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Themed Section: Immune Targets in Hypertension

REVIEW ARTICLE

Immune cell trafficking, lymphatics and hypertension

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Activated immune cell infiltration into organs contributes to the development and maintenance of hypertension. Studies targeting specific immune cell populations or reducing their inflammatory signalling have demonstrated a reduction in BP. Lymphatic vessels play a key role in immune cell trafficking and in resolving inflammation, but little is known about their role in hypertension. Studies from our laboratory and others suggest that inflammation-associated or induction of lymphangiogenesis is organ protective and anti-hypertensive. This review provides the basis for hypertension as a disease of chronic inflammation in various tissues and highlights how renal lymphangiogenesis is a novel regulator of kidney health and BP.

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Abbreviations

APC, antigen-presenting cell; DC, dendritic cell; DOCA, deoxycorticosterone acetate; IAL, inflammation-associated lymphangiogenesis; LEC, lymphatic endothelial cell; LHTN, L-NAME-induced hypertension; LT, lymphotoxin; PVAT, perivascular adipose tissue; SFO, subfornical organ; SHR, spontaneously hypertensive rat; SSHTN, salt-sensitive hypertension; TonEBP, tonicity-responsive enhancer-binding protein; UUO, unilateral ureteral obstruction

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Introduction

The increasing prevalence of hypertension in children and adults is a significant public health issue. A major concern in hypertensive disease and what makes its investigation necessary is target organ damage. Hypertension actively contributes to the development of heart, kidney, brain, eye and peripheral vascular diseases. Endothelial cell activation by inflammatory factors and attenuated endothelium-dependent vasodilatation are underlying events in the pathogenesis of hypertension (Sprague and Khalil, 2009). Blood vessels undergo remodelling wherein the lumen diameter decreases and the ratio of medial to intimal thickness increases. Chronic hypertension increases the workload on the heart, which eventually undergoes hypertrophy and fibrosis leading to increased wall stiffness and dysfunction (Drazner, 2011). The systemic elevated BP load eventually reaches the renal microvasculature leading to nephrosclerosis and renal damage. Changes in the microvascular structure of the cerebral circulation are associated with an increased risk of death, ischaemic stroke and decline in cognitive abilities (Gąsecki *et al*., 2013).

Over the years, a better understanding of the pathophysiology of hypertensive end-organ damage has been attained, which aids the design of better therapeutics. However, the incidence of hypertension continues to rise globally, indicating the need to revisit current treatment options. Hypertension and cardiovascular diseases have gained appreciation as a low-grade inflammatory disease, and studies on human hypertension support this association (Solak *et al*., 2016). Aberrant immune system activation and inflammation is now recognized to have a mechanistic role in the progression of hypertension and should pave the way for designing more effective therapeutics to attenuate end-organ damage in hypertensive patients (Miguel *et al*., 2015; Lopez Gelston and Mitchell, 2017).

Immune system activation in hypertension

The contribution of both the innate and adaptive immune systems in the pathogenesis of hypertension has been recognized for the past 50 years. Pioneering studies by White and Grollman showed that immunosuppression blunted hypertension in rats with partial renal infarction (White and Grollman, 1964; Okuda and Grollman, 1967) and that the transfer of lymphocytes from lymph nodes of rats with renal infarction triggered the elevation of BP in normal recipient rats (Okuda and Grollman, 1967). Subsequently, studies by Olsen (1970) reported the presence of inflammatory cells in the vasculature of **angiotensin II**-infused rats. In another study, it was demonstrated that thymectomized or athymic nude mice with renal infarction did not maintain hypertension (Svendsen, 1976). Transplant of the thymus from Wistar–Kyoto rats to spontaneously hypertensive rats (SHRs) lowered BP (Bendich *et al*., 1981), and similar results were obtained by treatment with anti-thymocyte drugs or the immunosuppressive drug cyclophosphamide (Dzielak, 1991). Studies also demonstrated that transfer of splenocytes from deoxycorticosterone acetate (DOCA)-salt hypertensive rats to normal recipient rats triggered hypertensive responses in the recipient rats (Olsen, 1980).

Following the evidence of immune system activation in hypertension, immunosuppression became a popular tool of choice to lower BP in experimental models of hypertension. Administration of the immunosuppressive agent mycophenolate mofetil blunts the development of several forms of hypertension including salt-induced hypertension after angiotensin II infusion (Rodriguez-Iturbe *et al*., 2001), SHRs (Rodriguez-Iturbe *et al*., 2002), salt-induced hypertension after **NOS** inhibition (Quiroz *et al*., 2001), Dahl salt-sensitive rats (Mattson *et al*., 2006) and hypertensive patients (Herrera *et al*., 2006). All of these studies had demonstrated renal injury associated with the increased BP; the treatments reduced renal inflammation and injury and were accompanied by a lowered BP.

