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DIFFERENCES IN LYMPHATIC AND BLOOD CAPILLARY PERMEABILITY: ULTRASTRUCTURAL-FUNCTIONAL CORRELATIONS

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ABSTRACT

The major structural features of lymphatic capillaries, as they contrast with blood capillaries and as they pertain to endothelial permeability, are reviewed briefly with special emphasis on intrarenal vessels. The most characteristic structural feature of lymphatic endothelium is the discontinuity of the basal lamina. Basal laminae of blood vessels, such as renal glomerular capillaries, are prominent and are known to play a role in preventing extravasation of plasma proteins. By analogy, the lack of a basal lamina around lymphatic capillaries can be considered to be of major functional importance in facilitating access of interstitial macromolecules to the abluminal surface of endothelial cells and thus to the transport pathways that provide entry to the lymph. Tracer studies with horseradish peroxidase, for example, reveal that the protein enters the intraendothelial cytoplasmic vesicular system suggesting that this system may provide a transport pathway. Tracer is also seen between adjacent endothelial cells but in the kidney, liver and thyroid these intercellular channels comprise relatively narrow spaces of about 20 nanometers or less and do not form prominent gaps such as are seen in lymphatics of the diaphragm and skin. Evidence that macromolecular transport across endothelial cells may be asymmetric, favoring movement from interstitium to lymph, is derived from 1) studies using isolated perfused lymphatics, 2) differential luminal and abluminal membrane staining with cationic stains, 3) the presence of charged microdomains on lymphatic endothelial cell surfaces revealed with macromolecules of different charges, and 4) studies on cultured monolayers of porcine arterial endothelial cells. It is concluded that significant differences exist between blood and lymphatic capillary endothelial cells, especially as they relate to permeability, and that future research using molecular probes will highlight these differences.

The major ultrastructural differences between blood and lymphatic vessels have been well documented (1,2). Indeed, they now provide the means by which these vessels are distinguished in routine electron microscopic studies. However, the extent to which these structural contrasts reflect differences in function, especially in permeability, is not known. In the past it has been easy to adopt the simplistic view that endothelial cells, whether they be lymphatic or blood vascular, have the same basic structure (exhibiting only minor ultrastructural differences), perform the same basic function in that they line vessels--and

thus possess no fundamental differences. A level of knowledge has now been reached when this view can no longer be sustained, and it must be recognized that cells that look alike even ultrastructurally may have widely divergent functions.

The purpose of this brief review is to correlate some of the structural differences between blood vascular and lymphatic endothelium with known dissimilarities in permeability. The emphasis will be on renal lymphatic endothelium and its permeability since much information has accumulated on this topic (3). It is, however, important to keep in mind that this review deals primarily with the kidney because it has become increasingly clear that major differences in lymphatic endothelial permeability and mode of action exist among the different organs and regions of the body. For instance, the lymphatics of the diaphragm (4), with their intercellular gaps and rhythmic compressions induced by respiratory movements, appear to function in quite a different manner from lymph vessels of the liver (5), kidney (3) and thyroid (6).

General structure

At the light microscopic level there is little if anything to distinguish, with any degree of certainty, most peripheral lymphatic vessels from blood capillaries or venules. Many of the criteria that have been advocated as distinguishing features in routine histological sections have their fallacies, and all must be treated cautiously. In an organ such as the kidney there is no sure way to tell them apart other than to examine them at the ultrastructural level. For example, the thinness of the wall does not distinguish a lymphatic from a vein because in the kidney the walls of veins merely comprise an endothelial lining and a delicate connective tissue coat (7). Nor does the apparent presence of a valve define a lymphatic vessel since a confluence of tributaries can mimic this appearance. Similarly a lymphatic can rarely be identified with any level of certainty in tissue sections by the appearance of its luminal contents for the latter are altered variously as the tissue is processed.

