FINE FILAMENTS IN LYMPHATIC ENDOTHELIAL CELLS

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Several and various types of cells contain fine cytoplasmic filaments closely resembling the myofilaments of muscle cells (2, 18, 23, 24). In many of these cells and especially when cultured, it has been demonstrated that some of these filaments react with heavy meromyosin (HMM) in the same way as do the actin filaments of muscle cells (3, 6, 7). This suggests that these filaments may be actinoid and form part of a contractile system.

As fine intracytoplasmic filaments do occur in lymphatic endothelial cells (2, 14), we undertook an electron microscope investigation of their fine structure and their reaction on incubation with HMM and EDTA. We postulated that lymphatic endothelial cells possess a contractile filamentous system to which these filaments belong.

MATERIAL AND METHODS

The pulmonary peribronchovascular lymphatics of 20 neonatal rabbits, up to 2 days of age and sacrificed with an intraperitoneal injection of nembutal, were investigated. In total, 186 lung tissue blocks not exceeding 1 mm in thickness were studied.

Glycerination and Incubation with HMM

130 tissue blocks were glycerinated according to the procedure of Ishikawa et al. (7) successively in 50%, 25%, and 5% glycerol. All glycerol solutions were prepared by dilution with the standard salt solution (0.1 M KCl, 0.005 M Mg Cl2, 0.006 M phosphate buffer, pH 7.0).

70 glycerinated tissue blocks were incubated in a HMM solution (17.5 mg/ml) 40 h at 0°C. The myosin was isolated from rabbit psoas muscle (6, 17, 20). HMM
was obtained by trypsin digestion of the myosin and separated from the light meromyosin fraction by dialysis and centrifugation (16).

40 glycerinated tissue blocks were incubated with the HMM solution containing 0.01 M ATP, and 20 glycercinated tissue blocks were incubated with the standard salt solution without HMM (40 h at 0°C).

**Incubation with EDTA**

36 tissue blocks were incubated with the standard salt solution containing 5 mM EDTA for 1 h at 4°C (4).

**Electron Microscopy**

All tissue blocks were fixed in 2.5% glutaraldehyde (pH 7.2, 2 h, 4°C), postfixed in 1% OsO₄, and embedded in Epon. The ultrathin sections were stained with uranyl acetate and lead citrate (22) and investigated with a Philips 300A electron microscope. The effect of the HMM and EDTA upon the filaments of the lymphatics was compared with the reactions of the myofilaments of the peribronchovascular smooth muscle cells in the same tissue blocks.

**RESULTS**

**Normal Morphology and Topography**

The endothelial cells of the peribronchovascular lymphatics contain a large number of fine cytoplasmic filaments (Fig. 1 a, b), measuring either 9-10 nm (thick filaments) or 5-6 nm (thin filaments).

The thick filaments lie mainly in the middle of the cell, but may also occur in the other cell parts. They are usually grouped into variably oriented bundles, but sometimes form a rather loose network. On cross-section the thick filaments exhibit a less electron-dense core and appear tubular (Fig. 1 b, inset).

The thin filaments usually form bundles, too, but these are always located close to the abluminal cell membrane (Fig. 1 a, b). In longitudinally sectioned bundles the individual filaments are difficult to distinguish from each other. The bundles of thin filaments are sometimes longer than 5 μm and often exhibit variably sized, unevenly distributed dense and fusiform areas (Fig. 1 a, b).

**Morphology after Glycerination and Incubation with HMM**

As a result of the glycerination, the cytoplasm of the lymphatic endothelial cells is much less electron dense, so that the intracellular filaments are better distinguishable. The 5-nm filaments often lie dispersed and disoriented. Otherwise, the morphology of the thick and thin filaments is as in unglycerinated tissues.

After incubation with HMM, the thin filaments are thicker and irregularly outlined (Fig. 2 a). Favorable longitudinal sections reveal characteristic arrowheads with an axial periodicity of about 36 nm (Fig. 2 a), and all arrowheads of the same filament point in the same direction. On cross section, a fuzzy coat surrounds the thin filaments. The diameter of the entire filament complex approximates 21–26 nm (Fig. 2 b). After incubation either in a HMM solution containing ATP or in the pure standard salt solution, the thin filaments remain unchanged.

The morphology of the thick filaments does not change after incubation with HMM (Fig. 2 a), HMM plus ATP, or standard salt solution.

**Morphology after EDTA**

The thin filaments appear labile and the thick filaments remain the major filamentous component of the cytoplasm (Fig. 3).

**Myofilaments of Smooth Muscle Cells**

After glycerination and incubation with HMM, the actin filaments are thicker (20–25 nm) and reveal arrowheads with a periodicity of about 36 nm. The myofilaments remain morphologically unchanged after incubation either with HMM plus ATP or with the standard salt solution. Treated with EDTA the myofilaments are labile, leaving the 10-nm filaments as the major filamentous component of the sarcoplasm.