Inflammation in the cardiovascular system and the brain is also an important event in the pathogenesis of hypertension. Immune cells accumulate in the adventitia and perivascular adipose tissue (PVAT) of the larger vessels and of the smaller resistance vessels during hypertension. The PVAT then releases factors that modulate the tone of these vessels and also secretes factors leading to inflammation (Guzik *et al*., 2007). Several forms of hypertension are associated with increased vascular wall expression of **chemokines** like **CCL2 (also known as MCP-1**) and adhesion molecules including **VCAM-1** and **ICAM-1** (Ebrahimian *et al*., 2011). Another important target of activated immune cells in hypertension is the heart. Activation of T-cells found in the heart of angiotensin II-infused hypertensive mice caused cardiac inflammation, hypertrophy and fibrosis (Kvakan *et al*., 2009). BP regulation is also governed by central mechanisms, in part, through innervation of the blood vessels and the kidney. Strong evidence for the involvement of the CNS was demonstrated by the study showing that renal denervation abolishes hypertension in humans (Schlaich *et al*., 2009). Intracerebroventricular administration of the anti-inflammatory antibiotic minocycline reduces levels of **TNF-α**, **IL-1β** and **IL-6** in the paraventricular nucleus and reduces angiotensin II-dependent hypertension (Shi *et al*., 2010).

The immune system is constituted of both the innate immune system, consisting of macrophages, dendritic cells (DCs), mast cells, granulocytes etc, and the adaptive immune system, consisting of T and B lymphocytes. Components from both classes of the immune system are implicated in hypertension (Norlander *et al*., 2018). Guzik *et al*. (2007) conducted a study that dissected the role of T and B lymphocytes in hypertension. They demonstrated that angiotensin II-induced hypertension was blunted in $Rag1^{-/-}$ mice that are deficient in T and B lymphocytes. Vascular superoxide production and endothelial dysfunction were also blunted in these mice. The adoptive transfer of T but not B lymphocytes restored hypertension, indicating the importance of T-cells in the initiation of hypertension. T-cells are also required for the development of DOCA salt-induced and noradrenaline-induced hypertension (Marvar *et al*., 2010). Similarly, Crowley *et al*. demonstrated that severe combined immune deficiency mice deficient in lymphocytes are protected against hypertension. The lymphocyte-deficient mice displayed diminished cardiac damage and renal injury in response to angiotensin II infusion. More recently,

Mattson *et al*. (2013) used Dahl salt-sensitive rats with deleted Rag1 gene to demonstrate that these rats have attenuated BP, kidney damage and albuminuria. Mice lacking the macrophage colony-stimulating factor, also called osteoporotic mice (Op/Op), have blunted hypertensive responses to chronic angiotensin II infusion. The endothelial dysfunction, vascular remodelling and oxidative stress associated with wild-type controls were reduced in these Op/Op mice (De Ciuceis *et al*., 2005). Deletion of monocytes using diphtheria toxin prevented hypertension and reduced the expression of markers of aortic inflammation (Wenzel *et al*., 2011). Harwani *et al*. (2012) demonstrated that the cholinergic agonist nicotine promoted the development of pro-inflammatory responses in splenic macrophages from SHRs and also induced toll-like receptor-mediated cytokine release. Most recent studies have also demonstrated the involvement of the gut microbiome in hypertension. Karbach *et al*. (2016) reported that germ-free mice had reduced BP and reduced leukocyte infiltration in the kidney and vasculature in response to angiotensin II infusion when compared to normal mice.