Basal lamina

Ultrastructurally the most obvious difference, and the one most frequently used to identify lymphatics, is the more or less complete absence of a basal lamina coupled with the lack of fenestrations in lymphatic endothelium. The blood vessel, on the other hand, is often highly fenestrated and surrounded by a complete and prominent basal lamina. With such fenestrations present, one has no difficulty in seeing how macromolecules can escape from blood to interstitium: indeed, the question is not how proteins escape from peritubular capillaries, but rather how their escape is limited. Presumably--as with the glomerulus--the basal lamina plays a major role in curtailing extravasation of protein, not only by providing a physical restraint to its passage but also by the mutually opposing effects of the negative electric charges carried by the basal lamina and the plasma proteins.

If this is a function of the basal lamina around blood vessels then the absence of a comparable structure around lymphatic vessels seems logical. Indeed, were it to be present it could be little short of disastrous for it might shield, both structurally and electrically, the extravasated plasma proteins (and other macromolecules) from their endothelial transport pathways and thus restrain the passage of these substances from interstitium to lymph. In the absence of a lymphatic basal lamina the interstitial protein can reach not only the intercellular regions but also the plasma membrane of the lymphatic endothelium. However, despite this freedom for protein to approach the lining cells, the lack of fenestrations or gaps would still seem to pose an appreciable barrier to the passage of macromolecules between interstitium and lymph.

How then can macromolecules gain entry to the lymph? While this question

is by no means new and has received much study, it has not yet been answered satisfactorily.

Intercellular pathways

The most commonly held theory is that hydrostatic pressure differences between lymph and interstitium, brought about through rhythmic active contraction or passive compression of lymphatic vessels, causes the intermittent movement of fluid through sizeable gaps between adjacent endothelial cells. While gaps such as this are not unusual in lymphatics of the skin and are the pattern in lymphatics of the diaphragm, they have almost never been seen in renal lymphatics despite repeated observations on kidneys from different species and under differing conditions of renal function (1,8,9).

Lymph vessels in the kidney, as well as in the liver (5) and thyroid (6), reveal several endothelial contacts in any one cross section but none form gaps wider than the usual intercellular space of roughly 20 nanometers. Even under conditions of increased lymph formation (e.g., mannitol diuresis (8), ureteric obstruction) intercellular gaps are rarely seen. Only when trauma to the tissues has occurred do gaps between adjacent endothelial cells appear. Thus, the lymphatics in these organs seem to be different from those in the diaphragm (4) and skin, which do reveal gaps between adjacent endothelial cells, at least as far as their permeability and the mechanism of lymph formation is concerned.

Intracytoplasmic vesicular system

Horseradish perioxidase (HRP), which can been seen under the light and electron microscope, has frequently been used as a tracer for macromolecules, especially for plasma albumin which it resembles in molecular size. When injected intravenously, HRP appears rapidly in the lymph (10) and in such studies the most obvious ultrastructural finding in the lymphatic endothelial cells is the presence of tracer within elements of the vesicular system. This occurs within a matter of seconds and spans the cell from abluminal to luminal surface. Although this finding suggests that the vesicular system is involved in transport, it does not provide proof, since protein could enter the system without there being net transport into the vessel lumen.

Further suggestive evidence that the vesicular system serves as a transport route for macromolecules has been derived in isolated perfused renal hilar lymphatics (11). In this type of preparation, when HRP is added to the bathing fluid, it enters the vesicular system and appears within the lumen of the vessel within seconds. It is, of course, possible that this process occurs along a concentration gradient only, but transport in the opposite direction, that is from luminal to bathing fluid, in these isolated vessels does not seem to occur. Rather, when HRP is added to the perfusing luminal fluid, the tracer remains within the lumen and lines the luminal plasma membrane and its invaginations but does not cross the cell.

Recently the integrity of intracytoplasmic endothelial vesicles has been questioned. Ultrastructural studies using either serial thin sections (12) or specific outer leaflet membrane staining (13) have shown that a majority of what appear to be isolated free cytoplasmic vesicles in fact connect with one or other surface of the cell as well as with the neighboring vesicles. Such evidence undermines the theory that vesicles can transport macromolecules by shuttling across the cell between interstitial and luminal surfaces. However it does not negate the view that the configuration of the vesicular system and its lining membrane is constantly changing. For example, invaginations could begin as caveolae and progress to form a complex interconnecting system that loses its attachment with the forming surface and establishes a new connection with the opposing surface of the cell. Under such circumstances macromolecules could be transported either by their attachment to the invaginating membrane

or simply within the lumen of the branching complex.

