Stereomicroscopic Funnel-like Architecture of Pulmonary Lymphatic Valves*

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Introduction

Although lymphatic valves are basically important in channeling lymph and establishing the lymphatic microcirculation, not much attention has been paid to their structure in the past. They are still classically, and as will be shown, erroneously considered to occur as two semilunar cusps, attached to opposite sides of the vessel wall, and hanging into the vessel lumen like two closely apposed swallow's nests. This widely accepted textbook description (1, 2, 4–7, 15–17, 19, 20) has been largely extrapolated from observations on venous valves situated in the large veins (i. e. the saphenous veins) of the body. Also the appearance of routine histologic tissue sections falsely suggests and mimicks a bicuspid architecture, as our preliminary graphic anatomic reconstructions of 26 pulmonary lymphatic valves from serial (6 μ) histologic tissue sections have revealed (3, 11, 14). Reconstructed valves seemed to have the shape of a cone or a funnel, oriented obliquely in relation to the axis of the vessel and attached over its entire length to the wall of the vessel. The lumen appeared to be situated near the deepest point of the funnel.

These anatomic graphic reconstructions were however only an indirect, probably a more or less biased approach to the real architecture of the pulmonary lymphatic valves. Indeed, due to their overall dimensions and their specific "intravascular" localization, it was impossible to locate accurately the reference points which are an essential condition in anatomical reconstructions. Even if major deformations could be avoided due to the inherent complexity of the lymphatic vessel network and of its valves, and because of the rather limited number of the 6 micron sections used for the actual reconstructions (18), slight lateral deformations were unavoidable. Moreover (3), still another cause of artefactual lateral deformation, lenghtening and stretching of the graphic models, occurred because we reconstructed in perspective under a very large angle rather than by orthogonal projection.

Hence our graphic anatomic reconstructions did not constitute an exact replica of reality and were undoubtedly subject to various artefacts to some extent. Therefore the present study was undertaken in order to correct these earlier results. As only a direct observation of the lymphatic valvular architecture seemed appropriate, we studied lymphatic valves stereomicroscopically on single and serial 150–250 micron thick histologic sections through the entire lung lobes.

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Materials and Methods

At the moment of the postmortem examination, the lungs of twelve human infants dying within 5 days after birth were fixed in toto with Bouin's fluid for 48 hrs. Only babies in whom autopsy was performed within several hours to one day after death were studied. The infants' gestational ages ranged from 26 to 40 weeks, their birth weights from 800 gm to 3800 gm. The autopsies included detailed gross and microscopic examination, the babies having died from a wide variety of neonatal diseases. Infants with congenital malformations were not considered in the present investigation.

Thick (1–2 cm) lung tissue slices, representing a complete transsection of every lung lobe, were then cut with a sharp dissecting blade and further fixed by immersion in Bouin's fluid for 1–2 weeks. These were embedded in paraffin under vacuum and cut into single and serial sections of 150–250 micron thickness with a large sledge microtome adapted for entire organ tissue sections (Jung-Tetrander microtome type I.). The sections were mounted on glass slides, deparaffinized, monochromatically stained with eosin, or light green, or hematoxylin and covered with glass cover slips. Then they were examined using a stereomicroscope (Zeiss) with incident light.

Results

Studied stereomicroscopically on these thick, glass mounted histologic tissue sections, the architecture of the lymphatic valves as well as of the lung parenchyma could be directly observed. Valves were present in the pleural, peribronchial, periarterial and perivenous lymphatics, which form the two main sets of lymphatic vessels that occur in the human lung: the superficial or pleural and the deep or parenchymatous (peribronchovascular) lymphatic plexus (8, 10, 12–14).

On these thick sections the lumina of the lymphatic vessels are characteristically quite large and when viewed axially or in cross section appear circular to oval (fig. 6). Often they are sectioned along their long axis and viewed more laterally, appear elongated and sometimes slightly curved to tortuous. The lumen, which usually does not contain erythrocytes, is lined by endothelial cells and appears empty or contains faintly stained lymph. Lmphatics are easily differentiated on these thick sections from blood capillaries by several characteristics of the latter: a lumen of much smaller calibre, a less tortuous and more straight, sometimes more angulated course within the connective tissue compartment of the lung and the presence of many, closely packed, intensely stained erytrocytes in the lumen (fig. 3).

