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Regulation of Lymph Node Vascular-Stromal Compartment by Dendritic Cells

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Abstract

During normal and pathologic immune responses, lymph nodes can swell considerably. The lymph node vascular-stromal compartment supports and regulates the developing immune responses and undergoes dynamic expansion and remodeling. Recent studies have shown that dendritic cells (DCs), best known for their antigen presentation roles, can directly regulate the vascular-stromal compartment, pointing to a new perspective on DCs as facilitators of lymphoid tissue function. Here, we review the phases of lymph node vascular-stromal growth and remodeling during immune responses, discuss the roles of DCs, and discuss how this understanding can potentially be used for developing novel therapeutic approaches.

Keywords

Lymph node; Vascular; Stromal; Dendritic cells

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The Lymph Node Vascular-Stromal Compartment

Although lymph nodes can appear to resemble bags of cells, the lymphocytes within are supported by a vascular-stromal compartment that provides survival factors, guidance cues, regulatory molecules, and structural support [1–4]. The blood vessels can be viewed as the “bones” of this compartment, entering as a feeding arteriole that branches continuously until they become the fine vessels of the capillary beds [5, 6] (Figure 1). These capillaries then recombine to become postcapillary high endothelial venules (HEVs), which lead to veins that join and exit at the hilus, near where the feeding arteriole enters [5] (Figure 1). These blood vessels are intertwined with a series of interconnected lymphatic sinuses, and both types of vessels are encased in a reticular network comprised of a collagen-rich fibrils ensheathed by mesenchymal reticular cells, most of which are podoplanin (PDPN)-expressing fibroblastic reticular cells (FRCs) [7–12] (Figure 1, inset A). The potential space between the fibrils and FRCs serves as a conduit that is connected to afferent and efferent lymphatic flow for rapid delivery of small soluble molecules to and from distal locations and across the lymph node. This reticular network is also functionally contiguous with the vessels, as molecules can be delivered through the reticular network to the endothelial cells [8, 13]. FRCs in the reticular network also supports endothelial cells through vascular-endothelial growth factor (VEGF) production [10, 14, 15]. The vascular-stromal compartment, while comprised of blood vessels, lymphatic sinuses, and the reticular network, can thus be viewed in some respects as a single structural compartment and its distinct entities might be predicted to grow and remodel together.

Together, the components of the vascular-stromal compartment play a critical role in shaping immune responses, by bringing together antigen, antigen-presenting cells and lymphocytes and regulating immune cell survival and function (see Box 1). Here, we will focus on the vascular-stromal growth and remodeling that occurs during immune responses and the emerging role of DCs in this process.

Vascular-Stromal Growth and Remodeling During Immune Responses

Lymph nodes can grow to many times their normal size within days, and this growth is accompanied by a proliferative expansion and remodeling of the vascular-stromal compartment. Multiple investigators have observed that this process is comprised of several different phases [5, 11, 34–36]; here we synthesize the observations made with several immunization regimens such as cutaneous or subcutaneous administration of bone marrow-derived DCs [11, 37], OVA/CFA [11, 36], OVA/Alum [38], oxazalone [34], KLH/CFA [39], and LCMV [35]. We use the nomenclature derived from our own studies.

Initiation

The initiation phase occurs within about two days after immunization. Lymph nodes enlarge by 2–5 fold during this phase [11, 35, 37, 38, 40, 41] (Figure 2A), due at least in part to rapid feeding arteriole remodeling and increased blood flow [42–44]. There is also an increase in blood vascular permeability [5] and HEV endothelial cells can rapidly upregulate VCAM-1 expression, allowing naïve lymphocytes to enter the lymph node parenchyma at a higher rate

[11, 42]. The lymph node enlargement is also due in part to reduced egress, attributable mainly to downregulation of S1P receptor 1 on lymphocytes [18].

During this period, blood vessel endothelial cells (including HEV endothelial cells), lymphatic endothelial cells, and PDPN⁺ reticular cells (comprised mainly of FRCs but also including FDCs (Figure 1)), begin to upregulate their proliferation rate (Figure 2B). Endothelial and PDPN⁺ reticular cell numbers may begin to increase [11, 36, 37, 45] (Figure 2C). Morphologically, reticular cells including FRCs appear less tightly wrapped around vessels, which may be linked to the increased vascular permeability. [5, 11, 46] (Figure 2E). There is also relaxation of the FRC network by this time [45] (Figure 2E).

