clone Dy8/6C5) and clone MANDRA-1 (kindly supplied by Dr. G. W. Nigro, University of California San Diego, and all procedures were conducted in accordance with institutional guidelines. Cell lysates were harvested at 13,000 × g for 10 min at 4°C. Protein concentration was determined by the Bio-Rad protein assay, using BSA as standard. Cell lysates were mixed with 6× protein sampling buffer (final concentration of buffer: 50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 100 mM diethyethanol, 0.0001% bromophenol, loaded on 4–20% Tris-glycine gel, and SDS-PAGE performed at 15 mA constant current. Proteins were then

Preparation of cell lysates, immunoblotting. Normal and BIO14.6 hamster heart tissues at 6 wk were homogenized by a polytron grinder and lysed on ice for 30 min in 2 ml of ice-cold RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.1% deoxycholic acid, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail purchased from Sigma). Cell lysates were collected by centrifugation at 13,000 g for 10 min at 4°C. Protein concentration was determined by the Bio-Rad protein assay, using BSA as standard. Cell lysates were mixed with 6× protein sampling buffer (final concentration of buffer: 50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 100 mM diethyethanol, 0.0001% bromophenol, loaded on 4–20% Tris-glycine gel, and SDS-PAGE performed at 15 mA constant current. Proteins were then
transferred from SDS-PAGE gels to nitrocellulose membranes at 20 V overnight. Membranes were blocked in a western buffer (0.05% Tween-20 in Tris-buffered saline, pH 7.4) with 5% skim milk for 1 h, and then incubated with primary antibodies for 2 h at room temperature. After extensive washing with Western buffer, the membranes were incubated for 1 h with horseradish-conjugated goat anti-mouse or rabbit IgG secondary antibody diluted at 1:1,000 in the Western buffer with 0.5% milk. The horseradish peroxidase-conjugated protein complex was detected by enhanced chemiluminescence according to the manufacturer’s protocol (ECL kit, Amersham).

Light microscopic immunohistochemistry. Heart tissues (n = 5) were frozen in isopentane precooled with liquid nitrogen and sectioned at a thickness of 10 µm on a cryostat. After air-drying for 30 min, sections were incubated for 30 min in 1% BSA/3% normal donkey or goat serum (depending on the secondary antibody). Sections were incubated with primary antibody (dystrophin, β-dystroglycan, and α-sarcoglycan) for 60 min, fixed with 4% formaldehyde for 30 min, and then labeled with secondary antibody conjugated to FITC or Cy5. Omission of the primary antibody and omission of both primary and secondary antibodies provided negative controls. The subcellular structure of the dystrophin-dystroglycan complex was visualized with a laser scanning confocal microscope (MRC-1024, Bio-Rad).

Measurement of immunolabeling intensity. For measurement of immunolabeling intensity, images were taken as eight-bit-intensity digital image (intensity value = 0–255) from each channel using the same laser excitation and photo-multiplier conditions used for laser scanning confocal microscopy in BIO14.6 and normal hamsters. To compare intensities of membrane immunolabeling, six cross-sectional areas were randomly chosen from multiple sections of each heart (n = 5). To determine the intensity of sarcolemma and T tubules, areas showing an intensity over the threshold set at 25–30 were automatically detected by SigmaScan Pro (Jandel Scientific), and the average membrane intensity (arbitrary units) was computed as the average of those areas.

Electron microscopic immunohistochemistry of dystrophin. Tissue sections were fixed with 2% formaldehyde + 0.001% glutaraldehyde (n = 2) and treated with a denaturing buffer, 6 M guanidine hydrochloride in 50 mM Tris HCl. Sections were then incubated with blocking buffer (1% BSA/3% normal goat serum/1% cold fish gelatin in 0.1 M glycine/PBS) and subsequently incubated with the primary antibody for 2 h, washed six times with PBS, and then incubated with secondary mouse IgG conjugated to 1-nm immunogold (NanoGold, Molecular Probes). After several washes in PBS, sections were fixed with 2.5% glutaraldehyde, incubated with a silver enhancement solution (Intense M), followed with 0.05% gold chloride solution, then osmicated with 1% OsO₄, dehydrated, embedded into Epon resin (Arcturus, ACM Resin), and then polymerized by baking. Polymerized thick sections were further sectioned onto an electron microscope grid at a thickness of 0.1 µm. Thin sections were stained with 1% uranyl acetate and 1% lead solution (1% lead acetate, 1% lead nitrate) and observed on an electron microscope (JEM 100-CX, J EOL, Tokyo).

