

Thrombus Formation in Lymphatic Vessels Associated with *Brugia malayi*

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Abstract:

Lymph thrombi within affected afferent lymphatic vessels of cats infected with *Brugia malayi* were examined by scanning electron microscopy. Thrombi were made up of alternating layers of fibrin strands and cells, many of which were intact erythrocytes. The endothelial lining of the vessel wall near the site of thrombus attachment showed transition from normal endothelial cells to a flattened syncytium in which individual endothelial cells could not be distinguished. Imprints of cells, including erythrocytes, were visible in the vessel wall at the point of thrombus attachment. Damage to the endothelial lining may be a factor in the initiation of thrombus formation.

Lymphatic filariasis is a common infection of man in many tropical and subtropical areas. The two major species of nematodes responsible for this disease are *Wuchereria bancrofti* and *Brugia malayi*. Although there are some variations in the clinical manifestations of the disease produced in different geographical areas, lymphatic dysfunction is a constant feature. In previous studies using an animal model (1), we observed that in lymphatic vessels inhabited by *Brugia malayi*, pools of stagnant lymph resulted from vessel dilatation and valve incompetence. Lymph thrombi were frequently seen in these enlarged afferent vessels. The present study describes some of these lymph thrombi. Emphasis is on characteristics revealed by scanning electron microscopy, especially at the site of thrombus attachment to the endothelial surface.

Materials and Methods

Animal model. Domestic cats were infected with *Brugia malayi* larvae in such a way that the developing and adult filarial parasites localized in the afferent lymphatic vessels between the popliteal lymph node and the hind foot where the infection was initiated (1). At various intervals from 4 to 18 months after infection, 0.2 ml of 0.4% sky blue dye was injected into the interspace of an anesthetized cat's hind foot. The cat was then killed with an overdose of sodium pentobarbitol and the skin covering the afferent vessels was reflected. The two major lymph vessels that drain into the popliteal lymph node were examined in each cat using a dissecting microscope with a fiber optic light source that provided intense illumination without rendering the vessel wall opaque due to excessive heating. Lymph thrombi, in addition to living and dead worms, were observed in many of the vessels.

Tissue preparation for scanning electron microscopy. Lymphatic vessels that contained thrombi were excised and fixed at 4°C in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0). After fixation, the vessels were cut to expose the thrombi, rinsed twice in cacodylate buffer and post-fixed in 0.1% osmium tetroxide. After two additional rinses in cacodylate buffer, the specimens were dehydrated in increasing strengths of ethyl alcohol. Each specimen was critical-point dried (Polaron,

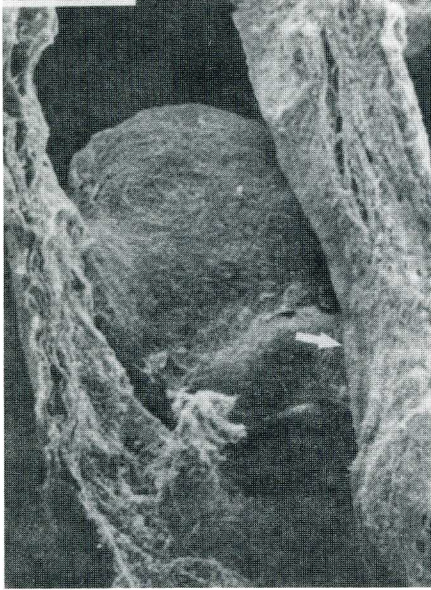


Fig. 1 Excised lymphatic vessel cut to expose a thrombus. Arrow points to a site of thrombus attachment to the vessel wall. Bar = 750 μm .

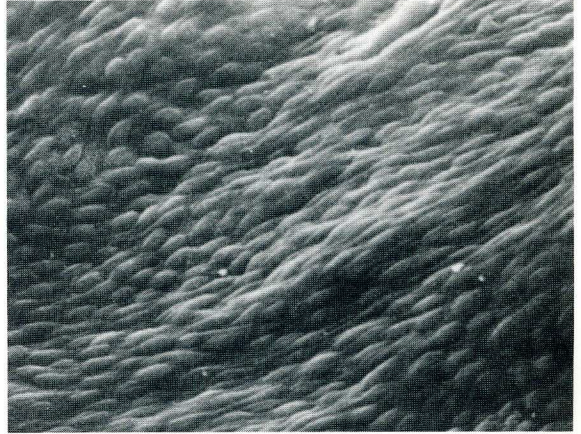


Fig. 2 Normal lymphatic endothelium. The cells are aligned longitudinally in the direction of the lymph flow. Bar = 100 μm .