Expansion

The expansion phase occurs from about days 2 and 5. During this phase, the proliferation rates of endothelial cells and PDPN⁺ reticular cells continues to increase, leading to more noticeable increases in cell numbers with most immunization strategies [11, 15, 36, 38, 40, 41, 47–49] (Figure 2B–C). Consistent with the increased endothelial cell numbers, HEVs show elongation and increased branching by optical projection tomography [35, 50]. The vascular expansion is associated with a less mature phenotype on HEVs, with downregulation of HEC-6ST, the sulfotransferase that is critical for luminal PNAd expression, and upregulation of the immature HEV marker MAdCAM-1 [34]. In viral and bacterial infection models, stromal CXCL13 and CCL21 are transiently downregulated, suggesting a loss of mature FDC and FRC state as well [51].

Re-Establishment of Vascular-Stromal Quiescence

After day 5, there is a re-establishment of vascular-stromal quiescence, whereby endothelial cell and PDPN⁺ reticular cell proliferation rates begin to drop from their peak rate at day 5 but are maintained at above baseline levels for up to a few weeks [11, 38] (Figure 2B). Endothelial cell and PDPN⁺ reticular cell numbers may stay stable at high numbers or, as proliferation rates can remain quite high even after the peak, continue to increase during this time [11, 38, 41] (Figure 2C, E). HEVs begin to show a recovery of a more mature HEV endothelial cell phenotype [11, 40]. Perivascular reticular cells regain a tighter organization around the vessels but remains less well organized than at steady state [11] (Figure 2E). These changes suggest the beginning of a vascular maturation and stabilization that occurs with physiological angiogenesis [46]. PDPN on reticular cells, which is upregulated by day 2 after immunization, remains high [41, 50]. Note that lymph nodes are still enlarged during this phase (Figure 2A), and this phase is associated with continued germinal center development and plasma cell accumulation [11, 38].

The re-establishment of quiescence is theoretically followed eventually by regression of the expanded vascular-stromal compartment after resolution of the immune response. A partial regression has been documented for the expanded lymphatics after 1–3 months [15, 52], but further work will be needed to follow the fate and phenotypic changes of endothelial cells and reticular cells over time to understand when, if ever, complete regression is achieved.

Role of DCs in Vascular-Stromal Growth and Remodeling

Conventional DCs in homeostatic lymph nodes are comprised mainly of 1- CDP-derived, Flt3-dependent resident DCs that develop from circulating pre-DCs which enter via HEV and 2- migratory DCs comprised of CDP-derived, Flt3-dependent DCs and a smaller population of monocyte-derived DCs that enter in a CCR7-dependent manner via afferent lymphatics from the draining tissue [53, 54]. Resident DCs in particular are closely associated with FRCs and can pick up antigen flowing through the conduits [8] (Figure 2E). Some DCs are also situated tightly around the perimeter of HEVs and lymphatics [11] and can acquire antigen from afferent lymphatic sinuses [55], suggesting DCs are likely to have direct contact with the endothelial cells as well as with the perivascular reticular cells. Thus, DCs are situated to be able to directly regulate elements of the vascular-stromal compartment.

Here we will discuss recent studies that have delineated roles for DCs in vascular-stromal regulation at homeostasis and during immune responses. We will keep in mind several issues during this discussion. First, the use of CD11c and MHCII to designate DC subset (CD11c^{hi}MHCII^{med} cells for resident DCs and CD11c^{med}MHCII^{hi} cells for migratory DCs) has some limitations for multiple reasons [54]. CD11c^{med}MHCII^{hi} cells as a population are comprised of migratory DCs and Langerhans cells that develop from yolk sac-derived progenitors [53, 54]. Thus we will refer to the CD11c^{med}MHCII^{hi} population as CD11c^{med}MHCII^{hi} antigen-presenting cells (APCs) when discussing them as a whole. Also, the distinctions between the CD11c^{hi}MHCII^{med} cells and CD11c^{med}MHCII^{hi} cells can blur during inflammation, as the CD11c^{hi}MHCII^{med} population may also include migratory APC and monocyte-derived cells [54, 56, 57]. We will thus specify the populations being depleted without labeling them as resident or migratory DCs unless there is other evidence for one or another population. Second, CD11c can also be expressed by cells such as monocytes, NK cells, and T cells [54]. As some of the studies that we will review in this section used CD11c + cell depletion strategies, we will present additional evidence when possible for a role of DCs.

