

EVOLUTION OF LYMPH THROMBI IN EXPERIMENTAL *BRUGIA MALAYI* INFECTIONS: A SCANNING ELECTRON MICROSCOPIC STUDY

R.C. Fader, A. Ewert

Department of Microbiology, The University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas

ABSTRACT

Lymph thrombi in cats experimentally-infected with *Brugia malayi* were examined by scanning electron microscopy. A variety of morphological forms were noted and it appeared that thrombi undergo a maturation process characterized by at least three transitional phases. Initially, the thrombus consists of erythrocytes encased in fibrin (Phase I). Phase II thrombi are characterized by the appearance of phagocytic cells and fibroblasts on the surface of the thrombus. At the end of the maturation process, the thrombus surface consists solely of fibroblasts or endothelial cells, perhaps derived from the vessel wall (Phase III). Occlusion of the lymphatic lumen by thrombi and the accompanying inflammatory response triggered by *B. malayi* infection may be a major factor in the pathogenesis of lymph stasis in this disorder.

Previous studies have shown that lymph thrombi are frequently present in domestic cats experimentally infected with the filarial nematode, *Brugia malayi* (1,2). Because these thrombi are present in superficial vessels, it is possible to fix the thrombi *in situ* and determine how they interface with vessel walls and valves as well as examine their composition. The current study is an extension of earlier work which showed that endothelial cells lining lymph vessels were

altered at the site of thrombus attachment and that erythrocytes layered between sheets of fibrin made up a sizable portion of some thrombi at a given point in time (1). Evidence is presented in this report which suggests that lymph thrombi undergo a progression of morphological forms while in the lymph vessels of experimentally-infected cats.

MATERIALS AND METHODS

The method of infecting domestic cats with *Brugia malayi*, processing tissues and equipment used have been described earlier (1). In the current studies, the lymph vessels containing thrombi were fixed *in situ*. After injection of lymph-staining dye and reflection of the skin a 30 gauge lymphangiography set was used to cannulate the affected vessel distal to the site of the thrombi, which could be detected through the vessel wall. The set was attached to a 3 ml syringe held by a manually operated syringe injector. Fixative was then slowly injected to minimize distortion of the vessel walls. Simultaneously, the area surrounding the vessel was covered with gauze soaked in fixative. After fixation for 30-45 minutes, the tissues were excised and processed as previously described (1). Scanning electron microscopic identification of various cells was based on published description and micrographs found in the *SEM Atlas of Cells and Tissues* (3).

RESULTS

Consistent findings during light and electron microscopic examination of lymphatic vessels of cats infected with *B. malayi* suggest that a predictable sequence of events occurs in the formation of lymph thrombi (Table 1).

Initial events: In many of the cats used for these studies, filarial worms could be observed through the vessel wall when the overlying skin was reflected. After *in situ* fixation, the vessel wall could be carefully dissected away, revealing the worm (Fig. 1). Closer examination of the worm revealed the striated cuticle and also suggested that a part of the host response to the worm consisted of monocytes attached to the worm surface (Fig. 2).

When the worm in Fig. 1 was removed from the vessel, the endothelium could be observed directly. A micrograph of normal endothelium from a site proximal to the worm is seen in Fig. 3. The endothelial cells are aligned longitudinally in the direction of the lymph flow and few cells besides endothelial cells are present. In contrast, examination of the endothelium associated with the worm reveals massive cell infiltration (Fig. 4a).

A closer examination of this area reveals that these cells are predominantly erythrocytes and leukocytes, either polymorphonuclear leukocytes (PMNs) or lymphocytes (Fig. 4b). Whether the erythrocytes arise from mechanical damage to the endothelium by the worm is difficult to ascertain. In some areas however, damage to the integrity of the vessel could be observed (Fig. 5), and erythrocytes and fibrin could be seen on the vessel surface. Damage to the endothelium, followed by clot formation involving erythrocytes is probably the initial event in lymph thrombus formation.

