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An Unusual Familial Cardiomyopathy Characterized by Aberrant Accumulations of Desmin-Type Intermediate Filaments

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Summary. Immunofluorescence microscopy using antibodies specific for different intermediate filament types has been used to study a rare familial cardiomyopathy characterized electron microscopically by massive accumulations of unordered intermediate filaments. The results show that the inclusions in cardiac muscle cells are composed of the desmin type of intermediate filament characteristic of muscle tissues, and draw attention to the importance of these filaments in maintaining normal cardiac ultrastructure and function.

Key words: Cardiomyopathy – Intermediate filaments – Immunofluorescence – Desmin

Introduction

A rare form of cardiomyopathy found in three brothers with progressive myocardial deficiency has been described by Porte et al. (1980). Histologically this cardiomyopathy is characterized by large proteinaceous inclusions in cardiac muscle cells. Under the electron microscope these inclusions consist largely of accumulations of filaments 7–10 nm in diameter.

Sera to different intermediate filament proteins can be used as cell type specific markers (Franke et al. 1978a; Bennett et al. 1978). Currently such sera distinguish five different tissue types, muscle, epithelia, mesenchyme, glial (astrocytes) and neuronal tissue (for review see Lazarides 1980; Weber and Osborn 1981). Thus it was of interest to try to identify the type of intermediate filament present in this cardiomyopathy by immunofluorescence techniques. The inclusions were strongly stained by antibodies to desmin (skeletin), the intermediate filament type typical of muscle tissue (Cooke 1976; Lazarides and Hubbard 1976; Small and Sobieszek 1977). In normal cardiac muscle, antibodies to desmin stain Z bands and intercalated discs; in this cardiomyopathy, although the

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Z bands were stained, very little decoration of intercalated discs was observed. Thus an abnormality either of desmin expression or turnover, or of the incorporation of desmin into the supramolecular structures seems to characterize this new type of cardiomyopathy.

Material and Methods

Two brothers, K.M., born in 1948 and K.J., in 1954, whose case histories have been documented along with ultrastructural observations on cardiac and skeletal muscle biopsies (Porte et al. 1980) died of heart failure in July and December 1980, respectively. A few hours after death, samples of myocardium and of skeletal muscles were fixed by conventional techniques for light and electron microscopy, or were frozen in liquid nitrogen and subsequently stored at $-70°$ C in preparation for immunofluorescence microscopy. Samples obtained at autopsy under the same conditions from a patient with no history of heart trouble served as controls.

Microscopy. Frozen sections approximately 5 μ in thickness were cut on the cryostat and transferred to clean glass slides. The sections were dried for 1 h at room temperature and fixed by immersion in acetone at $-10°$ C for 10 min. They were then treated with the primary antibody (see below) for 30 min at 37° C, washed well with phosphate buffered saline, and treated with the appropriate fluorescein labeled second antibody for 30 min at 37° C. After a further wash in phosphate buffered saline, the specimens were mounted in Mowiol 4–88 (Hoechst, Frankfurt). For further details of the immunofluorescence procedures see Osborn and Weber (1981). For details of the electron microscopical procedures see Porte et al. (1980).

Antibodies. The antibodies to prekeratin, vimentin and desmin used in this study have been fully characterized on cells in culture and on frozen sections of rat tissues (see for example Franke et al. 1978, 1979, 1980; Osborn et al. 1980). They were used at a concentration of approximately 50 μg/ml. The desmin antibody was elicited in rabbits against desmin purified from chicken gizzard. The antibody was further purified by affinity chromatography on the antigen, bound to Sepharose 4B. The vimentin antibody was elicited in guinea pigs against vimentin purified from mouse 3T3 cells (Franke et al. 1979) and affinity purified on vimentin from rabbit chrondrocytes bound to Sepharose 4B. The prekeratin antibody was made in guinea pigs against prekeratin purified from cow hoof (Franke et al. 1978b) and affinity purified on the same antigen bound to Sepharose 4B.

Fluorescein labeled goat anti-rabbit IgGs, or rabbit anti-guinea pig IgGs were purchased from Miles-Yeda, Israel, and were used at a final concentration of approximately 0.5 mg/ml.

