

NEW STRUCTURAL DETAILS OF DERMAL LYMPHATIC VALVES AND ITS FUNCTIONAL INTERPRETATION

Judit Daroczy, M.D.C.Sci.

Dermatology Clinic of the Semmelweis Medical University, Budapest

ABSTRACT:

Dermal lymphatics (rat and human) were studied electronmicroscopically and changes within the lymphatic wall including neof ormation of intralymphatic valves were followed by serial sections. Two new forms of intralymphatic valves were observed — unicellular valve or single endothelial cell extension deep into the lymphatic lumen and bunch valve, or a connective tissue core with numerous tip-cells at its cuspidal edge. These structures functioned as "flap" or "helper" valves and like inlet (intramural) valves, they were variable structures appearing and disappearing with changes in the local interstitial environment. Lymphatic capillaries probably act as microscopic pistons to pump and suction lymph fluid in a centripetal direction.

Dynamic function of the lymphatic system is only partially known (5,9,13,17). Normally, tissue hydrostatic pressure is subatmospheric or less than that in lymphatic capillaries. While it appears that lymphatic capillaries take up materials against the hydrostatic pressure gradient, in point of fact minute amounts of fluid and protein are pushed out from blood capillaries into the interstitium. The overall effect is to locally raise tissue hydrostatic pressure (17) which temporarily exceeds intralymphatic atmospheric pressure and forces interstitial fluid into lymphatic capillaries. At the inception of this "filling phase" other factors such as colloid osmotic pressure of lymph, total tissue pressure, contractions of adjacent muscles and pulsation of nearby arteries probably also facilitate lymphatic filling. Other evidence suggests that a constant pressure gradient does not maintain centripetally directed flow of lymph. Nonetheless, local and sporadic forces

favor lymphatic pumping with flow dependent on microcirculatory dynamics (15).

This work demonstrates new structural details of lymphatic valve systems. The results provide a new dimension to interpreting the dynamic morphology of dermal lymphatics.

MATERIALS AND METHODS

Eight male Wistar rats weighing 150-250 gms were sensitized to dinitrochlorobenzene (DNCB) by application of a 30% acetone solution to back skin. Seven days later a contact dermatitis was elicited by application of 5% DNCB to the lower paws and a 2x2 cm area of shaved back skin. Mild erythema was observed at 1 h, when tissue samples from back skin and lower paws were taken from each rat under ether anesthesia and fixed by immersion using Karnovsky's fixative and postfixed with OsO_4 (6).

Human material was selected from 8 patients undergoing biopsy for diagnostic electron microscopy. These included pityriasis rubra pilaris (back) (3 patients), benign acanthosis nigricans (neck) (3 patients), and ichthyosis vulgaris (scapular region) (2 patients). Biopsy specimens were fixed in 3% buffered glutaraldehyde (phosphate buffer pH 7.4) at 4°C for 2 hours and postfixed with OsO_4 . Tissues were uniformly treated for 2 hours with uranyl acetate en bloc and embedded in Epon-812. Semithin sections (1 μm) were stained with toluidine blue for orientation purposes. Ultra-thin sections were obtained with Reichert UmO₃ ultramicrotome, stained with uranyl acetate and lead citrate

and examined with Siemens Elmiskop IA and Philips 400 electron microscope.

The structure of dermal lymphatic valves in both rat and human material were identical. *Inlet valves* or intraluminal valves were visible along the lymphatic vessel wall with open junctions between neighboring endothelial cells. Four other types of intralymphatic valves were also observed: 1. one cell or unicellular, 2. bunch, 3. joining, 4. segment valves. Unicellular and bunch valves were demonstrated primarily in lymphatic capillaries.