Phase I Thrombus (Immature): We previously reported the results of a scanning electron microscopic examination of what is probably a thrombus in an immature state (1). The thrombus surface is characterized by fibrin overlying layers of erythrocytes (Fig. 6a). The inner structure is predominantly composed of erythrocytes and platelets

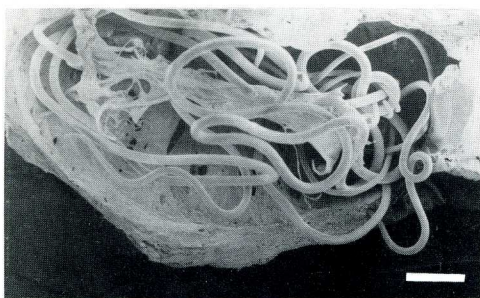


Fig. 1: *B. malayi* within a lymphatic vessel. Bar = 500 μ m.

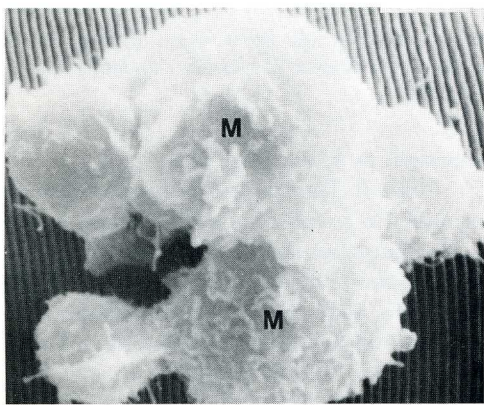


Fig. 2: Monocytes (M) on the worm surface. Note the striated cuticle of the worm and the pseudopodia on the monocyte. Bar = 5 μ m.

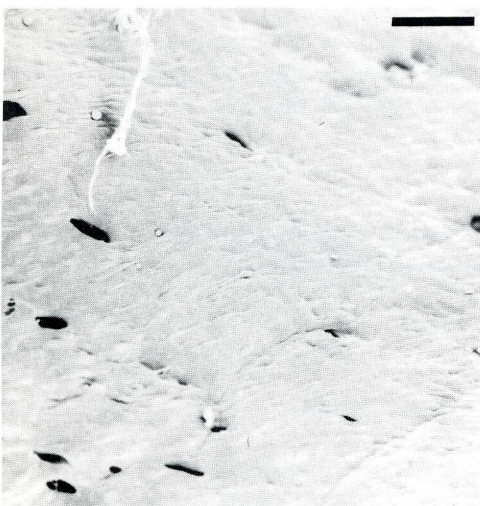


Fig. 3: Normal lymphatic endothelium. Compare to Fig. 4. Bar = 50 μ m.

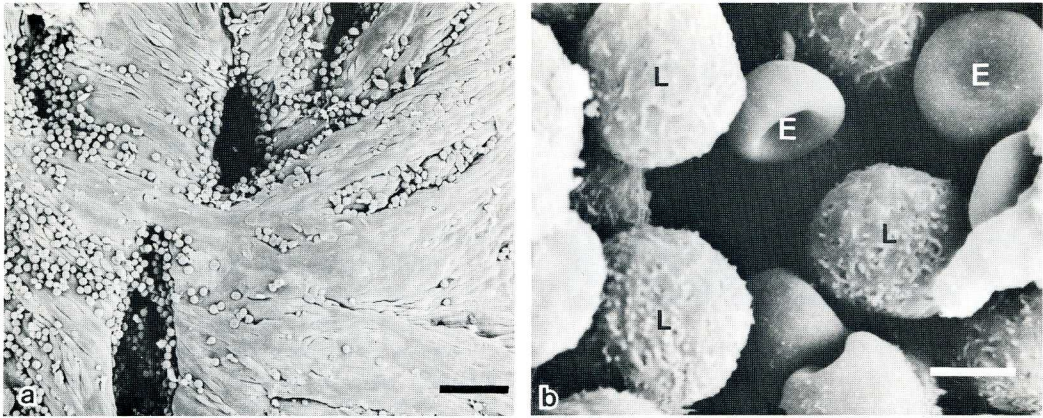


Fig. 4: a) Endothelium associated with worm in Fig. 2. Note the adhering cells. Bar = 50 μ m. b) High power magnification of the adherent cells in Fig. 4a. Erythrocytes (E) and leukocytes (L), either circulating PMNs or lymphocytes, are the predominant cell types found. Bar = 3 μ m.