Results

Cardiac Muscle

The main features previously described in cardiac muscle cells from heart biopsies of both pathological cases (Porte et al. 1980) were still clearly recognizable despite the poor ultrastructural preservation due to post mortem changes. Large chromophobic or slightly acidophilic inclusions were present in most muscle fibres in all areas of the myocardium that were examined (Fig. 2). Under the electron microscope, these inclusions were seen to consist of large numbers of randomly oriented intermediate filaments in which sarcoplasmic reticulum (SR) components and glycogen were often included (Fig. 1). Other conspicuous changes included the disruption of myofibrils, the presence of rod-like bodies presumably derived from the Z-bands and extensive development of the sarcoplasmic reticulum. This frequently showed signs of massive involution resulting in the accumulation of myelin bodies.

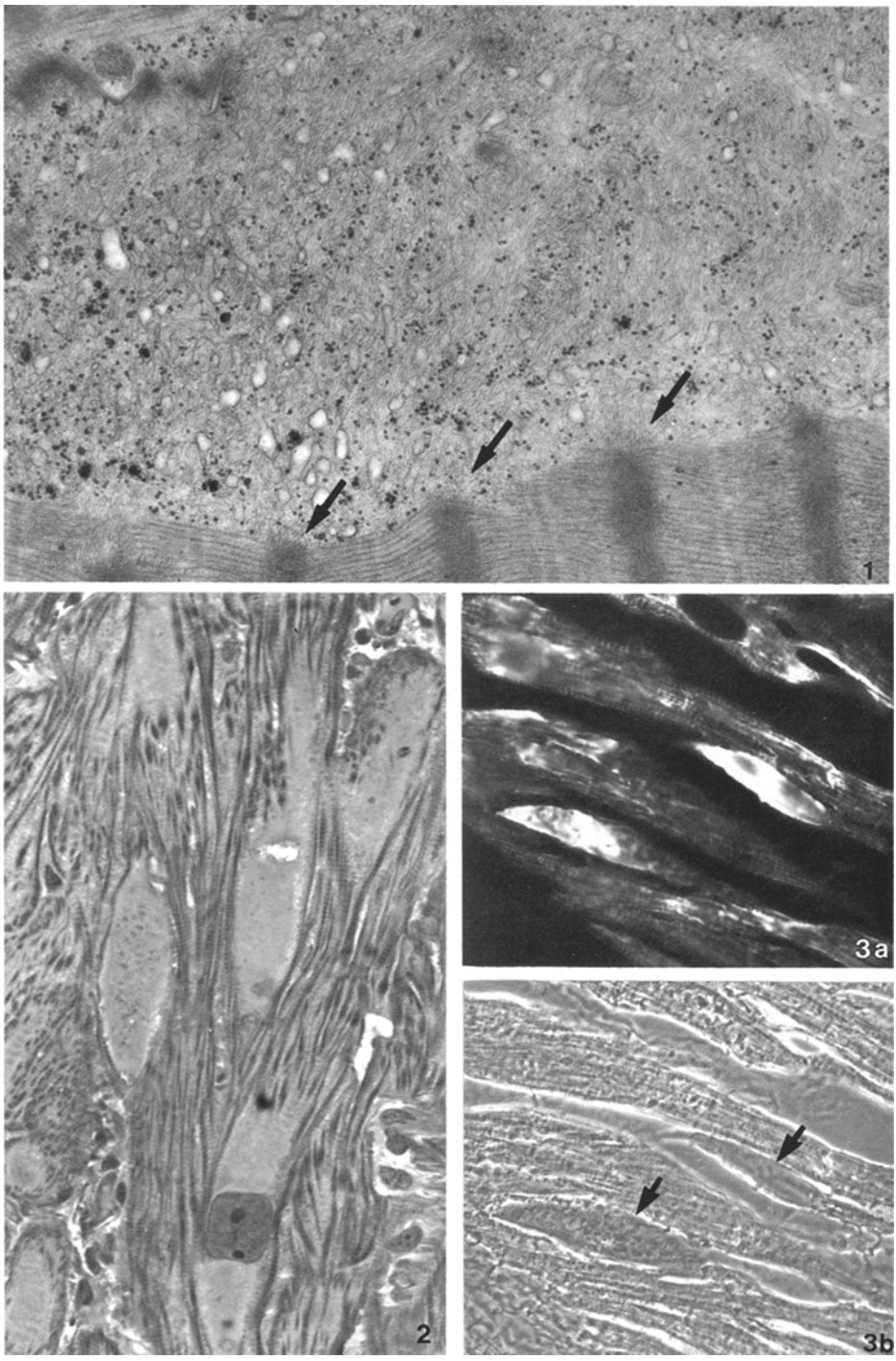


Fig. 1. Case K.J. Thin section through a filamentous inclusion. Note the large number of randomly oriented intermediate filaments, as well as the SR elements and glycogen particles visible in this cardiac muscle cell. The arrows indicate tufts of intermediate filaments connected with the Z bands of an isolated myofibril. ×21,500

