

FACTOR VIII-ASSOCIATED ANTIGEN IN HUMAN LYMPHATIC ENDOTHELIUM

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ABSTRACT

Lymphatic vascular endothelium both on tissue section and in culture exhibits positivity for Factor VIII-associated antigen although staining is generally less intense and more spotty than in comparable blood vascular endothelium. Lymphatic endothelium also exhibits Weibel-Palade bodies. Neither marker, therefore, reliably distinguishes blood vascular endothelium from lymphatic endothelium.

INTRODUCTION

Whereas Factor VIII-associated antigen (F8AA), a key component of vascular hemostasis, is now recognized (1,7) as a distinctive synthetic product of blood vascular endothelium (BVE), its existence and importance in the lymphatic circulation, which transports tissue fluid, macromolecules, and wandering cells, is either disputed or seldom considered. Based on our previous identification (6) of F8AA in lymphatic vascular endothelium (LVE) lining collecting lymphatic channels in dogs, we now examined LVE directly for F8AA in tissue sections and tissue culture and indirectly (ultrastructure) for F8AA synthesis-storage sites (Weibel-Palade bodies) in patients with a variety of disorders involving the lymphatic system.

MATERIALS AND METHODS

Tissue specimens were obtained after

operation or necropsy in 14 patients in the following categories: dilated lymphatics from fluid overload or congenital or malignant lymphatic obstruction and benign lymphatic vascular tumors (cystic hygromas and cavernous lymphangiomas) in various sites. Subject age ranged from 20-week fetus to adult. Tissues fixed in 10% formalin were assayed for F8AA using primary rabbit antiserum to human F8AA (Accurate Chemicals, Westbury, NY) and secondary swine anti-rabbit IgG in a sensitive indirect peroxidase-antiperoxidase method. Tissues snap-frozen in isopentane precooled by liquid nitrogen and cultured lymphatic endothelial cells grown on cover slips were assayed by an indirect immunofluorescent technique using the same primary antibody and fluorescent goat and anti-rabbit antibody (Cappel Worthington, Malvern, PA). Some tissues and cultured cells were processed for transmission electron microscopy (TEM) and examined for the presence of Weibel-Palade bodies. Primary cultures of lymphatic endothelial cells were grown in monolayer culture from explants of 3 resected lymphangiomas by a modification of previously described methods involving physical cloning and selective trypsinizations (1).

RESULTS (Figs. 1-8)

In tissue sections, LVE lining typical thin-walled lymphatic channels or cysts containing clear or chylous fluid constantly displayed F8AA reactivity, albeit usually

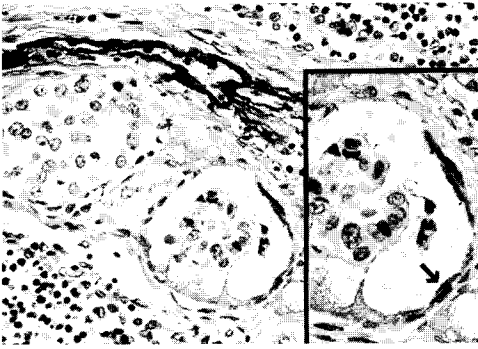


Fig. 1. Lymph node capsule showing metastatic adenocarcinoma of the breast within dilated lymphatics (x370). Note intense F8AA reactivity in capsular venule and less intense focal reactivity in distended lymphatic. Inset shows details of granular F8AA (arrow) in lymphatic endothelium (x800).

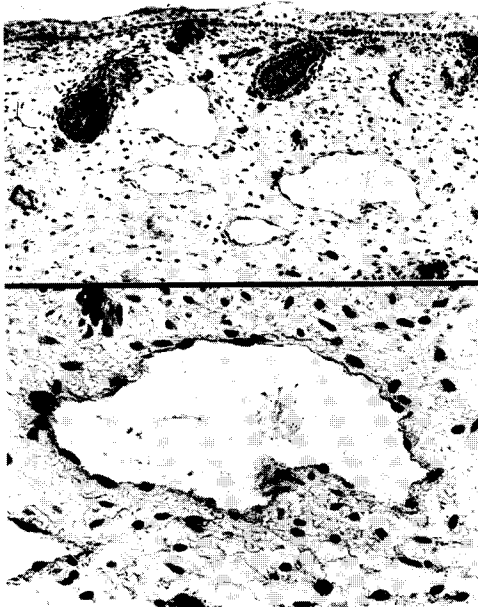


Fig. 2. Fetal strangulating cystic hygroma, Down syndrome. Note section of skin with dilated lymphatics within reticular dermis (x110). Lower micrograph shows greater detail with granular F8AA reactivity in lymphatic endothelium (x265).

