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VASCULAR ENDOTHELIAL MARKERS OF THE HUMAN THORACIC DUCT AND LACTEAL

D. R. Gnepp

Department of Pathology, St. Louis University School of Medicine, St. Louis, Missouri

ABSTRACT

Factor VIII-related antigen (F8) and Ulex europaeus lectin (UEL) are accepted markers for human blood vessel endothelium. However, disagreement exists as to whether lymphatic vessels stain for F8, and accordingly this study was undertaken to address this issue. Moreover, another vascular endothelial marker, angiotensin converting enzyme (ACE) was also examined in lymphatics. Segments of human thoracic duct and portions of small bowel containing lacteals with post-mortem intervals of less than 15 hours, were removed at autopsy and fixed in B5 or formalin. The specimens were processed routinely and sections examined by indirect immunohistochemical techniques for F8 (Dako Corp.), ACE and for UEL (EY Lab). F8, UEL, and ACE positivity was uniformly found in thoracic ducts and lacteals; however, the staining intensity was less in lymphatic vessels with F8 and UEL than with comparable arteries or veins. ACE staining intensity, on the other hand, was similar in blood vessels and lymphatics. Both formalin and B5 fixation preserved antigenicity; however, background staining was greater with B5 fixation whereas tissue staining was slightly more intense with formalin fixation.

Factor VIII-related antigen (1-4) is a well-accepted marker for human blood vessel endothelium. However disagreement exists as to whether lymphatic vessels stain for Factor VIII-related antigen. This study was undertaken to address this issue and, moreover, two other vascular endothelial markers, Ulex europaeus lectin (3,5) and angiotensin converting enzyme (6) were also examined in lymphatics and comparable blood vessels.

MATERIALS AND METHODS

Segments of eight human thoracic ducts having a postmortem interval of less than 15 hours were isolated in the right chest just proximal to the diaphragm, adjacent to the azygos vein and anterior to the spine. They were excised and placed in 10% buffered formalin or B5 fixatives overnight. Segments of nine small intestines containing lacteals and having a postmortem interval also of less than 15 hours were similarly prepared. A few of the specimens taken from the same autopsy were fixed in both formalin and B5 fixatives, and these were considered as two separate specimens. The tissues fixed in B5 were then placed in 70% ethyl alcohol for 30 minutes. Each specimen was routinely processed and embedded in paraffin. Five micron, nonheat fixed sections were cut and stained with hematoxylin and eosin for Factor VIII-related antigen (F8) (Dako Corp., Santa Barbara, CA), Ulex europaeus lectin (UEL) (EY Laboratories, San Mateo, CA), and for angiotensin converting enzyme (ACE) (kindly supplied by Dr. R. Auerback at the University of Wisconsin) using the peroxidase-antiperoxidase technique. Positive and negative controls were utilized throughout. Immunohistochemical staining was done using the following methods:

UEL

Deparaffinized sections were hydrated through graded ethanol solutions, placed in two changes of distilled water followed by two changes of phosphate-buffered saline (PBS), pH 7.5, each for 5 min and then incubated in 2% H_2O_2 solution. They were then incubated with UEL-I at a concentration of 10μ/ml diluted in tris buffer for 1 hour. They were washed in PBS and incubated with 5% normal swine serum (NSS) in tris buffer for 15 min and then with anti-UEL-I (EY Laboratories) at a dilution of 1:20 in 2% NSS in tris buffer for 30 min and washed in PBS for 10 min. The sections were then incubated with swine anti-rabbit IgG (Dako Corp.) diluted 1:20 in 1% NSS in tris buffer for 30 min, washed in PBS for 10 min, and incubated with rabbit PAP (Dako Corp.) at a dilution of 1:100 in 1% NSS in tris buffer for 30 min and then washed in PBS for 10 min. The sections were incubated with diaminobenzidine with 0.02% H_2O_2 in tris buffer for 5 to 8 min, washed in distilled water, counterstained with Lillie-Mayer hematoxylin for 3 min, dehydrated and cover-slipped with permount.

