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Electron microscopic observations on the formation of elastic fibres in cultures of aortic medial cells and adventitial cells

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Summary. The formation of elastic fibres was observed in the cultured cells derived from the tunica media and the tunica adventitia of mouse aorta.

Bundles of myofilaments with dense bodies were abundantly observed in the cytoplasm of the cultured medial cells, and numerous bundles of microfibrillar components were present in the intercellular spaces. Fine granules of approximately 50 nm in diameter were observed in the bundles of microfibrillar components. It was supposed that these fine granules of elastin fused with each other and formed elastic aggregates and then formed large elastic clumps.

Numerous bundles of microfibrillar components were also present in the intercellular spaces of the cultured adventitial cells. Elastic aggregates were scarcely observed in the bundles of microfibrillar components. However, large elastic clumps as observed in the medial cell culture could not be found in the adventitial cell culture.

It is suggested that the formation of large elastic clumps might be related to the sheet structures or lamellae of elastic fibres in the tunica media.

Key words: Elastic fibres, Cell culture, Aorta, Electron microscopy

Introduction

Ross and Bornstein (1969) indicated that elastic fibres consist of two distinct structural components: the central amorphous component (elastin) and the peripheral microfibrils, and they suggested that microfibrils play a primary role in the elastogenesis.

It is well known that elastic fibres in the tunica media of aorta represent sheet structures or lamellae, but those in the tunica adventitia do not represent such structures. Baba et al. (1985) reported that aortic elastic fibres in the tunica adventitia consisted of central amorphous elastin surrounded by many microfibrils. However, elastic fibres in the tunica media were associated with few microfibrils.

Since the morphological differences are present between elastic fibres in the tunica media and those in the tunica adventitia *in vivo,* we investigated the formation of elastic fibres at the ultrastructural level in the mouse aortic medial and adventitial cells *in vitro.*

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Materials and methods

Culture technique

The aortae were obtained from two-day-old mice. The tunica adventitia was separated from the aorta under direct vision of a dissecting microscope. The aortic medial cells were enzymatically isolated using trypsin (0.2% in Hanks' solution at 37°C for 1 to 2 hours). Freshly isolated cells were seeded into Falcon culture dishes (3.5 x 10 mm) at an initial density of 1 x 10^5 cell/dish and grown in Ham's F-12 medium (Seromed Biochem HG) supplemented with 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 µg/ml). A plastic cover slip (Miles Lab.) was put on the bottom of the culture dish. The cultures were maintained at 37°C in an atmosphere of 5% CO_2 in air. Medium was changed at 2 to 3 day intervals.

The tunica adventitia separated from the aortae were also cultured in the same manner as described above.

Electron microscopy

Samples for electron microscopy were taken at 10 and 21 days after the start of culture. The cell layers on a plastic cover slip were fixed in 1% tannic acid-2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature for 1 hour and postfixed in 1%

phosphate-buffered osmium tetroxide at 4°C for 30 minutes. After dehydration in graded ethanol the specimens were embedded in Epon 812. Thin sections were cut with a LKB Ultrotome, stained with uranyl acetate followed by lead citrate and examined in a JEL 100-C electron microscope.

Results

Medial cell culture

The enzymatically isolated aortic cells after removal of the tunica adventitia were grown, and formed a multilayer on a plastic cover slip. In the cultured cells, bundles of myofilaments with dense bodies were observed. Weibel-Palade bodies were not seen in the cultured cells. Numerous microfibrillar components were present in the intercellular spaces (Fig. 1). At 10 days, masses of electron dense material presumed to be elastin deposit were scarcely seen. At three weeks, many elastic aggregates which were masses of electron dense material were observed (Fig. 2). Large elastic clumps which were not surrounded with microfibrillar components were seen (Figs. 3, 4). Fine elastic granules were present in the microfibrillar components. These elastic granules were approximately 50 nm in diameter (Fig. 5).

Adventitial cell culture

The bundles of myofilaments with dense bodies were not seen in the culture cells derived from the tunica adventitia of aorta (Fig. 6). Microfibrillar components were present in the intercellular spaces, as observed in the medial cell culture. Elastic aggregates were scarcely present in the microfibrillar components (Fig. 7). However, large elastic clumps, which were observed in the medial cell culture, were not seen even at three weeks.

