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Editorial Review

The role of lymphatics in renal inflammation

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Abstract
Progressive renal diseases are characterized by tubulointerstitial inflammatory cell recruitment, tubular atrophy and fibrosis. Various aspects of the recruitment of leukocytes have been extensively studied, but the exit routes (i.e. the lymphatic vessels and their biology) have only recently found attention. Similar to the recruitment of inflammatory cells, the exit is coordinated by an orchestrated interaction of chemotactic cytokines and adhesion molecules. During inflammatory injury, new routes are created by the de novo formation of lymphatic vessels, i.e. neolymphangiogenesis. These newly formed lymphatic vessels help to cope with the increase in interstitial fluid related to inflammation. Here, we review some aspects of lymphatic biology and the current knowledge about lymphatic vessels in renal inflammation.

Keywords: lymphatic endothelial cells; lymphatic vessels; renal allograft; renal inflammation; tubulointerstitial inflammation

Introduction

The lymphatic vessel network drains interstitial fluid and returns it to the blood (Figure 1). During the last decades, our knowledge of this system has evolved substantially. The focus has long been on immune surveillance and response to infectious agents, but new functions in lipid transport and fat metabolism as well as in the regulation of salt storage and hypertension have emerged (reviewed in [3, 4]). The interest in lymphatic vessels has also reached the kidney. The first part of this review gives a general introduction into the biology of lymphatic vessels, whereas the second part will focus on the role of lymphatic vessels in the kidney.

Anatomy and physiology of the lymphatic system

The majority of vascularized tissues (except for the central nervous system and bone marrow) contain a lymphatic capillary network, which returns an estimated 1–2 L of lymph to the venous circulation every day. The lymphatic vasculature is a unidirectional open system and displays many features which discriminates it from the blood vascular system. It is composed of blunt-ended capillaries (30–80 µm in diameter) devoid of a basement membrane or pericytes (Figure 1, [5]). They consist of single-layered 'oak leaf'-shaped partly overlapping cells, which are interconnected via discontinuously arranged 'button-like' junctions and mostly lack the continuous 'zipper-like' interendothelial tight junctions present in the endothelial layer of blood vessel endothelial cells (BECs). The resulting interjunctional gaps are thought to represent sites of entry for interstitial fluid, macromolecules and immune cells [6, 7]. Lymphatic endothelial cells (LECs) are highly endocytic and permeable to proteins thereby allowing transcellular uptake of fluid and macromolecules. Filaments connect the lymphatic capillaries to the perivascular matrix leading to an increase of the vessel lumen and widening of the intercellular gaps in tissue swelling, facilitating fluid entry in this context [8].

Lymphatic capillaries drain into precollecting vessels, followed by larger collecting lymphatics [9]. Precollectors exhibit some perivascular smooth muscle cells (SMCs), whereas bigger collecting vessels contain continuous endothelial junctions, a basement membrane, an SMC layer and bileaflet valves. These allow for the centripetal flow of lymph augmented by rhythmic contractions [10] (Figure 1).

Characterization of LECs and formation of lymphatic vessels

LECs express a distinct set of genes which discriminates them from BECs [11, 12]. Unfortunately, these markers are not specific and also expressed in various other cell types (see Table 1). The most widely employed LEC markers are podoplanin [13, 14], the lymphatic vessel hyaluronan receptor (LYVE-1, [15]), vascular growth factor receptor 3 (VEGFR-3), which is the receptor for