Photo at right:

Fig. 4. Lymphangioma of soft tissues near knee in a child. Note dilated lymphatic channels in upper figure (x210). Details of intense F8AA reactivity in endothelium lining cystic structures shown in lower micrograph (x325).

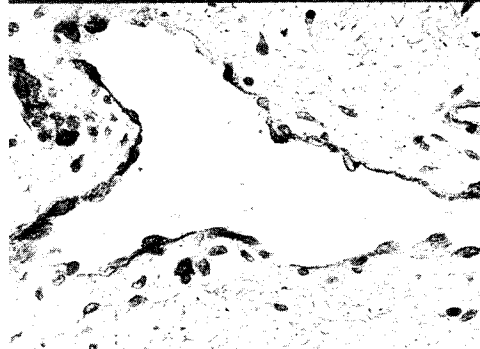
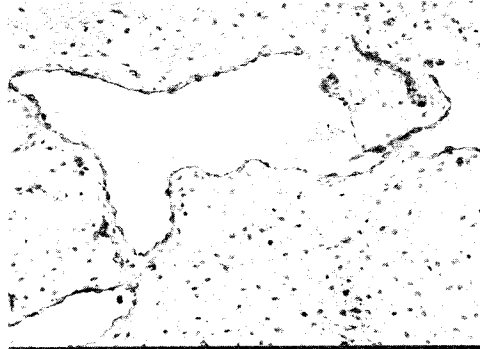
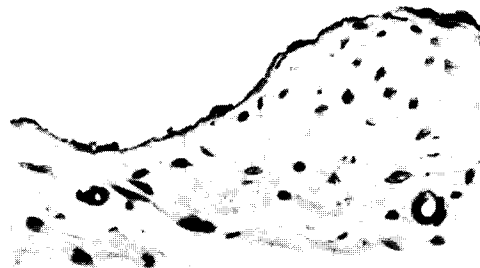
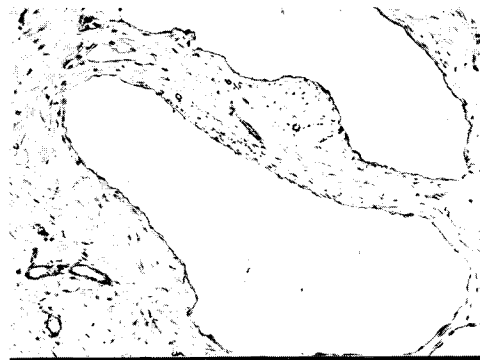


Fig. 3. Fetal strangulating cystic hygroma, hydrops, Turner's syndrome. Note dilated lymphatic structures (x85). Lower photomicrograph shows detail of F8AA reactivity in endothelial lining cells (x325).