Statistics. Echocardiographic values are represented as means ± SD and other data as means ± SE. To compare echocardiographic data, a two-way ANOVA was used. The factors for ANOVA were the hamster strain and ages. When overall variances associated with those factors were significantly different, multiple comparison tests between normal and BIO14.6 CM hamsters, or between 4 wk and the other ages were performed using the Student-Newman-Keuls test. A P value < 0.05 was regarded as significant.

RESULTS

Echocardiographic findings. The body weight in CM hamsters was smaller but increased significantly with age from 4 to 14 wk in both normal and CM hamsters. The LV mass index, estimated by echocardiography divided by the body weight, tended to be smaller in BIO14.6 hamsters than in normals (difference significant only at 10–14 wk of age, data not shown). As shown in Fig. 1, the LVDD increased significantly with age in both strains of hamsters, but in the CM hamsters LVDD increased substantially more and was significantly larger than in normal hamsters between 8 and 14 wk. The LV systolic function, measured as %FS and corrected VCF, gradually decreased with age in CM hamsters and was significantly reduced compared with normal hamsters between 6 and 14 wk (Fig. 1; significant between 8 and 14 wk compared with 4 wk of same strain). The thickness of the posterior LV wall increased with age in normal hamsters but was significantly reduced in CM hamsters compared with normals after 8 wk (Fig. 1). All of these findings are consistent with progression of the cardiomyopathy. The heart rates were not significantly different between CM and normal hamsters at baseline (448 ± 54 vs. 465 ± 45 beats/min, respectively) and did not differ at subsequent time points.

In vivo assessment of membrane permeability. As shown in Fig. 2, at low and high magnification myocytes with increased sarcolemmal permeability showed two types of intracellular staining with EBD. In one pattern (type A), sarcolemmal and T tubular structures could be identified by WGA staining, and such blocks of cells exhibited a diffuse, uniform pattern of intracellular EBD staining (Fig. 2, A and B, asterisks) without abnormalities on the adjacent HE-stained sections (Fig. 2B). In the second pattern (type B), the structure of the sarcolemma and T tubules could not be identified by WGA staining, and EBD-stained areas were irregular and less intense (Fig. 2, E and H, pound sign). In the adjacent HE-stained sections, clusters of necrotic myocytes with infiltration by mononuclear or polymorphonuclear cells were observed (Fig. 2, A and B). In normal tissues (Fig. 2C), there was no intracellular staining with EBD, as shown in Fig. 2, F and I.

The percentage of the entire LV cross-sectional area stained by EBD (including both staining patterns) in normal and CM hamsters is shown in Fig. 3. In normal hamsters, no myocytes were stained with EBD at 5 wk (rare myocyte noted at 9 wk), whereas in CM hamsters 1.42 ± 0.88% of myocytes were EBD positive at 5 wk with a significantly further increase to 5.17 ± 1.64% at 9 wk.

Because cardiac myocytes exhibit autofluorescence under the fluorescent microscope, we also observed sections unstained with EBD in which the autofluorescent effect on EBD positivity was negligible and similar.
results were obtained. Heart sections from advanced CM hamster hearts (3–5 mo) show calcified areas in the myocardium, which exhibit strong emission at red fluorescent channels, but this effect was negligible at 5 and 9 wk.

Immunoblotting and immunohistochemistry by light microscopy. On immunoblot analysis, the expression of dystrophin protein was not altered. However, β-dystroglycan was reduced (~9% reduction compared with normals), and α- and δ-sarcoglycan proteins were completely deficient (Fig. 4).

To assess the subcellular distribution of the DGC and its spatial relationship to damaged myocardial areas, we compared immunoblotting and immunohistochemical myocardial labeling of dystrophin, β-dystroglycan, and α-sarcoglycan at 5, 9, and 14 wk of age in normal and CM hamster hearts. α-Sarcoglycan was considered representative of all the sarcoglycans, which have been shown to be absent or markedly reduced in the BIO14.6 hamster (25). As shown in Fig. 5, E and F, α-sarcoglycan was found to be distributed in the sarcolemma in normal hamster hearts, whereas it was not detected in the BIO14.6 hamsters even at 5 wk, suggesting that the sarcoglycan complex was lost consequent to the deficiency of δ-sarcoglycan. The transmembrane component of the DGC, β-dystroglycan, was localized at the sarcolemma and T tubules as a continuous staining pattern both in normal and CM hamster as shown in Fig. 5, A–D. However, in CM hamster hearts the intensity of β-dystroglycan was reduced by 19% (47.03 ± 3.51 vs. 37.14 ± 1.96 arbitrary units, P < 0.05) compared with normal hearts (Fig. 5).