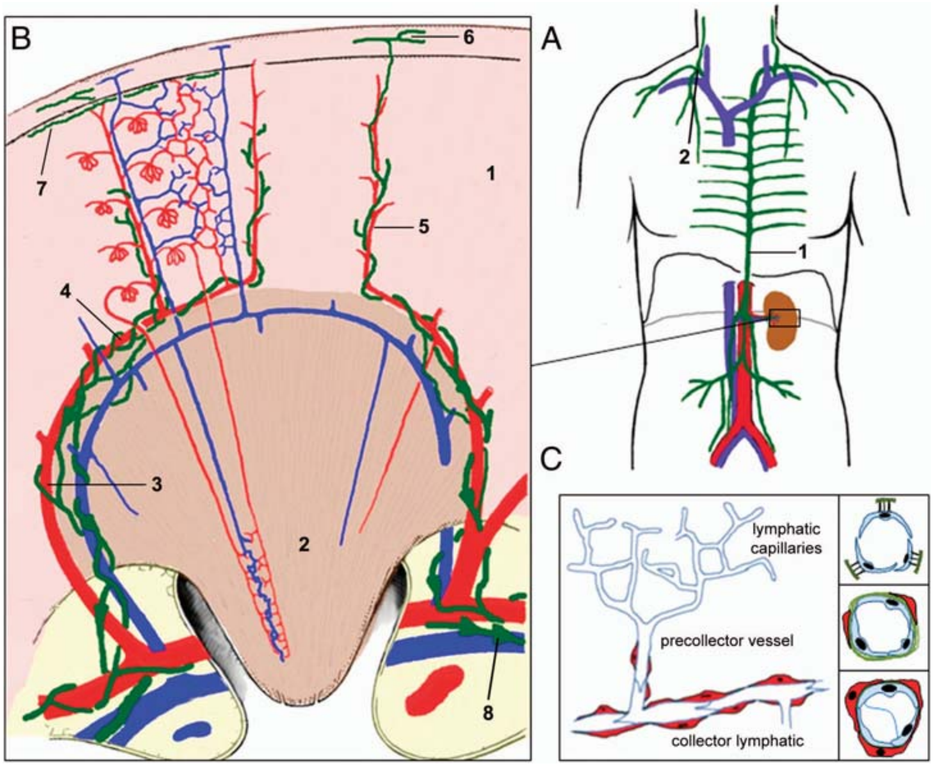

Fig. 1. Structure of the lymphatic vascular system. (**A**) Organization of major lymph trunks. Lymph drained from lower extremities, abdomen and left side of the body collects through the thoracic duct, which empties into the left subclavian vein (1 in A). Lymph from the right arm, hemithorax and right side of the head drains into the right lymphatic duct (2 in A), which anastomoses with the right subclavian vein. (**B**) Insert from (A). Organization of the renal lymphatics. Blind-ended lymphatic capillaries run in close proximity to the interlobular arteries. They empty into lymphatic precollectors, which follow the arcuate vessels along the bases of the pyramids, the interlobar blood vessels of the renal columns and finally drain into hilar collector lymphatics. Green: lymphatics, red: arteries, blue veins, 1: renal cortex, 2: renal medulla, 3: interlobar artery, 4: arcuate artery, 5: interlobular artery, 6: lymph vessel of capsule, 7: subcapsular lymph vessel, 8: valve (modified according to [1, 2]). (**C**) Structural organization of lymph vessels. Lymphatic capillaries are 30–80 µm in diameter, blind-ended and form a capillary plexus in many tissues (left). LECs of initial capillaries are loosely interconnected via button-like junctions and are not surrounded by a basement membrane or pericytes. They are anchored in the surrounding connective tissue by radial filaments. Precollectors have a diameter of ~100 µm; endothelial cells are more tightly connected and surrounded by a loose connective tissue and sparse SMCs. They may also contain valves. Collectors are surrounded by a basement membrane and SMCs and contain bileaflet valves. Endothelial cells are more closely interconnected since collector lymphatics do not absorb fluid from surrounding tissue.

VEGF-C and VEGF-D, and the prospero-related homeobox transcription factor 1 (Prox1, [16]).

LECs do not only differ from BECs in their gene expression pattern but are also heterogeneous among themselves depending on the segment of the lymphatic tree and the tissues they reside in [11, 17]. In human skin, for example, two functionally different subpopulations of LECs were demonstrated, characterized by their expression levels of podoplanin and transcripts such as CCL21, CCL27 and the atypical chemokine receptor DARC [11].

The migration of LECs and formation of the lymphatic vasculature (termed lymphangiogenesis) are orchestrated by a large set of genes. VEGFR-3 and its main ligand VEGF-C are essential in this process and embryonic deletion of VEGF-C results in a lack of the lymphatic vasculature in mouse embryos [18]. VEGFR-3, while also expressed in BECs in early development, becomes restricted to LECs in later life [19]. Podoplanin is essential for the separation of the developing lymphatic vasculature from blood vessels via interaction with platelets. Gene targeting of podoplanin in mice resulted in dilation and malfunction of lymphatic vessels [20].

Neolymphangiogenesis in inflammation

Neolymphangiogenesis in the adult occurs in various conditions associated with inflammation, such as acute and chronic infections, disorders of immune regulation (e.g. rheumatoid arthritis and psoriasis), wound healing, tumour growth/metastasis and transplant rejection [4, 9]. The functional role of neolymphangiogenesis is not quite understood, but it seems to be primarily a protective response and aids in clearance of tissue oedema and inflammatory infiltrates [21–24]. Neolymphangiogenesis shares many pathways with developmental lymphangiogenesis including the pivotal role of the VEGFR-3/VEGF-C axis in stimulating lymphatic proliferation.

In tissue inflammation, pro-inflammatory cytokines, such as interleukin-1α, -1β and tumour necrosis factor (TNF), result in the release of VEGF-C and VEGF-D by

Table 1. Proteins expressed by lymphatic endothelial cells[a]

Marker	Subcellular localization	Protein function	Expression in other cell types	Species
Podoplanin	Membrane/cytosol	Cell adhesion, tube formation	Podocytes, Schwann cells, perineurial cells, mesothel cells, myoepithelial cells, osteocytes, FDCs, ovarian granulosa cells, stromal reticular cells, ependymal cells	H, M, R
Prox1	Nucleus	Transcription factor, control gene for lymphatic development/ differentiation	Hepatocytes/HCC, lens epithelia, cortex, dentate gyrus and hippocampus; can be down-regulated in models of inflammation in LECs	H, M, R
LYVE-1	Membrane/cytosol	Less strongly expressed in collector lymphatic vessels, down-regulated by TNFα	Subset of macrophages, certain pulmonary blood vessels, liver sinusoids, HEVs	H, M, R
VEGFR-3	Membrane/cytosol	Receptor for VEGF-C and -D; stimulates proliferation of LECs	Tumour blood vessels, fenestrated capillaries, stem cell subset, tumour cells	H, M, R
FOXC2	Nucleus	Transcription factor	Podocytes	H, M, R
CCL21/SLC	Cytosol, secreted	Chemokine, ligand of CCR7	HEVs, endothelial cells in inflammation, podocytes, ischaemic neurons	H. M, R
D6	Membrane	Decoy chemokine receptor	B-cell subset, other leukocytes, haemopoetic cells	H, M, R

[a]CCL, chemokine (C–C motif) ligand; CCR, chemokine receptor; HEV, high endothelial venule; FDC, follicular DC; FOXC2, Forkhead box protein C2; HCC, hepatocellular carcinoma; LEC, lymphatic endothelial cells; LYVE-1, lymphatic vessel hyaluronan receptor 1; Prox1, prospero-related homeobox transcription factor 1; SLC, secondary lymphoid organ chemokine; H, human; M, mouse; R, rat.