Cytokines and antigens in hypertension

Once localized in the target organs, immune cells release inflammatory cytokines such as TNF-α, IL-1β, IL-6, **IL-17** and **IFN-γ**. In generalized terms, T helper 1 (Th1) cells secrete pro-inflammatory cytokines, and Th2 cells secrete anti-inflammatory cytokines. Th1 cytokines mediate the pathogenic effects leading to end-organ damage, and evidence exists for elevated levels of these cytokines in hypertensive models (Trott and Harrison, 2014). Direct infusion of IL-17A, for example, has been reported to induce hypertension and endothelial dysfunction in mice (Nguyen *et al*., 2013). IL-17$^{-/-}$ mice exhibited an initial increase in BP in response to angiotensin II, similar to wild-type mice; however, BP dropped in IL-17$^{-/-}$ mice after a week. Increases in vascular oxidative stress and endothelial dysfunction were also blunted in IL-17$^{-/-}$ mice (Madhur *et al*., 2010). IL-6 promotes the polarization of T-cells towards Th17 cells that secrete IL-17. IL-6$^{-/-}$ mice also display blunted responses to angiotensin II infusion (Lee *et al*., 2006). **Etanercept**, a TNF-α antagonist, prevents vascular dysfunction and the development of hypertension (Guzik *et al*., 2007). Following angiotensin II infusion, IFN-γ is elevated in the kidneys of hypertensive mice, and inhibition of IFN-γ prevents the end-organ damage induced by angiotensin II infusion (Garcia *et al*., 2012). Accordingly, adoptive transfer of anti-inflammatory regulatory T-cells did not affect hypertension but attenuated the cardiac hypertrophy, fibrosis and inflammation induced by chronic angiotensin II infusion (Kvakan *et al*., 2009). **IL-10** is an anti-inflammatory cytokine that stimulates the differentiation of regulatory T-cells and is also produced by the same cells; studies have reported that carotid arteries from IL-10$^{-/-}$ mice have marked endothelial dysfunction in response to angiotensin II. Vascular superoxide production is also increased in IL-10$^{-/-}$ mice (Kassan *et al*., 2011).

The complete mechanisms by which inflammatory cytokines mediate end-organ damage are not well understood. In the kidneys, the accumulation of inflammatory cytokines leads to a loss of peritubular capillaries resulting in medullary hypoxia and increased oxidative stress (Rodriguez-Iturbe *et al*., 2013). The infiltrating lymphocytes also express angiotensin II, and increased renal interstitial angiotensin II and oxidative stress are factors well known to impair pressure-induced natriuresis, thereby affecting renal function. Inflammation and oxidative stress are inextricably linked, and the generation of ROS reduces the bioavailability of **NO**. Chabrashvili *et al*. (2002) demonstrated that mRNA levels of the components of NADPH oxidase were increased in the kidneys of SHRs prior to the development of hypertension. The kidney of the SHR has an exaggerated tubuloglomerular feedback response, which may be due to the diminished availability of NO. In the vasculature, inflammatory cytokines released around blood vessels can alter the rates of synthesis and degradation of vasoconstrictors and vasodilators, including NO. TNF-α inhibits **endothelial NOS** and reduces the capacity of the endothelium to produce NO leading to an impairment of vasodilator responses. Accordingly, inhibiting TNF-α restored endothelium-dependent vasodilatation (Zhang *et al*., 2006). IL-17 has also been demonstrated to cause inhibition of endothelial NOS activity, thereby increasing vascular tone and leading to endothelial dysfunction (Nguyen *et al*., 2013). Vascular collagen deposition and aortic stiffening happen as a consequence of increased oxidative stress (Wu *et al*., 2014). IFN-γ has been reported to induce the expression of angiotensinogen in renal proximal tubular cells (Satou *et al*., 2012), which when converted to angiotensin II promotes sodium reabsorption in the nephron.

What activates the immune system in hypertension and causes the infiltration of immune cells in target organs is a more recent subject of investigation. The concept of self-antigens promoting hypertension was introduced following the discovery of agonistic antibodies to **adrenoceptors** and **angiotensin receptors**. Endogenous antigens like **heat shock proteins** and γ-ketoaldehydes (isoketals) generated by the lipid peroxidation of arachidonic acid are increased in the kidneys of hypertensive animals (Kirabo *et al*., 2014). Isoketals can react with intracellular proteins and form protein adducts, which could then be taken up by DCs and presented to MHC type I receptors, thus activating the immune system. The production of isoketals also promotes the production of cytokines like IL-1β, IL-6 and IL-23 by DCs, which further affect the polarization of T-cells (Kirabo *et al*., 2014). Studies have also examined the effects of antigen recognition by specifically blocking the interaction between DC **CD80** and **CD86** with the T-cell receptor **CD28**; this reduced vascular T-cell accumulation and prevented hypertension (Vinh *et al*., 2010). Seminal work by Zimmerman *et al*. (2004) reported that hypertension is caused in part by increased sympathetic outflow following elevated levels of ROS in the subfornical organ (SFO) of the brain in response to angiotensin II infusion. Adenoviral overexpression of cytoplasmic SOD in the SFO reduced ROS and lowered BP as potently as direct i.c.v. infusion of losartan, highlighting the crucial role of CNS inflammation in hypertension. Collectively, mounting evidence has changed the previously existing notion that immune cell infiltration and inflammation are a consequence of hypertension but, instead, suggest that the inflammation in the kidneys and other organs are central to the development of hypertension.