Watford, England), coated with gold-palladium (Hummer VI, Technics, Springfield, VA), and examined with an ISI Super III scanning electron microscope (International Scientific Instruments, Mountain View, CA).

Results

In a number of vessels examined *in situ*, lymph thrombi were present. These thrombi were sometimes observed floating free in the lumen but were most often attached to the vessel wall (Fig. 1). Examination of the normal endothelial lining of the lymphatic vessel revealed spindle-shaped endothelial cells aligned parallel to the longitudinal axis of the vessel (Fig. 2).

Another thrombus observed in a lymph vessel was fixed *in situ*, then was removed for closer examination after critical-point drying. A piece of the thrombus remained attached to the vessel wall when the bulk of the thrombus was removed, as indicated by the schematic drawing (Fig. 3). Scanning electron micrographs of the thrombus (Fig. 4a) and the portion remaining on the vessel wall (Fig. 4b) were made for orientation of subsequent pictures. The cleavage plane of the thrombus allowed us to examine the interior and exterior structure (Fig. 5).

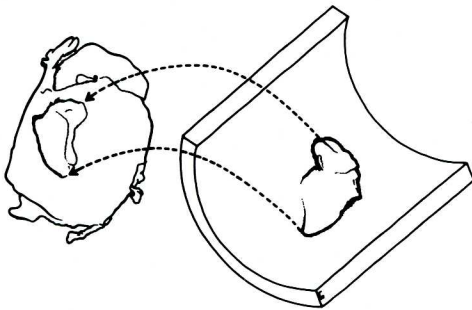


Fig. 3 Diagram of thrombus removal from vessel wall. See Figure 4.

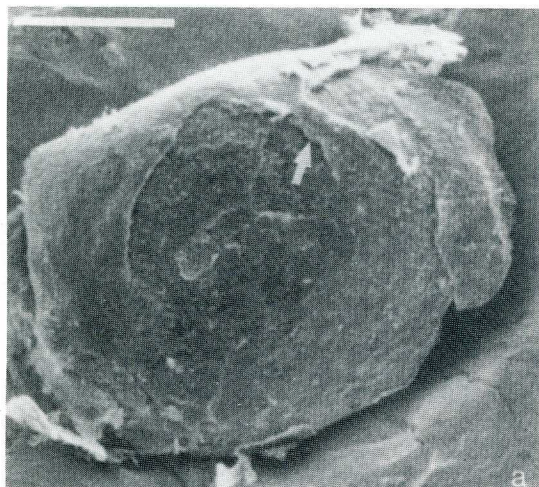
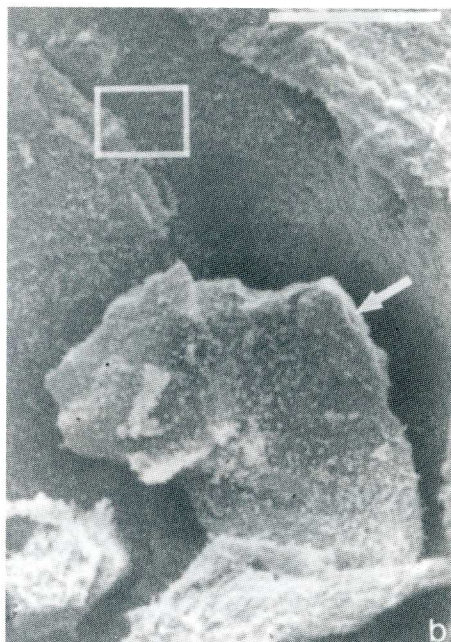


Fig. 4a Thrombus removed from a lymphatic vessel after critical-point drying. Arrow points to area depicted in Figure 5. Bar = 750 μ m.

b Lymphatic vessel after removal of the thrombus in a. Arrow points to a piece of thrombus remaining on the vessel surface. The outline of this piece can be seen on the thrombus in a. Area within the box is depicted in Figure 6. Bar = 1.5 mm.



Removal of the thrombus also allowed us to examine the adjacent vessel wall (Fig. 6). To determine if the thrombus was attached to the underlying vessel wall, the remaining piece was removed and the vessel wall was examined (Fig. 7).