As was observed previously on the usual 5–6 micron histologic sections of infant lungs, there were also no lymphatic capillaries visible on these 150–250 micron thick sections at the level of the air-blood barrier or in the intersaccular septa, but lymphatic capillaries were frequently situated between the saccular wall and the interlobular, pleural, peribronchial or perivascular connective tissue sheets. These juxta-saccular and juxta-alveolar lymphatic capillaries (fig. 6) do constitute the most distal or peripheral, endothelially lined and truly visible and identifiable endings of the lung lymphatics. (The terms and definitions "air-blood barrier", "intersaccular and interalveolar septum", "saccular and alveolar wall" have been extensively described and defined in our earlier studies [12–14].)

The data confirm in a general way the above-mentioned graphic reconstructions (3, 11, 14), though not being identical. They contrast with the commonly accepted opinion, for only exceptionally (i. e. twice) did valves appear to be bicuspid, i. e. composed of two semilunar cusps attached to opposite sides of the vessel wall. In these twelve lungs all other lymphatic valves exhibit the shape of a slightly bent funnel (fig. 1) or cone (fig. 4), which may best be compared on lateral thick sections to either a "wind tunnel" (fig. 2), half an "hourglass" (fig. 3) or a "trailing net" (fig. 2) opened at its bottom, the more or less curved shape of the funnel being probably due to some shrinkage and retraction which inevitably occurs during the preparation of the tissues (fixation, embedding, staining) prior to the light optical investigation itself. Perhaps this slight variability in appearance may also occur during the *in vivo* lymphatic microcirculation and organ motion.

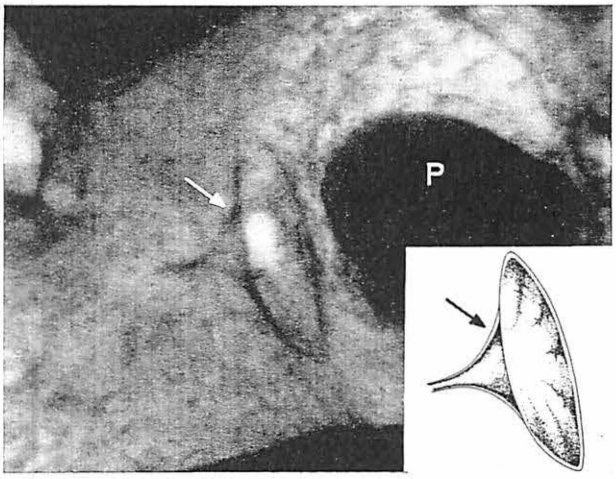

Fig. 1
Funnel-like valve (arrow) localized in the interlobular connective tissue, surrounding a pulmonary vein (P). 161×; Photomicrograph of a thick section of a human infant lung.

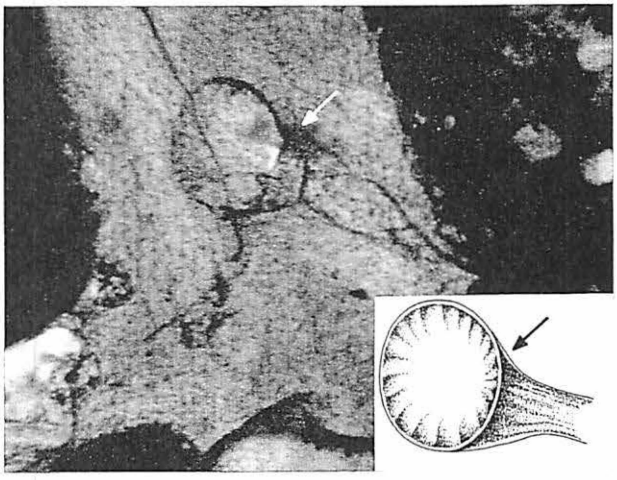

Fig. 2
Funnel-like valve (arrow), mimicking the architecture of a "windtunnel" or a "trailing net" and localized in the interlobular connective tissue. 95×; Photomicrograph of a thick section of a human infant lung.