macrophages, dendritic cells (DCs), granulocytes, mast cells and fibroblasts [25–29]. The induction of nuclear factor-kappaB by inflammatory stimuli activates Prox1, and both nuclear factor-kappaB and Prox1 activate the VEGFR-3 promoter. This can enhance the sensitivity of lymphatic endothelium to VEGF-C and VEGF-D [30]. The cytokine lymphotoxin α, mainly secreted by T cells, also contributes to neolymphangiogenesis in mice [31]. Data from renal transplant inflammation in humans and inflamed cornea in mice have shown that lymphangiogenesis does not solely occur by continuous sprouting from neighbouring lymphatics but also includes incorporation of lymphatic progenitor cells into the growing lymphatic vessels [32, 33]. In salt-induced lymphangiogenesis, stimulation of tonicity-responsive enhancer-binding protein (TonEBP) leads to VEGF-C secretion by macrophages in the skin, stimulating lymphatic vessel growth [34]. A secreted splice variant of VEGFR-2 acts as a VEGF scavenger and prevents lymphangiogenesis in tissues devoid of lymphatics, such as the cornea [35].

Role of the lymphatic vasculature in inflammation

Afferent lymphatic vessels transport antigens and immune cells, mostly conventional DCs and T cells, from tissue to draining lymph nodes [36]. They also serve as drains for non-cellular structures. Peripheral tissue antigens can enter the lymph node via afferent lymphatics directly [37, 38].

Proteins expressed by LECs can directly modulate the local inflammatory milieu. The atypical chemokine receptor D6 takes up chemokines which results in digestion and local chemokine clearance [39, 40]. The deletion of this receptor in mice leads to chronic inflammation of the skin [39]. DARC is another atypical chemokine receptor expressed by lymphatic precollector vessels [41]. In contrast to D6, DARC is involved in the transcellular transport and presentation of chemokines [42]. Therefore, lymphatic vessels are important players in the regulation of both local as well as systemic inflammatory reactions.

Mediators of cell recruitment and cell movement into lymphatic vessels

During DC mobilization and migration from peripheral tissue to lymph nodes, the interaction of the chemokine receptor CCR7 with its ligands CCL19/21 is thought to be a key mechanism. LECs in inflamed tissues, but also under non-inflammatory conditions, secrete the chemokine CCL21/SLC, thereby establishing a gradient which attracts CCR7-positive activated DCs to the afferent lymphatic vessels aiding in relocation to lymph nodes [43–45]. Podoplanin, which has been shown to bind CCL21 along the basolateral side of the LECs and the perivascular stroma, seems to be closely involved in creating the perivascular gradient. Interestingly, CCR7 expression on DCs is absolutely required for intravasation into initial lymphatics [46]. CCR7 is also crucial for the exit of CCR7-positive effector/memory T cells from peripheral tissues via afferent lymphatics, suggesting that CCL21 might be involved in CCR7-positive T-cell trafficking [47, 48].

A subtype of LECs (characterized by low expression of podoplanin and high expression of DARC) in precollector vessels of human skin secrete CCL27, thereby leading CCR10-positive T cells through precollector lymphatics [41].

In a mouse model of contact hypersensitivity, it was demonstrated that lymph node migration of CXCR4 expressing dermal DCs and Langerhans cells could be substantially reduced using a CXCR4 antagonist, suggesting a role of the CXCR4 ligand CXCL12, a molecule expressed by dermal lymphatics [49].

Sphingosine-1 phosphate (S1P) produced by LECs causes egress of T cells expressing the corresponding receptor via efferent lymphatics, whereas increased tissue S1P results in the arrest of T cells in inflamed tissues [50, 51].

The mechanisms of transmigration of inflammatory cells into lymphatic vessels are still incompletely understood. *In vitro* assays suggest a function for the adhesion molecules intracellular adhesion molecule 1 and vascular cell adhesion molecule 1, both of which are strongly up-regulated upon TNF stimulation in cultured murine and human dermal LECs. Blocking antibodies to both molecules inhibited adhesion and transmigration of lipopolysaccharide-activated macrophage-derived DCs [52]. Incubation of DCs with CCL21 led to dramatic acceleration of the otherwise slow process of lymphatic transmigration [44]. Remarkably, the adhesion molecule JAM-A appears to impede DC trafficking via afferent lymphatics [53].

Anatomy of lymphatics in the normal kidney

The anatomy of renal lymphatics has been studied in various mammals e.g. rabbit, rat, mouse, sheep, dog and cat [54–58]. No prominent differences between species were described [55]. Early studies used dye injection techniques, which can be difficult in their interpretation and resolution. Studies using the modern markers (e.g. podoplanin) confirmed many aspects of this early work [58].

The initial lymphatic capillaries run in close proximity to the interlobular arteries (Figure 1). They empty into lymphatic precollectors, which follow the arcuate vessels along the bases of the pyramids. Then, lymphatics follow the interlobar blood vessels of the renal columns and finally drain into hilar collector lymphatics [57].

In the renal cortex, lymphatics come close but do not enter the glomeruli [57, 58]. Towards the outer cortex, the number of lymphatics decreases. No lymphatic vessels are present in normal renal medulla [54, 58]. It was hypothesized that fluid from the medullary interstitium moves to lymphatics associated with arcuate or possibly interlobar blood vessels.

Human kidney

While reviewing the literature, we noted that the staining patterns of endothelial markers along the vascular tree of the kidney were not well described. The most commonly used markers for lymphatic vessels were podoplanin, LYVE-1 and Prox1 [59]. Podoplanin can be localized very reliably in human paraffin-embedded tissue after heat-based antigen retrieval using the monoclonal antibody D2-40 [59–62]. LYVE-1 has been described to be less reliable, which is consistent with our experience [63]. LYVE-1 also stains some endothelial cells of glomerular capillaries in the mouse [58]. The interpretation of the nuclear staining of Prox1 is sometimes difficult particularly when only single nuclei are present on a cross section of small lymphatic vessels.