Fig. 2. Case K.J. Semi-thin section stained with ferric haematoxylin-eosin. Note the large chromophobic inclusions in the cardiac muscle fibres. ×340

Fig. 3a, b. Case K.J. **a** Frozen section of cardiac muscle treated with purified antibodies to desmin in indirect immunofluorescence microscopy. The inclusions are strongly fluorescent. **b** Same area as in (**a**), but viewed with phase contrast optics. The inclusions are indicated by arrows. ×400

On myocardium cryostat sections of both cases desmin antibodies revealed immunofluorescent inclusions in most cardiac muscle cells as well as Z band staining of muscle fibres (Figs. 3a, 4).

Observations of the same section with epifluorescence and phase contrast showed that the inclusions which stained most strongly had an amorphous structure, while less intensely fluorescent inclusions had a granular aspect (Fig. 3). From electron microscopal studies it is known that the smooth inclusions are almost exclusively formed of intermediate filaments, while the "granular" inclusions contain other components such as SR tubules, glycogen, myelin bodies and myofibrillar residues in variable amounts. Thus the immunofluorescence intensity seems to reflect, at least qualitatively, the amount of intermediate filaments present. Besides the large inclusions, smaller fluorescent areas were observed scattered throughout the heart fibres (Figs. 3a, 4).

Other antibodies were also tested on frozen sections of the myocardium to see if they reacted with the inclusions. Prekeratin antibodies stained neither the inclusions nor the muscle fibres. Vimentin antibody stained neither the inclusions nor the muscle fibres but stained fibroblasts lying between the muscle fibres and vascular endothelial and smooth muscle cells, the latter being also stained strongly with desmin antibodies. Thus the inclusions are positive for desmin, but negative for two other intermediate filament subunits, i.e., vimentin and prekeratin. Antibodies against actin and the microfilament associated proteins α-actinin and tropomyosin did not stain the inclusions.

When formalin-fixed paraffin embedded samples of myocardium from the pathological cases were deparaffinized and treated in immunofluorescence microscopy with desmin antibody, inclusions were visible and showed similar variations depending on their amorphous or granular structures. Z bands were not clearly defined by desmin antibodies in such preparations (Fig. 6).

Fig. 4. Case K.J. Frozen section of cardiac muscle treated with antibody to desmin. Immunofluorescent inclusions and diffuse deposits are stained but intercalated discs are not apparent (cf. control Fig. 5). ×300

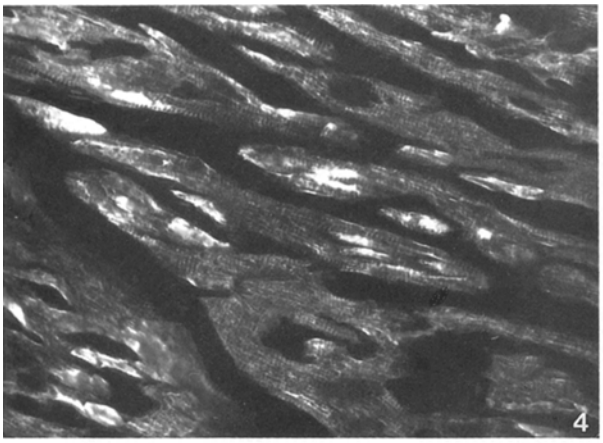
Fig. 5. Normal heart. Frozen section treated in indirect immunofluorescence microscopy with antibody to desmin. Z bands and intercalated discs are positively stained. ×300

Fig. 6. Case K.M. Formalin fixed, paraffin embedded section treated after deparaffinization in indirect immunofluorescence microscopy with antibody to desmin. ×480

Fig. 7. Case K.J. Frozen section of skeletal muscle treated in indirect immunofluorescence microscopy with antibody to desmin. Subsarcolemmal areas and intermyofibrillar spaces are strongly stained. ×560

