Z Band Abnormality Characterized by Interwoven Structure in Human Cardiac Muscle

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Summary. The fine structure of a Z band abnormality which closely resembled that of nemaline body was studied using myocardial biopsies. The abnormality was found in 10 out of 103 examined patients with various cardiac muscle diseases. Ultrastructurally, they were characterized by interwoven textures of fine filaments in a form similar to the nemaline body. However, they differed in two points from the nemaline body: first, most of them were interposed between the sarcomeric arrangement as an intramyofibrillar architecture, and second, two parallel filaments with periodic bridges were demonstrated in this abnormality. In the present study, the interwoven Z band abnormalities occurred regardless of the disease's sort, the patient's age, and the hypertrophic grade in the muscle cell. Meanwhile, some degenerative changes, myofibrillar lysis and mitochondrial degeneration, were frequently detected in the cardiac muscles with the abnormalities. Pathologically, the interwoven form of the Z band abnormality seemed to indicate an unbalance of the compensative mechanism at the cell level.

A Z band abnormality which closely resembled the nemaline body in the skeletal myopathy (Shy et al., 1963) has recently been reported in the human cardiac muscle by a few investigators (Legato, 1970; Ferrans et al., 1975; Maron et al., 1975). In the previous papers (Izumi et al., 1978a,b) we had proposed that the alteration should be regarded as an interwoven form of Z band abnormality. Thus, the present study aims at investigating the fine structure of the alteration and elucidating what effect this condition would reflect in the cardiac muscle cell biology.

MATERIALS AND METHODS

Cardiac muscle specimens of 103 patients were examined. The specimens were obtained from the right ventricular wall using Konno-Sakakibara endomyocardial biopsy. The examined cases were composed of: 47 patients with a primary myocardial disorder, such as idiopathic cardiomyopathy and myocarditis; 37 patients with secondary muscle damage, such as valvular or congenital heart disease; and 19 patients with hearts exhibiting electrical failure, such as atrioventricular block and
ventricular tachycardia. The age of those cases ranged from 2 to 72 years: 41.5 ± 15.1 years old (mean ± standard deviation). The biopsy specimens were fixed in 2.5% glutaraldehyde (0.1M phosphate buffer, pH 7.4) immediately after the sampling, and postfixed in 2% osmium tetroxide, dehydrated by ethanol and embedded in epoxy resin according to the conventional method. Both cross and longitudinal semithin sections of muscle fibers were cut from those epon blocks and successively stained with malachite green, toluidine blue and basic fuchsin (tribasic staining originated by Kurotaki, 1972). Muscle cell diameters were measured by the pen-digitizer computer system (Ohsawa, Oscon) using our method shown elsewhere (Izumi et al., 1978). After a series of diameter calibrations on 200 muscle fibers of the cross sections, the median and 95% range of cell diameters were calculated from only the measured values of the fibers containing nuclei. On the other hand, the longitudinal semithin sections of muscle fibers were observed to estimate the pathological lesions. Ultra-thin sections, cut from the blocks presenting the longitudinal aspects of the fibers, were stained with uranium and lead acetate and observed by a transmission electron microscope, Hitachi HU 125DS. Moreover, fine structures of the abnormalities were re-examined by an electron microscope equipped with a goniometer specimen stage (Hitachi H-500).

RESULTS

A cardiac Z band abnormality which looked like the nemaline body was characterized in morphology under two types of microscopes. Under the light microscope, the abnormal structures were dyed as darkly at the Z band by the tribasic staining. They existed in a rod-like shape and were interposed at the Z band position between the regular sarcomeric arrangements (Fig. 1A). Under the electron microscope, the abnormalities were composed of fine filaments with the same electron density as the Z band. Those filaments were woven into a structure which contacted to the thin myofilaments on both sides. Most of the structures with a length that corresponded to just one sarcomeric unit appeared singly in the sarcomeric arrangement as shown in Fig. 1B. At high magnification, parallel lines in a regular interval (7-15 nm) and periodic lines (10-20 nm) perpendicular to the former were found in those structures (Fig. 1C). The parallel lines were arranged generally in the same direction as the myofibrils. In cross sections, tetragonal lattice structures appeared (Fig. 1D). The interwoven structures were often variable in the cardiac muscles where the disorders were frequently found. For example, some of the structures varied for several sarcomeric units in length (Fig. 2A); the fine filaments differed in direction from the myofibrils in a few instances (Fig. 2B); and the abnormal structures occasionally clustered in the cytoplasm (Fig. 2C). A peculiar pattern of the texture was demonstrated at high magnification. Figure 3A reveals a couple of the filaments with periodic bridges perpendicular to the long axis. The same pattern was also conformed in ultrastructural observations of the interwoven structures using a goniometric specimen stage (Fig. 3B). In the left part, at +24° inclination angle to the myofilaments, the fine filaments spiral and align parallel with each other; in the middle part, at +6°, other parallel lines are detected in the texture; in the right part, at −30°, coupled filaments form numerous transverse bridges with periodic intervals.
Besides, whenever the interwoven structure appeared, the cardiac muscle specimens revealed either the Z band widening, or the clumped Z band materials or both (Table 1).