ACE

Paraffin sections were heated in a 58°C oven for 30 min, placed in two changes of xylene for 20 min, and then hydrated through graded ethanol solutions for 1 min each. They were placed in .025% H_2O_2 in methanol for 20 min, followed by a 5 min wash in PBS. Sections were then incubated with 1-3 drops of the blocking reagent from a universal monoclonal peroxidase staining kit (Ortho Diagnostics, Raritan, NJ) for 20 min in a humidity chamber, with anti-ACE reagent for one hour using 50 lambda for each slide at a dilution of 1:500 in tris buffer, and rinsed in PBS for 10 min. Slides were incubated for 20 min with the rabbit anti-mouse IgG linking reagent (Ortho Diagnostics) from the universal monoclonal peroxidase staining kit, rinsed in PBS for 10 min and then incubated for 20 min with PAP from the same universal monoclonal kit. Slides were washed in PBS for 10 min, then incubated with diaminobenzidine with .02% H_2O_2 in tris buffer for 5-8 min, washed in distilled water and counterstained with Lillie-Mayer hematoxylin for 3 min, dehydrated and cover-slipped with permount.

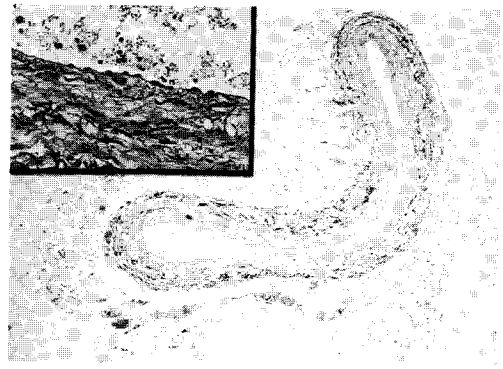

Fig. 1. Thoracic duct. Inset: detail of inner half of the wall (H&E).

Factor VIII-related antigen

Paraffin sections were heated in a 58°C oven for 30 min, deparaffinized in two changes of xylene for 20 min and hydrated through graded ethanol solutions for 1 min each. They were placed in a .025% solution of H_2O_2 in methanol for 20 min, washed in PBS, pH 7.5, for 5 min and incubated in 5% normal swine serum (NSS) (Dako Corp.) in tris buffer for 15 min. This was followed by incubation of the primary antibody, Factor VIII-related antigen, at a dilution of 1:100 in tris buffer and 1% NSS for one hour, a 10 min wash in PBS and then a 30 min incubation with swine anti-rabbit IgG (Dako Corp.) at a dilution of 1:20 in 1% NSS and tris buffer. The sections were then washed in PBS for 10 min, incubated with rabbit PAP (Dako Corp.) at a dilution of 1:100 in 1% NSS in tris buffer for 30 min and washed again in PBS for 10 min. Sections were then incubated with diaminobenzidine with .02% H_2O_2 in tris buffer for 5-8 min, washed in distilled water, counterstained with Lillie-Mayer hematoxylin for 3 min, dehydrated and cover-slipped with permount.

RESULTS

Eight thoracic ducts were collected at

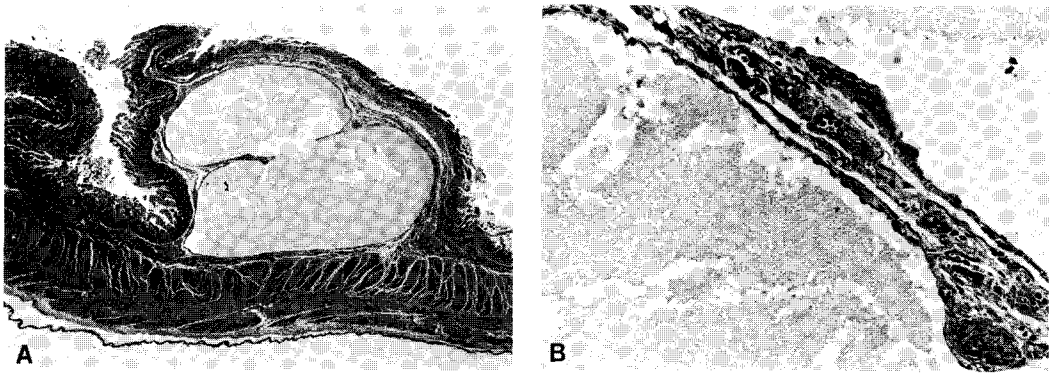

Fig. 2. a) Dilated lacteal (H&E). b) Detail of infolding of lacteal wall from Fig. 2a (H&E).