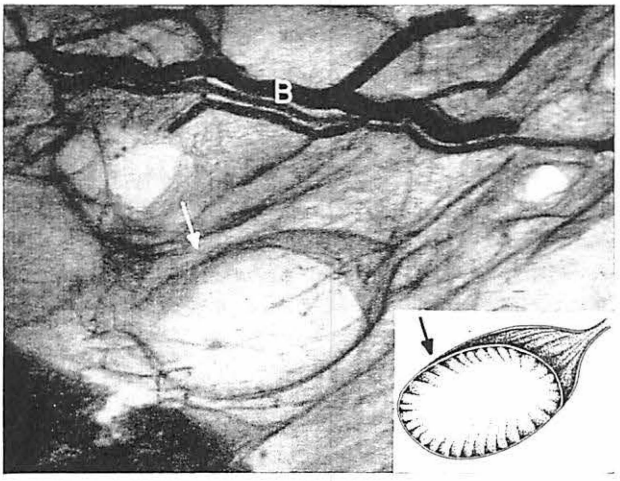

Fig. 3
Funnel-like valve (arrow), whose shape may be compared to half an „hourglass". Blood capillaries (B) do occur as well; they are characterized by a lumen of smaller calibre, the presence of numerous intensely stained erytrocytes in the lumen, and a more straight course. 71×; Photomicrograph of a thick section of a human infant lung.

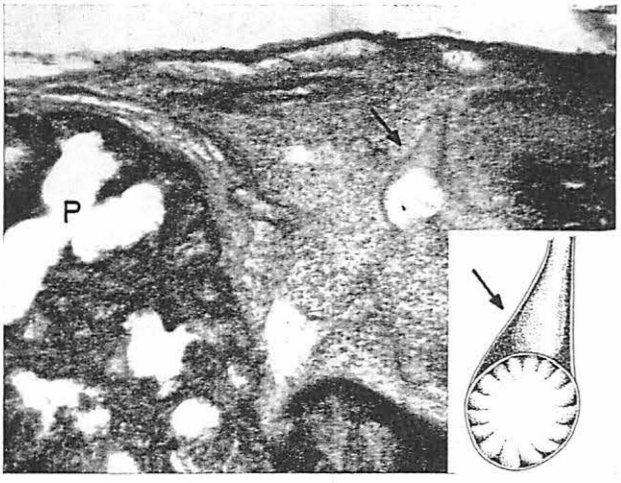

Fig. 4
Cone-like valve (arrow) situated at the junction between the pleural and the interlobular connective tissue. 85×; Photomicrograph of a thick section of a human infant lung. (Pulmonary alveolar parenchyma: P.)

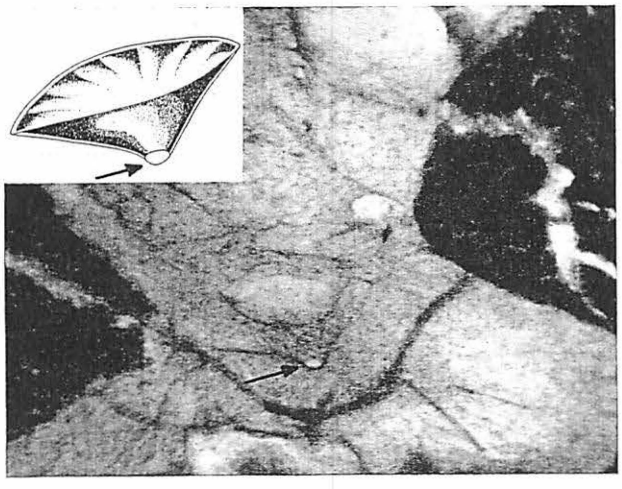

Fig. 5
Axial view of a funnel-like valve situated in the interlobular connective tissue and whose small or distal opening (arrow) is especially visible. 80×; Photomicrograph of a thick section of a human infant lung.

Lymphatic "valves" thus appear to be a simple cone-or funnel-like formation which is axially or longitudinally suspended in the lymphatic vessel lumen and whose distal or small opening (fig. 5) is localized at the deepest point of the funnel. At its large or proximal opening (and only there) the cone- or funnel-like sac is concentrically attached or fused to the entire circumpherence of the lymphatic vessel wall itself. Measurements of the cross-sectional diameter of the proximal or large opening of the valvular funnel reveal the same variability in dimensions as we reported in our earlier studies of the lymphatics of newborn lungs (i. e. from 30 up to more than 250 micron, 9), while the distal or small opening may be estimated to measure around 15 micron. Thus, valves may be considered to be "monocuspid" or, even better, cone- or funnel-like protrusions or folds of the intimal layer of the lymphatic vessel wall itself. They seem to be lined by a double layer of endothelial cells supported by a central framework of connective tissue. The existence of a lateral triangular septum by which the lymphatic valve seemed to be fused axially over practically its entire length to the lymphatic vessel wall itself, as was suggested by our earlier graphic reconstructions (3, 11, 14), is not confirmed by these stereomicroscopic investigations, which permitted a more real and direct observation. Some variants on this general scheme and valvularlike diaphragms seem to occur sometimes as well.