Fig. 8. Normal heart. Thin section through intercalated disc showing normally folded appearance and high concentration of fasciae adherentes. ×16,800

Fig. 9. Case K. Thin section through intercalated disc region. Note the unfolded appearance of this region in comparison with that seen in the control heart (Fig. 8). ×16,800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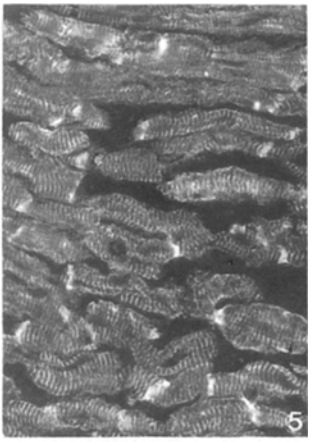
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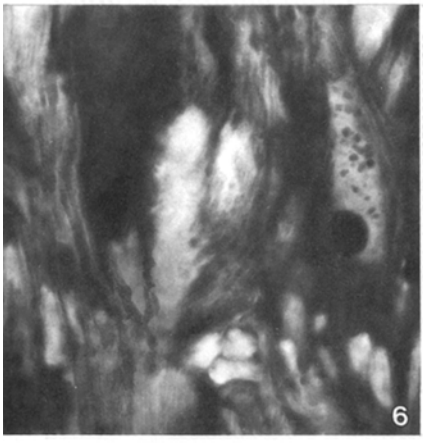
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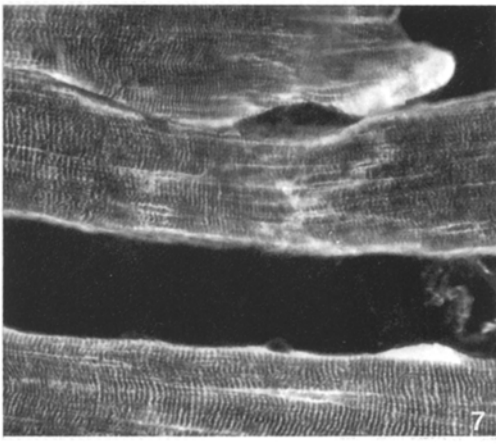
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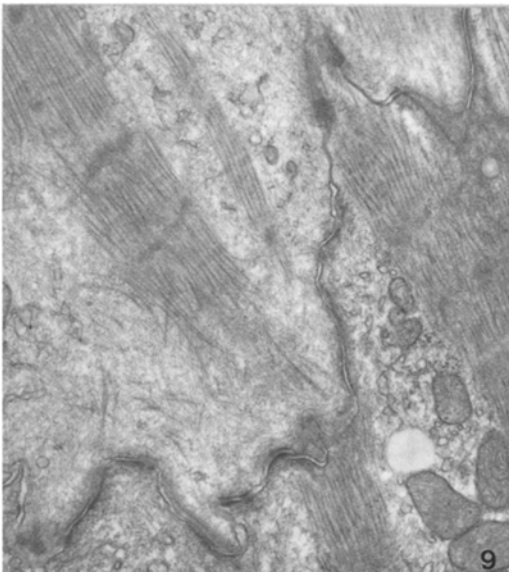
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Intercalated Discs

The Z bands in the muscle fibres in both pathological cases were strongly fluorescent (Figs. 3, 4) whereas the intercalated discs present in cardiac muscle were not clearly revealed. Only in rare fibres did a faintly fluorescent line suggest their presence, although they were easily detected on adjacent sections treated with haematoxylin-eosin. Moreover, control human heart sections taken at autopsy from a patient with no history of heart disease and treated with the same desmin antibody (Fig. 5) showed strongly fluorescent intercalated discs.

An attempt was made to compare the ultrastructure of the intercalated disc in normal heart (Fig. 8) with that seen in the cardiomyopathy (Fig. 9). The results show that although the control specimen displays the normally folded appearance typical of this structure, the specimen from the cardiomyopathy is much more unfolded. In addition there is an apparent difference in the concentration of fasciae adherentes in the two cases.