The lymphatic system

Lymphatic vessels transport immune cells and soluble antigens out of the peripheral interstitium to the draining lymph nodes, where further acquired immune responses are initiated. Soluble antigens reach the lymph nodes faster than they reach antigen-presenting cells (APCs) like DCs and are thought to prime the lymph node for the arrival of APCs (Randolph *et al.*, 2017). By also taking up fluid extravasated from the blood vasculature, lymphatic vessels regulate tissue fluid homeostasis (Levick and Michel, 2010; Aspelund *et al.*, 2016). Lymphatic capillaries, also called initial lymphatic vessels, are blind-ended structures present in nearly all tissues. They consist of a single layer of oak leaf-shaped lymphatic endothelial cells (LECs) and are equipped with thin fibrillar structures called anchoring filaments that permit expansion of these vessels with increased interstitial fluid pressure. The capillaries lack mural cell coverage, have little basement membrane coverage, and button-like cell–cell junctions (Trzewik *et al.*, 2001; Baluk *et al.*, 2007). As such, lymphatic capillaries are the preferred route for uptake of fluid and macromolecules in what is generally assumed to be a nonselective process (although mechanisms for selective uptake may exist, Triacca *et al.*, 2017).

Lymphatic capillaries coalesce into larger collecting vessels, which unlike the capillaries have tight zipper-like junctions with a continuous basement membrane (Wiig and Swartz, 2012). The collecting vessels have intraluminal valves and also possess smooth muscle cell coverage. The interendothelial flaps in the capillaries and the intraluminal valves in the collecting vessels are responsible for the unidirectional transport of lymph. Unlike the extrinsic contraction of veins, lymphatic smooth muscles cells provide an intrinsic pump to propel fluid along against a gradually increasing pressure before returning lymph to the blood circulation (Zawieja, 2009; Wiig and Swartz, 2012). Given these important roles of the lymphatic vessels, defects in their function disrupt fluid and immune homeostasis causing oedema and inflammatory diseases. Indeed, the respiratory and gastrointestinal systems, which are readily exposed to foreign antigens, have a dense network of lymphatic vessels stressing their importance in immune surveillance and defence mechanisms (Aspelund *et al.*, 2016).

Lymphatic vessels are not passive conduits for the transport of antigens and leukocytes, but rather, the process is actively regulated by LECs (Card *et al.*, 2014). LECs express an array of chemokines and most notably, and nearly LEC-specific, are **CCL21** and **CCL19**. The receptor for these chemokines, **CCR7**, is expressed on DCs and other leukocytes, and this ligand–receptor interaction aids the recruitment of immune cells into and through the lymphatic vessels to the lymph nodes (Forster *et al.*, 2008; Card *et al.*, 2014). Recent studies have also reported that LECs can themselves participate in the induction of peripheral immune tolerance through antigen archiving and presentation through MHC class I and class II molecules (Cohen *et al.*, 2010; Dubrot *et al.*, 2014; Tamburini *et al.*, 2014). LECs have also recently been demonstrated to have direct interactions with DCs and T-cells altering their maturation, differentiation and cytokine repertoires (elegantly reviewed in Maisel *et al.*, 2017).

Inflammation-associated lymphangiogenesis

Inflammation is a complex biological process that occurs as a protective mechanism against harmful agents and tissue remodelling. Inflammation is associated with the migration and activation of leukocytes, increased vascular permeability and an accumulation of excess interstitial fluid. To relieve the tissue of this hostile micro-environment, excess fluid, cells and antigens must be cleared and hence there is an increased demand for lymphatic drainage. To keep up with this need, lymphangiogenesis occurs at sites of persistent inflammation given that lymphatics are structurally suited to be the physiological route for removal of increased antigens, fluid, cytokines and macromolecules from the site of inflammation. The process has been termed inflammation-associated lymphangiogenesis (IAL) (Medzhitov, 2010; Lim *et al.*, 2013; Kim *et al.*, 2014). The rate at which these new vessels grow and the nature of these new vessels are highly tissue-specific and stimulus-specific. IAL has been observed in inflammatory conditions in several organs including the skin, intestine, heart, kidney and airway tract, and the consequences of IAL vary with the disease state and type of tissue in question (excellently reviewed in Kim *et al.*, 2014; Abouelkheir *et al.*, 2017).

