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AN ELECTRON MICROSCOPIC STUDY OF MICROFIBRILS OF BONE MARROW

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Abstract

After fixation of bone marrow with glutaraldehyde, tannic acid and saponin, a delicate network of microfibrils (10 nm) was observed in the extracellular space. Masses of microfibrils were most frequently observed between the endothelial cells of the sinusoidal wall and adventitial reticular cells, but were also observed at other sites throughout the marrow stroma. Microfibrils are an important component of the extracellular material of bone marrow and appear to provide an anchoring substrate for the endothelium.

KEY WORDS: Bone marrow, endothelium, reticular cells, microfibrils, collagen, basement membrane.

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Introduction

With light microscopic techniques, the extracellular space of bone marrow contains a delicate network of argyrophilic fibers (see Bloom and Fawcett, 1968). These fibers are related closely to sinusoidal endothelial cells and radiate from the sinusoids outward into the marrow stroma. Electron microscopic studies have reported a few fibrils having periodicity typical of type I collagen associated with marrow sinusoids (e.g., DeBruyn et al., 1971; Campbell, 1972). Other fibrillar material related to sinusoids and within the marrow stroma also has been observed frequently, but this material lacks periodicity and often has been poorly defined (see Tanaka and Goodman, 1972; Weiss, 1976).

During ultrastructural studies on bone marrow of rats using techniques to preserve cytoskeletal elements of fixed marrow cells, it became apparent that these same techniques provided better resolution of some components of the extracellular material of bone marrow. This paper describes the ultrastructural arrangement of microfibrils of marrow following fixation with glutaraldehyde, tannic acid and saponin.

Materials and Methods

Bone marrow from male Wistar rats weighing 150 to 200 gm was used in this study. The thoracic aorta was cannulated, the inferior vena cava cut, and the vascular bed was flushed with isotonic Krebs' buffer containing one unit of heparin per 100 ml. After the vascular bed cleared, 100 ml of fixative consisting of 2% glutaraldehyde in 0.1 M cacodylate was infused into the aorta. Pieces of marrow were removed from the femora, cut into small blocks and placed for 1 h in fixative containing 2% glutaraldehyde, 2% tannic acid and 1%

saponin (after Maupin and Pollard, 1983). After fixation, blocks of marrow were rinsed twice for 30 minutes in buffer, stained overnight in 1% aqueous uranyl acetate, osmicated, dehydrated and embedded in Polybed. Thick sections were stained with azure II and examined by light microscopy to determine suitable blocks for electron microscopic study. Thin sections were stained with lead citrate and examined in a Philips 201 electron microscope operating at 60 kV.

Results

Examination of marrow blocks with electron microscopy showed a wide range of ultrastructural features. Near the surface of a block so much tannic acid had penetrated the cells and extracellular space that ultrastructural detail was largely obscured. On the other hand, the central region of a block was penetrated by saponin as judged by disruption of plasmalemmas but not by tannic acid. Therefore, regions lying between these two extremes were selected for electron microscopic study. In these regions the plasmalemmas of endothelial cells and reticular cells were partially or largely removed by saponin treatment and the organelles were stained intensely (see Figs. 1, 2).

Examination of the extracellular space near the abluminal surface of the endothelial cells showed regions seemingly devoid of extracellular material or containing only some poorly defined amorphous material. However, at other scattered sites, banded collagenous fibrils and smaller fibrils (microfibrils) largely filled the extracellular space (Fig. 1). The larger collagenous fibrils, about 40 nm in diameter, were relatively few in number as compared to the microfibrils. The microfibrils were most numerous in the space between the endothelial cells and adjacent reticular cells, but also were observed commonly along the abluminal surface of reticular cells. Small bundles of similar microfibrils were observed frequently throughout the marrow stroma typically in close association with reticular cells. The distribution of these bundles of microfibrils seemed to correspond well with the argyrophilic material reported with light microscopy. In cross section (Fig. 2) these small fibrils appeared as hollow cylinders about 10 nm in diameter and were bound together by amorphous material of moderate density. The microfibrils often ran parallel to the surface of endothelial or reticular cells as shown in Figure 2, but in other sections (Fig. 3) microfibrils were directed more or less perpendicular to the surface of these cells, and seemed to terminate near the plasmalemmma when part of a plasmalemma was still present. Microfilaments of endothelial cells (Fig. 3) and reticular cells were particularly apparent with the present techniques. Some of these microfilament bundles within endothelial cells (Fig. 3) and adventitial reticular cells appeared to terminate along the cell surface at sites where microfibrils were present in the extracellular space.

