MORPHOMETRIC ANALYSIS OF ELASTIC FIBERS IN HUMAN SKIN LYMPHATIC CAPILLARIES

R. Gerli, L. Ibba, C. Fruschelli

Institute of Histology and General Embryology, University of Siena, Siena, Italy

ABSTRACT

In contrast to their absence near dermal blood capillaries, elastic fibers are commonly seen adjacent to dermal lymphatic capillaries under light microscopy. Based on morphometric analysis, the elastic fiber network that surrounds these skin lymphatic capillaries is predominantly oriented longitudinally to the lymphatic vessel wall. Quantitative analysis reveals that the density of these pericapillary elastic fibers are almost twice that of the intercapillary elastic fibers but only about one-half as thick. These data suggest that dermal lymph capillaries are surrounded by a specific elastic network of functional significance, morphologically distinct from that seen in the intercapillary dermis. Because lymphatic capillaries are often difficult to identify especially when collapsed, this elastic network may facilitate the positive identification of dermal lymphatic capillaries by light microscopy and thereby help differentiate them from blood capillaries. The possible role of this lymphatic elastic network in the absorptive activity of the dermal lymphatic system is also discussed.

As part of the lymphatic vascular system, dermal lymphatic capillaries aid in the removal of interstitial fluid and, in conjunction with macrophages, also transport dermal tissue proteins. The initial lymphatics enable antigen-presenting Langerhans cells and other macrophages to reach regional lymph nodes to initiate immune processes (1-3). The skin lymph capillaries also provide an exit route for T-lymphocytes circulating in the dermis (4). The initial lymphatic network of the skin is tortuous and irregular and consists of lymph capillaries also known in clinical lymphology as "initial lymphatics" (5) localized principally in the superficial "papillary" zone of the dermis and in the deep "reticular" zone near the dermo-subcutis junction (6). The lumen of the lymphatic capillaries is generally larger (20-70μm) than that of the blood capillaries (5-10μm), and unlike blood capillaries, lymphatic capillaries contain valves (7-10). Lymphatic endothelial cells consist of a thin cytoplasm except in the vicinity of the nucleus. Moreover, lymphatic endothelial junctions are sometimes of the "open" type and the endothelial basal membrane is characteristically thin and discontinuous (11-15). Thin fibrous structures known as "anchoring filaments" emanate from the abluminal surface of the endothelial cells of the lymphatic capillaries and project into the surrounding connective tissue which is rich in collagen and elastic fibers (11,16).

Thus, the lymphatic system is reasonably well understood with respect to the functions of lymph formation, lymph propulsion, and the behavior of endothelial junctions in normal and pathological states. The elastic sheath which surrounds lymphatic but not blood capillaries may further explain certain phenomena related to the initial lymphatic network.

An elastic perivascular lymphatic network exists in the human dermis (17-19)

and this network probably plays an important role in the absorptive and peristaltic activity of initial lymphatics. It has also been claimed that the elastic sheath helps distinguish lymphatic from blood capillaries using light microscopy. However, a systematic study of these dermal elastic fibers has not as yet been performed in the skin of man or experimental animals. Accordingly, we qualitatively studied the elastic fibers in human skin lymphatic capillaries, quantitatively analyzed their morphology, and then examined the correlation between the occurrence of these elastic fibers in lymphatic and blood capillary membranes.

MATERIALS AND METHODS

Twelve lateral arm skin biopsy specimens taken during operation from both male and female patients (18-45 years of age) were used. Histological examination of adjacent specimens were performed to exclude the possibility of skin abnormalities. The specimens were divided into minute fragments and fixed in Karnovsky fixative solution (20) for 3 hours at 4°C, postfixed in 1% osmium tetroxide in 100mM Na Cacodylate buffer pH7.4, dehydrated and embedded in Epon-Araldite. Semithin sections were cut with an LKB Nova ultramicrotome, oxidized with 5% H_2O_2 for 10 minutes to remove osmium, etched with sodium methylate, washed in 70% ethanol and stained with resorcin-fuchsin for 1-3 hours at 50°C. Photographs were taken with a Zeiss Axiomat light microscope fitted with a x50 or x100 immersion lens. The final enlargement of the prints was x1280 or x2240. The elastic fibers in the lymph and blood perivascular areas of the papillary dermis were analyzed, along with the elastic fibers of the intercapillary areas, excluding the band lying within 50μm of the dermo-epidermal junction. Morphometric analysis was carried out with a Kontron Ibas I semiautomatic image analyzer. The elastic fibers were divided into two categories: "perilymph-capillary elastic fibers" (PEF) within an arbitrary radius of 12μm from the endothelial profile; and "interlymph-

capillary elastic fibers" (IEF) which included all the other elastic fibers but not those within 50μm of the dermo-epidermal junction. Only capillaries clearly defined in transverse or longitudinal section were studied; ambiguous sections were disregarded. We determined the thickness (putative diameter) of the elastic fibers in the peri- and intercapillary areas (because the sections of the elastic fibers were random, the minor axis was taken), the distance of the elastic fibers from the endothelial cells in the lymphatic pericapillary area and the density (number/area and area/%) and "form factor" of the peri- and intercapillary elastic fibers. The diameter of lymphatic capillaries was also determined.