Stereomicroscopically, we were also able to confirm the existence of so-called complex microcirculatory lymphatic networks or circuits (fig. 6), whose existence was already revealed radiologically and by graphic reconstructions (14). They are composed of several lymphatics and valvular funnels which point in various directions in a given and rather limited area of the lung parenchyma, constituting the organ's very complex microcirculation.

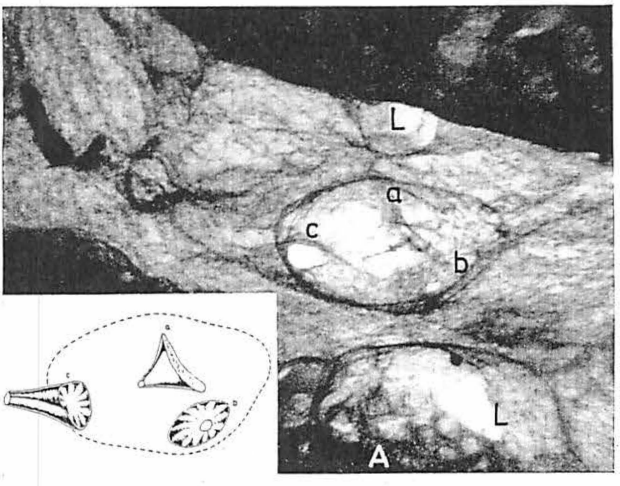

Fig. 6
Complex microcirculatory "lymphatic network or circuit" composed of several lymphatics and valvular funnels occurring in the interlobular connective tissue. As is observable stereomicroscopically, the valvular funnels "a" and "b" are both oriented axially; however funnel "b" is oriented upside-down, while funnel "a" is down-upside. The valvular funnel "c" ist not seen axially but laterally, its distal opening being oriented to the left of the picture. Juxta-alveolar lymphatic capillaries with large circular to oval lumen (L); Alveolar parenchyma (A). 81×; Photomicrograph of a thick section of a human infant lung.

Discussion

These stereomicroscopic observations on thick tissue sections made possible a direct investigation of entire lymphatic valves and confirmed the basically monocuspid funnel- or cone-like architecture of the pulmonary lymphatic valves. They were also more realistic than our earlier anatomical graphic reconstruction studies (3, 11, 14), which were subject (see introduction) to several possible artefacts, especially lateral deformation, and lengthening and stretching of the graphic models. This may explain the oblique orientation in relation to the axis of the vessel and the lateral fusion to the lymphatic vessel wall over the entire length of the graphically reconstructed valves. Indeed, stereomicroscopy does reveal valves to be axially suspended within the lymph vessel lumen and to be fused only at their large or proximal opening to the vessel wall circumpherence, providing at the same time a simpler, less sophisticated valvular architectural model than the anatomical graphic reconstructions had suggested.

The cone- or funnel-like lymphatic valvular architecture revealed by stereomicroscopy, also harmonizes entirely with the radiographic and microradiographic appearances of lymphatic valves, as they were seen after injection of the pleural lymphatics with a barium sulphate (Micropaque-Damancy) suspension (14) (fig. 7).

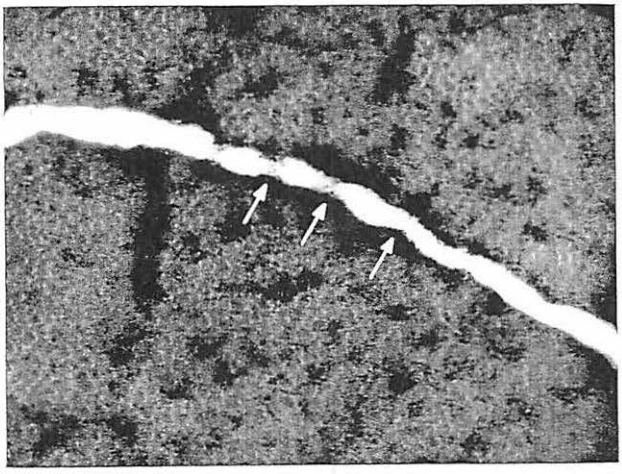

Fig. 7
Injection specimen of a pleural lymphatic with a barium sulphate suspension in a human adult lung. Localized and partial constrictions (arrows) do occur along the injected lymphatic; their appearance corresponds to the funnel- or cone-like valvular concept. Photograph of the pleural surface of the lower lung lobe.

