Lymphatic Vessels, Inflammation, and Immunity in Skin Cancer

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INTRODUCTION

Skin is the largest organ in mammals, serving as a physical and immunologic barrier to the external environment (1). As an immune organ, skin coordinates rapid responses to external challenge through constitutive immune surveillance involving both resident and recruited leukocytes (2). Exquisitely spatiotemporal regulation of immune cells is required for homeostatic tissue maintenance—loss of function is associated with a variety of dermatopathologies, including chronic infection, inflammation, autoimmunity, and cancer (1). The interplay between local inflammation, tissue remodeling, and antitumor immune responses is of particular interest given the recent success of immunotherapies, namely blockade of immune checkpoint molecules (3). Because tumor microenvironments in part regulate antitumor immunity, these also present targets for release of local immunosuppression that may synergize with checkpoint therapies (4). Although tumor-associated blood vessels are appreciated for their role in regulating leukocyte infiltration and function (5), tumor-associated lymphangiogenesis has lagged behind with respect to recognition of its role(s) in tumor-associated inflammation and immunity. Recent revelations, however, have highlighted these important roles with respect to coordination of local inflammation (6) and immunity (7–11), indicating that their deregulation may similarly influence inflammation-induced tumor progression and metastasis.

In this review, we discuss clinical and experimental evidence revealing the significant inflammatory and immunomodulatory functions of lymphatic vasculature, and suggest how tumor-associated remodeling may alter regional inflammation and immunity, thereby contributing to disease progression and metastasis. In addition, we suggest that lymphatic vessel morphology and function may be a biomarker of cutaneous immune status, and discuss how lymphatic biomarkers may provide insight into diagnosis and therapy for cutaneous malignancy.

INFLAMMATION AND CANCER

It is now well appreciated that environmental exposures account for the occurrence of many different types of cancer, with 15% to 20% of cancer-related deaths linked to inflammation or infection (12). Environmental factors include...
infectious agents, physical trauma (nonhealing scars, burns), industrial toxins (e.g., tar, arsenic, radiotherapy), as well as those associated with lifestyle (tobacco, alcohol, diet, ultraviolet exposure; ref. 13). As one might expect, tumors whose etiology is associated with environment-induced inflammation often occur in tissues with the greatest surface area, including lung, skin, and gastrointestinal tract (14). Inflammation is considered one of 10 hallmarks of cancer and is increasingly appreciated for its complex role in tumor initiation, promotion, malignant conversion, and metastasis (15). Furthermore, immune infiltrates provide prognostic biomarkers (16) and potent targets for cancer therapy (17). We are just beginning to understand, however, how complex interactions between environmental agents, genetic factors, and tissue microenvironments coordinate local inflammation and subsequent tumorigenesis.

**SKIN INFLAMMATION AND CANCER**

Skin is a stratified tissue composed of epidermal, dermal, and subcutaneous adipose layers functioning in concert as a barrier to the external environment (1). Keratinocytes are the primary cell type in the epidermis and undergo continuous cycles of homeostatic proliferation and differentiation to maintain a cornified layer protecting against water loss and microbe penetration (18). Melanocytes reside in basal layers of the epidermis. The dermis is largely composed of collagenous and noncollagenous (e.g., elastic fibers and structural proteoglycans) connective tissue components that provide substratum support for blood and lymphatic vessels, nerves, mesenchymal support cells (fibroblasts), and resident immune cells. The cellular components of these layers critically regulate immune surveillance to facilitate rapid responses to insult while, importantly, maintaining immune tolerance (2, 19)—breakdown of this balance is associated with a diverse set of immune-mediated dermatopathologies (1).

There are four major types of skin cancer: basal cell carcinoma, squamous cell carcinoma, melanoma, and nonneoplastic skin cancers (e.g., Merkel cell carcinoma, Kaposi sarcoma). Predisposition to skin cancer is associated with chronic cutaneous inflammation (e.g., discoid lupus erythematosus, chronic wounds, dystrophic epidermolysis bullosa), viral infections [e.g., human immunodeficiency virus, human herpes virus 8, and human papillomavirus (HPV)], and inflammatory environmental agents [e.g., UV radiation (UVR), viral infection, aging, diet, smoking, radiotherapy, phototherapy, physical trauma; ref. 13], with inflammation playing a well-documented role in regulating progression of these cutaneous malignancies (20); however, skin cancers are also associated with chronically injured or nonhealing scars, burns, and chronic friction, with incidence of malignancy in scar tissue being low (0.1%–2.5%; ref. 13).

Furthermore, cells in skin secrete diverse inflammatory mediators, including Toll-like receptor (TLR) ligands, polypeptide growth factors, for example, TNFα, as well as chemokines that direct trafficking and subsequent activation of recruited leukocytes, many of which have been implicated as mediators of tumor progression in skin (20). For example, mast cells foster skin carcinogenesis through the release of prosurvival and proangiogenic polypeptide growth factors and matrix remodeling proteases that activate proliferative programs in keratinocytes, fibroblasts, and endothelial cells (21). B cells secrete (auto)antibodies that form immune complexes with complement proteins—these accumulate in skin and influence resident and recruited myeloid cells’ activation and function (20). Tumor-infiltrating leukocytes play an important role in regulating angiogenic responses, which, in turn, regulate their entry (22), and thereby influence metastatic potential (23). Although much focus has been placed on blood vasculature with respect to leukocyte infiltration and function, the lymphatic endothelium may similarly participate in these complex interactions. In support of this, using a murine model of chemically induced skin carcinogenesis, it was recently reported that lymphatic vessels and their subsequent drainage are required for induction of local leukocytic infiltration, and consequently their absence resulted in the initiation of fewer tumors (6). Given our current understanding of how lymphatic vessels participate in regulating local inflammation and immunity, as described below, it is reasonable to hypothesize that tumor-associated lymphatic remodeling participates in shaping immune microenvironments of solid tumors.