The data were statistically analyzed and compared (p=0.05). The biopsy specimens were not analyzed according to age but the mean values were taken.

RESULTS

The semithin sections stained with resorcin-fuchsin revealed the constant presence of elastic fibers near dermal papillary lymphatic capillaries (Fig. 1). By

Fig. 1. Section of dermis. In the upper part, a lymphatic capillary (L) in transverse section. Many elastic fibers surround the endothelial profile. In lower part, elastic fibers of the dermis. x50.
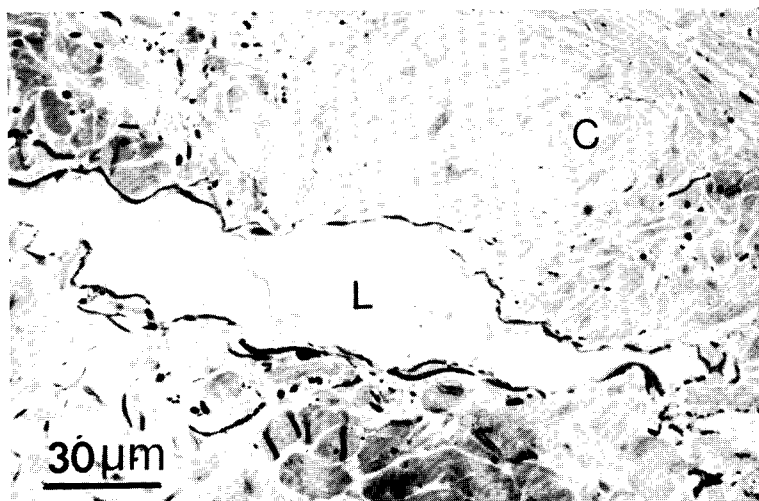
Fig. 2. Section of dermis. In the center a lymphatic capillary (L) in longitudinal section. Near the endothelium many elastic fibers are arranged longitudinally to the vessel. At the upper right, a blood capillary (C) is seen without surrounding elastic fibers. x50.

contrast, practically no elastic fibers were found near blood capillary loops (Fig. 2). The papillary area more than 50μm from the dermo-epidermal junction showed a uniform distribution of elastic elements arranged in apparently random fashion. In the vicinity of the dermo-epidermal junction there were extremely few elastic fibers and at the junction itself there were none. The mean diameter of the lymphat-

ic capillaries was $38\pm16\mu$m; neighboring elastic fibers were mostly disposed longitudinally to the capillaries and were clearly differentiated from the intercapillary fibers.

Morphometric findings, summarized in *Table 1*, show that the PEFs of transversely or longitudinally sectioned lymphatics had a thickness of about 1.6μm whereas IEFs were twice as thick. The

Table 1
Morphometric Parameters of Dermal Perilymph-Capillary (PEF) and Interlymph-Capillary (IEF) Elastic Fibers.

Fiber type (section)	Counts vessels/fibers	Thickness μm	Distance[1] μm	Density #/100μm^2	Density μm^2/100μm^2	Form PE[2]
PEF (T)	42/2800	1.60±1.2*	5.37±3.2*	3.52±1.2**	7.10±2.3*	0.78±0.2**
PEF (L)	54/1210	1.61±1.3**	5.20±3.6*	0.73±0.2**	7.50±3.8*	0.43±0.3**
IEF	3000	3.20±1.8**	---	1.80±0.5**	7.20±3.1*	0.61±0.3**

PEF   perilymph-capillary elastic fibers within a radius of 12μm from the endothelium
IEF   interlymph-capillary elastic fibers outside the perivascular area
(T)   lymphatic capillaries sectioned transversely
(L)   lymphatic capillaries sectioned longitudinally
[1]   distance from the endothelium
[2]   Form factor: circles = 1, irregular shapes <1
*   not significantly different from similarly marked values in the same column (p=0.05)
**   significantly different from the other values in the same column (p=0.005)

Measurements are given as mean value ± SD.

density of PEFs was about 3.5 elastic fibers per $100\mu m^2$ of tissue in lymphatic capillaries sectioned transversely, whereas the density of IEFs was about half this value and the density of PEFs was approximately only 0.7 in the lymphatic capillaries sectioned longitudinally. However, when we considered the fiber density expressed as area per cent, the data was found to be very similar, about $7\mu m^2/100\mu m^2$. The distance of the PEFs from the endothelial profile was approximately $5\mu m$. There were practically no PEFs around blood capillaries. The form factor of PEFs was nearly 0.8 in transverse sections of lymphatic capillaries and nearly half this value in longitudinal sections. The form factor of IEFs was intermediate between the two.