Fig. 5: Area showing alteration of damage of the endothelium. Erythrocytes are the predominant cell type in the area. Note the erythrocyte trapped in the fibrin strands (arrow). Bar = 10 μ m.

enmeshed in fibrin (Fig. 6b).

Phase II Thrombus (Maturing): A lymph thrombus in a maturing state is shown in Fig. 7. The thrombus appears attached to a valve. Examination of this thrombus and others like it revealed that the surface is composed mainly of fibroblast-like cells intermixed with macrophages, PMNs, lymphocytes, and platelet aggregates (Fig. 8a). Very few erythrocytes, the most common cell type seen in the smaller thrombi, are noted. However, fibrin could be observed in some areas on the thrombus surface, again entrapping various cell types into the thrombus (Fig. 8b).

Examination of the endothelium adjacent to the thrombus suggests cell migration through the endothelial wall (Fig. 9).

Table 1.
Classification of Transitional Forms of Lymph Thrombi Observed During Experimental Filariasis

Phase	Condition	Surface Composition
Phase I	Immature	Fibrin, erythrocytes, platelets
Phase II	Maturing	PMNs, lymphocytes, macrophages, fibroblasts, fibrin, few erythrocytes
Phase III	Mature	Fibroblasts or endothelial cells

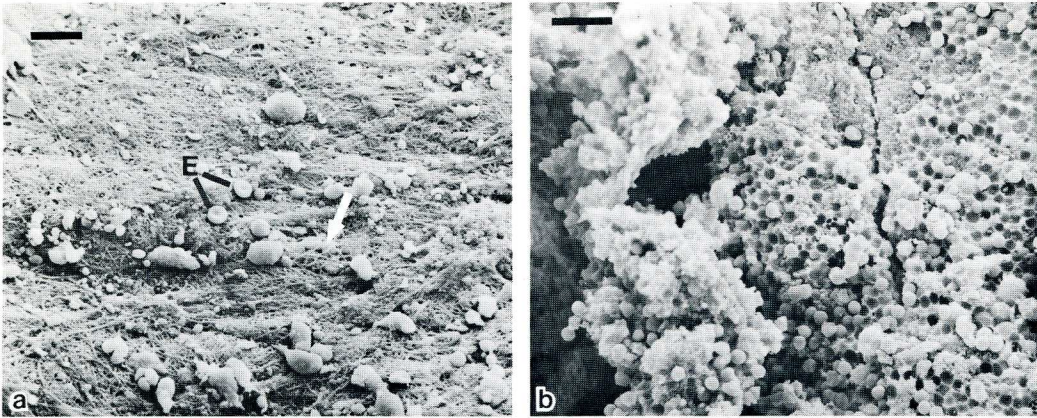


Fig. 6: a) Surface of a small, immature thrombus (Phase I). Note the erythrocytes (E) and the fibrin matrix. Erythrocytes can be observed under the fibrin (arrow). Bar = 10 μm . b) Interior of an immature thrombus. Erythrocytes, compressed and misshapen by their encasement in fibrin, are the main components of these thrombi. Bar = 15 μm .