**LYMPHATIC VESSELS AND REGIONAL INFLAMMATION**

Lymphatic vessel architecture dictates local flow and the unidirectional transport of peripheral information to the secondary lymphoid system (24). Lymphatic vessels develop as a hierarchical, one-way drainage system functioning to transport cells and fluid into the lymphoid system, and as a result of this architecture, lymphatic vessels lie at the interface of tissue biology and immunology (24). In skin, lymphatic vessels form two plexuses, one superficial extending into dermal papillae, and the other near the subpapillary arterial network (Fig. 1A). Initial lymphatic capillaries are small, blind-ended vessels with minimal basement membrane that directly attach to extracellular matrix (ECM) through anchoring filaments, allowing for rapid response to changes in local interstitial fluid pressure (IFF); swelling of ECM due to edema pulls on these filaments, opening the loose, button-like interendothelial junctions (Fig. 1B; refs. 25–28). Lymphatic capillaries drain into collecting vessels characterized by a continuous basement membrane, smooth muscle coverage, and a system of valves that prevent retrograde fluid flow (Fig. 1C). Lymphatic dysfunction results in accumulation of protein-rich interstitial fluid (lymphedema), leading to progressive fibrosis, adipose deposition, and inflammation (29). Although lymphatic vessels are required for drainage of soluble antigen and trafficking of activated dendritic cells (DC) to local lymph nodes (LN), their contribution to other aspects of inflammation and immunity is just being explored. There have been several excellent reviews highlighting current knowledge of the contribution of lymphatic vessels to inflammation (30) and tolerance (31). Herein, we highlight their roles in immune induction and resolution or tolerance (Fig. 2A–D), and discuss how their dysfunction contributes to tumor progression and metastasis. Of note, as many of these studies were not performed in skin, it remains to be seen how many of these mechanisms will translate across tissue systems.
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Figure 1. Structure and function of initial and collecting lymphatic vessels.

A, the lymphatic vessels of skin are composed of two plexuses, one superficial which extends into the dermal papillae near the subpapillary arterial network, which drains vertically into the deep lymphatic plexus below the second arterial network. B, initial lymphatic capillaries are blind-ended vessels with discontinuous basement membrane and no associated smooth muscle cells (SMC). At resting state, the lymphatic endothelial cells that comprise the initial capillaries are characterized by unique overlapping, button-like junctions that allow for passive flow and leukocyte trafficking through interendothelial gaps in an integrin-independent manner. Local inflammation results in vascular leakiness driving increased IFP and enhanced flows. At least in the mouse respiratory tract, inflammation is associated with a remodeling of the interendothelial junctions of initial capillaries into tight, zipper-like junctions. Lymphatic capillaries are anchored directly to the ECM through anchoring filaments, such that under high levels of IFP, stretching of ECM results in distension of initial capillaries and enhanced fluid flows and cellular trafficking both by intercellular and transcellular mechanisms. C, collecting vessels are larger vessels that have both a continuous basement membrane and SMC coverage. Collecting vessels are notably defined by the presence of a system of valves, which separates the vessel into functional units or lymphangions. SMCs mediate contraction of individual lymphangions that drives the opening of downstream valves while closing valves immediately upstream. This system of local contraction and relaxation drives unidirectional fluid flows from peripheral tissues to draining LNs.
Lymphatic vessels, inflammation, and immunity. A, homeostatic lymphatic capillaries support immune surveillance through steady-state homing of resident immune cells, including DCs and some subsets of memory T cells. B, local inflammation and damage activate a series of danger signaling as well as increased IFPs that activate initial lymphatic capillaries, resulting in remodeling (either proliferative or nonproliferative), upregulation of adhesion molecules, and enhanced expression of the homing chemokine C–C motif ligand 21 (CCL21). Altered adhesions and CCL21 coordinate to facilitate entry of activated CCR7+ DCs into afferent lymphatic vessels and migration toward draining LNs where they interact with and activate naive T cells. The decoy receptor D6 ensures proper presentation of homeostatic chemokines by lymphatic endothelial cells (LEC) by scavenging inflammatory chemokines to specifically facilitate mature over immature DC migration. Changes in lymphatic flows that result from altered signaling in both initial capillaries and collecting vessels may influence accumulation of inflammatory cytokines that help to perpetuate local inflammation leading to infiltration and accumulation of leukocytes in tissue, which further drive lymphatic remodeling. C, although important for immune induction, evidence also indicates that lymphatic capillaries importantly regulate resolution of local inflammation and immunity through leukocyte egress and chemokine sequestration. Both macrophages and some T cells exit peripheral tissue through draining lymphatic capillaries using CCL21 and sphingosine kinase (SPHK) conversion of sphingosine into sphingosine-1-phosphate (S1P) as signals for their exit, all produced by initial lymphatic vessels. Cellular exit is required for resolution of disease. ICAM1, intracellular adhesion molecule 1; LFA1, lymphocyte function associated antigen 1. D, novel immunomodulatory roles of LECs have been described, largely in the context of lymphoid organs. LECs inhibit both antigen-dependent and independent T-cell activation through production of nitric oxide (NO) and nonspecific inhibition of DC–T-cell interactions. Inflamed LECs inhibit maturation of DCs through ICAM1 and receive peptide-loaded MHCII complexes from mature DCs. In addition, LECs promiscuously present endogenous and scavenge exogenous antigen for cross-presentation on MHCI molecules and direct deletion of antigen-specific CD8+ T cells.