CD31 and CD34 are commonly used markers for BECs [59]. Both are not specific for BECs, and the expression of these markers along the lymphatic network and the percentage of positive lymphatic vessels is poorly described. We think that further studies are necessary using the three markers for LECs in comparison with the markers for BECs to clearly describe the human renal vascular tree.

The anatomy of the lymphatic tree in the human kidney seems to be very similar to what was described in the above-mentioned animal studies [54, 64]. Immunohistochemistry for podoplanin in well-preserved human kidneys demonstrated lymphatic vessels in the midcortex and along interlobular and arcuate arteries [65, 66]. No podoplanin-positive vessels were present in the superficial cortex, in glomeruli and in the interstitial tissue between tubuli [65]. Towards the corticomedullary junction, podoplanin-positive vessels became more common. No lymphatics were present in the inner or outer medulla. The largest lymphatic vessels were described within the renal sinus [65]. These were no longer associated with the arteries [65]. Numerous lymphatics were also described to be present within the media of the muscular sinus veins [65].

The role of lymphatic vessels in animal models

The role of lymphatics in normal kidneys was studied through lymphatic ligation experiments in rats. This resulted in proteinuria and in a reduced creatinine clearance in the second week after ligation. These changes were associated with tubular damage, tubulointerstitial fibrosis and mesangial expansion [67].

All chronic renal diseases result in the histopathological pattern of tubulointerstitial inflammation, tubular atrophy and widening of the interstitium through the deposition of extracellular matrix (interstitial fibrosis) [68, 69]. The remnant kidney model in the rat reflects some of these aspects [70]. In contrast to the normal rat kidney, a prominent accumulation of lymphatic vessels (illustrated by immunohistochemistry for podoplanin and LYVE-1) was present in remnant kidneys in association with fibrotic regions and moderate infiltration of mononuclear inflammatory cells in the tubulointersitium of the cortex [70]. By immunohistochemistry and *in situ* hybridization, an increased VEGF-C expression (potentially mediating neo-lymphangiogenesis) was detected mainly in interstitial mononuclear cells, presumably macrophages [70].

Unilateral ureteral obstruction is a model of rapid interstitial fibrosis. In the normal non-obstructed kidneys, podoplanin- and LYVE-1-positive vessels were present adjacent to large- and intermediate-sized vessels, but not within the tubulointerstitium of the cortex [71]. In the obstructed kidney, the number of podoplanin-positive lymphatics increased in the cortex but also in medulla and in the renal pelvis [71]. Induction of VEGF-C parallelled TGF-β1 expression. *In vitro* TGF-β1 induced significant up-regulation of VEGF-C in proximal tubular epithelial cells (human) as well as in mouse collecting duct cells and macrophages [71]. Administration of a TGF-β Type-I receptor inhibitor (LY364947) to rats with unilatral ureter obstruction resulted in significant reduction of VEGF-C and LYVE-1 messenger RNA expression and reduced the number of lymphatic vessels. This study illustrates an important new link between lymphangiogenesis and interstitial fibrosis via TGF-β-mediated induction of VEGF-C.

Furthermore, these studies illustrate the importance of lymphatic vessels under normal and inflammatory conditions. The formation of new vessels might well be a response to the increased interstitial fluid and inflammatory cell accumulation in the disease process.

The role of lymphatic vessels in inflammatory diseases of the human kidney

Consistent with the data in the rat remnant kidney model, lymphatics were described to be scattered throughout the interstitium in the cortex of human end-stage kidneys ($n = 3$), with an increased number compared to normal renal cortex [66].

We localized podoplanin-positive lymphatic vessels in renal biopsies from patients with acute interstitial nephritis, chronic interstitial nephritis and patients with IgA nephropathy [60]. Sites of interstitial inflammation were associated with a high number of lymphatic vessels. The mean number of podoplanin-positive vessels was significantly higher in renal biopsies from patients with chronic interstitial nephritis or chronic IgA nephropathy as compared to biopsies with acute tubulointerstitial nephropathy [60]. This illustrates that the inflammatory process has to persist for some time before neolymphangiogenesis becomes apparent. Tertiary lymphatic organs, which are structured accumulations of B cells, T cells and DCs, were surrounded by lymphatic vessels [60]. The functional role of these structures is still obscure.

In a study on patients with renal involvement in multiple myeloma, Zimmer *et al.* [72] used a similar approach as ours, but combined it with morphometry. The investigators compared renal biopsies from patients with multiple myeloma ($n = 37$) to biopsies from patients with acute kidney injury ($n = 12$) and controls (biopsies from allograft donors taken before implantation; $n = 15$). Patients with multiple myeloma had a significantly higher lymph vessel length density than the two other groups. Lymph vessel length density was not associated with the degree of fibrosis but significantly associated with the degree of interstitial inflammation [72]. Active proliferation of lymphatic vessels in patients with multiple myeloma was demonstrated by staining for the proliferation marker Ki-67. Acute kidney injury biopsies demonstrated lymphatic vessels similar to normal controls.