1. *Unicellular valve*: Formed by a single endothelial cell, this valve was separated in part from neighboring endothelial cells. It extended deeply into the lumen while maintaining connections with adjacent endothelial wall cells and had an extremely short contact with nearby connective tissue. The shape of these intraluminal-appearing endothelial cells varied, being round, oval or rod-shaped. Nuclei also varied being oval, spindle-shaped or indented. This unique endothelial cell projected like a short bud into the lumen. Sometimes it spanned the lumen to approach the endothelium of the opposite side. On the cell surface there were prickle-like or longer pseudopodia projecting into the lumen. The cytoplasm contained short tubules of rough endoplasmic reticulum, free ribosomes, mitochondria, vesicles and abundant filaments at 6-9 nm in diameter. They were often bundled at the cell base. The basal lamina was better developed and the hemidesmosome-like densities were more frequent at the connective tissue base of the unicellular valve than in other parts of the wall (Fig. 1).

2. *Bunch Valve*: The stem of the "bunch" consisted of a connective tissue fold covered by a single layer of endothelial cells. Along its free edge endothelial cells budded into the lumen, but differed from other endothelial cells (Fig. 2a). These "tip-cells" had extremely short connections with the connective tissue component of the valve, and were characterized by well developed basal lamina and numerous hemidesmosome-like membrane densities. Intracellular nexus junctions

between adjoining tip-cells were also encountered. Open intercellular junctions (inlet valves) were not visible at the bunch valve region. The tip-cells had elongated pseudopodia-like projections, and the cytoplasm was rich in 6-9 nm filaments (Fig 2b).

In our material the highest number of budding tip-cells was seven; however, sometimes nuclei were detected only by serial sections. They varied in form, being oval, elongated and frequently deeply indented. Unicellular and bunch valves often were observed together in the same section (Fig. 2a). 3. *Joining Valve*: Detectable at the point where lymphatics join one another, these valves were usually bicuspid, derived from opposite walls, and varied in length. The valve cusp contained a core of connective tissue and a surface of endothelial cells which joined directly with the wall endothelium (Fig. 3). There were no differences in organelle content between wall and valvular endothelial cells. The basal lamina was thicker and the hemidesmosome-like plaques more frequent in the valve than in the wall and there were no open intercellular junctions along the valvular endothelium. At the edges of the valve cusps, tip-cells displayed morphological properties distinguishable from other endothelial cells including budding into the lumen, short connections with connective tissue core of the cusp, elongated pseudopodia-like projections, indented nuclei, abundant cytoplasmic filaments of 6-9 nm in diameter, and connections of adjoining tip-cells characterized by increased densities of adjacent cytoplasm (Fig. 4a, b). The number of tip-cells varied from 1-5. 4. *Segment Valve*: These valves consisted of folds of connective tissue which extended into the lumen and divided them into discrete segments. The luminal surface was covered by endothelial cells, occurred in pairs and emanated from facing walls. Their morphology were identical to that of joining valves. The tip-cells of the cusps were able to seal the lumen due to nexus junctions between them (Fig. 5).

Functionally, dermal lymphatic capillaries are found in different states of contraction and dilation (Fig. 6).



Fig. 1: Lymphatic capillary. Unicellular valve extends into the lumen (Lu). Its cytoplasm is rich in microfilaments (mf) and the basal lamina and the hemidesmosome-like densities (arrows) are well developed, arrowheads = contacts between the endothelial cells. Ichthyosis vulgaris, (2500x)