Steady State

In mice that express diphtheria toxin receptor (DTR) in CD11c+ cells and that allow for inducible depletion of these cells with DT injection, CD11c+ cell depletion for one day increased basal endothelial cell division, as detected by bromodeoxyuridine (BrdU) uptake. As endothelial cell numbers were not examined over a longer period [11], it is unknown whether the change in BrdU uptake reflects the beginning of an expansion response or an injury response to compensate for loss of endothelial cells. However, consistent with the idea that CD11c+ cells maintain vascular homeostasis, depletion of CD11c+ cells for eight days caused reversion of HEV to an immature state, with loss of PNAd and lymphocyte entry and upregulation of MAdCAM. Injection of cultured bone marrow derived dendritic cells (BMDCs) restored HEV phenotype, suggesting that the relevant CD11c+ cells that maintained HEV phenotype were DCs. Molecularly, CD11c+ cell-derived lymphotoxin (LT) $\alpha 1\beta 2$ was necessary to maintain HEV function, and culture of CD11c+ cells with HEV

endothelial cells maintained some aspects of HEV phenotype, suggesting a direct regulation of HEV by CD11c+ cells [58].

Whether this effect reflects the activities of migratory or resident DCs or potentially other CD11c+ cells is not well understood. Mice that express functional human CCR7 on T cells but are otherwise CCR7-deficient have a deficiency in migratory APCs but a normal complement of resident DCs in lymph nodes. These mice show about 50% less cumulative PNAd+ HEV vessel length, suggesting an HEV defect. However, the HEV mediated T cell entry normally, and the T cell deficiency seen in these lymph nodes could also be attributed to the reduced CCL21 expression by FRCs and faster T cell egress seen in these mice [59]. How much the HEV phenotypic maintenance by CD11c+ cells reflects activities of migratory APCs, resident DCs, and/or potentially other CD11c+ cells will be of interest to better understand in the future, at least in part because it may also help the field to understand regulation in stimulated lymph nodes (see next section).

Initiation

Upon immune stimulation in the skin by immunization, infection, or contact sensitizers, local DCs and Langerhans cells become activated and migrate in large numbers to the draining lymph node, bringing in antigen [53, 54]. This results in a spike in relative CD11c^{med}MHCII^{hi} cell numbers in the node (Figure 2D–E). Footpad injection of cultured mature or immature bone marrow-derived DCs resulted in the accumulation of the injected DCs in the draining node and lymph node enlargement by 1 day, suggesting the idea that endogenous migratory DCs could induce lymph node enlargement [37, 60]. Supporting this idea were CD11c+ cell depletion studies, whereby CD11c+ cell depletion just before immunization with OVA/CFA, OVA/Montanide, or HSV-2 infection prevented lymph node growth [37, 41, 44]. CCR7 deficiency in non-T non-B radiosensitive cells had normal cellularity in popliteal lymph nodes but showed blunted lymph node growth after immunization with OVA/CFA [36]. Injection of DCs could stimulate initial lymph node enlargement even in RAG1^{−/−} mice [37] and the CD11c+ cell-dependent lymph node enlargement with OVA/CFA at day 2 was also T and B cell-independent [36], suggesting that DCs could mediate lymph node growth independently of antigen presentation.

DCs could potentially contribute to lymph node enlargement by modulating lymphocyte trafficking, but this has not been thoroughly examined. DCs were implicated but not directly shown to modulate the feeding arteriole remodeling in draining lymph nodes after vaginal infection with HSV-2 [44] and the role of DCs on the initial HEV activation that would allow increased entry or on lymphocyte egress is not known. However, DC modulation of FRC structural dynamics to allow tissue expansion has been recently shown. C-type lectin domain family 1 member B (CLEC-2) on DCs can interact with its ligand PDPN on FRCs. PDPN normally promotes FRC myosin light chain (MLC) phosphorylation, actomyosin contractility, and cell adhesion via Rho kinase [40, 45], a well-known driver of contractility and adhesion [61]. PDPN can also limit proliferation in vitro and in vivo [45]. Binding of CLEC-2 inhibits PDPN-mediated contractility [40, 45] by causing sequestration of PDPN with CD44 in lipid rafts [40], leading to elongation and reduced spreading in vitro [40, 45]. In vivo, mice deficient in CLEC-2 in CD11c+ cells did not show changes at homeostasis as

young adults but showed reduced FRC elongation and blunted lymph node growth upon immunization [40, 45]. While these results may reflect CLEC-2 on multiple CD11c+ cell types, skin DCs express high levels of CLEC-2 [62], and the large influx of skin DCs upon immunization would suggest that migratory DCs are likely to be a major source of CLEC-2 signals. Thus, the data suggest that migratory DCs contribute to initial lymph node growth at least in part by inhibiting PDPN-mediated FRC contractility, allowing the FRC network to relax, and thus accommodate the increased number of lymphocytes (Figure 2E).

