Histochemical and Electron Microscopic Studies of the Human Cutaneous Lymphatic Capillary

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MATERIALS AND METHODS

Under local anesthesia with 1% hydrochloric procaine, skin specimens were taken from the left upper arms of a 30- and a 46-year-old Japanese man, and from the scrotum of a 27- and a 70-year-old male. For the light microscopic observations the materials were fixed in 10% formalin and stained with hematoxylin-eosin, PAS, PAS after digestion with diastase, orcein, toluidine blue, colloidal iron, mucicarmine, aldehyde fuchsin, resorcin fuchsin-picrofuchsin, azan-Mallory, congo-red, Giemsa, sudan III, sudan black, Masson's ammoniacal silver nitrate, Weigert's staining for fibrin, alcian blue, Berlin blue reaction, or silver staining for reticulum fiber. Some 1 μm thick sections were cut from Epon blocks and stained with toluidine blue.

For the electron microscopic observations, 1 mm sized specimens were fixed in 2.5% 0.1 M phosphate-buffered glutaraldehyde (pH 7.4) at 4°C for 2 hr, washed, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for further 2 hr, dehydrated in ethanol and embedded in Epon 812 as usual. The ultrathin sections were placed directly on grids without supporting film and stained with uranyl acetate followed by lead citrate. The lymphatics were first looked for in the 1 μm thick sections stained with toluidine blue.

RESULTS

Light microscopic observations. The specimens with elastic staining sometimes show elastic fibers which are frequently arranged radially around the lymphatics (Figs. 4 and 5). Their irregular lumina (Fig. 1) change in shape with serial sectioning (Figs. 2 and 3). But if these characteristic findings are not found, it is very difficult to identify them. All the other staining methods have revealed no special characteristics to differentiate the lymphatics from the blood vessels. The lymphatics can be smaller than the blood capillaries and some are collapsed in

Fig. 1. A lymphatic capillary (L) with a more irregular shape of the lumen than that of the blood vessel (>). CT, connective tissue. H-E, ×50.
Fig. 2. Two lymphatic capillaries (L) are dilated in the dermis. No special characteristic findings are seen in comparison with the blood vessel (†). CT, connective tissue; EP, epidermis. PAS, × 120.

**Table 1. Light microscopic characteristics of the human cutaneous lymphatic capillaries**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
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<tbody>
<tr>
<td>Lumen</td>
<td>Larg, RBC almost not seen</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Thinner, unrecognizable, not sharply bordered, less frequent nuclei, wall defect (artifact †), luminal and abluminal projection</td>
</tr>
<tr>
<td>Pericyte</td>
<td>Absent</td>
</tr>
<tr>
<td>Connective tissue area</td>
<td>Elastic fiber (+) or (−) around lymphatics</td>
</tr>
<tr>
<td>Serial sections</td>
<td>Rapidly changing contour of lumen</td>
</tr>
<tr>
<td>Distribution</td>
<td>None in dermal papilla</td>
</tr>
</tbody>
</table>

The results are summarized in Table 1.

*Electron microscopic findings.* In the lymphatic lumina there are many fine electron dense particles (Fig. 6) which probably originate from serum. In the cytoplasm of the endothelium one observes usual cell organelles including vesicles, coated vesicles and filaments (Fig. 8). There are two types of infolded projections of the lymphatic wall into the lumen. The one is associated with collagen fibers and the ground substance between the two endothelial layers and the other lacks the collagen. The endothelium possesses no, or only a poorly developed basal lamina. Elastic fibers are not always seen in the adjoining connective tissue area (Figs. 3 and 7). However, the larger is the vessel, the more the fibers are observed. Destroyed cells and cell-organelles are noticed in the lymphatic lumen and the endothelium is defective here and there. No lymphatic is found in the papilla,
Fig. 3. Elastic fibers are not especially dense around the lymphatics. Note a change in contour of the lumen as revealed with serial sections (Figs. 2 and 3). CT, connective tissue; EP, epidermis. Orcein, x 120.

Figs. 4 and 5. Elastic fibers are thick and some of them are radially arranged around the lymphatic capillary (L), which is not the case with the blood capillary (†). CT, connective tissue; EP, epidermis. Elastica van Gieson, x 120 (Fig. 4); x 300 (Fig. 5).

as Araya (1970) has already suggested. The electron microscopic characteristics are listed in Table 2.

DISCUSSION

The staining of elastic fibers and serial sections are often, if not always, useful for identification of the lymphatic capillary under the light microscope. However, the elastic fibers are not always found, as also confirmed by the electron micro-
**Table 2. Ultrastructural characteristics of the human cutaneous lymphatic capillaries**

<table>
<thead>
<tr>
<th>Lumen</th>
<th>Fibrin, destroyed cells and cell-organelles (all artifacts?), larger, irregular-shaped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium</td>
<td>Open junction, end-to-end and end-to-side typed junction, wall defect (artifact?), thinner, luminal and abluminal projection</td>
</tr>
<tr>
<td>Basal lamina</td>
<td>Absent or scanty, half-desmosome</td>
</tr>
<tr>
<td>Pericyte</td>
<td>None</td>
</tr>
<tr>
<td>Fiber</td>
<td>Fibrils and collagen fibers attached to the endothelium, sometimes elastic fibers around the lymphatic</td>
</tr>
<tr>
<td>Localization</td>
<td>Absent in dermal papilla</td>
</tr>
</tbody>
</table>

Fig. 6. A lymphatic capillary (L) is seen with plenty of elastic fibers (ef) in the adjacent connective tissue area. Note rich luminal and abluminal projections of the endothelium and fine electron dense particles in the lumen as well as an open junction (∩) and a wall defect (∩), cf, collagen fiber; E, endothelial cell of lymphatic capillary. Electron micrograph, × 7,500.

Scopic observations. The author could find no useful histochemical staining methods for the identification of the lymphatics; even PAS staining was not helpful. Whether the lymphatic is larger than the blood capillary, is considered to be a matter of extent of dilatation as well as of direction of sectioning. The lymphatic may contain a small number of red blood corpuscles in the lumen, which is most
Fig. 7. An undilated lymphatic capillary (L) around which no elastic fiber is detected. cf, collagen fiber, E, endothelium. Electron micrograph, × 15,000.

Fig. 8. The endothelial cell (E) of lymphatic capillary contains filaments. The cell appears to have been desquamated from the connective tissue area (CT) with a vacant space between them (↑) which is not considered to be an artifact produced by sectioning. The numerous fibrils (f) are in the connective tissue area. No basal lamina can be proved. Electron micrograph, × 26,000.
likely to be artifactual (Ohkuma 1974). When a large part of the wall is
defective, the lymphatic can hardly be differentiated from the spaces which are
artificially produced in the connective tissue. Dilatation of the lymphatics could
be produced by the edema of the tissue induced by local anesthesia, circulatory
disturbance, bleeding or liberated chemical substances during the biopsy proce-
dures.

As for the fine structure, the destroyed cells and cell-organelles found in the
lymphatic lumen and the wall defects are thought to be artifacts caused by the
biopsy. But this does not allow us to decide whether or not open endothelial
junctions exist in the tissue under the normal condition. If the connective tissue
between the endothelial cells in an infolded projection of the wall is abundant, and
the protrusion is marked, the projection may be similar to the valve which has
the same structure.

Since the blood vessels are always surrounded by a continuous basal lamina,
regardless of sectioning-direction, the non-existant or scanty basal lamina of the
lymphatic capillaries is the most important criterion to identify them.

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