DISCUSSION

These findings indicate that unlike blood capillaries, human skin lymphatic capillaries are uniformly surrounded by elastic fibers. This demonstration not only confirms previous reports (17-19), but other noteworthy points emerge from morphometric analysis.

The lymph capillaries are not only surrounded by a network of elastic fibers, but close to the lymphatic endothelium, fiber density as number/area is significantly greater than in the intercapillary areas. The ratio of these two densities is 2:1. Although there is greater density of elastic fibers around lymphatic capillaries, the thickness of their profiles is significantly less than that of intercapillary fibers in the ratio 1:2. These two facts taken together indicate that the lymphatic capillary membrane has its own elastic network which is distinct from that of the connective tissue and is quite original and unique, especially since blood capillaries are devoid of an elastic fiber network. In the area of the initial lymphatics, the elastic fibers are finer and form a denser mesh than the coarse elastic component of the surrounding dermis. However, the density data, expressed as percentage area, are similar. In other words, the "sum" of areas of elastic fibers is the

same, at any distance from the lymphatic capillary membrane. It seems likely that the larger but sparser intercapillary elastic fibers branch out near the lymphatic capillaries to form a finer but denser pericapillary elastic network. The "number" of fibers increases but the "mass" remains the same.

Quantitative analysis shows that the hypothetical branching of IEFs into PEFs occurs according to the ratio 1:2. This ratio agrees with the two morphometric parameters of these categories of fiber: both density, as number/area, and thickness area correlated in the highly significant complementary and symmetrical ratio of 1:2 and 2:1.

Quantitative analysis also confirms the morphological structure of the elastic fibers and their prevalently longitudinal arrangement relative to the lymphatic capillaries. The positive staining perivascular profiles give a form factor of about 0.8 in transversely sectioned capillaries and nearly half this value in longitudinal sections. The first value is similar to the form factor of transversely sectioned elastic fibers (nearly circular) and the second to that of longitudinally sectioned fibers (non-circular), exactly as analyzed and as they seem to be arranged in the lymphatic capillaries examined by us. This arrangement seems in close agreement with the report of Daroczy (15) who suggests that the elastic fibers around the dermal lymphatic capillaries lie mainly parallel to the long axis of the capillary lumen or are arranged in a spiral pattern.

The IEFs, obviously cut randomly, yield a value of form factor which is intermediate between the extremes obtained for PEFs.

The function of the elastic fibers near lymphatic capillaries is not clear. There are no in depth studies on the relationship between elastic fibers and the endothelial membrane. In animals, "anchoring filaments" have been correlated with elastic fibers in the surrounding connective tissue by some authors but without further comment (11,21). Others conclude that collagen and elastic fibers in human dermis are connected to the "anchoring

filaments" which favors opening of the endothelial junctions (5,14,22,23). Leak (24) claims that the elastic fibers are directly connected to the abluminal endothelial membrane and Daroczy (15) suggests that the connections are made by "elastic microtubules." Jones et al (25) maintains that elastic fibers in pig skin arise from the elastic fiber envelope of the superficial lymphatics of the dermis and branch towards the epidermis with their fine terminal ends inserting into the dermal-epidermal zone.

Preliminary results of a study in progress indicate that lymph capillaries in the skin and elsewhere, are enveloped in a true "fibrillar-elastic apparatus" which exerts force and leverage on the perivascular elastic network and binds the endothelial membrane directly to the elastic fibers of the perivascular connective tissue. The anchoring filaments turn out to be the proximal linkage between fibrillar-elastic apparatus and endothelial cells.

In some patients with skin diseases there are severe alterations of the elastic fibers and impairment of the absorbing lymphatic network (18,19,26). Acne rosacea with elastosis and lymphedema, leprosy and mycosis fungoides with severe immune deficiency and destruction of dermal elastic fibers are indicative of a relation between pathologic processes, lymphatic system, and dermal elastic fibers. It seems likely therefore that the elastic network around the lymphatic capillaries performs certain basic functions: 1) storing of elastic "kinetic" energy during stretching, dilatation, and filling of the capillary; this energy is returned to the vascular wall in the phase of relaxation, compression, and emptying of the lymphatic capillary to propel the newly formed lymph monodirectionally (ensured by intraluminal valves); 2) transmission of extracapillary tensions (arterial pulse, muscle massage, respiratory and peristaltic movements, mechanical stress), damped and elastically compensated, to the endothelial junctions so that they can better open. Thus, tissue fluid, protein molecules, migrating cells, for example, move from the interstices into the initial lymphatic system and are propelled proximally. These actions minimize the likelihood of local edema and when circulating antigens in lymph contact specific lymphocytes in the lymph nodes, the immune defenses are triggered.