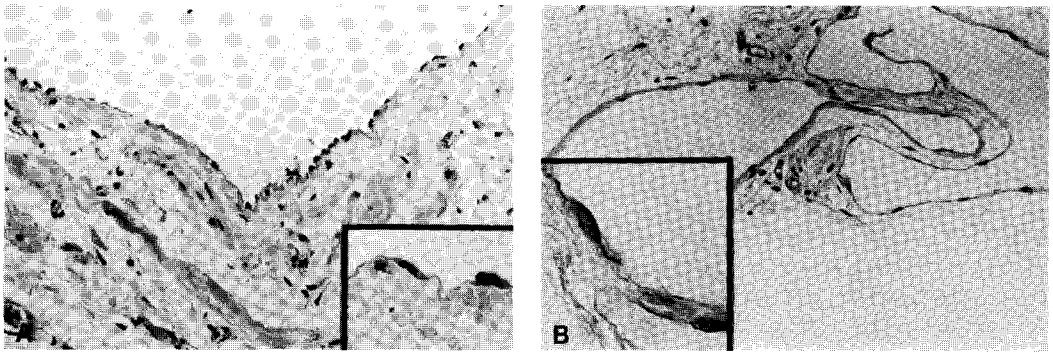

Fig. 3. a) Factor VIII-related antigen immunoperoxidase stain of the thoracic duct. Inset: detail of surface endothelial staining from another area. b) Factor VIII-related antigen immunoperoxidase stain of a lacteal. Inset: detail of multifocal, noncontinuous staining of endothelial surface from an adjacent area.

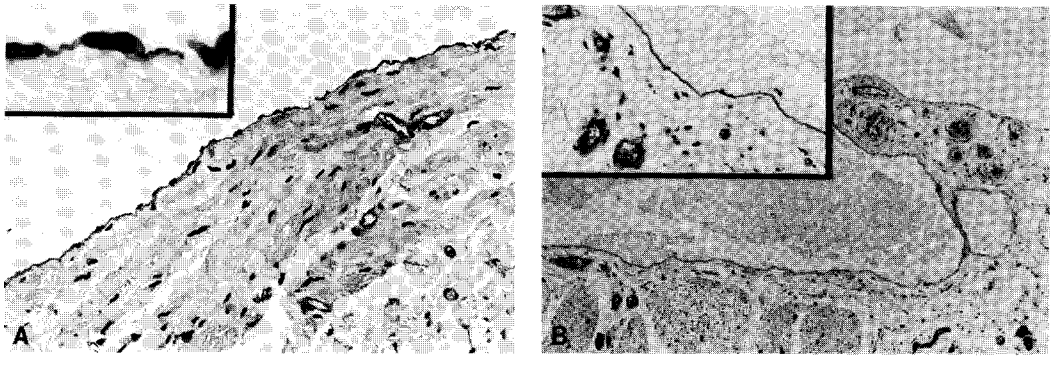

Fig. 4. a) Ulex europaeus lectin immunoperoxidase stain of the thoracic duct. Note continuous endothelial surface positivity as well as positive staining of several blood vessels in the media. Inset: detail of endothelial staining. b) Ulex europaeus lectin immunoperoxidase stain of a lacteal. Note continuous surface staining and several adjacent positive staining blood vessels. Inset: detail of endothelial surface staining.

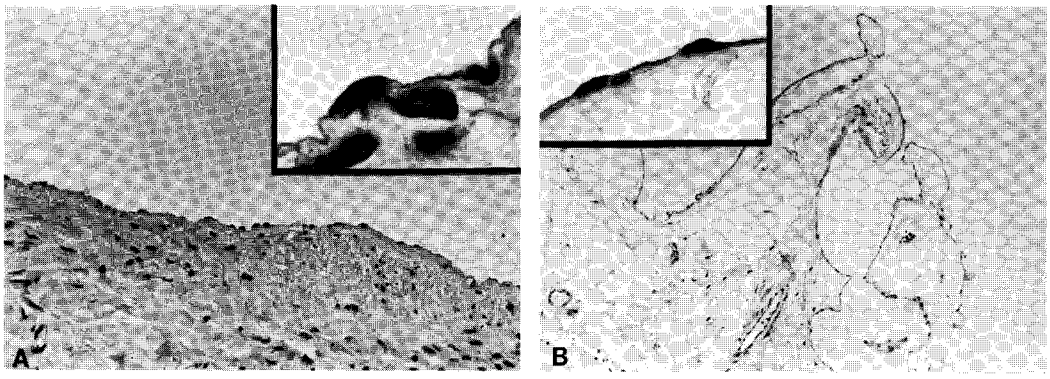

Fig. 5. a) Angiotensin converting enzyme immunoperoxidase stain of the thoracic duct. Inset: detail of an area with continuous endothelial surface staining. b) Angiotensin converting enzyme immunoperoxidase stain of a lacteal. Inset: detail of endothelium with continuous surface staining from an adjacent area.