The Z band abnormality, reminiscent of a woven texture, was found in 10 out of 103 examined patients (9.8%). Those cases were composed of 4 out of 22 congestive cardiomyopathies, 2 out of 15 hypertrophic cardiomyopathies, and one each out of the followings: 2 cases of aortic stenosis, 12 cases of atrial septal defect, 2 cases of constrictive pericarditis and 4 cases of ventricular tachycardia. Among them, the interwoven structure appeared conspicuously in four cases (2 congestive cardiomyopathies, an atrial septal defect and a ventricular tachycardia). The interwoven structure did not change in morphology regardless of type of cardiac muscle disease. The mean age of patients showing its presence was $49.7 \pm 10.0$ years (mean $\pm$ standard

Fig. 1. A. An interwoven form of Z band abnormality under the light microscope. Tribasic staining. $\times 900$. B. A general appearance of the Z band abnormality under the electron microscope. $\times 25,000$. C. and D. High magnifications at the longitudinal section (C. $\times 73,000$) and the transverse section (D. $\times 150,000$)
Fig. 2. Legend on the opposite page.
deviation), while that of non-presence was 40.6±15.3 years. There was no obvious difference in age between both groups. Analysis of the muscle cell diameters produced the following results: a congestive cardiomyopathy showed the maximal diameter (31 μ in median); the minimum diameter (17 μ) was found in a ventricular tachycardia; and the other cases were scattered between the two values (Fig. 4). In brief, the interwoven structure appeared regardless of the hypertrophic level in the muscle cell. Moreover, a degenerative degree of the muscle cell was estimated by following four cytopathological indices: myofibrillar lysis, mitochondrial degeneration, nuclear deformity and sarcoplasmic vesicular change (Table 1). All of those cases showed various degenerative changes in grade. Especially, conspicuous degeneration was found in 6 cases.

DISCUSSION

The nemaline body, a specific rod shaped structure in the diseased skeletal myofiber was first noticed by Shy et al. (1963) and characterized morphologically by the following 5 features (Engel, 1966): 1) origin in Z band, 2) tetragonal filaments, 3) structural continuity with thin filaments, 4) periodic lines perpendicular to the long axis, and 5) periodic lines parallel to the long axis. At the present time, the body is considered to be laterally polymerized Z bands, and is composed of alpha-actinin and tropomyosin (Stromer et al, 1976). Concerning the cardiac muscle, an abnormality like the nemalin body was initially reported both in the right ventricular muscle of a dog (Maunelle and Getty, 1968) and in the papillary muscle of a cat (Fawcett, 1968). Thereafter, the abnormality was found also in the human cardiac muscle by

### Table 1. Summary of cellular alterations. The left part shows the combinations among Z band abnormalities; degenerative changes are exhibited in the right

<table>
<thead>
<tr>
<th>Z-band abnormality</th>
<th>Mitochondrial degeneration</th>
<th>Nuclear deformity</th>
<th>Sarcoplasmic vesicular changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widened form</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Clumped form</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Interwoven form</td>
<td>+</td>
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<thead>
<tr>
<th>Congestive cardiomyopathy</th>
<th>Myofibrillar lysis</th>
<th>+</th>
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<tbody>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Atrial septal defect</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constrictive pericarditis</td>
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<td>Ventricular tachycardia</td>
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Fig. 2. Variations of the interwoven abnormality. A. The abnormal structure extends over several sarcomere units. ×11,000. B. The filament's direction differs from the myofibril axis. ×15,000. C. The abnormal structure forms a cluster. ×5,000
Fig. 3. A. Filaments coupled with numerous bridges. ×64,000. B. Observations with a goniometric specimen stage at the inclination angles indicated under the figures. ×73,000
Interwoven Form of Z Band Abnormality

LEGATO (1970), Ferrans et al. (1975) and Maron et al. (1975). Goldstein et al. (1977) noted that the abnormality would be useful for close analysis of the Z band structure because a common lattice structure was seen in foundation between those structures.