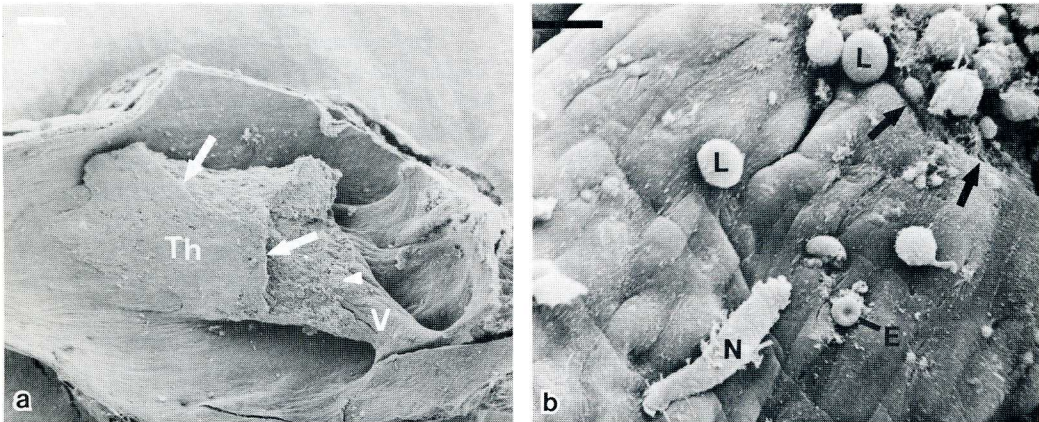


Fig. 7: a) Maturing, Phase II thrombus (Th) in a lymphatic vessel. A portion of the thrombus was broken off to examine the internal structure (arrows). The thrombus appears to be attached to a valve (V). The arrowhead points to the valve/thrombus interface, shown at higher magnification in 7b. Bar = 200 μm . b) Valve/thrombus interface. The edge of the thrombus can be seen at the arrows. Numerous cell types are present in the area. N - neutrophil; E - erythrocyte; L - lymphocyte. Bar = 10 μm .

Phase III Thrombi (Mature): A thrombus occluding a vessel wall can be observed in Fig. 10a. Examination of the surface of this thrombus reveals a smooth surface of fibroblast-like cells with a complete absence of fibrin, macrophages and other cell types (Fig. 10b). Another mature thrombus with a very pleomorphic shape is seen in Fig. 11a. The surface of this thrombus appears to be made up of endothelial cells (Fig. 11b). A

deposition of other cell types can be observed on the thrombus surface, but the cells do not appear to be part of the thrombus.

DISCUSSION

Examination of lymphatic vessels containing *Brugia malayi* shows that a wide variation of host reactions may be seen. One of the most interesting host reactions is the presence of lymph thrombi that are attached

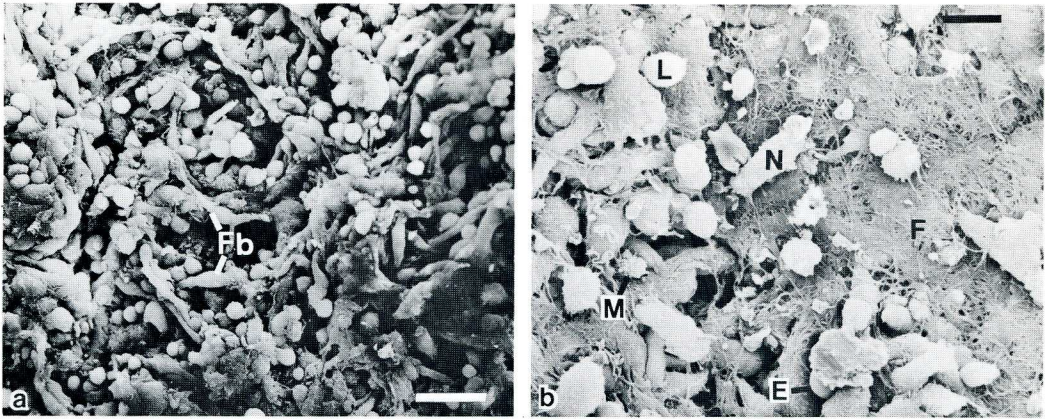


Fig. 8: Micrograph of two surface areas of the thrombus from Fig. 7. a) Surface area containing numerous cell types but very little fibrin. Note the appearance of fibroblast-like cells (Fb) in the area. Bar = 20 μm . b) An area in which many cell types are associated with layers of fibrin (F). L - lymphocyte; M - macrophage; N - neutrophil; E - erythrocyte. Bar = 10 μm .