In the search for the mechanisms of such postnatal lymphangiogenesis, several mediators of IAL have been found in different inflammatory diseases (Tan *et al.*, 2014; Maisel *et al.*, 2017); however, the most common of these are the potent lymphangiogenic VEGFs **VEGFC** and **VEGFD**. VEGFC and VEGFD signal through the receptor **VEGFR-3**. The sources of these mediators may be either leukocytes or stromal cells. Macrophages in particular have been demonstrated to be sources of VEGFC in several cases. In a mouse model of tail lymphoedema, $CD68^+$ macrophages were reported to be sources of VEGFC (Gousopoulos *et al.*, 2017), and depletion of macrophages reduced IAL in a mouse model of acute colitis (Becker *et al.*, 2016). More recently, DCs and neutrophils have also emerged as sources of lymphangiogenic signals (Baluk *et al.*, 2005). Besides leukocytes, epithelial cells (Wuest and Carr, 2010), keratinocytes (Halin *et al.*, 2007) and fibroblastic reticular cells (Chyou *et al.*, 2008) have also been reported to be sources of VEGFC in inflammation. In the kidney, VEGFC staining was observed in tubular epithelial cells during unilateral ureteral obstruction (UUO) (Lee *et al.*, 2013). Other inflammatory stimuli like **LPS** (Kang *et al.*, 2009), lymphotoxin [**α (TNFSF1)** and **β (TNFSF3)**) and inflammatory cytokines like IL-17 and IL-8 (Chauhan *et al.*, 2011; Choi *et al.*, 2013) can also mediate IAL. Some cytokines like IFN-γ exhibit inhibitory effects on IAL, and **TGFβ** has been implicated in both stimulation and inhibition of lymphangiogenesis in disease states (Zampell *et al.*, 2012; Kinashi *et al.*, 2013). Inhibition of TGFβ induces lymphangiogenesis in a mouse model of chronic peritonitis (Oka *et al.*, 2008) and also improves lymphatic function in a model of lymphoedema (Avraham *et al.*, 2010), whereas in a model of peritoneal fibrosis, inhibiting the TGFβ receptor **TGFBR2** suppressed lymphangiogenesis (Kinashi *et al.*, 2013). Thus, the outcome of IAL is dependent on the balance of pro-lymphangiogenic and anti-lymphangiogenic factors and cytokines (Zampell *et al.*, 2012). The new lymphatic

vessels that are formed may proliferate from existing vessels (Wirzenius *et al*., 2007), sprout as new vessels (Kataru *et al*., 2009; Flister *et al*., 2010) or form from the transdifferentiation of bone marrow-derived cells (Maruyama *et al*., 2005; Kerjaschki *et al*., 2006). Interestingly, this seems to depend on the type of lymphangiogenic stimulus. Although controversial, evidence exists for the transdifferentiation of macrophages into LECs in LPS-induced peritonitis (Hall *et al*., 2012) and corneal inflammation (Maruyama *et al*., 2005). Moreover, activated murine peritoneal $CD11b^+$ macrophages *in vitro* were reported to form tube-like structures and express LEC-specific markers (Maruyama *et al*., 2005).

Is inflammation-associated lymphangiogenesis good or bad?

Whether IAL serves to resolve or exacerbate inflammation remains a matter of debate. In the skin, IAL has been demonstrated to have functional implications in both acute and chronic inflammation. In acute models of skin inflammation, blocking IAL by the inhibition of VEGFR-3 signalling increased inflammation (Kajiya and Detmar, 2006), and overexpression of VEGFC induced lymphangiogenesis and limited inflammation in chronic inflammatory conditions (Huggenberger *et al*., 2011). It has also been demonstrated that in contact hypersensitivity, lymphatic vessels are necessary for the regulation of long-term immune responses and fluid balance. Upon initial contact, K14-VEGFR3-Ig mice, which lack dermal lymphatic vessels, not only develop increased oedema but also fail to tolerize to hypersensitization upon subsequent challenges (Thomas *et al*., 2012). In the gut, alterations to the lymphatics result in changes not only to immune function but also to lipid transport. Deficiencies in the structure or function of lacteals affect lipid absorption and lead to the leakage of lymph. A proliferation of lymphatic vessels is associated with chronic inflammatory bowel diseases like Crohn's disease, ileitis and colitis, and IAL is unlikely to improve lymphatic drainage as oedema, and lack of DC migration to the draining lymph nodes is evident in such cases (Acedo *et al*., 2011; Abouelkheir *et al*., 2017). In airway inflammation, infection leads to robust lymphangiogenesis, and blocking IAL during infection resulted in increased mucosal oedema (Aurora *et al*., 2005; Baluk *et al*., 2005). Conversely, stimulating IAL also increased oedema in severe pulmonary lymphangiectasia (Yao *et al*., 2014). In rat cardiac allografts, newly formed lymphatic vessels participate in the trafficking of immune cells, and presumably donor antigens, to the secondary lymphoid organs; inhibiting VEGFR-3 using adenoviral VEGFR3-Ig was reported to increase cardiac allograft survival. Interestingly, this was not linked to reduced lymphangiogenesis but rather to reduced CCL21 production and entry of $CD8^+$ T-cells into the allograft (Edwards *et al*., 2018). Thus, the functional implications of IAL are highly disease- and tissue-specific.