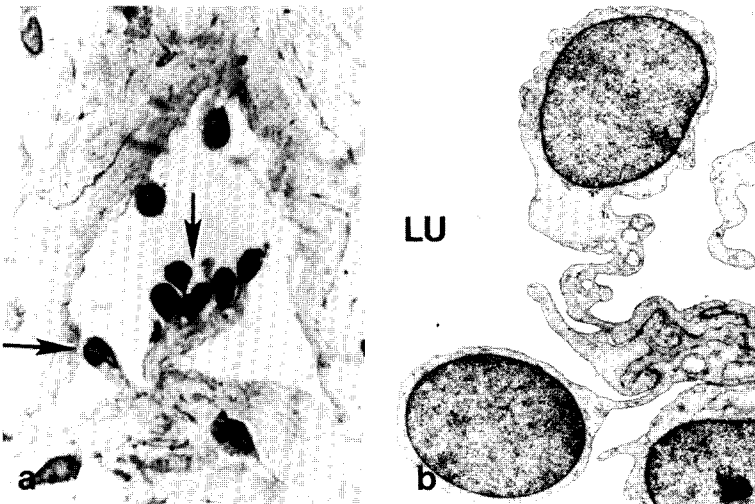


Fig. 2: Lymphatic capillary with an unicellular (arrowhead) and a bunch valve. a. The tip-cells are seen (arrow) along the edge of the connective tissue core of the bunch valve. Toluidine blue, (630x) b. The tip-cells of the bunch valve extend into the lumen (Lu). Well developed basal lamina and hemidesmosome-like plaques in the valve's core, (5500x) Pityriasis rubra pilaris.

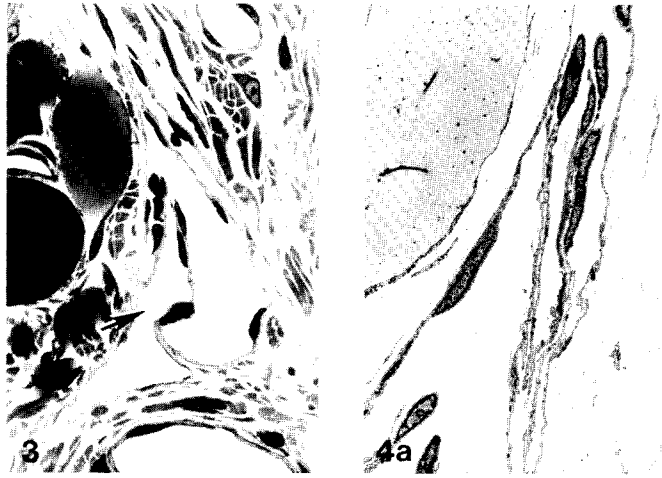


Fig. 3: Two pairs of intralymphatic valves are seen, arrowhead = joining valve, Rat paw, Toluidine blue, (1000x)



Fig. 4: Intralymphatic valve with typical tip-cells. a. 1170x b. Dense cytoplasmic plaques (arrows) between the valvular tip-cells, Rat paw, (2500x)

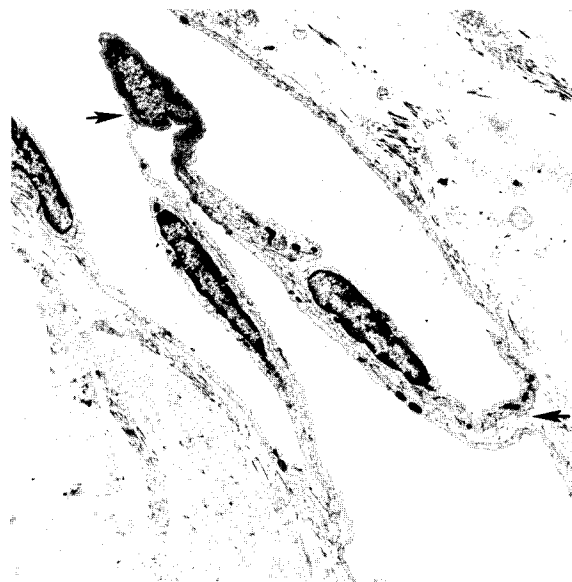


Fig. 5: Lymphatic capillary. Segment valve sealing the lumen (arrow) shows the typical structure of the intralymphatic valves, arrowhead = connective tissue core, Rat paw, (1950x)



Fig. 6: Submicroscopical segments of a dermal lymphatic capillary are found in different dilatation periods, arrowhead = multiple wrinkles of the intraluminal structure in a collapsed segment, *Pityriasis rubra pilaris*, (4300x)