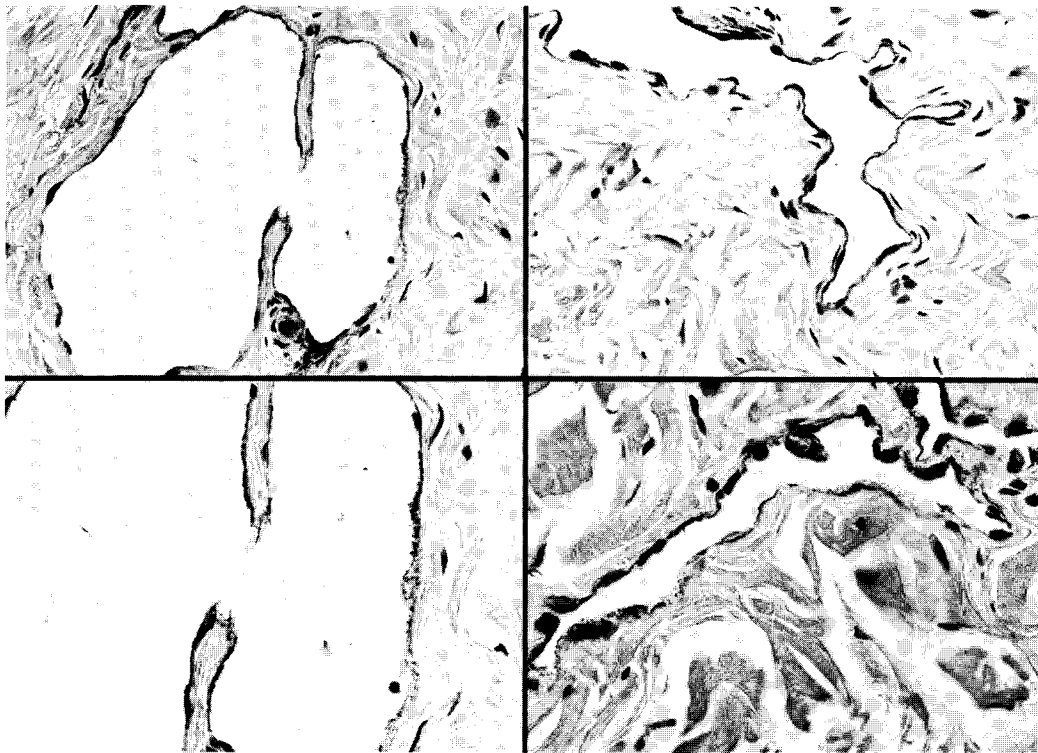


Fig. 5. Lymphangioma of soft tissue of right foot showing tortuous and dilated lymphatic channels. Top two figures x250. Bottom two figures show details of the granular F8AA reactivity of the lining endothelium (x385).

less intense and more focal and spotty than BVE lining arteries, veins, and blood capillaries. In culture, LVE showed strong F8AA reactivity, and TEM in one patient exhibited numerous classical Weibel-Palade bodies.

COMMENT

Lymphatic vascular endothelium lining normal, obstructed, and neoplastic lymphatics shares a number of histologic and histochemical features with comparable blood vascular endothelium in tissue section and in tissue culture including presence of the distinctive endothelial marker Factor VIII-associated antigen confirming and extending earlier more limited studies in man and experimental animals (1,5,6,8). Possible explanations for other reports of F8AA immunohistochemical negativity in lymphatic endothelium may relate to differing fixation and tissue processing, failure to employ ap-

propriate controls, variability in antibody specificity using different commercial antisera, or variable endothelial differentiation in pathologic states including retrodifferentiation or reversion to a more primitive cell type incapable of F8AA synthesis (2).

Weibel-Palade bodies (9), proposed synthesis-storage sites for F8AA (10) previously considered unique ultrastructural features of blood vascular endothelium, are also described here in cultured lymphatic endothelial cells from a lymphangioma. While these cytoplasmic organelles were not found in tissue sections or cultured LVE cells from other patients, their absence may reflect more slowly proliferating endothelium. Indeed, classical Weibel-Palade bodies were also just described in endothelium lining obstructed lymphatics in cats with *Brugia malayi* filariasis (3).

Demonstration that LVE contains F8AA and associated cytoplasmic organelles *in vitro* as well as *in vivo*, in con-

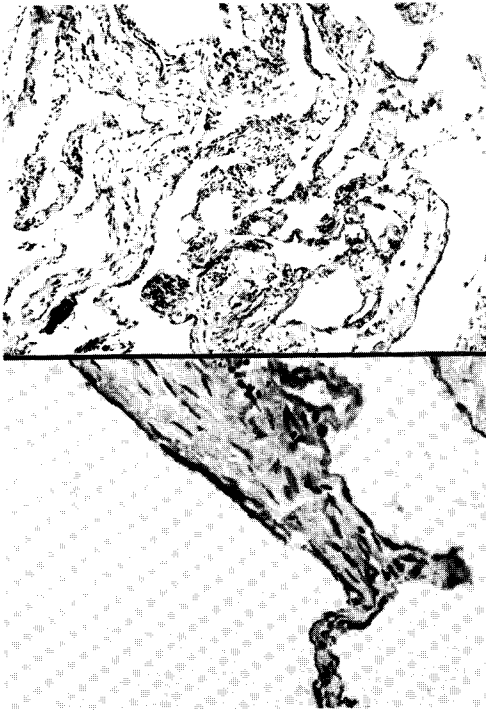


Fig. 6. Mediastinal lymphangioma. Low-power micrograph on top shows complex pattern of dilated lymphatic spaces with intervening connective tissue (x85). Bottom figure shows higher magnification revealing positive reactivity of the endothelium for F8AA (x210).