Sakamoto *et al.* [59] localized podoplanin (by D2-40 staining) in 124 human kidney biopsy specimens. Podoplanin-positive lymphatic vessels in control kidney biopsies, taken 1 h after renal transplantation ($n = 9$), were limited to the periarterial space of the interlobular arteries and infrequently found in tubulointerstitial areas of the normal cortex [59]. Podoplanin-positive lymphatics were present in cortical areas of tubulointerstitial inflammation and fibrosis in biopsy specimens of IgA nephropathy [60], diabetic nephropathy, lupus nephritis, anti-neutrophil cytoplasmic antibody-related glomerulonephritis and tubulointerstitial nephritis [59]. Lymphatic vessels were described to be frequently filled with inflammatory cells, suggesting that they are functioning. In diabetic nephropathy, the number of lymphatics was significantly higher in areas of tubulointerstitial fibrosis as compared with non-diabetic renal disease with a similar severity of tubulointerstitial fibrosis [59]. The authors confirmed a low number of lymphatics in acute tubulointerstitial nephritis [59]. The number of lymphatics correlated significantly with the severity of tubulointerstitial fibrosis [59]. Mononuclear cells (monocytes/macrophages/DCs) were found to express VEGF-C in the inflamed tubulointerstitium [59]. The majority of these cells were CD68 positive. Additionally, a strong expression of VEGF-C was detected in tubular epithelial cells, predominantly proximal tubular epithelial cells, which may contribute to lymphangiogenesis in the outer cortex [59]. Therefore, in various endogenous kidney diseases, neolymphangiogenesis has now been well described. The cause of injury does not seem to matter as neolymphangiogenesis was found in very different renal diseases. It is associated with the presence of inflammatory cells which release factors known to promote lymphangiogenesis (e.g. VEGF-C). Besides CD68-positive macrophages/DCs, activated tubular epithelial cells seem to be able to trigger lymphangiogenesis.

Lymphatic vessels in human renal allografts

During the explantation of a kidney, the lymphatics are dissected; thus, early after transplantation, the renal allograft has no lymphatic drainage. The lymphatic regeneration is fast, starts within the first week and a competent lymphatic off-stream is present within 2–3 weeks [73–75]. The lymph flow from a normal sheep kidney was shown to be ∼1–3 mL/h [56]. After renal transplantation, the lymph production continuously increased in volume, which illustrates the importance of a functional lymphatic system to prevent interstitial oedema in the graft and consequent injury [76].

Kerjaschki *et al.* [77] were the first to describe 35 renal allograft biopsies, containing podoplanin-positive lymphatic vessels and nodular infiltrates, of a preliminary screen of 350 archival biopsies. Nodular mononuclear infiltrates in biopsies were associated with extensive (>50-fold) neolymphangiogenesis as compared with normal control kidney cortex ($n = 6$) or biopsies taken in the acute phase of rejection with diffuse mononuclear infiltrates (six biopsies with acute interstitial rejection and four with vascular rejection). The presence of lymphatics was confirmed by additional immunohistochemical staining for LYVE-1 and Prox1. The nuclear proliferation marker Ki-67 was expressed in numerous LECs in the peritubular space and by mononuclear cells within the nodular infiltrates [77]. VEGF-C was found to be expressed by macrophages within the nodular infiltrates [77]. The LECs also expressed the lymphoid chemokine SLC/CCL21 [77]. Within the nodular infiltrates, numerous CCR7-positive cells were detected.

In 162 sequential protocol biopsies taken 6, 12 and 26 weeks after transplantation, podoplanin-positive lymphatic vessels were described in areas of cellular infiltration in about two-third of the biopsies [78]. The lymph vessel density was significantly increased at sites of inflammation independent of the morphologic pattern of the inflammatory reaction (nodular versus non-nodular) compared with areas of well-preserved tissue [78]. Patients with an acute rejection episode over the course of time tended to have a lower lymph vessel density in the infiltrates of their first biopsy. The graft function at 1 year after transplantation was significantly better in patients who had a high lymph vessel density in the areas of cellular infiltration in their biopsies [78]. The authors hypothesized that lymphatic vessels serve as an exit route for inflammatory cells thereby reducing the inflammation in transplant kidneys [78]. Although the numbers of lymphatic vessels may vary, the increased numbers of lymphatic vessels are similar to the results found in endogenous renal diseases. Whether the newly formed lymphatic vessel in areas of fibrosis are of negative functional impact leading to fibrosis is unclear at the moment. Depending on the time point during the course of the allograft, the functional consequences of the lymphatics might be different. Early after implantation, the intense neoangiogenesis might be a response to injury (increased efflux of lymph). Later, in the course, the persistence of a high number of lymphatic vessels in the outer cortex might be a sign of ongoing inflammation in the graft or an increased allogenic response mediated through the increased number of lymphatics.

We recently studied the atypical chemokine receptor CXCR7 in renal allograft biopsies. CXCR7 is a receptor for two ligands (CXCL11 and CXCL12), both known to be involved in lymphatic trafficking in allografts [62]. CXCL11 and CXCL12 were significantly elevated in allografts with acute rejection [62]. CXCR7 was localized in a series of 64 indication and 24 protocol biopsies [62]. In control biopsies, CXCR7 was found on smooth muscle and on endothelial cells of a small number of peritubular vessels. A significant increase of CXCR7-positive vessels was demonstrated in acute rejection and a subset of these CXCR7-positive endothelial cells was identified as lymphatic vessels. Both CXCR7-positive blood and lymphatic vessels increased during allograft rejection. The impact of these CXCR7-positive lymphatic vessels on allograft survival is currently unknown and the focus of ongoing studies. CXCR7 is the third atypical chemokine receptor expressed by LECs, which might modulate the local inflammatory milieu within the lymphatic tree, but in doing so also the systemic immune response.

Lymphangiogenesis and macrophages

A common feature in both endogenous kidney diseases and renal allografts was the close association between inflammation and neoangiogenesis. The human renal tubulointerstitium contains a mixed population of CD68-positive cells, i.e. DCs and macrophages potentially involved in lymphangiogenesis [79].