Asymmetrical transfer

Recent evidence that macromolecular transport across endothelium may be direction-oriented and may thus not be the strictly passive process that it is commonly assumed to be, supports these tracer protein studies. Three lines of evidence will be cited here.

One has been derived in our laboratory from the isolated perfused lymphatic preparation (11). It demonstrates that the rate of albumin transport from bathing fluid to luminal fluid is influenced by temperature to a greater extent than can be accounted for by viscosity change alone and without any change in the size or appearance of the intercellular channels. Thus when the lymphatic preparation is cooled to 4°, protein transport is reduced by about 40%. When the temperature is cooled to 24°C or 30°C, albumin transport is reduced by about 30% and when the temperature is raised to 40°C the rate is increased by about 50%. No change in the rate occurs during the course of the experiment when the temperature is maintained throughout at 37°C.

The second line of evidence points to a charge differential between the luminal and abluminal surfaces of the endothelial cells. This has been shown by the use of tracer macromolecules of known charge (14,15) as well as by the use of stains with different charges (15). For example, a marked difference in staining characteristics between the two surfaces of a renal hilar lymphatic endothelial cell has been demonstrated using cationic stains such as ruthenium red or alcian blue (15). These stains have a greater attraction for the luminal surface indicating that it carries a more anionic charge than does the abluminal surface. Such an interpretation is appealing for it could mean that the interstitial surface, bearing cationic sites, affords an attraction for anionic plasma proteins. Were this membrane to be incorporated into the vesicular system and thus move across the cell, its charge characteristics would alter (becoming more anionic) as confluence with the luminal surface becomes established, and the attached proteins would be released into the vessel lumen.

The third and most recent line of evidence is derived from *in vitro* studies on blood vessel endothelium (16). Porcine arterial endothelial cells were grown as a monolayer on a permeable support and used to examine the transendothelial transfer of albumin. This transfer was found to be asymmetric, even against a concentration gradient, in that the movement of albumin from interstitial to luminal surface was greater than in the opposite direction. This asymmetric transfer was abolished when NaCn was added to the culture. It is interesting that in this preparation of arterial cells, net transport was in the same direction as with lymphatic cells--that is from interstitium to lumen.

It should not be inferred from these three lines of evidence that protein transport by lymphatic endothelial cells is limited to the vesicular pathway. Tracer studies reveal that HRP, when added to the bathing fluid of an isolated perfused lymphatic preparation, enters the intercellular space, even though that space is no wider than the normal gap between adjacent cells. Unlike blood vessel endothelium, many of these spaces between lymphatic cells do not possess a junctional complex and thus present no barrier to transport. This transport could simply occur along a concentration gradient, but might include solvent drag, and could be enhanced by a differential charge across the cell.

CONCLUSION

Current evidence indicates that there are numerous important structural differences between blood and lymphatic endothelial cells reflective of their permeability.

The most obvious is the basal lamina which restricts the escape of protein from the blood vessels and by its

absence allows protein and fluid access to the lymphatic endothelium.

Another is the presence of fenestrations in blood capillaries designed for major fluid transport. The low rate of fluid transfer across lymphatic endothelium does not call for fenestrations.

Transport across the intercellular pathways is limited in blood capillaries since every intercellular space reveals a junctional complex. Conversely, lymphatic endothelium presents a structurally leaky interface between interstitium and vessel lumen.

Fourthly, there are molecular differences pertaining to the cell membrane that influence permeability through electric charge, receptors or other as yet unknown effects. Current research in several laboratories is focusing on histochemical and immunochemical staining differences between blood and lymph capillaries and it seems clear that molecular probes will increasingly be used to elucidate the function of endothelial cells. As these techniques become more refined, it seems inevitable that more differences will become evident, not only between blood vascular and lymphatic endothelium, but also among lymphatic endothelia from different sites.

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