Inhibition of PDPN function by DC CLEC-2 can potentially also play a role in the early increase in vascular permeability after immunization [5]. This may be in part attributable to the role of PDPN in stimulating platelet production of S1P, to promote barrier function [63]. However, perivascular mesenchymal cells can also act as a physical barrier and act directly on endothelial cell to limit vascular permeability [46]. Inhibition of PDPN contractility and adhesion by DC CLEC-2 could potentially cause the observed loosening of the FRCs around the HEVs [11] (Figure 2E), leading to reduced communication with the endothelial cells and reduced barrier function, thereby contributing further to the increased vascular permeability. Thus DC-derived CLEC-2-PDPN interactions could potentially also contribute to the increase in lymph node mass upon immunization by affecting vascular permeability and allowing more fluid into the lymph node.

CD11c+ cells also mediate the initial upregulation of vascular-stromal proliferation that characterizes this phase. CD11c+ cell depletion greatly reduced the upregulation of endothelial cell and PDPN+ reticular cell proliferation in skin-draining lymph nodes [36, 37, 41]. Immunization with OVA/CFA upregulated vascular-stromal proliferation even in *Rag1*^{-/-} mice [36], suggesting that the initiation of vascular-stromal growth is independent of antigen presentation and could reflect direct interactions between DCs and the vascular-stromal compartment. Migratory APCs are not necessary for upregulating vascular-stromal proliferation, as CCR7 deficiency did not affect the upregulation of vascular-stromal proliferation [36]. However, they can have redundant roles with CCR2-dependent monocytes. Both CD11c^{med}MHCII^{hi} APCs and Ly6C+ monocytes secrete IL-1 β by 1 day after immunization with OVA+CFA, which contributes to stimulating FRCs to upregulate VEGF expression to induce endothelial cell proliferation [57]. The interaction between the myeloid cells and the FRCs appears direct, as myeloid cells could induce FRCs to upregulate VEGF in culture [14, 57]. Loss of both CCR2-dependent and CCR7-dependent cells was needed to blunt the upregulation of endothelial cell proliferation. [36, 57], suggesting that lymph-borne APCs entering from the skin during inflammation can work in conjunction with blood-borne monocytes to stimulate FRCs to initiate vascular growth.

More work will be needed to more clearly delineate the roles of DCs in the initiation of lymph node vascular-stromal growth and remodeling and to separate these functions from that of homeostatic vascular-stromal regulation. For example, does the prevention of vascular-stromal proliferation by CD11c+ cell depletion reflect in part the role of CD11c+ cells in maintaining vascular health and function during homeostasis? It should be emphasized that CD11c+ cell depletion just prior to OVA/CFA immunization did not result in loss of PNA⁺ HEV detected by flow cytometry by day 2 [36, 37], but it is a possibility that CD11c+ cell depletion perturbs endothelial (and perhaps FRC) function enough so that

they cannot be induced to proliferate upon immunization. Relevant for understanding all the phases of lymph node vascular-stromal growth and remodeling also will be to better understand the roles of specific DC subsets even within the resident and migratory DC populations. For example, both resident and migratory DCs are comprised of CD103/XCR1+ and CD11b+ subsets that differ in their antigen-presentation and T cell-stimulatory activities—do these subsets have specific functions in regulating aspects of vascular-stromal function? This understanding would also allow us to better understand how T cell-stimulatory functions are related to vascular-stromal regulatory functions. For example, is the ability to process antigen linked to the ability to regulate vascular-stromal growth and remodeling? These insights would enrich our understanding of how differing elements of the immune system are coordinately regulated.

Expansion

The role of DCs has not been examined for the expansion phase of the vascular-stromal compartment, at least not separately from their role in the initiation phase. In contrast to the initiation phase, however, relative CD11c^{med}MHCII^{hi} migratory APC accumulation is reduced [11, 38](Figure 2D) and it is clear that T and B cells are required for the expansion of the blood and lymphatic endothelial cells and PDPN+ reticular cells. B cells in particular are required for lymphatic and HEV expansion [34–36, 48], and T cells play a role in FRC expansion [36, 41]. Interestingly, T cells also have a regulatory role in limiting lymphatic expansion [47]. At the molecular level, B cell derived lymphotoxin (LT) $\alpha 1\beta 2$ is needed for HEV expansion and LT β R ligands contribute to full FRC proliferation and expansion [35, 41]. These studies, together with the role of DCs in the initiation phase suggest a scenario whereby innate immune activation begins the process of vascular-stromal growth. After this initial phase, the vascular-stromal growth can proceed to accommodate the expansion of adaptive cells, and this expansion is regulated through a direct feedback from those cells. However, in the absence of sufficient numbers of adaptive cells, the vascular-stromal growth will be interrupted. This feedback mechanism ensures that the size of the niche in the lymph node remains adapted to the size of the ongoing immune response.