On the usual 6 micron-tissue sections prepared for routine light microscopy, only a small part of the funnel-like lymphatic valve is present and this does moreover occur after the tissues have been cut under a variable angle, as illustrated by some examples in fig. 8. As a result, structures are obtained which falsely mimic a "classic" bicuspid valvular architecture, suggest two independent lymphatic vessels, or present bizarre formations which are usually interpreted by the microscopist as cutting artefacts of the thin and delicate lymphatic "valves". This demonstrates the futility of attempting to deduce the direction of lymphatic flow from the orientation suggested by the "free border" of a lymphatic valve (fig. 8 b, A.B.C.) on only one (or even several) histological sections which lack tri-dimensional information. For such purposes tri-dimensional graphic reconstructions, stereomicroscopical investigation or *in vivo* microcirculatory studies are needed.

Moreover, we have observed lymphatic valves to exhibit analogous microscopic appearances as illustrated in fig. 8, in examining routinely processed 5–6 micron surgical tissue sections of many other body organs, such as in lymph nodes and their surrounding fibro-adipose capsular and hilar tissues, serosae, the appendix, mammary gland, kidney etc. Hence it may be extrapolated from these correlated stereomicroscopical and histological investigations of the pulmonary lymphatic valves, that the cone- or funnel-like lymphatic valvular architecture corresponds to a wide spread basic structural pattern occurring troughout the human body.

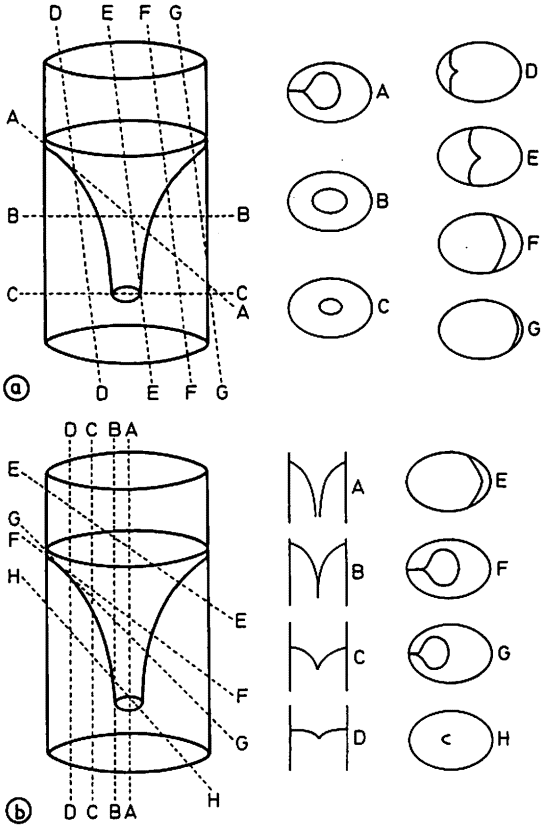

Fig. 8 a, b
Schematical drawings of a funnel-like lymphatic valve suspended into a lymphatic vessel; when cut at 6 μ under a variable incidence (as illustrated to the right), tissue sections are obtained which falsely mimick a "classic" bicuspid valvular architecture (a: D, E; b: A, B, C, D), suggest two independent lymphatic vessels (a: F, G; b: E) or appear as bizarre formations which are usually interpreted as cutting artefacts of the thin lymphatic "valves" (a: A; b: H).

Finally, it is reasonable that the cone- or funnel-like architecture of the lymphatic valves plays a basic role in channeling lymph and is well adapted to the establishment of a one-way flow and drainage of lymph, since the valve is probably occluded by a flow in a direction opposite to the normal.

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Summary

Stereomicroscopy of paraffin-embedded, 150–250 micron thick, glass-mounted tissue sections through the entire lobes of human infant lungs have revealed the pulmonary lymphatic valves not to be bicuspid, but to consist of one simple funnel-like or cone-like formation, which is fused to the lymphatic vessel wall circumpherence at its proximal or large opening.

Moreover, this basic monocuspid architectural valvular pattern seems to occur in other body organs and to play a basic role in the rheology of the lymphatic microcirculation, providing a well adapted one-way valvular system which results in a channeled flow and drainage of lymph.

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