In graft nephrectomies from 29 patients with chronic allograft injury, 17 of 29 graft samples showed interstitial lymphatic vessels, positive for podoplanin, LYVE-1 and VEGFR-3 [80]. In the interstitium of grafts, CD68 and VEGF-C double-positive cells were detected. In contrast, neither B cells nor T cells expressed VEGF-C [80]. In control tissue, there was no VEGF-C expression [80]. Therefore, CD68-positive DCs/macrophages might be an important trigger of neolymphangiogenesis.

To further define the source of LECs, six female donor kidneys were studied which had been transplanted into male recipients [32]. The tissue specimens showed a high rate of lymphatic endothelial proliferation as well as massive chronic inflammation. The nuclei of progenitor LECs were detected by co-localization of Prox1 by immunohistochemistry and the Y chromosome by *in situ* hybridization [32]. A total of 47 of 1005 nuclei (4.5%) of the Prox1-positive lymphatic endothelial nuclei demonstrated a single Y chromosome and were therefore derived from circulating progenitors of the recipient's genotype [32]. Potential candidates for lymphatic progenitors were described to be tissue macrophages [32]. In contrast, LECs in normal skin and gastrointestinal biopsies, taken from female bone marrow recipients who received a male donor graft, did not contain a Y chromosome [32]. Supportive evidence came from a study which demonstrated that podoplanin-positive interstitial lymphatic vessels contained CD68-positive cells in allograft nephrectomies [80].

Therefore, as already shown for other organs, CD68-positive cells can be involved in lymphangiogenesis in the kidney in two different ways. They can either promote lymphangiogenesis via the release of VEGFs or they can transdifferentiate and incorporate into a lymphatic endothelium [81]. It is currently unknown whether there is a difference in CD68-positive DCs or macrophages in the propagation of lymphangiogenesis.

Summary and outlook

Research on lymphatic biology is currently booming as summarized in the first part of the manuscript. In sharp contrast, our knowledge even of the physiological expression of the currently used markers of lymphatic vessels in the human kidney is still relatively limited. The current studies are consistent in the view that inflammation of the kidney (either in endogenous kidneys or renal allografts) results in the formation of new lymphatic vessels. Early studies demonstrated an enormous amount of lymph and cells to leave renal allografts via lymphatics. As the normal cortex is limited in the lymphatic drainage, new lymphatic vessels might be a necessary response to cope with this increased workload. In situations of interstitial oedema formation (e.g. allograft rejection, interstitial nephritis), these lymphatics would be important to decrease interstitial pressure. Therefore, in the early phase of inflammation, newly formed lymphatic vessels are most likely a positive response to injury. Neolymphangiogenesis seems to be promoted by lymphatic growth factors released by inflammatory cells, most likely CD68-positive cells (in the human kidney resembling macrophages and DCs). Additionally, bone marrow-derived cells are incorporated into lymphatic vessels of renal allografts in relatively low numbers.

The important question of whether neolymphangiogenesis is good or bad in the long run remains a matter of debate. It is possible that the higher number of lymphatic vessels might result in acquired immune responses promoted by the efflux of antigen and antigen-presenting cells. Blocking lymphangiogenesis has, for example, been shown to be beneficial in pancreatic islet transplantation [82]. It could further be hypothesized that the lymphatic drainage of sites, which normally have no direct access to the lymphatic tree, might change the normal interior milieu resulting in tissue injury. At the moment, there is little direct evidence for a negative role of lymphatics in chronic inflammation of the kidney. Neolymphangiogenesis can likely be seen as a double-edged sword promoting the increased efflux of lymph and inflammatory cells, thereby exerting a protective influence. On the other edge of the sword, the increased number of lymphatic vessels might promote acquired immune responses against the endogenous kidney or renal allografts, which might be detrimental in the long run.