Reestablishment of Quiescence

Whereas relative CD11c^{med}MHCII^{hi} migratory APC accumulation occurs rapidly and peaks early, CD11c^{hi}MHCII^{med} DCs steadily increase over time [11, 38](Figure 2D–E). Work in our lab has shown that depletion of about 50% of CD11c^{hi}MHCII^{med} cells during the re-establishment of quiescence led to an increase in BrdU incorporation by endothelial cells compared to undepleted mice but no increase in endothelial cell numbers even over the subsequent 11 days, suggesting the possibility that endothelial cells were dying but were also actively dividing to maintain their numbers. Consistent with the idea that DC depletion had injured the vascular-stromal compartment, HEV endothelial cells showed a more activated phenotype, with increased VCAM-1, and the blood vasculature was leakier. The perivascular reticular cells that normally reorganized more tightly around the vessels during this phase instead appeared more loosely bound, suggesting the possibility that DCs maintained vascular stabilization by acting on FRCs. The DC depletion over 2 days also reduced germinal center B cell numbers and plasma cells [11]. As FRCs can express survival factors for lymphocytes [26, 27] and DCs are associated with FRCs throughout the lymph

node [8], these data suggested that DCs might also regulate the function of reticular cells beyond that of the perivascular reticular cells during re-establishment of quiescence to support germinal center maintenance and the plasma cell response.

We used additional DC depletion strategies to understand the effects on reticular cells. In mature hematopoietic cells, expression of the transcription factor *Zbtb46* specifically marks CDP-derived DCs at homeostasis and also some monocyte-derived DCs during inflammation [64, 65]. Depletion of *Zbtb46*⁺ DCs or of non-T non-B *CD11c*⁺ cells at day eight after immunization with OVA+Alum resulted in loss of most of the *CD11c*^{hi}*MHCII*^{med} DCs and about 50% of *CD11c*^{med}*MHCII*^{hi} APCs. Within a day, these depletions led to a ~50% loss of PDPN⁺ reticular cells. When subsets were analyzed individually based on expression of CXCL13, CCL21, and CD35 (see Figure 1), it was apparent that all compartments were affected—FRCs of the follicles, T zone, and medulla as well as FDCs within germinal centers. DCs maintained PDPN⁺ reticular cell survival via *LTβR* ligands *LTα1β2* and *LIGHT*, and *LTβR* stimulation was sufficient to prevent DC depletion-induced loss of PDPN⁺ reticular cells and much of the loss of germinal center B cells and plasma cells. Taken together, these observations suggest that a DC-stromal axis supports ongoing B cell responses [38].

On the FRCs, *LTβR* maintained survival by maintaining high levels of PDPN [38]. This indicated that PDPN had a pro-survival function, which at first seemed to be unrelated to or even contradict its role in contractility [40] and limiting proliferation [45]. However, PDPN was also shown to promote cell-matrix adhesion [45, 66], and cell contractility is linked to cell adhesion [67]. The function of cell-matrix adhesion, which is mediated by integrins and downstream signaling to phosphorylate focal adhesion kinase (FAK) is context-dependent. Under certain conditions, cell adhesion and proliferation can be inversely correlated and adhesion can signal to limit proliferation [68–70]. In contrast, under stressful pro-apoptotic conditions, such as exposure to TNF or chemotherapeutics, cell adhesion can be upregulated to maintain survival [71, 72]. PDPN⁺ reticular cells showed increased phosphorylation of FAK at day eight after immunization compared to homeostasis. This result suggests that these reticular cells in the stimulated node were ‘stressed’ and could be more dependent on integrins for survival. Indeed, similar to the effects of blocking PDPN, *β1* integrin blockade in vivo at homeostasis and day eight had different effects. At homeostasis, *β1* integrin blockade for 24 hours did not reduce reticular cell numbers, and the spread of the numbers were suggestive of the beginning of an increase. Administration of anti-*β1* at day eight however disrupted reticular cell survival and caused a reduction in their numbers [38]. Together, these results suggested that, for reasons yet to be determined, the lymph node milieu is more stressful for reticular cells at day eight after immunization compared to homeostasis. In this context, DCs, via *LTβR* ligands, enable PDPN⁺ reticular cells to respond by upregulating PDPN, ultimately increasing integrin activation and cell matrix adhesion to maintain their survival.