A series of micrographs of the outer and inner structure of the thrombus are shown in Fig. 5a-d. A low power magnification of the broken edge of the thrombus illustrates the layering effect of erythrocytes and other cells that comprised this portion of the thrombus (Fig. 5a). The surface of the thrombus consisted of a thin layer of fibrinous strands (Fig. 5b) that covered layers of cells. These cells were held in place and often distorted by the strands (Fig. 5c). The inner surface of the thrombus consisted of a layer of cells, the majority of which were erythrocytes (Fig. 5d). These cells also appeared distorted and the imprint of fibrinous strands was evident on the cell surface. Imprints of cells dislodged from this surface were also apparent.

The vessel wall associated with the thrombus is shown in Figures 6a and 6b. The vessel lining appeared to have undergone a transition from a normal endothelial lining to a flattened syncytium in which individual endothelial cells could not be differentiated (Fig. 6a). A piece of thrombus was also visible in this area. A higher magnification of the area near the thrombus piece showed the interface between normal endothelial cells and the syncytium-like surface of the vessel wall (Fig. 6b). Imprints of cells dislodged from the vessel wall were also present.

A series of micrographs depicting a point of thrombus attachment to the vessel wall is illustrated in Fig. 7a-d. An area at the transition point between normal endothelium and the syncytium-like formation is shown in Fig. 7a. It was apparent that many cells were dislodged from the vessel wall when the thrombus piece was removed. High magnification showed that many of the remaining cells were

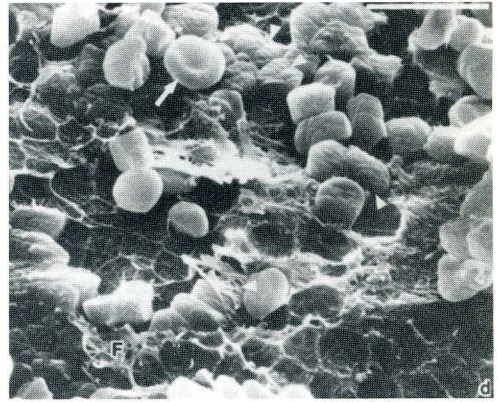
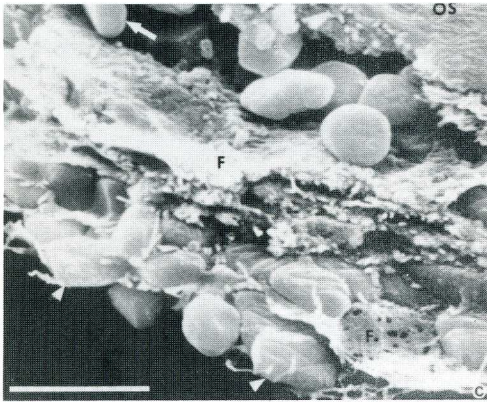
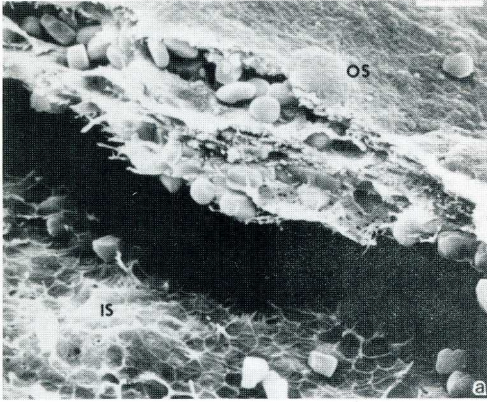


Fig. 5a Internal and external structure of a lymph thrombus. OS = outer surface; IS = inner surface. Bar = 15 μm .

b Outer surface of the thrombus. Note the criss-crossing layers of fibrinous material (arrow). BE = broken edge of the thrombus. Bar = 15 μm .

c Broken edge of the thrombus. Note the layering of erythrocytes (arrows) and fibrinous strands (F) that comprised this portion of the thrombus and the imprint of fibrinous strands on the surface of many cells (arrowheads). Bar = 15 μm .

d Inner surface of the thrombus. Note the layer of erythrocytes (arrows), fibrinous strands (F) and altered shapes of the cells. Imprints of strands are visible on the cell surfaces (arrowheads). Bar = 15 μm .

erythrocytes embedded in the vessel wall (Fig. 7b). A higher magnification of Fig. 7b shows an erythrocyte slightly distorted by its association with the vessel wall (Fig. 7c). Similar features were seen at other points in the area of attachment (Figure 7d) and the imprints of many cells dislodged from the vessel were also visible.