Inflammation and Immune Induction

Lymphatic vessels in adult tissues are typically quiescent, but may become activated when inflamed, resulting in lymphatic vessel remodeling within peripheral tissue and draining LNs (26). Lymphatic remodeling is defined as changes to lymphatic vessel structure and morphology through either mechanisms of proliferative lymphangiogenesis or lymphatic vessel enlargement (26). Lymphangiogenesis specifically describes the formation of new lymphatic vessels from pre-existing vessels and occurs in many experimental and clinical inflammatory diseases, including transplant rejection (renal and cornea), inflammatory bowel disease, chronic airway...
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VEGFs and their receptors are largely responsible for the development, sprouting, and remodeling of lymphatic vessels (26). Both hematopoietic and nonhematopoietic cells in skin orchestrate lymphangiogenic responses during inflammation. Epithelial cells in skin and fibroblastic reticular cells in LNs support VEGFA-dependent lymphangiogenesis (34, 36), whereas macrophages induce local lymphangiogenesis through secretion of VEGFC that, in turn, signals through where macrophages induce local lymphangiogenesis (40), but inflammatory lymphangiogenesis in draining LNs (40), but can be compensated for by neutrophils, likely through modulation of VEGFA bioavailability and some VEGFD production (41). In contrast, T cells can inhibit de novo lymphangiogenesis in an IFNγ-dependent manner, at least in LNs (42). Importantly, at least in the respiratory tract, inflammatory lymphangiogenesis is characterized by remodeling of the loose, button-like interendothelial junctions into tight, zipper-like junctions, characterized by firm adhesion along the length of two neighboring cells (Fig. 1A) that may have functional consequences for mechanisms of immune cell entry and fluid transport (25, 28).

Mobilization of DCs toward draining afferent lymphatic vessels is dependent on expression of the C–C chemokine receptor 7 (CCR7), which allows for active homing toward the C–C motif ligand 21 (CCL21) and CCL19-producing lymphatic vasculature (43). In addition to CCL21/CCL19, other chemokine signals required for cell entry into afferent lymph include chemokine C–X–C motif chemokine ligand 12 (CXCCL12), sphingosine-1-phosphate (SIP), CX3CL1, and the decoy receptor D6 (also known as CCBP2, ref. 44). Also involved are adhesion molecules, including intracellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), L1CAM (CD171), ALCAM (CD166), Cup-like lectin receptor (CLEC2), CD31, semaphorins, CD73, and the scavenger receptor CLEVER1 (44, 45). Whether integrins are absolutely required for DC transmigration is debatable, but is likely a function of inflammatory context and lymphatic endothelial cell (LEC) activation status (45–49). Cytokines, TLR ligands, and interstitial fluid flows alter expression of adhesion molecules on LECs in order to promote leukocyte migration (44), and LECs derived from various inflammatory contexts acquire distinct transcriptional programs (50), indicating that LEC function and leukocyte trafficking patterns may be inflammation specific. Furthermore, the endothelial glycoprotein plasminogen activator vesicle-associated protein (PLVAP) expressed by LECs controls entry of both lymphocytes and antigens into LNs (51). The PLVAP protein acts as a diaphragsm spanning transendothelial channels that transect sinus-lining LECs and confer selectivity of the sinus-parenchyma barrier (51). This selectivity may provide a way for the host to segregate small, likely inert proteins that enter the LN conduit system (52) from larger agents that might cause damage if disseminated systemically.

One class of proteins that regulate or tune innate and adaptive immunity are the chemokine decoy receptors that scav-enge and sequester chemokines to decrease local inflammatory signaling (53). This subfamily of “silent” chemokine receptors includes Duffy antigen receptor for chemokines (DARC), D6, and CCX-CKR (also known as CCR1L) and are strategically expressed in distinct cellular contexts (e.g., DCs and endothelial cells), where they regulate spatiotemporal inflammatory β-chemokine signaling (53). D6 is predominantly expressed by LECs (skin, gut, lung, and syncytiotrophoblast layer of the placenta)–D6-null mice are unable to properly resolve local acute inflammation following dermal challenge due to exaggerated cutaneous inflammatory responses characterized by an accumulation of β-inflammatory chemokines at sites of inflammation (54), a process that may prevent further leukocyte recruitment. Furthermore, D6, induced during inflammation by IL6 and IFNγ, facilitates selective presentation of homeostatic chemokines (e.g., CCL21) over inflammatory chemokines to prevent inappropriate inflammatory cell attachment to LECs and proper selection of mature over immature DCs (55). Extensive perilymphatic accumulation of leukocytes is observed in D6-null mice in both peripheral sites of inflammation and draining LNs, resulting in lymphatic congestion and impaired transport of antigen-presenting cells and fluid (56). Importantly, D6 is downregulated in several human malignancies, including Kaposi sarcoma, a cutaneous malignancy of lymphatic endothelial origin, where low levels of D6 are associated with disease aggressiveness and infiltration of proangiogenic macrophages (57).