Discussion

It was postulated that the bundles of microfibrils appear first and then the amorphous component (elastin)

Fig. 1. A ten-day-old culture. Numerous microfibrillar components are seen in the intercellular space. x 9,000

Fig. 2. A three-week-old culture. Elastic aggregate (arrow) is seen in the microfibrillar components. x 10,800

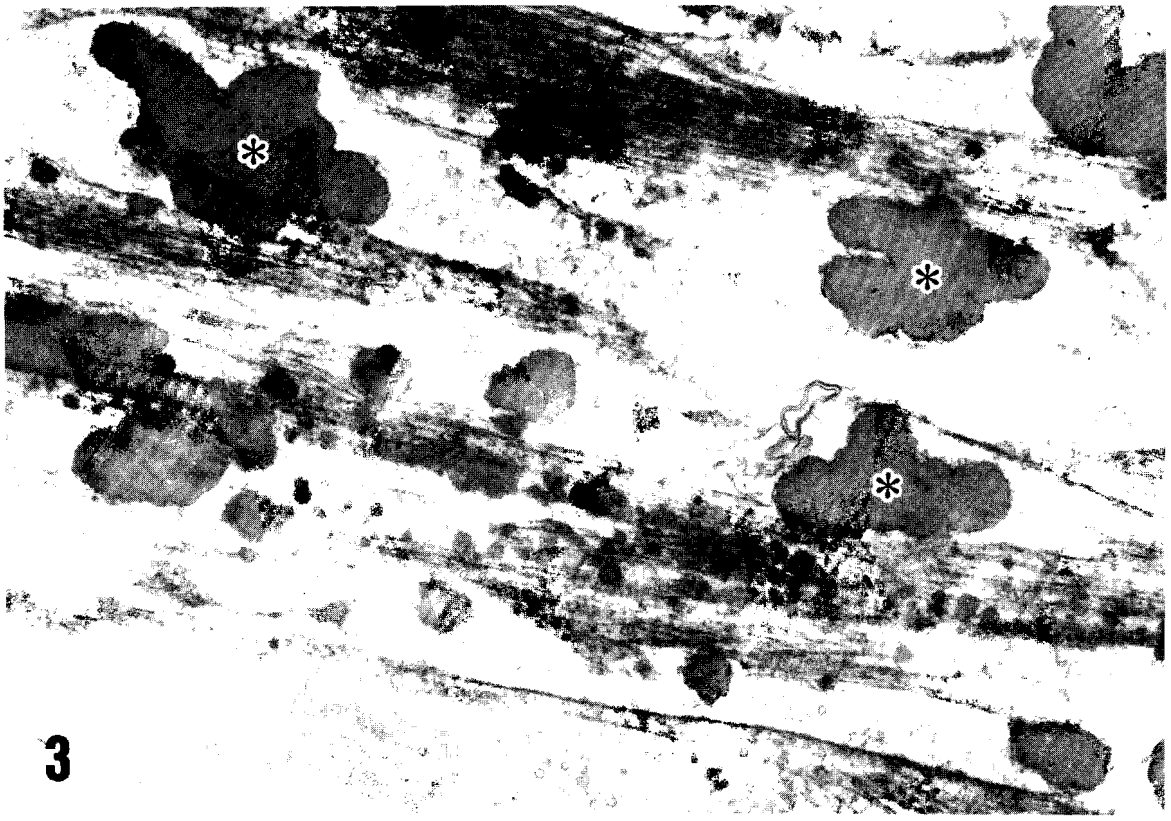

Fig. 3. A three-week-old culture. Large elastic clumps (asterisks) are observed. Elastic clumps are partly adheared to the microfibrillar components. x 18,000

Fig. 4. A three-week-old culture. Elastic clumps are formed by fusing of elastic aggregates. x 18,000

Fig. 5. A three-week-old culture. Fine granules of elastic material are seen in the microfibrillar components. x 36,000