Besides the macroscopic expansion, inflammation also affects lymphatic vessels at the cellular level. Under steady state, the expression of adhesion molecules like ICAM-1, VCAM-1 and L1CAM by LECs is quite low, whereas their expression is dramatically up-regulated by increased flow and inflammatory signalling presumably to facilitate the migration of DCs (Johnson *et al*., 2006; Maddaluno *et al*., 2009; Miteva *et al*., 2010; Vigl *et al*., 2011). Exposure of LECs to TNF-α *in vitro* up-regulates the expression of CCL21, and the up-regulation of CCL21 has also been demonstrated *in vivo* under inflammatory conditions (Johnson and Jackson, 2010; Miteva *et al*., 2010; Vigl *et al*., 2011). In addition to CCL21, **CXCL12** and **CX_3CL1** are other chemokines implicated in DC migration that are also up-regulated during inflammation (Johnson *et al*., 2006; Pegu *et al*., 2008). In response to inflammation, LECs also increase the expression of molecules that help remove inflammatory chemokines. For example, the chemokine-scavenging chemokine receptor **ACKR2 (also known as D6**) is up-regulated in inflammatory conditions, and in the absence of ACKR2, myelomonocytic cells accumulated around lymphatics vessels and impeded lymph flow (Lee *et al*., 2011; McKimmie *et al*., 2013). Inflammation also modulates lymphatic pumping activity. Systemic or i.d. injection of inflammatory cytokines IL-6, TNF-α and IL-1β decreased lymphatic pumping frequency and lymph flow velocity *in vivo* (Aldrich and Sevick-Muraca, 2013). However, **substance P**, a neuropeptide secreted by inflammatory cells, increases the pumping frequency of rat mesenteric vessels (Davis *et al*., 2008). How inflammation affects lymphatics and *vice versa* has been and continues to be investigated in great detail.

Lymphatics and hypertension

A previously unknown role for lymphatic vessels and lymphangiogenesis was described by Machnik *et al*. (2009) in the context of interstitial sodium homeostasis. This study demonstrated that the skin interstitium could act as a reservoir for sodium in order to buffer the effects of sodium overload on BP. In response to a high-salt challenge, the investigators observed that dermal lymphangiogenesis is associated with sodium accumulation in the skin interstitium. The resulting hypertonicity from sodium accumulation activates the transcription factor, tonicity-responsive enhancer-binding protein (TonEBP) in infiltrating macrophages. TonEBP binds to the gene encoding VEGFC and causes macrophages to secrete VEGFC, thereby leading to a robust expansion of the dermal lymphatic network. The lymphatic expansion was dependent on TonEBP secretion from the macrophages; macrophage depletion prevented dermal lymphatic hyperplasia and worsened sodium-dependent hypertension. Blockade of VEGFC signalling had similar effects in these mice (Wiig and Swartz, 2012). This study not only revealed a novel role for dermal lymphatic vessels but also demonstrated the extrarenal regulation of sodium homeostasis and BP control by the skin interstitium.

In another study, TonEBP-mediated cardiac lymphangiogenesis and macrophage infiltration was observed in the left ventricles of SHRs that were fed a high-salt diet (Yang *et al*., 2014; Yang *et al*., 2017). Retrovirus-induced overexpression of VEGFC led to enhanced lymphangiogenesis, reduced myocardial fibrosis and macrophage infiltration, decreased BP and preserved myocardial function. The opposite effects were observed after blocking VEGFC, where diminished lymphangiogenesis was accompanied by increased macrophage infiltration and pronounced left ventricular remodelling (Yang *et al*., 2014). In another study, it was

demonstrated that cardiac lymphangiogenesis also occurs in response to myocardial infarction; however, myocardial infarction induces dysfunction of the epicardial pre-collector and collecting lymphatic vessels leading to myocardial oedema (Henri *et al.*, 2016). Increasing cardiac lymphangiogenesis through the administration of VEGFC attenuates myocardial oedema, inflammation and fibrosis and improves cardiac function following infarction (Klotz *et al.*, 2015; Henri *et al.*, 2016).