Discussion

Following the development of animal models for studying lymphatic filariasis, Schacher and Sahyoun (2) showed that controlled studies could be used to systematically examine host reactions to lymphatic filariae. It became evident from their study (2) and reports of other investigators (3-5) that many of the observations made by Lichtenberg on human

filariasis (6) could also be seen in animal models. One very interesting phenomenon seen in both human and experimental filarial studies is thrombus formation within the affected, dilated lymph vessel. Since very little information has been reported on lymph thrombi, we decided to examine the thrombi and adjacent lymphatic vessels by scanning electron microscopy. From this study, several important observations can be made regarding thrombi within lymphatic vessels associated with filarial nematodes.

In situ examination of lymphatic vessels revealed that thrombus formation is a common sequela of lymphatic filariasis. Although at times thrombi were observed floating free within the lymph vessel, closer examination suggested that at least one surface had a fragmented appearance as if it had broken off a vessel wall or valve. In most instances, the thrombi were clearly attached. A closer examination of the attachment site revealed that the morphology of the vessel wall was altered in the vicinity of thrombus attachment.

The lining of normal lymph vessel walls was composed of endothelial cells aligned parallel to the longitudinal axis of the vessel as described by Gnepp and Green (7). These endothelial cells, which normally had raised areas corresponding to nuclei and were seen as distinct individual cells, became flattened and appeared as a syncytium near the point of thrombus attachment. It also appeared that numerous cells, including erythrocytes, were partially embedded in the vessel lining. Definite imprints of these cells can be seen in Fig. 7a-d. It is not known whether these cell-vessel associations were formed as a result of pressure exerted by an increase in the size of the thrombus, or if the initial reason for thrombus formation was endothelial damage and adherence of cells to the vessel surface. The appearance of the lymph vessel wall described here was similar in appearance to that of a blood vessel containing a thrombus as depicted by Wu and Mansfield (8).

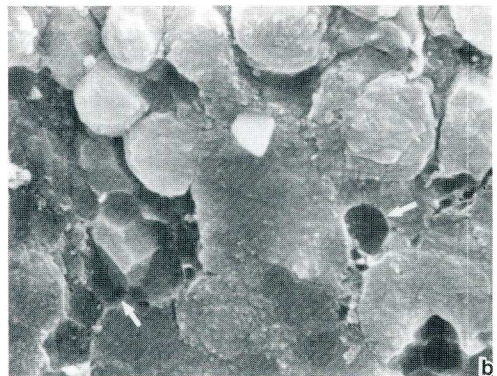
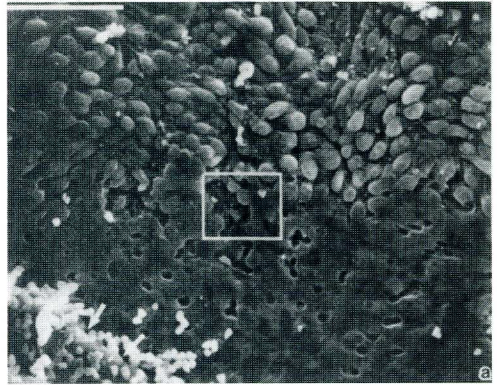


Fig. 6a Lymphatic vessel wall outlined by the box in Figure 4b. A transition from normal endothelium to a syncytium-like formation is seen near a site of thrombus attachment. A piece of thrombus is visible in the micrograph (arrows). Area outlined by the box is shown at higher magnification in b. Bar = 100 μ m.

b Interzone between normal endothelium and the syncytium-like formation. Note the imprint of cells dislodged from the vessel wall (arrows). Bar = 15 μ m.

In one thrombus examined in detail in this study, the major components were strands that appeared to be fibrin, and layers of cells — mainly erythrocytes and some lymphocytes. The fibrillar strands between cells and layers of cells (Fig. 5) appeared to be identical to the fibrin strands seen in blood thrombi (8). Some of these cells had a normal appearance, while others were distorted and the imprint of fibrin strands on the cell surface was evident.