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References

1. Földi M*Földi's Textbook of Lymphology for Physicians and Lymphedema Therapists*. 2nd edn. Munich, Germany: Elsevier, 2006.
2. Leonhardt H. *Taschenatlas der Anatomie. Innere Organe*. Stuttgart, Germany: Georg Thieme Verlag, 1991.
3. Wang Y, Oliver G. Current views on the function of the lymphatic vasculature in health and disease. *Genes Dev* 2010; 24: 2115–2126.
4. Alitalo K. The lymphatic vasculature in disease. *Nat Med* 2011; 17: 1371–1380.
5. Castenholz A. Functional microanatomy of initial lymphatics with special consideration of the extracellular matrix. *Lymphology* 1998; 31: 101–118.
6. Baluk P, Fuxe J, Hashizume H *et al*. Functionally specialized junctions between endothelial cells of lymphatic vessels. *J Exp Med* 2007; 204: 2349–2362.
7. Tammela T, Saaristo A, Holopainen T *et al*. Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. *Nat Med* 2007; 13: 1458–1466.
8. Solito R, Alessandrini C, Fruschelli M *et al*. An immunological correlation between the anchoring filaments of initial lymph vessels and the neighboring elastic fibers: a unified morphofunctional concept. *Lymphology* 1997; 30: 194–202.
9. Tammela T, Alitalo K. Lymphangiogenesis: molecular mechanisms and future promise. *Cell* 2010; 140: 460–476.
10. Florey H. Observations on the contractility of lacteals: Part I. *J Physiol* 1927; 62: 267–272.
11. Wick N, Saharinen P, Saharinen J *et al*. Transcriptomal comparison of human dermal lymphatic endothelial cells ex vivo and in vitro. *Physiol Genomics* 2007; 28: 179–192.
12. Podgrabinska S, Braun P, Velasco P *et al*. Molecular characterization of lymphatic endothelial cells. *Proc Natl Acad Sci U S A* 2002; 99: 16069–16074.
13. Breiteneder-Geleff S, Matsui K, Soleiman A *et al*. Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. *Am J Pathol* 1997; 151: 1141–1152.
14. Weninger W, Partanen TA, Breiteneder-Geleff S *et al*. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. *Lab Invest* 1999; 79: 243–251.
15. Banerji S, Ni J, Wang SX *et al*. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol* 1999; 144: 789–801.
16. Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. *Cell* 1999; 98: 769–778.
17. Kawai Y, Hosaka K, Kaidoh M *et al*. Heterogeneity in immunohistochemical, genomic, and biological properties of human lymphatic endothelial cells between initial and collecting lymph vessels. *Lymphat Res Biol* 2008; 6: 15–27.
18. Schulte-Merker S, Sabine A, Petrova TV. Lymphatic vascular morphogenesis in development, physiology, and disease. *J Cell Biol* 2011; 193: 607–618.
19. Tammela T, Enholm B, Alitalo K *et al*. The biology of vascular endothelial growth factors. *Cardiovasc Res* 2005; 65: 550–563.
20. Schacht V, Ramirez MI, Hong YK *et al*. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J* 2003; 22: 3546–3556.
21. Alexander JS, Chaitanya GV, Grisham MB *et al*. Emerging roles of lymphatics in inflammatory bowel disease. *Ann N Y Acad Sci* 2010; 1207Suppl 1E75–E85.
22. Kataru RP, Jung K, Jang C *et al*. Critical role of CD11b+ macrophages and VEGF in inflammatory lymphangiogenesis, antigen clearance, and inflammation resolution. *Blood* 2009; 113: 5650–5659.
23. Huggenberger R, Ullmann S, Proulx ST *et al*. Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. *J Exp Med* 2010; 207: 2255–2269.
24. Machnik A, Dahlmann A, Kopp C *et al*. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension* 2010; 55: 755–761.
25. Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. *Nature* 2005; 438: 946–953.
26. Baluk P, Tammela T, Ator E *et al*. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. *J Clin Invest* 2005; 115: 247–257.
27. Cursiefen C, Chen L, Borges LP *et al*. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004; 113: 1040–1050.
28. Yao LC, Baluk P, Feng J *et al*. Steroid-resistant lymphatic remodeling in chronically inflamed mouse airways. *Am J Pathol* 2010; 176: 1525–1541.
29. Ristimaki A, Narko K, Enholm B *et al*. Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. *J Biol Chem* 1998; 273: 8413–8418.
30. Flister MJ, Wilber A, Hall KL *et al*. Inflammation induces lymphangiogenesis through up-regulation of VEGFR-3 mediated by NF-kappaB and Prox1. *Blood* 2010; 115: 418–429.
31. Mounzer RH, Svendsen OS, Baluk P *et al*. Lymphotoxin-alpha contributes to lymphangiogenesis. *Blood* 2010; 116: 2173–2182.
32. Kerjaschki D, Huttary N, Raab I *et al*. Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. *Nat Med* 2006; 12: 230–234.
33. Maruyama K, Ii M, Cursiefen C *et al*. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J Clin Invest* 2005; 115: 2363–2372.
34. Machnik A, Neuhofer W, Jantsch J *et al*. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 2009; 15: 545–552.