**DISCUSSION**

Several authors have already reported that lymphatic endothelial cells contain intracytoplasmic filaments (10, 12, 15, 25). This study reveals that in the endothelial cells of the peribronchovascular lymphatics of the neonatal rabbit lung two types of filaments may be distinguished: thick filaments with a translucent core and a diameter of 9–10 nm, and thin filaments measuring 5–6 nm and closely resembling the actin filaments of muscle cells. Lauweryns and Boussauw (13) described analogous thick filaments in the endothelial cells of lymphatic valves of adult rabbit lungs, while Leak and Burke (15) reported 5- to 6-nm filaments in the endothelial cells of guinea pig dermal lymphatics.

Huxley (6) reported that HMM reacts with actin filaments isolated from striated muscle cells to
FIGURE 1 Details of a lymphatic endothelial cell (E) containing thin filaments (T) and thick filaments (F). The bundles of thin filaments are located close to the abluminal cell membrane and exhibit variably sized dense fusiform areas (small arrows). Fig. 1 b is a higher magnification of a part of (a). C, connective tissue; L, lymphatic lumen. (a) × 38,874, (b) × 75,240. Inset, cross section of a bundle of thick filaments. The thick filaments exhibit a less electron-dense core (arrow). × 200,000.

FIGURE 2 Details of lymphatic endothelial cells (E) after glycerination and incubation with HMM. (a) The thin filaments (T) are thicker and irregularly outlined. Favorable longitudinal sections reveal arrowheads (small arrows). The thick filaments (F) remain unaltered. X 79,800. (b) Cross section of thin filaments (T). A fuzzy coat surrounds the thin filaments (small arrow). R, extracellular anchoring filaments. × 51,350.
form arrowhead structures with a periodicity of about 36 nm. In the present study, 5-nm filaments of the lymphatic endothelial cells and actin filaments of smooth muscle cells of the same tissue blocks reacted with HMM to form similar arrowheads. This strongly suggests that the thin filaments in lymphatic endothelial cells may be actin-like or actinoid. Since ATP is known to dissociate actin-myosin complexes (5), the absence of arrowheads after incubation with HMM plus ATP could be expected.

Treated with EDTA the thin filaments appear labile while the thick filaments remain stable. This matches the observations of Cooke and Fay (4) who reported that under such circumstances the myofilaments of smooth muscle cells were labile while the 10-nm filaments remained stable. This, again, suggests that the thin lymphatic filaments are actin-like. It also implies that the lymphatic thick filaments resemble the 10-nm filaments of smooth muscle cells, which are thought to form a cell skeleton (4).

Longitudinally sectioned, the bundles of thin filaments sometimes revealed dense fusiform areas, which have till now not been described in lymphatic endothelial cells. Phelps and Luft (21) observed analogous areas in filament bundles of blood vessel endothelial cells and compared them with the fusiform densities of smooth muscle cells (4). Some important differences may be noted between the dense areas in smooth muscle cells and the dense areas in lymphatic endothelial cells. The former are connected to the 10-nm skeleton filaments and are stable in the presence of EDTA (4), while the latter relate to the actinlike filaments and are labile after incubation with EDTA.

Like the actin filaments in muscle cells, the actin-like filaments in endothelial cells probably form part of a contractile system, which may be based on a sliding of the filaments. This implies the presence of a myosin-like component (19), which could be represented by the dense areas of the thin filament bundles. The uneven distribution and scarcity of these dense areas could be explained by the instability of such a myosin-like component as occurs in smooth muscle cells (8).

The significance of a contractile system in lymphatic endothelial cells might lie in a mechanism for the active regulation of the endothelial intercellular gaps or junctions and hence the permeability of the lymphatic wall (11). It may also help modulate the lymphatic vessel diameter. Of course, it cannot be excluded that the thin filaments may also be involved in other cellular processes such as endocytosis (1), transport (9), and secretion (18).

As the thick endothelial lymphatic filaments resemble the 10-nm skeleton filaments of smooth muscle cells, they apparently form also an elastic cell skeleton. The chemical constitution of these filaments remains to be explored.

SUMMARY

The pulmonary lymphatic endothelial cells of neonatal rabbits contain 5-nm thin and 9-nm thick cytoplasmic filaments. The thin filaments react
with HMM-forming arrowhead structures, and when treated with EDTA they appear labile. The thick filaments do not react with HMM and are stable in EDTA. Comparison of the thin filaments with myofilaments of smooth muscle cells suggests that the thin filaments are actin-like and form part of a contractile system, while the thick filaments constitute a plastic cell skeleton. A contractile system in lymphatic endothelial cells might be involved in the active regulation of the permeability of the lymphatic endothelial cell lining.

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