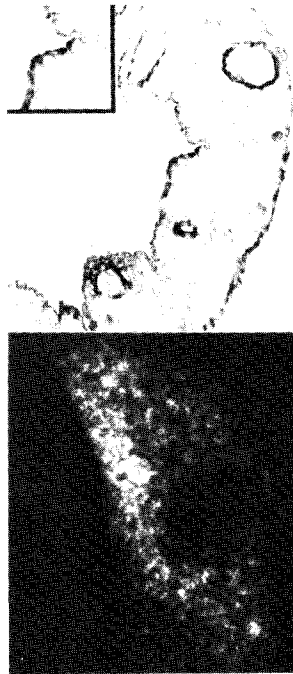


Fig. 7. Giant cervicomedial cystic hygroma. Above, low-power micrograph (x285) shows dilated lymphatic cystic spaces lined by endothelium giving a positive immunoperoxidase reaction for F8AA; greater detail (x700) shown in upper insert. Below, cultured lymphatic endothelial cells, derived from explants of the cystic hygroma, are decorated with anti-F8AA antibody on indirect immunofluorescence (x1125) (Modified from *Lymphology* 17 (1984), 15 (1)).

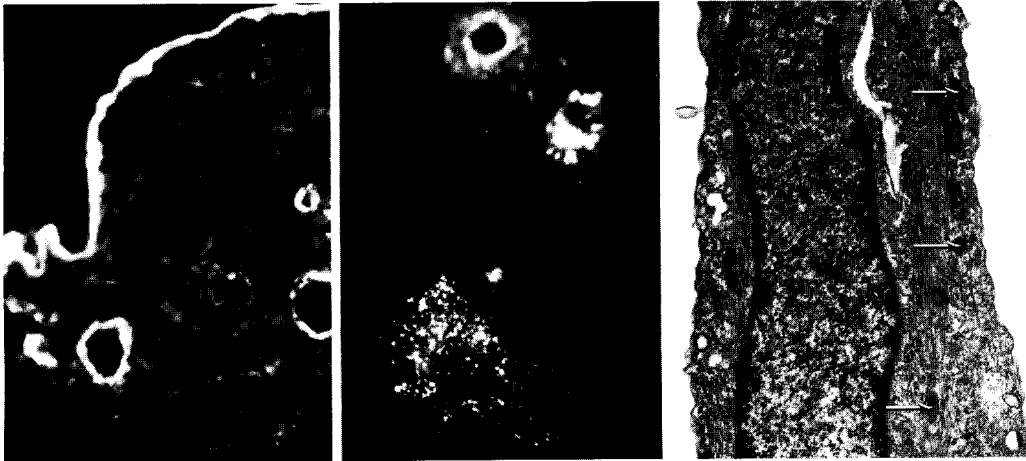


Fig. 8. Massive recurrent chyle-containing retroperitoneal lymphangioma. Left, strong immunofluorescent positivity for F8AA of endothelium lining large lymphogenous cyst (x235). Center, cultured lymphatic endothelial cells derived from the cyst lining show the characteristic granular pattern of F8AA (x550). Right, transmission electron microscopy of the cultured lymphatic endothelial cells shows abundant classical Weibel-Palade bodies (arrow) (x8300).

junction with other studies showing LVE rich in eicosanoids (prostacyclin, thromboxanes) and fibronectin, suggests that lymphatic-derived endothelial factors contribute to lymph and tissue coagulum (clots), an extracellular matrix phenomenon applicable not only to normal physiology but also to wound healing, containment of local infection, and lymphogenous spread of cancer.

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