There remain more questions to be answered. Because of increasing accumulation of *CD11c*^{hi}*MHCII*^{med} during the re-establishment of quiescence and because this subset was most completely depleted in the depletion models used, *CD11c*^{hi}*MHCII*^{med} DCs were implicated in maintaining reticular cell survival. However, this population can include both

resident DCs and migratory DCs—is one population more important? That DC-derived LT β R ligands are key mediators suggests that reticular cell survival is mediated by non-canonical NF- κ B signaling, which is dependent on prolonged receptor stimulation [73]. This would suggest that resident DCs may be more important, as resident DCs are more tightly associated with reticular cells [8] and thus may deliver more effective signals. Also, is there inhibition of PDPN function by DC-derived CLEC-2 during the re-establishment of quiescence? Whether this inhibition of PDPN function operates simultaneously with the increase in PDPN levels by DC-derived LT β R ligands remains to be determined.

Other outstanding issues include better understanding of FRC function and regulation during the re-establishment of quiescence—the specific changes that endow FRCs with DC-dependence, the environmental drivers that induce the DC-dependent state. Furthermore, as the stromal cell field better delineates the subsets of reticular cells and endothelial cells in secondary lymphoid organs and the specific functions they may have in supporting different aspects of immune responses—effector T cell function vs T cell tolerance or regulatory T cells, for example—it will be interesting to understand if regulation of growth and remodeling by DCs affects particular subsets more than others [31, 33, 49, 74–78]. This may then allow specific modulation of endothelial and reticular cell subsets that contribute to pathologic responses while preserving potentially important regulatory responses.

Implications for Disease Treatment

Although much remains to be understood about how DCs modulate the vascular-stromal compartment during immune responses, even our current state of understanding can potentially be harnessed to better understand and treat disease. For example, systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the generation of autoantibodies which can deposit and cause inflammation in end organs such as the kidneys and skin [79]. Reflective of systemic immune activation, SLE patients commonly have splenomegaly and lymphadenopathy (i.e. enlarged spleen and lymph nodes), with enlarged follicles, germinal centers, plasma cells, and vascular proliferation [79–81]. We studied the chronically enlarged lymph nodes in the MRL/lpr lupus mouse model to better understand the vascular alterations in this disease model. The endothelial cells appeared to be in a state of re-established quiescence, with expanded numbers and a low but above baseline level of proliferation. The accumulation of CD11c^hMHCII^{medi} cells added to this idea [82]. This suggests that the re-establishment of quiescence is a relevant phase to study for the understanding of chronic lymph node conditions and raises the possibility of a DC-stromal axis that maintains pathologic responses and can be targeted. Although this hypothesis needs to be directly experimentally tested, administration of SU5416, a receptor tyrosine kinase inhibitor that has activity against VEGF receptors, Flt3, and platelet derived growth factor (PDGF) receptors [83], among others, only transiently reduced endothelial cell numbers but was able to significantly prevent the accumulation of circulating autoantibodies and reduce autoantibody-secreting plasma cells in lymph nodes. SU5416 treatment also disrupted ectopic follicles comprised of a central germinal center and a corona of CD4–CD8- double negative T cells that are also found in human lupus and that can induce autoantibody generation [82, 84, 85]. The disruption of the ectopic follicles was associated with encroaching of the T zone stroma into the FDCs of the ectopic follicles, suggesting the

possibility that SU5416 had altered stromal integrity to disrupt autoantibody generation. Consistent with the idea of a DC-stromal axis that supports pathogenic B cell responses, constitutive ablation of CD11c⁺ cells in MRL/lpr mice did not affect T cell activation but markedly diminished plasma cell numbers and autoantibody production [86]. Of note, SU5416 is a precursor to orally available Sunitinib which is already FDA approved for renal cell carcinoma [83]. If experimental evidence indicates that there is a DC-stromal axis that can be targeted by Sunitinib, there is the potential that Sunitinib could be fast tracked for approval for an alternate disease such as lupus.

Tertiary lymphoid organs found at sites of chronic inflammation resemble inflamed secondary lymphoid organs and have a similar vascular-stromal compartment [87, 88]. DCs have been shown to support lymphangiogenesis in a thyroid tertiary lymphoid organ (TLO) model and to maintain TLOs in pulmonary infection models [89–91], suggesting a role for DCs in different phases of TLO formation and maintenance similar to the vascular-stromal regulation in lymph nodes. TLOs can generate potentially pathogenic antibodies or harbor pathogenic T cells and are a feature of Sjogren's syndrome, and detected in kidneys in lupus, inflamed synovium of rheumatoid arthritis, the central nervous system in multiple sclerosis, and in lung of rheumatoid arthritis patients and allergic airway disease [92–98]. Being able to target a DC-stromal axis, then, may have implications for disrupting an even wider range of pathologic conditions.