autopsy. Seven of these retained the bulk of the endothelium and were utilized for these studies *(Fig. 1)*. Small bowel specimens uniformly contained lacteals with areas of residual endothelial lining *(Fig. 2)*. Factor VIII-related antigen did not stain the lymphatic endothelium or endogenous blood vessel endothelial cells on two of the thoracic duct specimens. All other specimens examined stained positively to varying degrees for each of the antigens examined: F8 was found in 5 thoracic ducts and 9 lacteals, UEL was found in 7 thoracic ducts and 9 lacteals, ACE was found in 7 thoracic ducts and 9 lacteals *(Figs. 3-5; Tables)*. F8 and UEL appeared to stain blood vessels more intensely than the lymphatic vessels, while ACE stained blood and lymphatic vessels with similar intensity. F8, UEL, and ACE stained the cytoplasm of the endothelial cells in a diffuse fashion; however, F8 also demonstrated areas with just perinuclear F8 positivity. Areas of vessels without endothelium did not stain, accounting for much of the multifocality of the staining. However, areas with endothelium occasionally did not stain. Staining intensity in lacteals was slightly less than that found in the thoracic ducts. UEL stained lymphatic vessels slightly more intensely than did the F8 or ACE. Both B5 and formalin fixation preserved the antigenicity; however, background staining was greater with B5 fixation, while tissue staining was slightly more intense with formalin fixation.

DISCUSSION

Considerable controversy exists as to whether Factor VIII-related antigen (F8) is found in lymphatic vessels. Mukai et al (1), Howat and Variend (7), Ohkuma and Nishida (8) and others (9-11) did not find F8 staining in normal lymphatics. Stephensen and Mills (12) examined five lymphangioma circumscripta for F8 staining; all were negative. Capo et al (9) also found that two lymphangiomas were immunonegative for F8 in the endothelium of the lymphatic channels. However, Sehested and Hou-Jensen (13) were able to demonstrate F8 positivity of lymphatic endothelium in formalin-fixed paraffin embedded human tissue. Svanholm et al (14) examined human collecting lymphatic vessels from the skin of the dorsal foot for F8 using frozen sections and also formalin- and Bouin-fixed tissues. They found positive staining in all 29 sections examined; the frozen sections provided the strongest staining intensity. In addition, F8 positivity has been demonstrated in canine and human lymphatic vessels recently by several authors (2,3,15-18) in endothelial cells grown in tissue culture from human and canine thoracic duct (15) and from bovine mesenteric lymphatic vessels (19). F8 was also found in human lymphangiomas and lymphoceles (2,4,17,20,21).

Because it is difficult, using histology alone, to separate a lymphatic from a blood

FACTOR VIII-RELATED ANTIGEN

Thoracic Duct	*Fixation*	*Staining Intensity*	*Background Staining*
#1	B5	multifocal, 2^+	High
#2	B5	focal, 1-2^+	High
#3	B5	multifocal with diffuse areas, 2^+	High
#4	F	focal and diffuse areas, 2^+	Good
#5	F	focal, 1-2^+ in multiple areas	Good
#6	B5	multifocal, 2-3^-	High
#7	F	diffuse areas, 2-3^-	Good
#8	F	diffuse, 2-3	Good
#9	B5	focal, 1-2	High
Lacteal	*Fixation*	*Staining Intensity*	*Background Staining*
#1	F	focal, 1-2^+	Good
#2	F	focal, 1-2^-	Fair
#3	F	focal, 1-2^+	Good
#4	F	focal and diffuse areas, 2^+	Good
#5	B5	focal*, 1^+	Good
#6	B5	focal, 1^+	Good
#7	B5	multiple focal areas, 1-2^-	Good
#8	B5	focal, 1^+	Good

F = formalin

*Many lymphatic endothelial cells were sloughed.

vessel (14), I chose to examine the thoracic duct and intestinal lacteal in this study. The former is easily identified anatomically and the latter lymphatic capillaries can be conclusively identified using only light microscopy.