We have also observed such structures in a previous study on myofibril alterations of idiopathic cardiomyopathy, and called it an interwoven form of Z band abnormality (IZUMI et al., 1978a). The form could be categorized as one of three types of abnormality: the first one was represented by either Z band widening, branching or overriding; the second was indicated by clumping of Z band material; and the last was characterized by the interwoven structure of Z band filaments. In that report, we commented that the interwoven abnormality would appear in a few cases with idiopathic cardiomyopathy, and might be closely related to the first type of the Z band alteration because intermediate forms between the two types could be encountered. The present study has confirmed that the interwoven form had the same cardinal morphology as the nemaline body having the 5 features discribed above (Fig. 1). At the same time, there were two obvious differences between them. Firstly, most of the interwoven forms were interposed between the sarcomeric arrangements as an intramyofibrillar architecture (Fig. 1, 2A), while nemalin bodies were generally seen as a cluster in the cytoplasm (Fig. 2C); however, the appearance of the interwoven structure might differ greatly between the atrium and the ventriculus. In the atrial muscle, clustered structure seemed to be more prevalent than the interposed one (Mitsui et al., 1977). Secondly, the abnormality had an interesting fine structure: a couple of filaments with periodic bridge formation (Fig. 3) which has never been reported in the ultrastructure of the nemaline body. We dare to call this abnormality the interwoven form, though it has already been named Z band accumulation (LEGATO, 1970), or Z-rod (Goldstein et al., 1977). The reason is that the form was recognized as an intramyofibrillar Z band abnormality and the architecture reminded us of a woven texture at first sight. According to Goldstein et al. (1977, 1979), the interwoven structure had a three-dimensional structure closely similar to the Z band, which was presumed to be dynamic polymers of subunits forming \( 38 \times 24 \times 24 \) nm lattices. The subunits were proposed to be composed of an axial filament and four connecting filaments projected perpendicular to the former. The bridge lines of the texture, as shown in Fig. 3, could be predicted by his Z band model, because the periodic interval nearly corresponded to the subunit in length and the bridges seemed to be equivalent to the connecting filaments (Fig. 3). However, the coupled filaments could not be interpreted by his proposal. Further analysis seemed to be necessary to elucidate the three-dimensional structure of the interwoven abnormality.

There is no agreed opinion on cytopathological interpretations of the interwoven abnormality. Maunnel and Getty (1968), and Fawcett (1968) regarded it as cellu-
lar changes caused by aging. **LEGATO** (1970) claimed that it might be a stage in new production of the sarcomere. Further, **MARON** and **FERRANS** (1978) considered it to reflect an unbalance between the synthesis of Z band material and that of other sarcomeric components. In the present study, there was no disease specificity in the appearance of the interwoven abnormality. Therefore, it is not appropriate to propose a new concept of myocardial disease unlike the instance of the nemaline myopathy. In addition, there was also no obvious difference in age between the cases with the interwoven abnormality and those without it. Thus, we could not conclude that there is a significant correlation between the abnormality and the aging of the cardiac muscle. Concerning the sarcomerogenesis (**LEGATO**'s hypothesis), we examined the muscle cell diameter from the viewpoint that new synthesis of the sarcomere might be closely related to cell hypertrophy. However, **LEGATO**'s proposal could not be accepted because the interwoven forms appeared regardless of the hypertrophic level in the cardiac muscle cell (Fig. 4). Consequently, we agreed with the interpretation by **MARON** and **FERRANS** (1978): it reflects an unbalance in the synthesis of sarcomeric components, because some degenerative changes, myofibrillarlysis and mitochondrial degeneration were found more or less conspicuous in the cardiac muscle with the interwoven form (Table 1). Namely, a cardiac muscle presenting the interwoven form Z band abnormality seems to have lost the ability to synthesize new sarcomeric units which are necessary to counterbalance the loads given to the muscle, though it can produce only Z band materials. In the end, the materials may accumulate between sarcomeric arrangements or in the cytoplasm showing such appearance. From this viewpoint, the muscle with the interwoven form is considered to have lost a compensative power at the cell level. The cytopathological change may be caused from some factors such as a regression in cell activity or a deficiency in energy supply.

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**編目構造を呈するZ帯異常**

和泉 徹, 三浦和正, 服部 晃, 田村康二

ヒト心筋においても骨格筋でのネマリン小体に似たZ帯異常が存在する。本研究の目的は、その形態的特徴と心筋の病態との関連を明らかにすることである。各種心筋疾患患者103例の右室生検標本を電子顕微鏡で検索すると、10例（9.8％）に、この異常を認めた。形態上は細線維の繊りなす編目構造を主徴とし、ネマリン小体の基本構造と酷似していた。しかし、この異常の多くは、筋節配列間に介在する筋原線維内構築物である点や、編目構造を構成する細線維が対になり、その間に多数のブリッジ形成のみられる点が小体と異っていた。一方、この編目型異常は疾病の種類、患者の年齢、心筋細胞の肥大度と無関係に出現した。
ただ、その異常の出現した心筋においては、細胞の退行性変化の顕著なもののが多かった。すなわち、このZ異常は心筋細胞に生じた代償機構の破綻を反映すると考えられた。

REFERENCES