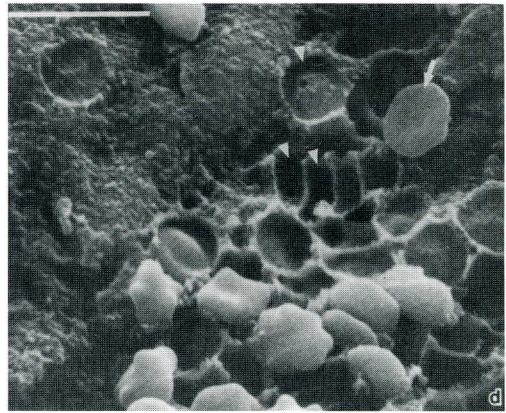
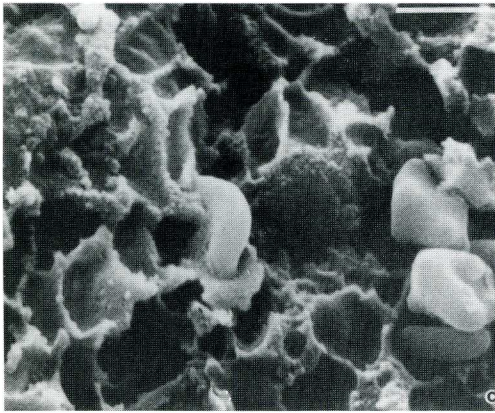
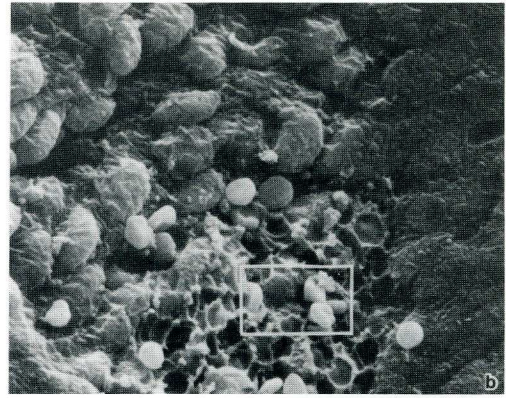
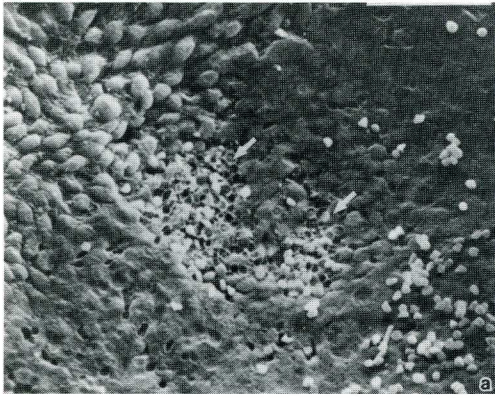


Fig. 7a Site of thrombus attachment to the vessel wall. Note the change in the morphology of the vessel wall near the attachment site. Arrows point to areas shown in b and d. Bar = 100 μm .

b Area at the transition between normal endothelium and a syncytium. Note the cells embedded in the vessel wall and imprints of cells dislodged from the wall. The area outlined by the box is shown in higher magnification in c. Bar = 15 μm .

c Thrombus attachment point showing erythrocytes embedded in the vessel wall and imprints of dislodged cells. Bar = 7.5 μm .

d Thrombus attachment point showing embedded cells, erythrocytes (arrow) and imprints of dislodged cells (arrowheads). Bar = 15 μm .

The appearance of the lymph thrombus examined in detail was similar to that described by Lichtenberg (6) who reported "plugs of clotted blood, fibrin, inflammatory cells or a mixture of these elements" while investigating the pathology of human lymphatic filariasis. Capillaries are presumed to be the source of erythrocytes in organized thrombi; however, the origin of erythrocytes in the unorganized thrombi of the present study is uncertain. Examination of tissues in related studies (unpublished data) have indicated considerable inflammation of lymphatic vessel walls, often with close apposition of blood spaces to the compromised lymphatic endothelium. Thus, the erythrocytes noted in this lymph thrombus may have entered into the lymphatic circulation by crossing the endothelial lining at points of inflammation.

Although the events leading to thrombus formation within the lymphatics are unknown, observations made in this study suggest that unorganized lymph thrombi are similar in composition to blood thrombi. Many questions concerning the origin of lymph thrombi remain unanswered; however, we now have another means to examine the formation of lymph thrombi in greater detail and to study the effect these thrombi have on disease production, especially in lymphatic filariasis.

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Whenever a new discovery is reported to the scientific world, they say first, "it is probably not true." Thereafter, when the truth of the new proposition has been demonstrated beyond question, they say, "Yes, it may be true, but it is not important." Finally, when sufficient time has elapsed to fully evidence its importance, they say, "Yes, surely it is important, but it is no longer new."

Michel de Montaigne [1533-1592]