Renal lymphatics and hypertension

In the kidney, lymphangiogenesis has been reported in association with inflammation and infiltrating immune cells in chronic inflammatory kidney diseases such as diabetic nephropathy, IgA nephropathy, tubulointerstitial nephritis and glomerulonephritis with the degree of fibrosis directly correlating to the extent of lymphatic expansion (Yazdani *et al.*, 2014). In acute kidney injury, renal lymphangiogenesis was associated with tubulointerstitial injury, and suppression of renal lymphangiogenesis reduced fibrosis in a mouse model of UUO (Lee *et al.*, 2013). Tubular epithelial cells along with macrophages were demonstrated to be sources of VEGFC in UUO. Hasegawa *et al.* (2017) were able to reduce inflammation and subsequent fibrosis by the administration of VEGFC for 2 weeks during UUO. In another study, it was reported that lymphangiogenesis might occur in parallel with the onset of proteinuria, yet prior to the development of fibrosis, with activated proximal tubular epithelial cells acting as sources of VEGFC (Yazdani *et al.*, 2012). Renal transplants are also associated with the formation of new lymphatic vessels; however, the consequences of these vessels are a matter of debate. The newly formed vessels could be beneficial by draining out fluid and for immune clearance immediately after operation. In fact, low lymphatic vessel density in the first post-procedural biopsy is associated with acute rejection. The persistence of these vessels on the other hand may eventually lead to transplant rejection as these vessels help transport APCs to the lymph nodes and initiate immune responses (Kerjaschki, 2004).

The importance of renal lymphatics in fluid balance was demonstrated as early as the 1960s. Renal lymphatic ligation was reported to result in renal oedema, increased urine volume and, surprisingly, increased BP in several studies (Barer and Ward-Mcquaid, 1957; Lilienfeld *et al.*, 1967). Zhang *et al.* (2008) later demonstrated that dual renal lymphatic ligation was as detrimental to kidney function as a nephrectomy. With renal immune cell infiltration and accumulation a part of the pathogenesis of hypertension, IAL in the kidney should be evident and necessary to remediate inflammation. Our laboratory investigated this relationship and reported that the kidneys of SHRs demonstrate increased renal lymphatic vessel density in association with inflammation and infiltration of $CD68^+$ macrophages (Kneedler *et al.*, 2017). Interestingly, a strain of SHRs (SHR-B2) that were hypertensive but resistant to renal injury displayed lower lymphatic vessel density than their age-matched controls. Fischer 344 rats are a strain of rats that are normotensive but display age-associated renal injury. Kidneys from these rats at 20 or 24-months of age demonstrated increased renal lymphatic vessel density accompanying age-associated renal injury