Lymphatic control of DC trafficking through both adhesion molecules and chemokines is a finely regulated, multiprocess (45, 49). Furthermore, given the intimate relationship peripheral afferent lymphatic vessels have with egressing leukocytes (45), it remains plausible that egressing leukocytes are educated by afferent lymphatic vessels as they exit peripheral tissue. The characterization of specialized three-dimensional structures at the interface of DC/LEC adhesions (58), intralymphatic crawling of DCs following entry into peripheral initial capillaries (59), acquisition of peptide:MHCII complexes by LECs from DCs (60), and LEC-mediated inhibition of DC maturation (61) all suggest that long-lived intercellular interactions between egressing leukocytes and peripheral afferent lymphatic vessels may be a novel control point for leukocyte function.

Tissue Egress and Immune Resolution

In addition to providing an initial route for entry of newly activated DCs into the afferent lymph, lymphatic vessels may also provide an important route of egress during resolution of acute and chronic inflammation. Consistent with this idea, induction of lymphatic vessel growth in skin is protective against acute and chronic skin inflammation (62, 63), and inhibition of vessel growth in experimental inflammatory bowel disease exacerbates inflammation and disease progression (35). In murine inflammatory bowel disease, macrophage egress from tissue in response to VEGFC-driven lymphangiogenesis is critical for limiting local inflammation (35). Similarly, in a mouse model of chronic respiratory tract infection, airway inflammation promoted bronchial lymphedema and airway obstruction when lymphangiogenesis was impaired (64). Furthermore, homeostatic gut leukocytes broadly migrate to draining LNs (65), whereas during...
cutaneous immunity, regulatory T cells preferentially egress from skin (66). Taken all together, dynamic patterns of leukocyte infiltration and egress appear to contribute to disease, and control over leukocyte trafficking and tissue egress may be a novel point of immune regulation (67). Such a strategy is used by viruses to mediate immune escape (67). IFNα production following viral infection (68) and local skin irradiation (69) drives accumulation of cells in lymphoid organs, resulting in decreased peripheral blood and thoracic duct lymphocytes and overall immune suppression. Egress from peripheral tissues appears tightly controlled by a combination of CCR7 and S1P signaling, although coordination of these signals is poorly understood. In models of lung and skin infection, T-cell egress from acutely inflamed peripheral tissue is dependent on CCL21/CCR7 chemotactic signals (70, 71), whereas chronic inflammation adopts CCR7-independent mechanisms for tissue exit (72). Tissue exit is likely required for recirculation of non–autograft-specific T cells recruited to sites of inflammation. Consistent with this, an interplay exists between antigen recognition and egress, where T-cell receptor (TCR) stimulation results in downregulation of CCR7 and tissue retention (67). In solid tumors, at least in some cases, where antigen recognition can be limited because of physical constraints of tumor microenvironments as well as lack of potent neoepitopes for TCR recognition (73), cellular egress from tissues may correlate with poor local immunogeneity.

SIP gradients also importantly regulate tissue entry and exit throughout the body (e.g., blood to lymphoid tissue to lymph to peripheral tissue; ref. 74). Lymphatic vessels express sphingosine kinase and maintain high levels of S1P in lymph-generating gradients to direct tissue egress at steady state (75). Increased levels of S1P are present in inflamed peripheral tissues, and this high tissue concentration may play a role in T-cell retention (76). The S1P receptor SIP1 inhibits migration of T lymphocytes into afferent lymphatic vessels both at steady state and during inflammation, resulting in their retention in nonlymphoid tissues at least partially through lymphocyte function associated antigen 1 (LFA1)/VCAM1 and very late antigen 4 (VLA4)/VCAM1 interactions (76). The interplay between these signals provides a novel mechanism of control over leukocyte accumulation within peripheral tissue and subsequent disease progression. All together, these mechanisms suggest that lymphatic vessel function influences accumulation and/or retention of leukocytes within inflamed tissue, thereby actively contributing to disease progression.

