Emphatically Lymphatic

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The centipede of science advances on many feet of tools, guided by serendipity and creativity, along a pathway made coherent only retrospectively by hypothesis. The tools may be big and singular, as Galileo’s telescope, or small and myriad, as the new molecules discovered by genetic and cellular analysis.

The article by Kerjaschki et al. (1) from Vienna, published in this issue of JASN, reveals otherwise invisible insights by using new definitive molecular markers for lymphatic endothelial cells (LEC). Previously, lymphatics had been distinguished from blood capillaries in tissue sections only by electron microscopy, which revealed their more prominent anchoring fibrils and vesicular transport system and their relative lack of basement membrane, fenestrations, and cell junctions.

Several new and reliable markers of LEC are now available, including antibodies to LYVE-1 (hyaluronate receptor) (2), Prox-1 (lymphatic vessel transcription factor) (3), VEGFR-3, ( receptor for VEGF-C, VEGF-D, lymphatic angiogenic factors) (4), and podoplanin. Podoplanin is a 38- to 43-kD mucoprotein normally expressed in podocytes (rat more than human) and on the luminal surface of lymphatic endothelium. Podoplanin got its serendipitous name from its association with transformation of arborized foot processes to flat feet (Latin, pes planus) (5). Podoplanin has been postulated to protect cell surfaces and/or to recruit and retain lymphocytes. These novel tools for demonstrating lymphatics in tissues have ushered in an era of rapid growth in knowledge of the biology and pathology of lymphatics (6–8).

The traditional functions of lymphatics are ascribed to circulatory physiology and immunology. Lymphatics return extravasated interstitial fluid to the blood and bring antigen and immunologically active cells to lymph nodes draining sites of inflammation. Lymphatics may be more than inert plumbing through their production of cytokines, such as SLC/CCL21, a chemokine for lymphocytes, and macrophage inflammatory protein-1α (9).

Renal lymphatics have received little attention until recently: Heptinstall’s Pathology of the Kidney devotes only four sentences and one figure to renal lymphatics (10). As described by Kerjaschki et al. (1) and others (11), renal lymphatics follow arterial branches to the level of the interlobular arteries, but not further—not in peritubular spaces or around glomeruli. The medulla has few, if any, lymphatics (11). The hilar lymphatic flow from normal kidneys has been measured in sheep and dogs in the range of 0.5–3.0 ml/h. When lymphatics are acutely obstructed in the rat, the kidney swells, urine flow increases, and there is increased loss of Na, K, ammonia, and urea (12,13).

Acute obstruction causes dilation and reflux into intrarenal lymphatics, providing an alternate pathway of drainage of an acutely obstructed kidney (14,15). In chronic obstruction, Tamm-Horsfall protein leaks from tubules into lymphatics and can be found in draining lymph nodes (16). Hobson noted in porcine reflux nephropathy that fibrosis follows the distribution of lymphatics. He postulated that an irritant got into the lymph and then extravasated into the interstitium and stimulated fibrosis (17). Massive proliferation of podoplanin+ lymphatic vessels was observed in fibrotic tubulointerstitial regions in rat remnant kidneys; these areas have greatly increased lymphatic density and reduced blood capillary density (18). The lymphatic vessels and interstitium contained mononuclear cells that expressed VEGF-C mRNA. Increased lymphatic vessel density was also noted by others in the fibrotic areas in cirrhosis (19). In malignant tumors, such as melanoma, increased local lymphangiogenesis predicts metastasis to lymph nodes, arguing that lymphatics provide an easy means of cell egress (8).

Lymphatics have been of special interest in renal transplantation from the beginning. The physiologic question was whether disruption of the lymphatic drainage caused by the transplant procedure would affect renal function. Studies by Murray et al. (20) using autografts in dogs proved that it did not. Subsequent studies provided visible evidence that the lymphatics reconnect (21). The complication of lymphocele, in which lymph drains into the tissues or peritoneum, is thought to be related to surgical trauma to the recipient lymphatics (22).

The immunological question was the role of lymphatics in allograft sensitization. Barker and Billingham (23) showed that depriving skin grafts of lymphatic drainage inhibited graft rejection. Lack of lymphatics was regarded as the basis for immunological privilege in the eye and brain. Studies in sheep by Niels Pedersen and Bede Morris (24) showed that the lymph draining a renal allograft increased remarkably in volume and in proliferating lymphoblasts (60 g of cells and 10 L of lymph over 10 d). However, removing this lymph did not alter the course of acute graft rejection. They concluded “all events which lead to the recognition of renal homografts can occur centrally within the graft itself.” This predicted the later un-
successful attempts to prevent rejection by thoracic duct drainage.

Kerjaschki et al. (1) raise a new question—what are the functional consequences of lymphangiogenesis? They report that 10% of the biopsies of renal allografts have a marked increase in cortical lymphatic density; these are located around the periphery of nodular infiltrates of T, B, and dendritic cells and macrophages. LEC expressed podoplanin, LYVE-1, and Prox-1. Podoplanin was on the abluminal surface, in contrast to the luminal location in normal LEC. Both LEC and lymphocytes were proliferating, as judged by expression of Ki67. Macrophages expressed VEGF-C, and the lymphocytes expressed the CCR7 chemokine receptor. SLC/CCL21 was detected in the subendothelial LEC space and was shown to bind to podoplanin by plasmon-resonance analysis. They reasonably speculated that lymphatic neoangiogenesis contributes to efflux of infiltrate and to the maintenance of alloimmune response to the graft, and therefore may be a useful therapeutic target.

A number of important questions remain to be answered. First, what is the clinical significance of the increased lymphatic density and the nodular infiltrates? The initial study did not address this question, although graft loss was high among the group with increased lymphatics. Second, what is the timing of the lymphatic proliferation and the pathogenesis of the lesions—are they the necessary consequence of macrophage infiltration? Protocol biopsy studies should provide answers to both of these questions. A third question is the functional role of the lymphatics, especially whether they have a causal relationship to the fibrosis and the recruitment of lymphocytes. Finally, the origin of the lymphatics is of some interest, since a recipient LEC precursor exists in the blood (25); conversely, if LEC are of donor origin, lymphatics could be a target of the immune response.

In any case, this elegant, creative paper is likely to stimulate exploration of the role of intrarenal lymphatics in other diseases, particularly those with an immunological basis.

References