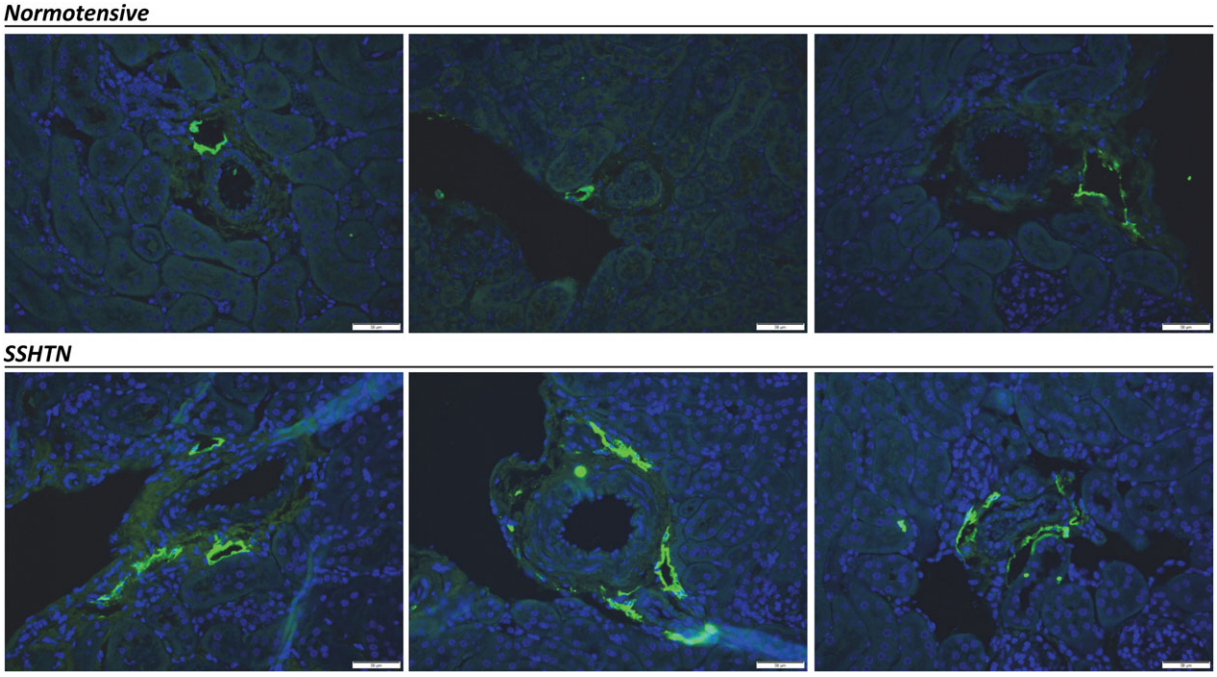

Figure 1

Increased renal lymphatic density in hypertension. In the normotensive kidney cortex, lymphatic vessels are limited to the interlobular arteries with 1–2 identifiable lymphatic lumen (green, Lyve-1) in each vascular bundle. During SSHTN, the endogenous lymphatic network expands, with increased numbers of lymphatic lumen. Blue, DAPI. Bars = 50 μm.

compared with 4-month-old controls (Kneedler *et al.*, 2017). Thus, the renal inflammation evident in hypertensive and aged kidneys is associated with an increase in renal lymphatic vessel density.

Following this genetic model of hypertension, our lab also tested if this increase in renal lymphatic density occurs in two other models of hypertension, salt-sensitive (SSHTN) and NOS inhibition-induced hypertension, induced by the administration of L-NAME (LHTN). In both hypertensive models, we observed a significant increase in renal lymphatic vessel density (Figure 1) (Lopez Gelston *et al.*, 2018). In SSHTN, the increase in lymphatic density was associated with renal infiltration of macrophages and Th1 cells, whereas in LHTN, this was associated with an increase in renal macrophages and DCs. Furthermore, we hypothesized that while minimal renal lymphatic expansion may be indicative of inflammation, it is insufficient to clear infiltrating immune cells from the cortical interstitium and that further augmenting renal lymphangiogenesis should therefore be beneficial. Enhancing renal lymphatic expansion prior to the onset of hypertension using an inducible genetic model of kidney-specific lymphangiogenesis (Lammoglia *et al.*, 2016) completely prevented the development of hypertension associated with high-salt diet and NOS inhibition (Lopez Gelston *et al.*, 2018). Importantly, in both of these models, augmenting renal lymphatic vessel density reduced the immune cell populations previously found to be increased, suggesting increased trafficking from hypertensive kidneys. This study emphasizes the role of renal lymphatics in BP regulation and provides a potential new therapeutic or diagnostic target for the treatment of hypertension.

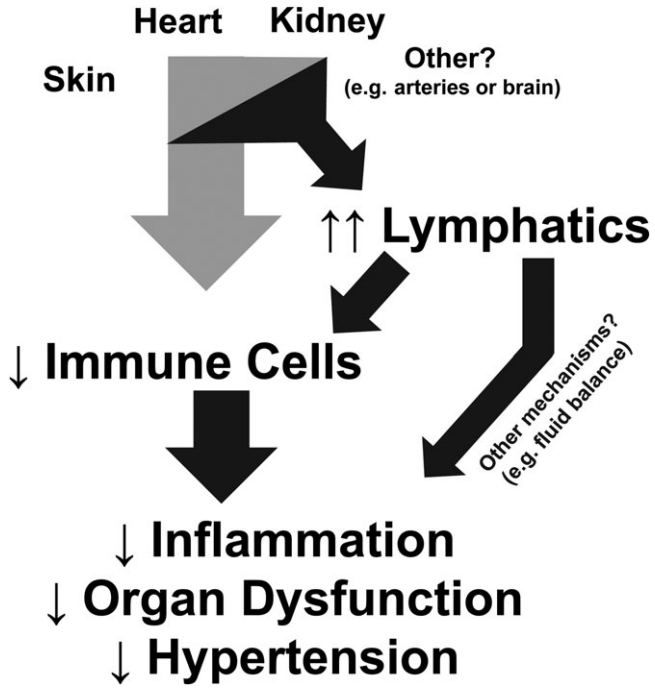

Figure 2
Mechanism by which increased lymphatics decrease inflammation, organ dysfunction and BP.

Conclusions

In conclusion, studies to date demonstrate that lymphatics and expanded lymphatic density are important not only in combating peripheral inflammation but are also key players in BP regulation (Figure 2). Targeting immune cell exfiltration from various tissues through the lymphatics may represent a unique method to reduce diseases of chronic inflammation and ameliorate hypertension.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

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Conflict of interest

The authors declare no conflicts of interest.

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