Tolerance

Although the role of the lymphatic vasculature in cellular trafficking is appreciated, novel functions of lymphatic vessels and endothelial cells comprising their structure are emerging and indicate a role for lymphatic vessels in local immunomodulation and tolerance (24). LECs inhibit T-cell activation and expansion through both antigen-dependent (7–10) and independent (77) mechanisms. Although central mechanisms of tolerance eliminate self-reactive T-cell clones during development in the thymus, education of the immune system is incomplete. As a consequence, tolerance must be maintained in the periphery to both environmental and self-antigens to prevent allergy and autoimmunity, respectively. Reminiscent of thymic epithelium, which deletes autoreactive T cells in the thymus, LECs express multiple peripheral tissue antigens and directly present to autoreactive T cells, albeit by a mechanism independent of the autoimmune regulator AIRE (9). Direct presentation of the melanocyte-specific protein tyrosinase on MHCI by LN LECs mediates deletion of tyrosinase-specific T cells (10). T-cell tolerance, in this context, is mediated by expression of the immune checkpoint molecule programmed death ligand 1 (PD-L1) on LECs and lack of co-stimulatory molecules; loss of PD-L1 activates tyrosinase-specific CD8 T-cell responses, resulting in experimental vitiligo (10). Although hyperactivation of melanocyte-specific T cells results in autoimmunity, vitiligo is prognostic for effective immunity during spontaneous regression of melanoma and following immunotherapy in patients with melanoma (78). Induction of anti-self responses, in either an autoimmune or neoplastic context, requires a break in natural mechanisms of peripheral tolerance in which lymphatic vessels and their expression of PD-L1 play at least some role. Overexpression of PD-L1 is a hallmark of adaptive immune resistance in melanoma and a potential barrier to immunotherapy (79). Although expression of PD-L1 is largely attributed to tumor cells, a wide variety of stromal cells also express PD-L1, and it remains to be determined how these players may contribute to immune suppression in tumor microenvironments (17).

In addition to direct presentation of peripheral antigens, LECs are phagocytic, scavenge lymph-borne antigen, and cross-present to MHCI in a transport associated with antigen processing 1 (TAP1)–dependent manner (7, 8). Again, presentation in this context results in lack of co-stimulation and dysfunctional T-cell activation characterized by reduced IFNγ production, high levels of PD-1 expression, and enhanced apoptosis (7, 8). Furthermore, overexpression of VEGF in murine melanomas results in immune protection against induced immunity, and specific cross-presentation of exogenous, tumor-associated antigens (ovalbumin; ref. 7). In both the tumor context and at steady state, presentation of the CD8 epitope for ovalbumin by tumor-educated LECs results in dysfunctional activation of antigen-specific CD8+ T cells (7, 8). Interestingly, phagocytic activity of LN-resident LECs has recently been reported in a vaccine model, where LECs were long-term depots for vaccine antigen (80). Although in these studies the authors did not demonstrate cross-presentation of antigen by LECs, this antigen capture and archiving provided enhanced protection against infection (80). Understanding the contextual cues driving LEC decision making with respect to antigen capture and presentation will be of undeniable importance moving forward with respect to vaccination strategies and understanding the role of persisting and chronic antigen exposure during vaccination, infection, and tumorigenesis.

ENVIRONMENTAL AGENTS AND LYMPHATIC FUNCTION

Under homeostatic conditions, lymphatic vessels facilitate acute immune induction followed by appropriate resolution as described above. However, environmental risk factors for malignancy (UVR, infection, and sustained trauma) also...
induce lymphatic vessel dysfunction and may disturb this critical immune balance in tissue (Fig. 3). In this way, regions of skin can acquire sectorial immune dysfunction, for example, immune compromised districts, over time due to accumulation of environmental insults even in the absence of systemic immune disorder (81). These “districts” exhibit enhanced risk for recapitulation of cutaneous disorders specifically at sites of trauma or insult (e.g., herpetic infections, irradiation, and burns; ref. 81), or instead can conversely be spared from systemic immune pathology (81), thus establishing regional discrepancies in immune function. How localized immune dysfunction may contribute directly to malignancy remains unclear; however, the coordinated dysfunction induced by these agents on both cells that acquire tumorogenic capacity and surrounding tissue stroma (including lymphatic vessels) is interesting to consider.

UVR is the most significant environmental risk factor for development of cutaneous malignancies (13). In addition to direct roles in initiating oncogenic mutations, UVR drives leukocyte infiltration of tissues, suppresses local T-cell function, and induces lymphatic vessel dysfunction (82). Clinically, lymphatic vessels are reduced in number and dilated in sun-damaged skin as compared with non-sun-exposed areas (83), and pathologically dilated lymphatic vessels are associated with severe photoaging (84). Similarly, in mice, acute exposure to UBV radiation induces leukocyte infiltration of skin associated with epidermal hyperplasia, erythema, and increased lymphatic vessel leakiness leading to edema (85, 86). Maintenance of lymphatic vessel integrity following UVR is, at least in part, due to tight junction molecules (claudin-5 and ZO-1), and administration of angiopeptin 1 (ANG1) improves integrity (85, 87). Disruption of claudin-5 junctions exacerbates not only edema associated with UBV exposure, but also accompanying leukocytic infiltrate (85), whereas overexpression of ANG1 is instead protective (87). Overexpression of VEGFC attenuates UBV-induced edema and leukocytic infiltrate through the promotion of local lymphangiogenesis (63).