Discussion

The combination of tannic acid and saponin used in the present study proved satisfactory for ultrastructural study of microfibrils of the extracellular space of bone marrow. In my previous studies using tannic acid alone, microfibrils were not an obvious feature of marrow, indicating that the addition of saponin to the fixative was a necessary step. The precise role of saponin in enhancing preservation of the microfibrils is unknown but appears to involve removal or alteration of substances associated with the microfibrils, thus allowing tannic acid to combine with the microfibrils. As reported by Maupin and Pollard (1983), tannic acid protects microfilaments from oxidation by osmic acid. In the present study it seems likely that tannic acid played a similar role in protecting microfibrils from damage during osmication and that saponin treatment enhanced binding of tannic acid to the microfibrils.

The microfibrils of the present study were morphologically identical to microfibrils described in extracellular materials of other organs (see Kawanami et al., 1978; Böck, 1983). These fibrils, often referred to as reticular or oxytalan fibrils, consist largely of type III collagen (Bornstein and Sage, 1980; Kleinman et al., 1981). In bone marrow, the larger and less frequently encountered fibrils having a banded appearance were presumed to be type I collagen. The observations of the present study would thus indicate a preponderance of type III collagen (microfibrils) associated with sinusoidal endothelium and reticular cells of bone marrow. This observation is in agreement with the studies of Bainton et al. (1986) who showed that bone marrow fibroblasts (reticular cells) in vitro synthesize type III collagen at a ratio of 3 to 1 over type I collagen.

With standard electron microscopic techniques, the basement membrane associated with marrow sinusoids is poorly visualized and is limited to regions where reticular cells cover the endothelium. It sometimes appears

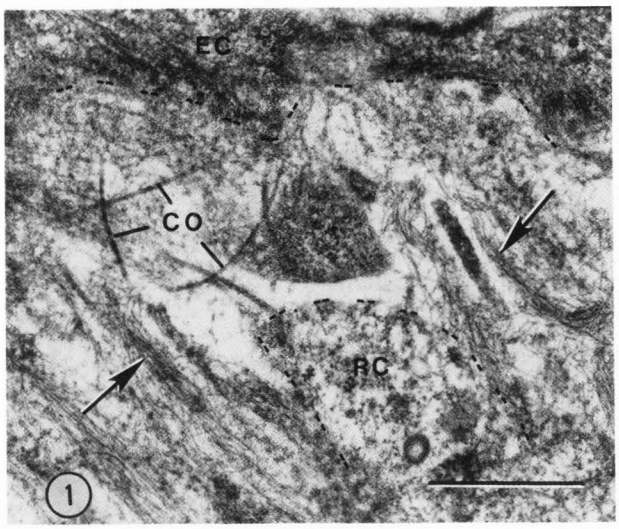

Figure 1. An endothelial cell (EC) of a sinusoid and an adventitial reticular cell (RC) from bone marrow of a rat are shown. Broken lines indicate cell boundaries. Banded collagen fibrils (CO) and clusters of microfibrils (arrows) occupy the extracellular space. Bar = 1 μm.

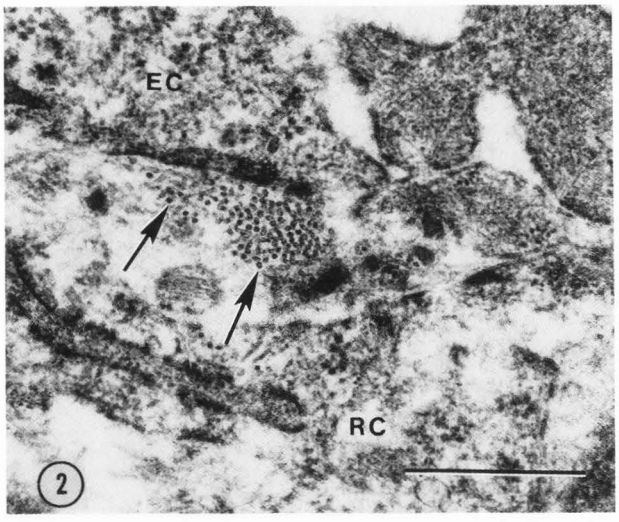

Figure 2. A group of microfibrils (arrows) cut in cross section lies between an endothelial cell (EC) of a sinusoid and an adventitial reticular cell (RC). Bar = 0.5 μm.