REFERENCES

1. Stingl, G, SI Katz, L Clement, et al: Immunologic function of Ia bearing Langerhans cells. J. Immunol. 121 (1978), 2005-2013.
2. Silberberg-Sinakin, I, GJ Thorbecke: Contact hypersensitivity and Langerhans cells. J. Invest. Dermatol. 75 (1980), 61-67.
3. Choi, K, DN Sauder: The role of Langerhans cells and keratinocytes in epidermal immunity. J. Leukocyte Biol. 39 (1986), 343-358.
4. Norris, DA, WP LeFeber: Mycosis fungoides and Sezary syndrome. In: *The Year Book of Dermatology*. Thiers, BH, RL Dobson (Eds.), London, Year Book Medical Publishers (1982), 9-79.
5. von Rautenfeld, DB, D Lubach, B Wenzel-Hora, et al: New techniques of demonstrating lymph vessels in skin biopsy specimens and intact skin with the scanning electron microscope. Arch. Dermatol. Res. 279 (1987), 327-334.
6. Rusznyak, I, M Foldi, G Szabo: *Lymphatics and Lymph Circulation*. Oxford, Pergamon Press (1960), 155.
7. Takada, M: The ultrastructure of lymphatic valves in rabbits and mice. Am. J. Anat. 132 (1971), 207.
8. Vajda, J, M Tomcsik: The structure of the valves of the lymphatic vessels. Acta Anat. 78 (1971), 521-531.
9. Braverman, IM, A Yen: Microcirculation in psoriatic skin. J. Invest. Dermatol. 62 (1974), 493.
10. Daroczy, J: The structure and dynamic function of the dermal lymphatic capillaries. Br. J. Dermatol. 109 (suppl. 25) (1983), 99-102.
11. Leak, LV, JF Burke: Ultrastructural studies on the lymphatic anchoring filaments. J. Cell Biol. 36 (1968), 129-149.
12. Jdanov, DA: Anatomy and function of the lymphatic capillaries. Lancet 25 (1969), 895-899.
13. Leak, LV: Studies on the permeability of lymphatic capillaries. J. Cell Biol. 50 (1971), 300-323.
14. Casley-Smith, JR: Are the initial lymphatics normally pulled open by anchoring

filaments? Lymphology 13 (1980), 120-129.

15. Daroczy, J: *The Dermal Lymphatic Capillaries*. Springer-Verlag (1988).

16. Pullinger, BD, HW Florey: Some observations on the structure and functions of lymphatics: Their behavior in local edema. Brit. J. Exp. Path. 16 (1935), 49-61.

17. Okhuma, M: Lymphatics of the skin. Proc. 8th Int. Congress of Lymphology, Montreal, Avicenum, Czechoslowak Medical Press, Prague (1981), 84-92.

18. Mortimer, PS, GW Cherry, RL Jones, et al: The importance of elastic fibres in skin lymphatics. Br. J. Dermatol. 108 (1983), 561-566.

19. Ryan, TJ, PS Mortimer, RL Jones: Lymphatics of the skin. Int. J. Dermatol. 25 (1986), 411-419.

20. Karnovsky, MG: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell. Biol. 27 (1965), 137a.

21. Böck, P: Histochemical staining of lymphatic anchoring filaments. Histochemistry 58 (1978), 343-345.

22. Leak, LV: The structure of lymphatic capillaries in lymph formation. Federation Proc. 35 (1976), 1863-1871.

23. Castenholz, A: Structural and functional properties of initial lymphatics in the rat tongue: Scanning electron microscopic findings. Lymphology 20 (1987), 112-125.

24. Leak, LV: The transport of exogenous peroxidase across the blood-tissue-lymph interface. J. Ultrastruct. Res. 39 (1972), 24-42.

25. Jones, RL, PS Mortimer, GW Cherry, et al: The dermal lymphatics: A possible structural connection with the epidermis and the influence of Langer's lines. Int. J. of Microcirculation Clinical and Experimental 5 (1986), 274.

26. Marks, R, J Harcourt-Webster: Histopathology of rosacea. Arch. Dermatol. 100 (1969), 683.

Professor Corradino Fruschelli
Istituto di Istologia ed
** Embriologia Generale**
Universita' degli Studi di Siena
Facolta' di Medicina e Chirurgía
Via Del Laterino, 8
53100 Siena, ITALY