Concluding Remarks

The studies described above have greatly increased our understanding of the role of DCs in lymph node vascular-stromal regulation. Cumulatively, the studies indicate that DCs, in addition to their well-known function as antigen-presenting cells, enable efficient immune responses by orchestrating the dynamic growth and remodeling of the vascular-stromal infrastructure and the lymph node niche. We are still in the early stages of our understanding of the vascular-stromal compartment and of the roles of DCs in modulating this compartment. Many exciting questions remain to be addressed (see Outstanding Questions box), and the insights gained will help us better understand the immune system and potentially allow us to better treat disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Box 1**Interactions of Immune Cells and the Vascular-Stromal Compartment**

Functionally, each component of the vascular-stromal compartment helps to promote and regulate immune responses in complementary ways. Besides supplying oxygen and micronutrients, the blood vessels bring in circulating cells via the HEVs. HEV endothelial cells express chemokines such as CCL21 and cell adhesion molecules such as peripheral node addressin (PNA^d) that allow for regulated entry of recirculating lymphocytes and, with inflammation, monocytes and NK cells [16]. HEVs also support the entry of pre-dendritic cells (pre-DCs), precursors of resident DCs that develop from common DC progenitors (CDPs) in a Fms-related tyrosine kinase 3 (Flt3)-dependent manner [17]. The afferent lymphatics relay the events occurring at the periphery, bringing in antigen-presenting cells, antigen, effector and memory T cells, and regulatory T cells from the draining tissue [16, 18, 19]. The efferent lymphatic sinuses, on the other hand, have high levels of sphingosine-1-phosphate (S1P) produced by lymphatic endothelial cells that promotes egress from the lymph node [16, 18]. Reticular cells express the matrix molecules of the reticular network but also provide guidance cues for cells within the lymph node parenchyma. CXCL13 localizes cells to the B cell follicles and is expressed by follicular dendritic cells (FDCs), specialized reticular cells that present antigen to B cells and that are required for germinal center maintenance [20] (Figure 1, inset B). CXCL13 is also expressed by a FRC subpopulation known as marginal reticular cells (MRCs), and, in inflamed lymph nodes, a subpopulation of follicular FRCs [21, 22] (Figure 1, inset B). FRCs in the T zone express CCL19 and CCL21 for localization of CCR7-expressing T cells and DCs and for lymph node retention of T cells in balance with egress-promoting S1P [18, 23–25] (Figure 1, inset A). Reticular cells also express survival factors such as B-cell activating factor (BAFF) for B cells [26] and IL-7 for T cells [27]. Lymphatic endothelial cells can also express IL-7 [28], and both FRCs and lymphatic endothelial cells can express regulatory molecules and self-antigen to further shape lymphocyte responses [29–33].

Outstanding questions

What are the exact DC subsets involved in vascular-stromal regulation during the different phases of vascular-stromal growth and remodeling?

During the re-establishment of quiescence, FRCs switch to a DC-dependent state. What are the phenotypic markers of this state? What are the environmental drivers involved in this switch?

Is there a DC-stromal axis in systemic autoimmune diseases such as lupus that can be targeted?

DCs also appear to play a key role in the regulation of the vascular-stroma compartment of TLOs. Can this interaction be targeted in therapeutic approaches?

Trends Box

The lymph node vascular-stromal compartment undergoes distinct phases of growth and remodeling during immune responses.

DCs contribute to the initiation of vascular-stromal growth and remodeling by mediating vascular-stromal proliferation and facilitating FRC relaxation, allowing lymph nodes to grow

During the re-establishment of quiescence, DCs maintain the niche for the ongoing immune response by promoting FRC survival

Figure 1. Lymph Node Organization and the Vascular-Stromal Compartment
Schematic of lymph node showing the architecture and vascular-stromal elements. Note that the blood flow enters at the hilus as a feeding arteriole that gives off branches in the medulla and T zone that then lead to capillary beds (represented by the loop where the red vessel becomes blue). Blood flows from the capillary beds into the postcapillary high-endothelial venules (HEVs) (represented by the thickened blue segment). Blood flows from the HEVs into veins that join together to exit at the hilus. Note proximity of the blood vasculature to

lymphatic sinuses, forming “paracortical cords” centered around these vascular-rich areas that run from the subcapsular sinus to the medulla [5, 9].

(Inset A) Schematic of cross sectional view of the vascular-stromal elements in the T zone. An HEV and lymphatic sinus are in close proximity. The HEV is ensheathed by pericyte-like cells and then by fibroblastic reticular cells (FRCs) [10]. The FRCs also ensheath reticular fibers and lymphatic sinuses [8, 9, 11]. The FRCs in the T zone express the chemokines CCL21 and CCL19 [23,24], directing CCR7-expressing T cells and dendritic cells to the T zone [25].