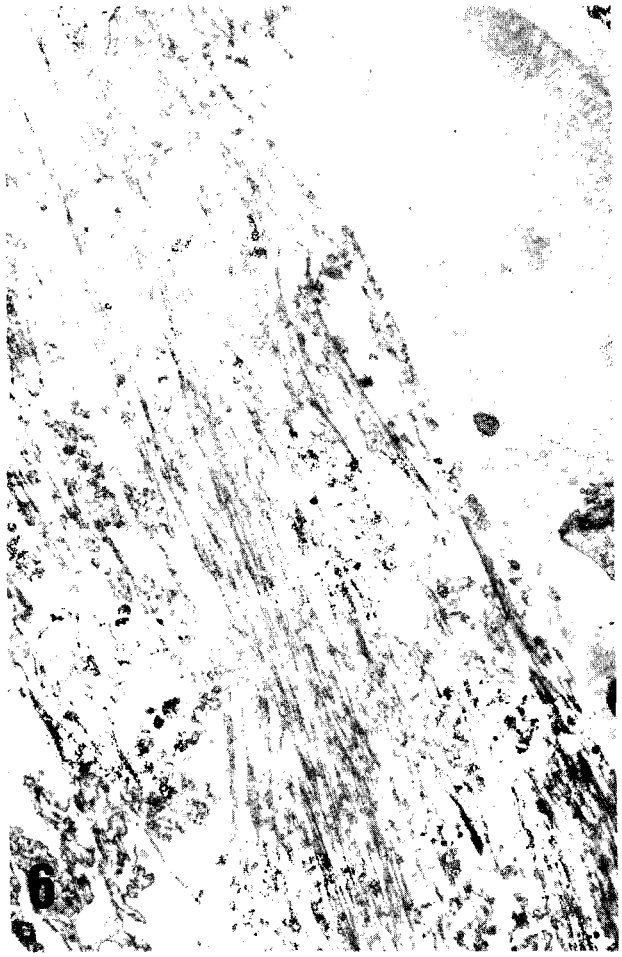

Fig. 6. A ten-day-old culture (adventitial cell culture). Numerous microfibrillar components are also seen in the intercellular space. x 5,400

Fig. 7. A three-week-old culture (adventitial cell culture). Elastic aggregates (arrows) are seen in the microfibrillar components. x 21,600

accumulates within the bundles in ligamentum nuchae (Ross and Bornstein, 1969). Karrer (1960) described a manner of elastic fibre formation in the aorta similar to that occuring in ligamentum nuchae. Recent evidence, however, questions the necessity of microfibrils for elastic fibre formation. Katsuda and Kajikawa (1977) pointed out that elastin deposit in the early stage of elastogenesis was not accompanied with microfibrils. Microfibrils are absent from elastic tissues of some fish species (Serafini-Fracassini et al., 1978).

Elastogenesis has been studied in cultured cells from arterial medial layer (Ross, 1971; Heineke and Tyberg, 1977; Kádár et al., 1981; Toselli et al., 1981) and intimal layer (Carnes et al., 1979). In studying cells derived from aortic medial cells, these authors described that microfibrillar components were present prior to the appearance of amorphous component (elastin). In this study, similar findings were obtained not only in the aortic medial cell culture, but also in the adventitial cell culture. Kádár et al. (1981) reported that electron-dense dots of 12 nm in diameter and electron lucent dots of 19.3 nm in diameter appeared, probably representing elastin aggregates at different stages of maturation in the aortic medial cell culture. We observed tannic acid-positive electron-dense elastic granules of 50 nm in diameter within microfibrillar components. In the in *vivo* system, Haust (1965) observed elastic granules of 50-150 nm in diameter in the fetal human aorta. Katsuda et al. (1977) reported that elastic granules of 25-50 nm in diameter were present in the aorta of experimental arteriosclerosis of rabbit. In this study, we could not find morphologically the fine dots of elastin described by Kádár et al. (1981). It seems that histochemical and immunohistochemical searches are needed to identify elastin, such as fine elastic dots.

The electron-dense elastic granules (50 nm in diameter) observed in this study aggregate with each other within the microfibrillar components, and then form larger masses of elastin. Elastogenesis in the medial cell culture may follow a similar process to that described by Ross and Bornstein (1969). However, in the medial cell culture large masses of elastin were present beside microfibrillar components. These large masses of elastin appeared to be linked with each other without bundles of

microfibrillar components, and then formed large elastic clumps. On the other hand, in the adventitial cell culture amorphous elastic aggregates were also observed within microfibrillar components, but large elastic clumps as observed in the medial cell culture, which were not surrounded with microfibrillar components, were not found. The formation of large elastic clumps observed in the medial cell culture might be related to the sheet structures or lamellae in the tunica media. These findings *in vitro* may prove the morphological differences of elastic fibres in the tunica media and adventitia *in vivo*. Although the regulating factor causing the difference of elastogenesis in the medial cells and adventitial cells are unknown, this culture system may provide useful information to analyze the regulating factor and to explain the morphological differences of elastic fibres in the tunica media and adventitia.

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