35. Albuquerque RJ, Hayashi T, Cho WG *et al*. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. *Nat Med* 2009; 15: 1023–1030.
36. Mackay CR, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990; 171: 801–817.
37. Itano AA, McSorley SJ, Reinhardt RL *et al*. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* 2003; 19: 47–57.
38. Roozendaal R, Mebius RE, Kraal G. The conduit system of the lymph node. *Int Immunol* 2008; 20: 1483–1487.
39. de la Torre YM, Locati M, Buracchi C *et al*. Increased inflammation in mice deficient for the chemokine decoy receptor D6. *Eur J Immunol* 2005; 35: 1342–1346.
40. Segerer S, Jedlicka J, Wuthrich RP. Atypical chemokine receptors in renal inflammation. *Nephron Exp Nephrol* 2010; 115: e89–e95.
41. Wick N, Haluza D, Gurnhofer E *et al*. Lymphatic precollectors contain a novel, specialized subpopulation of podoplanin low, CCL27-expressing lymphatic endothelial cells. *Am J Pathol* 2008; 173: 1202–1209.
42. Pruenster M, Mudde L, Bombosi P *et al*. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat Immunol* 2009; 10: 101–108.
43. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* 2008; 8: 362–371.
44. Johnson LA, Jackson DG. Inflammation-induced secretion of CCL21 in lymphatic endothelium is a key regulator of integrin-mediated dendritic cell transmigration. *Int Immunol* 2010; 22: 839–849.
45. Kriehuber E, Breiteneder-Geleff S, Groeger M *et al*. Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. *J Exp Med* 2001; 194: 797–808.
46. Pflicke H, Sixt M. Preformed portals facilitate dendritic cell entry into afferent lymphatic vessels. *J Exp Med* 2009; 206: 2925–2935.
47. Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol* 2005; 6: 895–901.
48. Debes GF, Arnold CN, Young AJ *et al*. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. *Nat Immunol* 2005; 6: 889–894.
49. Kabashima K, Shiraishi N, Sugita K *et al*. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. *Am J Pathol* 2007; 171: 1249–1257.
50. Ledgerwood LG, Lal G, Zhang N *et al*. The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics. *Nat Immunol* 2008; 9: 42–53.
51. Thangada S, Khanna KM, Blaho VA *et al*. Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines lymphocyte egress kinetics. *J Exp Med* 2010; 207: 1475–1483.
52. Johnson LA, Jackson DG. Cell traffic and the lymphatic endothelium. *Ann N Y Acad Sci* 2008; 1131: 119–133.
53. Cera MR, Del Prete A, Vecchi A *et al*. Increased DC trafficking to lymph nodes and contact hypersensitivity in junctional adhesion molecule-A-deficient mice. *J Clin Invest* 2004; 114: 729–738.
54. Albertine KH, O'Morchoe CC. An integrated light and electron microscopic study on the existence of intramedullary lymphatics in the dog kidney. *Lymphology* 1980; 13: 100–106.
55. Kriz W. [Lymphatic vessels of mammalian kidneys]. *Verh Anat Ges* 1969; 63: 25–32.
56. McIntosh GH, Morris B. The lymphatics of the kidney and the formation of renal lymph. *J Physiol* 1971; 214: 365–376.
57. Peirce EC, 2nd. Renal lymphatics. *Anat Ree* 1944; 90: 315–335.
58. Lee HW, Qin YX, Kim YM *et al*. Expression of lymphatic endothelium-specific hyaluronan receptor LYVE-1 in the developing mouse kidney. *Cell Tissue Res* 2011; 343: 429–444.
59. Sakamoto I, Ito Y, Mizuno M *et al*. Lymphatic vessels develop during tubulointerstitial fibrosis. *Kidney Int* 2009; 75: 828–838.
60. Heller F, Lindenmeyer MT, Cohen CD *et al*. The contribution of B cells to renal interstitial inflammation. *Am J Pathol* 2007; 170: 457–468.
61. Kalof AN, Cooper K. D2-40 immunohistochemistry—so far!. *Adv Anat Pathol* 2009; 16: 62–64.
62. Neusser MA, Kraus AK, Regele H *et al*. The chemokine receptor CXCR7 is expressed on lymphatic endothelial cells during renal allograft rejection. *Kidney Int* 2010; 77: 801–808.
63. Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006; 54: 385–395.
64. Kukk E, Lymboussaki A, Taira S *et al*. VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* 1996; 122: 3829–3837.
65. Bonsib SM. Renal lymphatics, and lymphatic involvement in sinus vein invasive (pT3b) clear cell renal cell carcinoma: a study of 40 cases. *Mod Pathol* 2006; 19: 746–753.
66. Ishikawa Y, Akasaka Y, Kiguchi H *et al*. The human renal lymphatics under normal and pathological conditions. *Histopathology* 2006; 49: 265–273.
67. Zhang T, Guan G, Liu G *et al*. Disturbance of lymph circulation develops renal fibrosis in rats with or without contralateral nephrectomy. *Nephrology (Carlton)* 2008; 13: 128–138.
68. Jedlicka J, Soleiman A, Draganovici D *et al*. Interstitial inflammation in Alport syndrome. *Hum Pathol* 2010; 41: 582–593.
69. Segerer S, Banas B, Wornle M *et al*. CXCR3 is involved in tubulointerstitial injury in human glomerulonephritis. *Am J Pathol* 2004; 164: 635–649.
70. Matsui K, Nagy-Bojarsky K, Laakkonen P *et al*. Lymphatic microvessels in the rat remnant kidney model of renal fibrosis: aminopeptidase p and podoplanin are discriminatory markers for endothelial cells of blood and lymphatic vessels. *J Am Soc Nephrol* 2003; 14: 1981–1989.
71. Suzuki Y, Ito Y, Mizuno M *et al*. Transforming growth factor-beta induces vascular endothelial growth factor-C expression leading to lymphangiogenesis in rat unilateral ureteral obstruction. *Kidney Int* 2012
72. Zimmer JK, Dahdal S, Muhlfeld C *et al*. Lymphangiogenesis is upregulated in kidneys of patients with multiple myeloma. *Anat Rec (Hoboken)* 2010; 293: 1497–1505.
73. Mobley JE, O'Dell RM. The role of lymphatics in renal transplantation. Renal lymphatic regeneration. *J Surg Res* 1967; 7: 231–233.
74. Murray JE, Lang S, Miller BF *et al*. Prolonged functional survival of renal autotransplants in the dog. *Surg Gynecol Obstet* 1956; 103: 15–22.
75. Malek P, Vrubel J, Kolc J. Lymphatic aspects of experimental and clinical renal transplantation. *Bull Soc Int Chir* 1969; 28: 110–114.
76. Pedersen NC, Morris B. The role of the lymphatic system in the rejection of homografts: a study of lymph from renal transplants. *J Exp Med* 1970; 131: 936–969.
77. Kerjaschki D, Regele HM, Moosberger I *et al*. Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J Am Soc Nephrol* 2004; 15: 603–612.
78. Stuht S, Gwinner W, Franz I *et al*. Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. *Am J Transplant* 2007; 7: 377–384.
79. Segerer S, Heller F, Lindenmeyer MT *et al*. Compartment specific expression of dendritic cell markers in human glomerulonephritis. *Kidney Int* 2008; 74: 37–46.
80. Adair A, Mitchell DR, Kipari T *et al*. Peritubular capillary rarefaction and lymphangiogenesis in chronic allograft failure. *Transplantation* 2007; 83: 1542–1550.
81. Kerjaschki D. The crucial role of macrophages in lymphangiogenesis. *J Clin Invest* 2005; 115: 2316–2319.
82. Yin N, Zhang N, Xu J *et al*. Targeting lymphangiogenesis after islet transplantation prolongs islet allograft survival. *Transplantation* 2011; 92: 25–30.

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