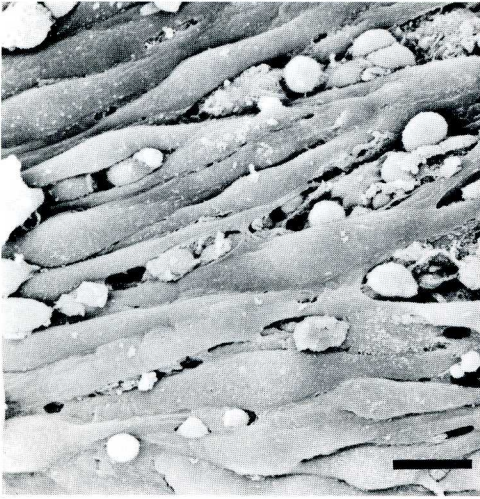


Fig. 9: Area of possible cell migration through the endothelium adjacent to the thrombus from Fig. 7. Bar = 10 μm .

by at least one point to a vessel wall or valve. During scanning electron microscopic studies of different thrombi, we have been impressed with the variation in their composition and arrangement. In this study we offer possible transition stages to show how lymph thrombi may develop and contribute to occlusion of the vessel lumen.

How lymph thrombus formation is initiated is a perplexing question. Damage to the endothelial surface, either by direct contact or by substances excreted by the worm, could trigger the appearance of components necessary for thrombus formation. Many observations have shown that inflammatory cells accumulate both inside and outside of lymph vessels harboring *B. malayi* (2). With inflammation comes increased vascularization of affected tissues. Thus, it is not surprising that erythrocytes, along with platelets, are available to interact with fibrinogen in lymph to initiate early thrombus formation. The subsequent concentric layering of cells, fibrin and amorphous material, some of which may be aggregates of platelets, shows that the thrombi grow in size and eventually may contribute to vessel occlusion. As the thrombus becomes more stable and has more contact with the vessel wall or valve, it may become covered with fibroblasts and endothelial cells. Histological sections of these thrombi have revealed that blood vessels and presumably lymphatic vessels invade the thrombus, at which time it becomes a permanent fixture in the lumen of the vessel. Lymph flow must then depend on small new vessels that follow blood vessels into the thrombotic mass or on collateral vessels that bypass the obstruction.

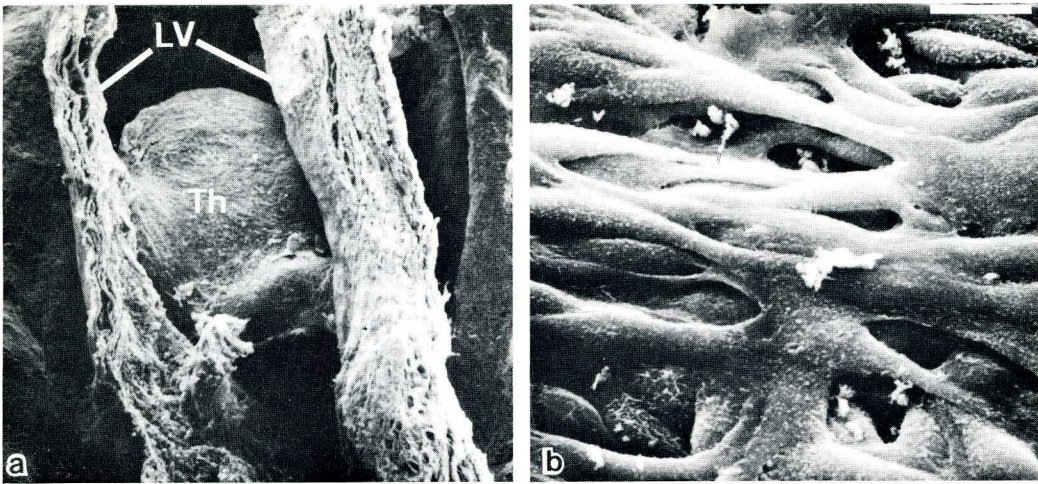


Fig. 10: a) Mature, Phase III thrombus (Th) within a lymph vessel (LV). Bar = 500 μm . b) Surface of the lymph thrombus showing fibroblast-like cells. Note the absence of other cell types and fibrin, characteristic of Phase I and II thrombi. Bar = 25 μm .