Dystrophin was identified in the sarcolemma and T tubules both in normal and CM hamsters, and the intensity of dystrophin in the CM hamster hearts was comparable to that in normals. However, in CM hamster hearts dystrophin also was strongly expressed at the intercalated discs as shown in Fig. 6, D and F (arrows), but it was not found at that location in normal hearts. There was no staining of β-dystroglycan at the intercalated discs of CM hamster hearts.

The distribution of dystrophin and dystroglycan in cardiomyocytes exhibiting increased sarcolemmal permeability was assessed in sections stained for both EBD and WGA. The membrane lipid and glycoprotein components showed staining by WGA, whereas there was no specific staining of dystrophin and β-dystroglycan in these areas, suggesting that the DGC was completely lost, despite some preservation of cellular architecture on WGA and HE staining in these areas.

Electron microscopic immunohistochemistry of dystrophin. Because dystrophin was abnormally localized at the intercalated disc in CM hamster hearts, confirmation by electron microscopic immunolabeling of dystrophin was performed. In normal hamster hearts dystrophin was clearly distributed at the sarcolemma, frequently at the invagination site of the T tubules at the Z lines (image not shown), but was not observed at the intercalated discs. In the CM hamster hearts, dystrophin was observed in association with the sarcolemma and with the sarcolemma and the intercalated discs as observed in Fig. 6.
DISCUSSION

Relationship of membrane damage to cardiac dysfunction. By quantifying the area of in vivo intracellular staining through the use of EBD in 5- and 9-wk-old hamster hearts, an increase of regions with enhanced membrane permeability and cell damage could be observed between 5 and 9 wk. Because the development of LV dysfunction also occurred during this period, increased membrane permeability could be the initiating event leading to myocyte damage and the progression of LV dysfunction.

HE staining of type B areas of WGA and EBD staining showed fracture and loss of the myocardial cell cytoskeleton as well as large numbers of inflammatory cells, findings consistent with necrosis, whereas areas with type A staining displayed relatively mild changes in myocardial structure and few inflammatory cells. Although the fate of type A EBD-stained myocytes is uncertain, apoptosis has been reported to have a significant role in mdx skeletal muscle that stained positive with EBD (16), occurring mainly in the early period before necrosis was evident (27). However, our preliminary results in the CHF147 hamster (a strain derived from the UMX7.1 CM hamster) indicated that the contribution of apoptosis to myocardial cell damage is

Fig. 2. Representative images of Evans blue dye (EBD) stained area. A, B: hematoxylin-eosin (HE) staining of area adjacent to wheat germ agglutinin (WGA) and EB sections. C: HE staining of normal field. Top half of A shows a necrotic area, and B is a high magnification of rectangular area in A. D, E: representative images of EBD intracellular staining or adjacent section on some regions. G, H: WGA staining of adjacent section of some regions to clarify sarcolemmal membrane structure of the cardiac myocytes. F, I: EBD/WGA staining from normal field, showing no intracellular EBD staining. In B, E, and H the * marks a typical type A EBD-stained myocyte; # indicates type B EBD-stained myocytes. Scale bar in A, C, D, F, G, I = 25 µm; bar in B, E, H = 10 µm.

Fig. 3. Quantification of EBD-positive area. Percent EBD-stained area of entire transverse LV sections at 5 and 9 wk in normal and BIO14.6 cardiomyopathic (CM) hamster hearts (n = 5, each group). *P < 0.05 vs. normal hamsters; #P < 0.05 vs. 5 wks.
minor compared with that resulting from necrosis early during the onset of heart dysfunction (8). Therefore, it can be hypothesized that the continuous contraction of the heart, which is enhanced in rate and strength during exercise, may promote progressively more sarcolemmal rupture with increasing age in hearts with cardiac δ-sarcoglycan deficiency. The absence of dystrophin and β-dystroglycan immunolabeling in type A-positive EBD-stained areas in which WGA staining of membrane lipid and glycoproteins were preserved is consistent with increased sarcolemmal permeability rather than cell rupture and suggests that loss of the DGC from the sarcolemma predisposes to cell rupture.

Other pathological mechanisms must also be considered, including the possibility that microvascular spasm described by Factor et al. (7) also may be involved in causing necrosis in blocks of myocardial cells. In this connection, the δ-sarcoglycan protein has been reported to be absent in vascular smooth muscle (6, 14).