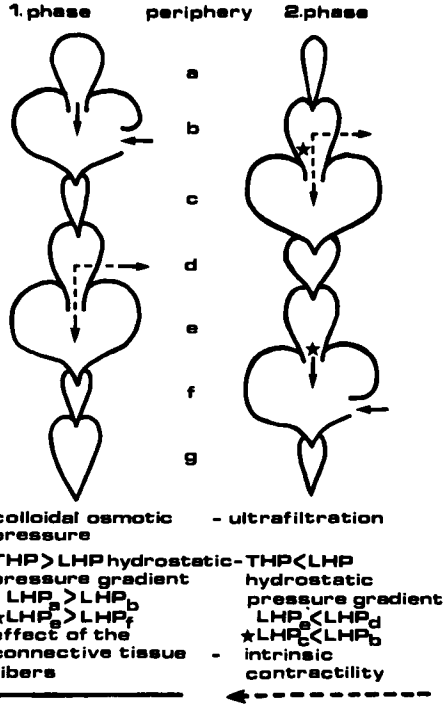


Fig. 7: Schematic drawing summarizing in two phases the piston function in identical segments (a-g) of the lymphatic capillary. Continuous arrow represents the forces (colloid osmotic pressure, hydrostatic pressure gradient, effect of the connective tissue fibers) causing the fluid to enter into the lumen and/or to flow further into a proximal segment. The dotted arrow indicates the forces causing the lymph to leave the lymphatic lumen and/or to flow into a proximal segment (ultrafiltration, hydrostatic pressure gradient, intrinsic contractility).

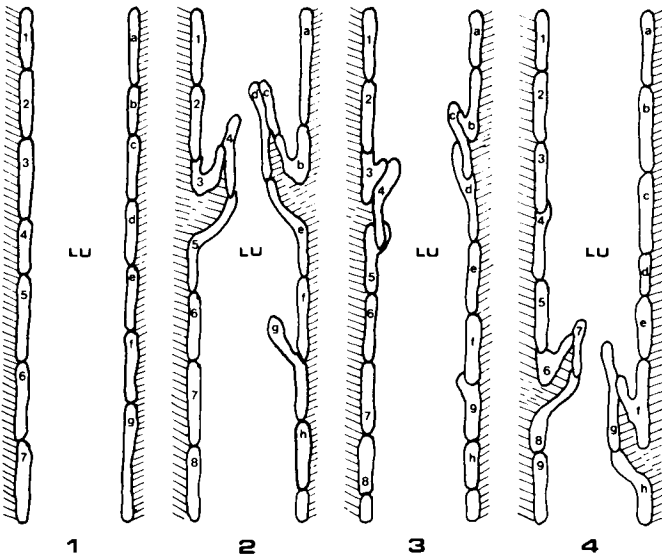


Fig. 8: Schematic drawing shows four different periods of activity and suggested new building and disappearance of temporary valves, 1 = inactive capillary, 2 = new valve is created, g = unicellular valve, 3 = temporary valve's structures (endothelial cells and connective tissue) are sliding back, 4 = new temporary valve is formed, Lu = lumen

DISCUSSION

Lymphatics are composed of draining-channels with centripetal streams of fluid travelling from peripheral tissues to the venous system. Rather than having a constant capacity, lymphatic capillaries adjust to a fluid challenge. Depending on the interstitial load lymphatic capillaries dilate by changing shape and altering their relationship to surrounding connective tissue. Structural properties facilitating dilatation of dermal lymphatics include: 1. special connections of the endothelial cell processes (end to end, overlapping, interdigitating, accordion-like wrinkles), 2. scanty, often interrupted or deficient basal lamina, 3. absence of pericytes, 4. collagen, elastic and anchoring fibers which directly connect with the abluminal membrane of the endothelial cells (4,11,12). During dilatation with loose connections of adjacent endothelial cell processes, overlapping cells slide on one another, interdigitations and foldings disappear and the lymphatic wall straightens as the lumen dilates. The close linkage of the lymphatic wall with connective tissue fibers make possible direct transmission of interstitial pressure to initial lymphatics.

Electron microscopy reveals valves in dermal lymphatic capillaries (3,7,8) that facilitate central lymph flow. The valve network includes inlet (intramural) and intralymphatic valves.