fibrillar but more often is largely amorphous (see Tanaka and Goodman, 1972; Campbell, 1972; Weiss 1976). The present study clearly demonstrated that a component of this basement membrane is a network of microfibrils. The network of microfibrils described here was less extensive than the basement membrane usually described in marrow and for this

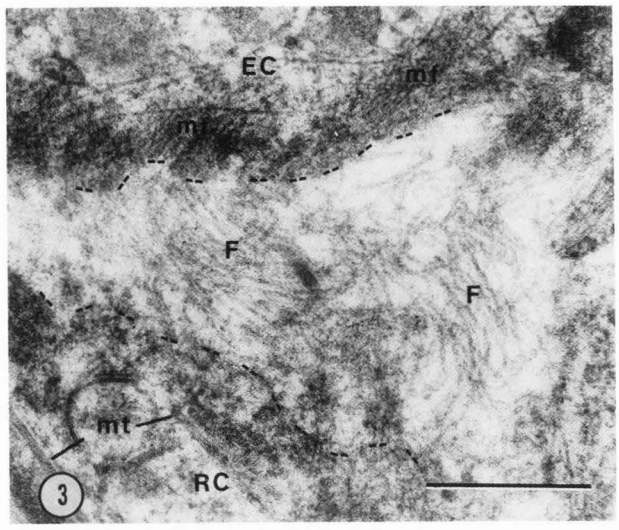

Figure 3. Microfibrils (F) are shown in the extracellular space between an endothelial cell (EC) of a sinusoid and an adventitial reticular cell (RC). Broken lines indicate cell boundaries. Microfilaments (mf) in the endothelial cell and microtubules (mt) in the reticular cell are labeled. Bar = 0.5 μm.

reason, microfibrils are thought to form only a part of the extracellular material making up the basement membrane. At some sites microfibrils form most of the basement membrane material, but at other sites, poorly visualized in the present study, amorphous material predominates.

A noteworthy observation of the present study was the close association sometimes observed between microfibrils and microfilaments within endothelial cells of the sinusoidal wall. The microfilaments appeared to be attached to the internal surface of the plasmalemma, as previously reported by Tavassoli (1977), while the microfibrils seemed to terminate near the external surface of the plasmalemma. This observation was interpreted as indicating that the endothelial cells are anchored to microfibrils of the extracellular space at these sites. In the absence of a typical basement membrane, microfibrils appeared to be an important attachment site for endothelial cells. In lymphatic capillaries, vessels that likewise lack a typical basement membrane, Leak (1970) has described a similar network of microfibrils (10 nm) that appears to anchor the endothelial cells of these vessels. Other studies (Macarak and Howard, 1983) have shown that endothelial cells in vitro can attach to several connective tissue components including type III collagen.

Acknowledgements

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Discussion with Reviewers

R.P. Becker: What is known of the glycosaminoglycan and proteoglycan content of the extracellular matrix in marrow?

Author: The extracellular material of marrow contains several glycosaminoglycans including hyaluronic acid and chondroitin sulphates A, B, and C (Noordegraaf and Ploemacher, Scand. J. Hematol., 22:327, 1979). The studies of McCuskey et al. (see Exp. Hematol., 3:297, 1975) suggest that the proportion of sulphated to unsulphated glycosaminoglycans may be important in regulating erythropoiesis in marrow. Also, the glycoprotein fibronectin forms an important part of the extracellular material of marrow (Bentley and Tralka, Exp. Hematol., 11;129, 1983). How these substances are related to the microfibrils of the present study is uncertain.

Mehdi Tavassoli: What is the relationship between extracellular microfibrils described here and the cytoskeletal elements of the cell? The 10 nm measurement reported here corresponds to the intermediate filaments, rather than microfilaments which are usually about 6 nm in diameter. Considering that intermediate filaments are also tissue-specific, and considering that these microfibrils must be produced intracellularly and released into the extracellular space, would it not be conceivable that these microfibrils are related to intracellular intermediate filaments?

Author: I do not believe there is a relationship between intermediate filaments of the endothelial and reticular cells and the extracellular microfibrils. Chemically the intermediate filaments likely consist of vimetin while the microfibrils are likely type III collagen. Furthermore, after treatment of cells with saponin and tannic acid, intermediate filaments are about 16 nm in diameter (see Maupin and Pollard, reference above) and hence are considerably larger than the microfibrils.