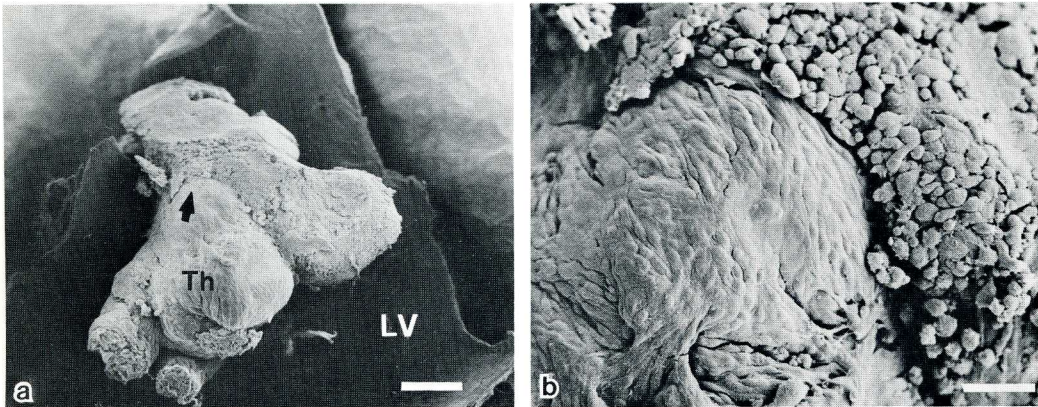


Fig. 11: a) Phase III thrombus (Th) in a lymph vessel (LV). Area at the end of the arrow is shown at higher magnification in b. Bar = 150 μm . b) Endothelial cells appear to make up the surface of this thrombus. (Compare to Fig. 3). A mass of cells have been deposited on the thrombus surface but do not appear to be part of the thrombus. Bar = 50 μm .

Substantiation that the photomicrographs are representative of transitional forms of lymph thrombi rather than different types of thrombi comes from multiple observations that the surface morphology of a single thrombus could be composed of Phase I and Phase II areas or Phase II and Phase III areas. A surface composed of a mixture of Phase I and Phase III areas on a single thrombus was never observed. The time frame involved

from initiation to maturation cannot accurately be estimated since it is not possible to know when a thrombus was initiated; however, the degree and duration of the inflammatory response may be significant factors in the rate of thrombus formation.

The role that lymph thrombi play in the pathophysiology of lymphatic filariasis is unknown. It is easy to see how the occlusion of a vessel by a lymph thrombus could result

in lymph stasis, a known factor in the process leading to elephantiasis. However, as previously mentioned, many observations have shown that fine collateral vessels will eventually bypass the occluded area or recanalize thrombi previously incorporated into the occluded vessel (4). It is likely that these smaller vessels are not as efficient in lymph transport as the initial vessel and contribute to lymph stasis. It is clear, however, that further study of lymph thrombi is necessary to clarify their significance in the disease process.

REFERENCES

1. Fader, R, A Ewert, D Folse: Thrombus formation in lymphatic vessels associated with *Brugia malayi*. *Lymphology* 17 (1984), 3.
2. Ewert, A, D Folse: Animal model of human disease. Lymphatic filariasis. *Amer. J. Pathol.* 115 (1984), 135.
3. Fujita, T, K Tanaka, J Tokunaga: SEM Atlas of Cells and Tissue. Igaku-Shoin, New York, 1981.
4. Bosworth, W, A Ewert: Lymphatic patterns of cats infected with *Brugia malayi* and *Streptococcus*. *Trans. Roy. Soc. Trop. Med. Hyg.* 71 (1977), 21.

Dr. Adam Ewert
Department of Microbiology
University of Texas Medical Branch
Galveston, TX 77550