Inlet valves are constant structures that develop along the capillary between wall endothelial cells and respond to local pressure variations and interstitial-intraluminal pressure gradients. Intralymphatic valves, on the other hand, are segment valves (3,16) and include bunch and unicellular valves (7,8). Because of nexus junctions these valves are able to seal the lymphatic lumen and like "lock gates" maintain unidirectional (centripetal) lymph flow.

When different forms of intralymphatic valves on serial sections are examined dermal lymphatic structure appears strikingly variable. In response to local pressure variation, intralymphatic valves vary in length and width. For example, in one phase rising interstitial pressure forces a connective tissue core to push the valve deeper into the lumen while in another phase increased

intraluminal pressure pushes connective tissue folds of the valve back into connective tissue of the wall (Fig. 6). Therefore, local pressure differentials create temporary valves. Moreover, changes in fluid flow modify the original endothelium by transformation into valvular endothelial and tip-cells. The final shape of unicellular valves and tip-cells probably depends on suction forces activated in the lumen which generate elongated, sometimes worm-like extensions and pseudopodia characteristic of intralymphatic valvular endothelial cells (Figs. 1,2,4,5).

Bunch and unicellular valves probably facilitate centripetal movement with increased fluid loads by acting as "flap" or "helper" valves (Figs. 1,2). As fluid demands recede, these "temporary valves" slide back into the wall's structure and are no longer evident (Fig. 6). Although a bunch valve theoretically may have actually been a single cusp of a bicuspidal joining or segment valve with the other cusp removed from the section plane, careful serial sections excluded this possibility.

Lymphatic capillaries with structured inlet and intralymphatic valves probably work as tiny pumps (5,9). At rest, tissue hydrostatic pressure (THP) is negative (-6 mmHg) and no hydrostatic pressure gradient exists toward lymphatic capillaries where intraluminal pressure (LHP) is atmospheric. As blood capillary pressure forces fluid into the interstitium THP rises and temporarily becomes higher than the LHP thereby generating a hydrostatic pressure gradient from the interstitial space to lymphatics. This pressure differential forces open the inlet valve and fluid enters and dilates the lymphatic lumen ("filling phase") (5,9,17). As rising intraluminal lymphatic pressure closes the inlet valves, backwash of lymph is prevented and lymph becomes more dilute. Centripetal lymph flow from one segment to the next (lymphangion) (13) is favored by both hydrostatic and colloid osmotic pressure differentials between distal and proximal lymphatic segments and intrinsic contractility of the lymphangion itself (10,13,14). Dilatation of the lymphatic wall probably triggers contraction of bundles of endothelial

microfilaments (1,13,18). During the next stage ("emptying phase") proximal intralymphatic valves open and distal valves close permitting some lymph to regurgitate into the intercellular space through cytoplasmic pores. Proteins and other macromolecules, however, are retained in the lumen. Thus, lymph flowing centrally is probably continuously concentrated (5,9).

In assessing function of lymphatic structures it is critical to take into account these forces exerted by submicroscopic segments of the lymphatic network in far smaller channels than usually studied physiologically. Moreover, in the maintenance of central lymph flow temporary and regional forces such as endothelial valve structures probably play an important role. While a "pressure wave" is likely lacking, periodic pumping and suction of initial lymphatics move lymph into larger lymphatic channels where they exert a suction effect (15,17). Finally, the shape of lymphatic valves vary considerably (2,4) and depend largely on the endothelial-connective tissue interconnections (12). Fig. 7 illustrates the postulated pumping and suction forces operating in the filling and emptying phases of comparable segments of a lymphatic capillary where on a submicroscopic level hydrostatic pressure forces interstitial fluid into the lymphatic lumen (dilated segment), while at the same time in an adjacent segment (collapsed lumen) very slight suction favors movement of lymph centrally (Fig. 6). These forces operate simultaneously, continuously and in variable phases. Although dysrhythmic, unlike an arterial pulse, these alternating pumping and suction phases of lymphatic capillary action resemble that of a piston (Fig. 8), which in turn, alters the lymphatic wall configuration and thereby gives rise to de novo lymphatic valves.

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