From the results of this study and those previously reported, it seems reasonable to conclude that most lymphatic vessels stain positively with Factor VIII-related antigen, although there is some variability. Perhaps differences in antisera, technique used by different laboratories or tissue fixation account for the variation in results. On the other hand, not even blood vessel endothelium invariably stains positive with F8 (1,3), and accordingly, there may be different expression of F8 in lymphatics from various locations. Our results conform to studies demonstrating F8 positivity in lymphatic endothelium, although F8 endothelial staining is more intense in blood vessels than lymphatics. Mukai et al (1) found, in general, that capillary endothelial cells showed the strongest positivity for F8; as the caliber of vessels increased, the positivity became weaker. Results in the present study, however, demonstrate that lacteals stain less intensely than the thoracic duct. This discrepancy may be attributed to thinning of the endothelium in the lacteal as it fills with chyle presenting a smaller cross-sectional surface area to stain in contrast to a blood capillary with plump

ULEX EUROPAEUS LECTIN

Thoracic Duct	*Fixation*	*Staining Intensity*	*Background Staining*
#1	B5	multifocal with many diffuse areas, 2^{+}-3	Good
#2	F	focal, 1-2^{+}	Good
#3	F	diffuse, 2-3^{+}	Good
#4	B5	focal, 1-2^{+}	Fair
#5	B5	focal, 1-2^{-}	Fair
#6	B5	focal*, 1-2^{-}	Fair
#7	B5	focal*, 1^{+}	High
Lacteal	*Fixation*	*Staining Intensity*	*Background Staining*
#1	F	diffuse, 2-3^{-}	Good
#2	F	diffuse, 2-3	Fair
#3	F	diffuse and multifocal, 1-2	Good
#4	B5	diffuse areas, 1-2^{-}	Good
#5	B5	multifocal with diffuse areas*, 1-3	Good
#6	B5	diffuse, 2-3^{-}	Fair
#7	B5	multifocal with diffuse areas, 2-3^{+}	Good
#8	B5	focal, diffuse areas, 1-2	Good

F = formalin *Many lymphatic endothelial cells were sloughed.

endothelium having a more exposed endothelial surface than arteries or veins.

In addition, this study also confirmed that Ulex europaeus lectin stains both the thoracic duct and lacteal. Others have previously demonstrated Ulex europaeus lectin in normal lymphatics (3,10,11) in a lymphangioma (10) and in endothelial cells from a lymphangioma grown in tissue culture (21). The staining of this marker usually appears a little less intense in lymphatic endothelium than in adjacent blood vessels. Nonetheless, the staining intensity of Ulex europaeus lectin in lymphatic vessels was slightly greater than staining for F8 and ACE in most of the preparations. Other observers (3) have found similar findings for F8 and UEL.

Angiotensin converting enzyme was demonstrated in all the specimens. Gnepp and Chandler (15) have previously demonstrated indirect immunofluorescent positive staining of angiotensin converting enzyme on canine thoracic duct endothelial cultures; however this current study is the first to document positive staining in human lymphatic endothelium.

Other investigators have recently demonstrated ABH blood group isoantigens, which mark human blood vascular endothelial cells (12), in breast lymphatics of patients with breast carcinoma (18). Moreover, Barsky et al (22) have demonstrated laminin and type IV collagen in human thoracic duct, but these markers were not found in the mouse intestinal lacteal, in two lymphangiomas or in two patients with intestinal lymphangiectasia.

ANGIOTENSIN CONVERTING ENZYME

Thoracic Duct	*Fixation*	*Staining Intensity*	*Background Staining*
#1	F	diffuse, 3+	Fair
#2	B5	diffuse, 2+	Fair
#3	F	diffuse, 3+	Fair
#4	B5	diffuse, 2-3+	Fair
#5	F	diffuse, 3+	Fair
#6	B5	diffuse, 2-3+	Fair
#7	B5	diffuse, 1-2	Fair
#8	B5	diffuse with multifocal areas, 2+	High
#9	B5	diffuse with multifocal areas, 2+	High
#10	B5	diffuse with multifocal areas, 2+	High

Lacteal	*Fixation*	*Staining Intensity*	*Background Staining*
#1	B5	diffuse, 2-3+	Fair
#2	B5	diffuse with multifocal areas, 2+	Fair
#3	B5	diffuse with multifocal areas, 2+	Fair
#4	B5	diffuse and multifocal, 2+-3	High
#5	F	mostly diffuse with focal areas, 2+	Good
#6	B5	diffuse, 2+	Fair
#7	F	diffuse with multifocal areas, 1-3+	Fair
#8	F	diffuse with multi-focal areas, 2-3+	Fair
#9	F	diffuse with multi-focal areas, 1-3+	Fair
#10	B5	diffuse with multi-focal areas, 1-2+	Fair

F = formalin

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Douglas R. Gnepp, M.D.
Department of Pathology
The University Hospital
1325 South Grand Boulevard
St. Louis, MO 63104