Skeletal Muscle

Electron microscopic examination of the skeletal muscles (intercostal, deltoid), from the pathological cases showed occasional intermediate filaments accumulations intermingled with glycogen and SR components. These were present mainly in subsarcolemmal areas but were also seen in intermyofibrillar spaces. They were in general very much smaller in size than those observed in cardiac muscle cells. When frozen sections of skeletal muscle from the cardiomyopathy cases were examined with desmin antibody (Fig. 7), fluorescent staining of subsarcolemmic areas and of intermyofibrillar trails was observed in some fibres. These presumably correspond to the filament accumulations seen in the electron microscope. In addition, Z band staining was observed. As in control muscle, no staining of the muscle fibres were detected with vimentin antibody, but strong staining of the fibroblasts lying between the muscle fibres was observed.

Discussion

The results of immunofluorescence confirm the presence of intermediate filaments in the inclusions accumulated in heart muscular fibres in this unusual familial cardiomyopathy. They identify the intermediate filaments as being of the desmin type. The inclusions are strongly stained by antibodies to desmin, the type of intermediate filaments typical of muscle tissues, but not by antibodies which are specific for other types of intermediate filaments, such as antibodies to vimentin or prekeratin. Heart intermediate filaments are normally abundant in the impulse-conducting system and in the force-producing fibres embedded in the connective tissue (see Viragh and Challice 1969). Purkinje fibres react strongly with antibodies agains desmin (Eriksson et al. 1979). In the pathological cases we studied, desmin filaments accumulated in large inclusions in fibres of the whole myocardium, and to a much lesser extent in skeletal muscle samples. This suggests a generalized disturbance in expression or turnover of desmin

or of a protein (or proteins) which control the assembly of desmin filaments into supramolecular structures, such as the Z band or the intercalated disc.

In normal muscle, desmin filaments play a cytoskeletal role, interconnecting myofibrils at the level of the Z bands and attaching them to the sarcolemma. In addition desmin probably serves to attach actin filaments to the Z bands and to faciae adherentes of the intercalated discs (see Lazarides and Hubbard 1978), thus helping to maintain normal myofibrillar architecture. The immunofluorescence results on the pathological material show apparently normal desmin incorporation into Z bands. However the myofibrillar disruption, with the particular modifications of Z band material observed in filamentous areas, suggests a relationship between the myofibrillar alterations and the 7–10 nm filament deposits. Immunofluorescence observations in nemaline myopathy (Thornell et al. 1980) suggests a dissociation between the occurrence of desmin and the rod-like bodies which appear to be derived from Z bands. In one case the nemaline rods have been shown to stain strongly with antibody to α-actinin whereas antibody to desmin stains only the region around the rods (Jockusch et al. 1980). However, in the familial cardiomyopathy we have studied the conspicuous accumulation of 7–10 nm filaments cannot be explained simply by polymerization of desmin detached from Z band material of disrupted myofibrils. More complex mechanisms affecting desmin regulation must be involved.

The intercalated discs in the cardiomyopathy were not clearly revealed by immunofluorescence with desmin antibody although they are clearly visualized in control specimens from normal human heart using the same antibody. When the cardiomyopathy specimens were studied under the electron microscope, only limited areas of the intercalated discs showed a classical deeply folded organisation; they were stretched by fibre dilation so that the faciae adherentes, with myofibrillar insertions, were much more dispersed in the junctional zones, although their fine structure appeared unaltered (Figs. 8, 9). These modification in the spatial organization of the faciae adherentes, together with abnormally high amounts of filaments in the sarcoplasm, might explain the poor definition of the intercalated discs by immunofluorescence. However the myofibrillar disruptions and filament accumulations, especially marked in the vicinity of disorganized intercalated discs, suggest a possible defect in actin fixation of the sarcolemma. This might be due to a deficiency in desmin renewal at the insertion sites.

Many aspects of this cardiomyopathy are difficult to evaluate because of the rarity of its occurence and the limited amount of material available for study. Nevertheless the results encourage the use of immunofluorescence techniques as an aid to diagnosis provided that highly characterized antisera recognizing one or other of the five intermediate filament classes are available. The pertubation of desmin expression, turnover, or of the incorporation of desmin filaments into structures such as the Z band or intercalated disc, results in the accumulation of 7–10 nm filaments visible in cardiac muscle cells in both light and electron microscope, and presumably also accounts for the progressive myocardial deficiency. Thus evidence is provided, at the genetic level, for the importance of the correct supramolecular organization of 7–10 nm filaments in maintaining normal cardiac function.

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