Distribution of dystrophin and β-dystroglycan in CM hamster hearts. It was recently reported (25) that all four sarcoglycans and α-dystroglycan are lost, whereas dystrophin and β-dystroglycan are preserved in the BIO14.6 hamster heart. In the present study, we correlated serial changes in cardiac function with the extent and type of myocardial damage and further investigated the subcellular distribution of dystrophin and β-dystroglycan in the young BIO14.6 hamster heart. In CM hamsters as young as 5 wk of age, we confirmed the absence of α-sarcoglycan, but unlike the previous study
we found β-dystroglycan to be reduced in the sarcolemma and T tubules. The use of a laser scanning confocal microscope to quantify β-dystroglycan may have allowed detection of the mild reduction in cardiac muscle. We also examined the expression level of β-dystroglycan in skeletal muscle and found no significant difference between normal and CM hamsters (data not shown), suggesting a possible contributing factor to the relatively more severe cardiac phenotype compared with that of skeletal muscle observed in BIO14.6 hamsters.

In CM hearts, dystrophin in the sarcolemma and T tubules was comparable to that in normal hamster hearts, but in addition it was strongly expressed at the intercalated discs at 5 and 14 wk. In skeletal muscle, dystrophin reportedly is found only at the sarcolemma, whereas in cardiac muscle it is also distributed in the transverse tubules but has never been reported to be present at the intercalated discs (13, 17). Decreased content of dystrophin protein has been reported previously on immunoblot analysis in BIO14.6 CM hamster hearts (11, 17), although this finding was evident in
animals older than those in our study. Our immunohistochemical data clearly indicate that dystrophin is preserved at 5 wk of age compared with β-dystroglycan. Iwata et al. (11) reported that the subcellular distribution of dystrophin in the B1014.6 CM hamster was not different from that in normals by using a rod domain anti-dystrophin antibody (Dy4/6D3). We used the same clone of anti-dystrophin antibody but found that it did not react as specifically with dystrophin in hamster tissue with immunoblotting and immunocytochemistry as the carboxy-terminal domain antibodies (Dy8/6C5 and MANDRA-1). Both light microscopic data and electron microscopic observations support our finding that dystrophin is abnormally distributed at the intercalated discs in the CM hamster hearts.

Dystrophin is a homolog of dystrophin localized to the intercalated discs in heart muscle; utrophin can replace the function of dystrophin in the dystrophin-deficient mdx mouse (21). Therefore, there is a possibility that the anti-dystrophin antibody used in the present study might have cross-reacted to some extent with utrophin localized at the intercalated discs, even if it exhibits a single band on Western immunoblotting, because the molecular weights are not distinguishable. However, we used specific monoclonal antibodies to dystrophin, and labeling by the antibody at the intercalated discs was absent in normal hamster hearts where utrophin is abundant (21). Therefore, cross-reactivity of the anti-dystrophin antibody with utrophin is a highly unlikely explanation for our finding.

Several lines of evidence suggest that alterations are present at the intercalated discs in the CM hamster. Kawaguchi et al. (12) reported decreased expression of desmin and increased expression of vinculin at the intercalated discs in CM hamsters. Luque et al. (15) described decreased expression of the gap junction protein connexin43 on immunohistochemical analysis in CM hamsters; connexin43 turns over several times a day (1), and it is possible that its expression might be affected by excessive dystrophin distribution at the intercalated discs. However, when and how the abnormal distribution of dystrophin occurs and its functional significance remain to be investigated. Two possible hypotheses might be considered. Dystrophin is reported to have interactions not only with the dystrophin-associated proteins but also with other cytoskeletal proteins such as the integrins (28), caveolin-3 (23), and aciculin (2) in cultured skeletal myocytes. Aculin, a cell adhesion regulatory protein, is localized mainly at the adherens junctions in cardiac myocytes. Because dystrophin is also localized at the intercalated discs near the adherens junction, we can speculate that in the CM hamster heart the cytoskeletal function of dystrophin may be partially mediated through adherens junction proteins, such as aciculin. Alternatively, the δ-sarcoglycan deficiency may cause abnormal dissolution of dystrophin protein into the cytosol, where it aggregates at the intercalated discs due to protein-protein interactions, without playing any functional role.

In summary, together with the known absence of the sarcoglycans and reduced β-dystroglycan, abnormal distribution of dystrophin may contribute to the observed loss of sarcolemmal integrity, which was associated with early progression of cardiac dysfunction in the CM hamster.

NOTE ADDED IN PROOF

Since this work was submitted, a study has been published (see Ref. 4a) on mice deficient in δ-sarcoglycan showing disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle, with evidence for a role of vascular dysfunction in the development of cardiomyopathy in that murine model.

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