A second environmental influence on lymphatic vessels is infection. Recurrent herpetic infections are a clinical cause of secondary lymphedema and are often associated with localized immune dysfunction (88). The majority of experimental data exploring neovascularization responses to viral infection in mice have focused on the cornea, a normally alymphatic tissue, where local induction of lymphangiogenesis is associated with wounding, graft rejection, and loss of immune privilege (32). In particular, herpes simplex virus 1 (HSV-1) infection induces genesis of lymphatic vessels into the cornea in a TLR-independent, VEGFA-dependent manner (89). In this model, lymphangiogenesis precedes angiogenesis and is maintained at later time points by VEGFC produced by local T cells (90). In addition to herpetic infections, skin infection by HPV similarly results in microscopic regions of lymphatic dysfunction marked by increased number and severe dilatation of local vessels (29). These regions are also associated with immune dysfunction and emergence of dermatopathology (clinical warts) associated with latent HPV infection following local trauma (29). HPV16-associated cancers exhibit lymphangiogenesis as well, and both remodeling of lymphatic vessels and expression of VEGFC are associated with increased risk and LN metastasis (91). In humans, lymphatic vessel density in squamous cell carcinomas is increased immediately adjacent to tumor nests (91). Similarly, in a model of murine de novo squamous cell carcinogenesis driven by HPV16 oncoproteins, premalignant lymphatic vessels are structurally altered but functional, and progressively lose drainage function in central regions of tumors, resulting in disruption of tissue hemodynamics (92).

Finally, surgical or other physical trauma results in disruption of regional lymphatic vessel networks (e.g., following radical mastectomy and LN resection), thereby leading to local lymphedema. The frequency of extremity (arm) lymphedema following surgical intervention for breast cancer ranges from 21% to 40%, depending on both the type of surgery and the use of adjuvant radiotherapy (29). Localized lymphedema in scar and adjacent tissues has been observed, and high incidence of bacterial and fungal infections at sites of amputation is indicative of impaired local immunity, with stump skin found to exhibit an altered microbiome as compared with nonamputated regions (88). The incidence of malignancy in scar tissue is 0.1% to 2.5% (13); however, the drivers of malignant conversion in these injured tissues and what role, if any, lymphatic function may play remains unknown.
Interestingly, VEGFC therapy may restore lymphatic function at least in some contexts to prevent surgery-associated lymphedema. Administration of growth factors along with LN transfer in pigs promoted robust growth of lymphatic vessels, helping to preserve transferred node structure and normalize lymphatic vascular anatomy (93).

LYMPHATIC VESSELS, INFLAMMATION, AND METASTASIS

Lymphatic vessels have been evaluated in the context of many solid tumors for their ability to predict locoregional, LN metastasis and overall survival (94). We limit our discussion here to the interplay between inflammation and tumor-associated lymphangiogenesis and direct the reader to recent reviews that cover mechanisms of lymphangiogenesis, lymphogenous tumor spread, and seeding in more detail (26, 27). The major molecular drivers of tumor-associated lymphangiogenesis are VEGFC and VEGFD, produced by both tumor and infiltrating myeloid cells (26). VEGFC and VEGFD exert their biologic effects through binding to VEGFR3 and VEGFR2, leading to activation of receptor-tyrosine kinase activity through autophosphorylation, which, in turn, activates aspects of LEC function and vessel formation (95, 96). VEGFC induces endothelial cell migration, permeability, proliferation, vessel enlargement, and enhanced trafficking of neoplastic cells to LNs, largely via VEGFR3-dependent mechanisms (96–100). VEGFR3 expression correlates with lymphatic metastasis in some human tumors, and high VEGFC and VEGFD expression is associated with increased lymphatic vessel density and lymphovascular invasion in both human tumors and animal models (97, 99–102), and consequently inhibition of either VEGFR3 or its coreceptor neuropilin 2 reduces incidence of LN metastasis (97, 103–105).

In skin cancer, the overwhelming majority of lymphatic vessel biomarker studies have focused on metastatic melanoma (94), where increased lymphatic vessel density correlates with VEGF expression in tumor microenvironments, lymphatic vessel invasion, and LN metastasis (39, 106, 107). Although several studies have demonstrated a positive correlation between lymphatic markers and survival (26, 106), other studies have failed to demonstrate a correlation (108, 109), thus generating some controversy over the true independent prognostic value of lymphatic vessel density and invasion biomarkers. In addition to melanoma, however, recent studies have reported increased lymphangiogenesis in dermis adjacent to tumor nests in squamous cell carcinoma that also exhibit enhanced levels of VEGFC produced by CD163+CD68+ macrophages (91). Similarly, an increase in the absolute numbers of lymphatic capillaries was observed in Merkel cell carcinoma, likely driven again by CD163+CD68+ tumor-associated macrophages (110), and increased levels of VEGFR3 cells (both tumor and stroma) and lymphatic (podoplanin+) vessels correlated with disease progression in one study of Sézary syndrome, an aggressive subtype of cutaneous T-cell lymphoma (111).

In addition to remodeling of vessels in the primary tumor, significant enlargement of lymph sinuses in tumor-reactive LNs is observed before the arrival of metastatic cells (112). Distal remodeling of lymphatic vessels, both structural and functional, is mediated, at least in part, by VEGFC and neuropilin 2 (98, 113, 114), and these changes may play a role in establishing premetastatic niches for locoregional dissemination. Importantly, lymphatic remodeling induced by these signals is also associated with altered fluid flows, which in turn correlate with LN metastasis (97–100, 115). Dynamic lymphoscintigraphy that traces accumulation of radionuclide over time in draining LNs reveals that increased rates of lymphatic flow associate with increased incidence of metastasis and lymphatic vessel density in primary tumors (116). Moreover, VEGFC overexpression further promotes lymph flow (116), and exogenous application of VEGFC reduces tumor IFPs (117).