(Inset B) Schematic of the stromal elements in B cell follicles. CXCL13 is expressed by a subset of FRCs known as marginal reticular cells (MRCs) and by follicular dendritic cells (FDCs) [20, 22]. There are also FRCs in the mantle zone (the follicular area around the germinal center) which express CXCL13 in secondary follicles [21]. CXCL13 localizes CXCR5-expressing cells such as B cells and T follicular helper cells to the follicles [99, 100]. In contrast to the T zone and B cell follicles, FRCs in the medulla express neither CXCL13 nor CCL21 [20, 101]. Note that “PDPN+ reticular cells” (PDPN+ RC) includes FRCs and FDCs. HEV BEC=HEV blood vessel endothelial cell; LEC=lymphatic endothelial cell.

Figure 2. Distinct Phases of Lymph Node Vascular-Stromal Growth and Remodeling During Immune Responses and Association with Distinct DC Subsets

(A–D) Shown are the changes that occur with immunization with ovalbumin + complete Freund's adjuvant (OVA/CFA) and OVA/Alum to illustrate common features of vascular-stromal growth across different immunization regimens. The graphs incorporate data from [11], [38],[36] and unpublished work (DCD, SC and TTL). (A) Lymph node cellularity. (B) Endothelial cell (EC) and podoplanin (PDPN)+ reticular cell proliferation rate as determined by the percentage of cells that take up bromodeoxyuridine (BrdU) over the 18–24 hours preceding sacrifice and flow cytometric analysis. Analysis of blood vessel endothelial cell

(BEC, CD45–CD31+PDPN–), lymphatic endothelial cell (LEC, CD45–CD31+PDPN+), and PDPN+ reticular cell (RC, CD45–CD31–PDPN+) analysis is shown. Note that fibroblastic reticular cells (FRCs) comprise the majority of the PDPN+ RC population detected by flow cytometry while follicular dendritic cells (FDCs) comprise a variable but smaller percentage (0% at homeostasis to nearly 30% in inflamed nodes (unpublished data and [38])). Thus the changes in PDPN+ RC proliferation and number likely reflects that of FRCs. (C) EC and PDPN+ RC numbers. (D) Percentages of CD11c_{hi}MHCII_{med} and CD11c_{med}MHCII_{hi} cells. (E) Illustration of the relative DC subsets and associated vascular-stromal states over time. During the initiation phase, CD11c_{med}MHCII_{hi} cell accumulate, the FRCs around high endothelia venules (HEVs) become more loosely organized, and the reticular network shows relaxation as the lymph node enlarges. Expansion is not depicted. During the re-establishment of quiescence, a more homeostatic-like balance between the two DC populations is restored, the endothelial cells and FRCs are now more numerous and the FRCs become more tightly associated with vessels.

Table 1

Summary of DC-mediated lymph node vascular-stromal regulation

cells	Effect	Mechanism	Direct effect of DC or mediator on vascular-stromal cells shown?
Homeostasis			
CD11c+ cells	Maintain low basal endothelial cell proliferation rate [11]	Not known	no
DCs	Maintain HEV phenotype and lymphocyte trafficking function [58]	DC-derived LT α .1 β 2 [58]	yes
Migratory DCs	Maintain HEV numbers and FRC CCL21 expression [59]		no
Initiation			
DCs implicated	Promote expansion of LN feeding arteriole [44]		no
Migratory DCs	Inhibit FRC contractility leading to reticular network relaxation [40, 45] and ?HEV permeability.	DC-derived CLEC-2 inhibits PDPN-mediated contractility and adhesion [40, 45]	yes
DCs T/B cell-independent	Upregulate EC and FRC proliferation [37]	DCs induce FRC VEGF expression that promotes EC proliferation [36]	yes
Migratory DCs/monocytes	Contribute to upregulating EC proliferation [57]	DC- and monocyte-derived IL1 β contributes to inducing FRC VEGF expression [57]	yes
Expansion			
DC role unknown; T cells	FRC proliferation and expansion [36] [41]	LT β R ligands [41]	no
DC role unknown; B cells	Lymphatic/HEV/BEC expansion [35, 36, 48]	B cell-derived LT α .1 β 2 is required for HEV expansion [35]	no
Reestablishment of Quiescence			
CD11c ^{hi} MHCII ^{med} DC	Mediate vascular quiescence and stabilization [11]	Mediate reorganization of reticular cells around vessels [11]	no
Zbtb46+ DC	Maintain the survival of FRCs and FDCs [38]	DC-derived LT α .1 β 2 and LIGHT maintains FRC PDPN, leading to β 1 integrin activation and increased survival [38]	yes