It was previously thought that functional lymphatic vessels were excluded from solid tumor parenchyma, thus negating their role in tumor progression. However, neither intratumoral lymphangiogenesis nor intratumoral lymphatic vessels are required in all cases for LN metastasis (118, 119). Furthermore, an impaired capacity of lymphatic vessels to transport fluid does not necessarily correlate with their role in transporting cells, and, instead, remodeling of peritumoral lymphatic vessels and activation of existing vessels is sufficient for altered homing properties and metastatic potential (98, 118, 119). The mechanisms by which lymphatic vessels actively recruit and promote seeding of tumor cells have been reviewed elsewhere (26). It is of note, however, that direct evidence for a sequential model of metastasis whereby lymphogenous spread precedes hematogenous colonization of distal organs is lacking (26, 120); thus, the relative contribution of tumor-associated lymphatic remodeling and metastasis to overall survival and disease progression remains somewhat unclear.

What may contribute to the association of lymphatic remodeling with poor prognosis is its relationship with local inflammation. Clinical and histopathologic studies have demonstrated a correlation between cyclooxygenase 2 (COX2) expression, lymphatic vessel density, and LN metastasis in human malignancy (121–124). Current data indicate that the prostaglandin pathway influences lymphangiogenesis through upregulation of VEGFC and VEGFD expression in tumor-associated macrophages (125), as well as in neoplastic cells (126); however, the role of prostaglandins in lymphangiogenesis is not tumor-specific. In a subcutaneous Matrigel plug assay of granuloma formation, lymphangiogenesis was also COX2-dependent, requiring the prostaglandin E (EP) receptors 3 and 4 and macrophage production of VEGFC (125). Similarly, COX2 in the microenvironment of involuting mammary glands contributes to normal involution-associated lymphangiogenesis as well as mammmary tumor-associated lymphangiogenesis, tumor cell invasion into lymphatic vessels, and distal metastasis (127). Interestingly, it was recently reported that LECs regulate prostaglandin degradation downstream of VEGFD–VEGFR3/2 signaling, resulting in the accumulation of tissue PGF2 and dilation of collecting lymphatic vessels draining the tumor (128). This dilation is associated with VEGFC expression and enhanced LN metastasis in both human and mouse models (128). Specifically in skin, UVR stimulates COX2 expression in epidermis (129)—the EP1, EP2, and EP4 receptors have been linked to UV-induced carcinogenesis (130), and notably
inhibition of COX2 via administration of celecoxib prevents both squamous and basal cell carcinoma development in mice and humans (131, 132).

**THERAPEUTIC IMPLICATIONS**

**Targeting Lymphangiogenesis**

Targeting of tumor-associated lymphangiogenesis as a potential therapy for solid tumors has been attempted in both preclinical and clinical trials (27, 94). It is difficult, however, to separate effects on the lymphatic vasculature from hematogenous vessels given overlap in mechanisms required for their remodeling and growth, namely VEGFR3 (104, 133, 134). Either way, direct targeting of tumor vasculature in the clinic with FDA-approved drugs, such as bevacizumab, sorafenib, and sutinib, that target VEGFA and kinase activity of VEGF family receptors (all of which may also interfere with signaling on tumor-associated lymphatic endothelium) provides only transient benefit, and ultimately tumors adapt and regrow (133).

Alternatively, it has been suggested that indirect inhibition of vascular remodeling may be induced through repolarization of immune microenvironments (135). Numerous studies have revealed that recruitment of myeloid cells facilitates the angiogenic switch through expression of VEGF and liberation of proangiogenic molecules from ECM (20, 21). Targeting recruitment of these cells into tissue parenchyma results not only in the release of local immunosuppression (i.e., increased effector T-cell activity), but also a simultaneous normalization of angiogenic vasculature (135). Given the role local inflammation also plays in lymphangiogenesis, it is reasonable to speculate that these strategies may similarly affect lymphatic vasculature. Use of NSAIDs for chemoprevention of cancer support this claim, as discussed above, where COX2-dependent tumor formation is also associated with lymphangiogenesis in tumor microenvironments and LN metastasis (128, 136, 137). Consequently, prophylactic treatment with inhibitors of COX1 and COX2 may halt tumor progression through simultaneous inhibition of local inflammation, lymphangiogenesis, and angiogenesis (Fig. 4A and B; refs. 130, 137).

One caveat to direct or indirect antilymphangiogenic therapy, however, is evidence indicating that lymphatic vessels are resistant to “normalization” therapy. Experimental evidence for this is provided by studies of *Mycoplasma pulmonis* infection of murine respiratory tracts where angiogenesis and lymphangiogenesis are associated with infiltrating leukocytes (34). Resolution of inflammation through dexamethasone administration resolved the angiogenic responses but failed to resolve changes in lymphatic vessels, indicating that aberrant lymphatic vessel responses are long-lived and do not require continued maintenance. Only when dexamethasone was administered prophylactically was local lymphatic vessel growth inhibited (34). Along these lines, in a corneal wounding
model, lymphatic vessel regrowth kinetics accelerated with sequential challenges, indicating potential memory responses facilitating rapid recall growth in the cornea (138). Although it remains to be seen whether similar observations can be made outside the respiratory tract and cornea, these reports present the very interesting idea that long-lived lymphatic vessel aberrancies may exist, and may predict functional consequences for future inflammation, therapeutic resistance, and recurrence. Clearly, if indeed refractory to normalization strategies, lymphatic vessels present an interesting challenge to clinical therapy, particularly with respect to therapeutic timing, that should be more systematically explored.

**Lymphatic Vessels as an Immune Biomarker**

Recent interest in the role of the immune system in tumor progression has justified a series of studies evaluating the potential for local infiltrates to provide prognostic value for risk stratification (16). Melanoma was one of the first tumors to be associated with spontaneous antitumor immunity leading to regression—appearance of autoimmune vitiligo (139), presence of intraskeletal tumor-infiltrating lymphocytes (140), expression of lymphocyte chemotactic factors (141), and a type I IFN transcriptional profile (142) enrich for a subset of clinical responders in this context. This idea has been formalized into a quantitative metric reflecting immune histopathology of tumors and is a significant prognostic indicator for disease-free survival and overall survival for colorectal cancer (16, 143). Evaluation of immune contexture of tumors, for example, “Immunoscore,” includes quantification of the density of CD8+ and CD4+ T effector and memory T cells, as a function of their location in central tumor regions as compared with invasive margins (143). The recognition that Immunoscore significantly reflects a prognostic biomarker for tumor progression and patient outcome has yielded a broader awareness of the importance of CD8+ T-cell infiltration as an indicator of an immunologically reactive tumor (4). What may further inform this type of immune-based stratification strategy is now an intense area of study, and efforts to include various suppressive components of tumor microenvironments might provide added value to current approaches. These efforts typically focus on infiltrating immune cells of myeloid origin; however, the potential role for nonhematopoietic stromal components, such as vasculature, both blood and lymph, deserves attention. In fact, it may be the close association between tumor-associated lymphangiogenesis and infiltrating myeloid cells that underlies controversy in clinical lymphatic vessel biomarker studies (39, 91). This, together with evidence that the degree of both intratumoral and peritumoral lymphatic vessel density is associated with the presence of tumor-associated macrophages, more advanced stage (i.e., increased tumor thickness, mitotic count, and ulceration; ref. 39), and decreased numbers of infiltrating lymphocytes (144), indicates that correlations between lymphatic vasculature and local immunity in clinical samples is significant to consider.

Current immunohistochemical mapping of immune infiltrates and other components of tumor microenvironments is necessarily reliant on biopsies of primary or metastatic disease. Solid tumors, however, are known to be heterogeneous with respect to both distribution of intrinsic oncogenic signaling and activation of microenvironmental factors (145). Consequently, biopsies may not capture the full complexity within a developing tumor. Although still the most valuable approach, identification of novel tissue biomarkers must be coupled to efforts to define correlative systemic biomarker signatures. The challenge in this approach is, however, exemplified by the fact that although VEGPC is prognostic when detected in tumor by immunohistochemistry (101), few studies have demonstrated a correlation between serum VEGF/C/D and LN metastasis (146). VEGF/C/D produced at high concentrations in local tissue may become diluted or unstable as they circulate through the lymphoid system back to the thoracic duct due to their short half-lives in circulation; as a consequence, it is not expected that serum levels will necessarily be diagnostic of LN metastases. A potential alternative source for biomarker identification is tumor interstitial fluid itself, which, unlike plasma, has a higher concentration of these proteins produced within tumor microenvironments and therefore may more reliably reflect altered biology within the tumor itself (147). Available methods for interstitial fluid isolation have recently been reviewed and include tissue centrifugation, tissue elution, capillary ultrafiltration, and microdialysis (147). Enhanced levels of known cancer biomarkers, including VEGF, can be detected in interstitial fluid when compared with plasma (147), supporting the idea that tissue interstitial fluid, the contents of which are closer to lymph than plasma, may be a more robust source of biomarkers predicting primary tumor behavior.

**CONCLUSIONS**

Our understanding of the roles lymphatic vessels play in regulating tumor immune microenvironments in skin and other solid tumors is still in its infancy. The interplay between the vasculature (both hematogenous and lymphogenous), local inflammation, and tumor progression sets up a far more complex story than that of nutrient delivery and waste removal (Fig. 4A and B). The lymphatic vascular coordinates local inflammation and immunity, and its dysfunction may contribute to deregulated local inflammation, a hallmark of cancer as described by Hanahan and Weinberg (15). However, where lymphatic vessels really come into play in a developing cutaneous malignancy remains unclear. Whether their early dysfunction predisposes tissue to altered immunity, thus leading to tumor immune escape, or if their predominant role comes later during metastasis and spread remains an open question. Likely, lymphatic vessels participate in a continuous feedback loop, responding to microenvironmental change in peripheral tissue to propagate a signal to LNs that then alters host responses to tissue and cyclically continues. Our continued understanding of the complexities of lymphatic vessel function in the unique contexts of normal and diseased skin will provide a model to understand how regional immune dysfunction can participate in cutaneous malignancy and potential targets for prognostic and therapeutic strategies.

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