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IMMUNE CELL INFILTRATION AND TERTIARY LYMPHOID STRUCTURES AS DETERMINANTS OF ANTI-TUMOR IMMUNITY[1]

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Abstract

Limited representation of intratumoral immune cells is a major barrier to tumor control. However, simply enhancing immune responses in tumor-draining lymph nodes, or through adoptive transfer, may not overcome the limited ability of tumor vasculature to support effector infiltration. An alternative is to promote a sustained immune response intratumorally. This idea has gained traction with the observation that many tumors are associated with tertiary lymphoid structures (TLS), which organizationally resemble lymph nodes. These peri- and intra-tumoral structures are usually, but not always, associated with positive prognoses in patients. Preclinical and clinical data support a role for TLS in modulating immunity in the tumor microenvironment. However, there appear to be varied functions of TLS, potentially based on their structure or location in relation to tumor, or the origin or location of the tumor itself. Understanding more about TLS development, composition, and function may offer new therapeutic opportunities to modulate anti-tumor immunity.

Introduction

Substantial intratumoral representation of T-lymphocytes, either spontaneously, or after vaccination or adoptive therapy, is generally well-correlated with immune mediated control of human cancers (1–5). Importantly, the subset of patients who respond clinically to new generation immunotherapies are those in which an immunological infiltrate is evident prior to treatment (6–10). Thus, enhancing the representation of intratumoral immune effectors holds the promise of improving clinical outcomes. However, simply enhancing the immune response in the tumor-draining lymph node, or infusing large numbers of tumor reactive T-cells through adoptive transfer, may not overcome the limitations of the tumor vasculature

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and microenvironment to support infiltration of these effectors. The desired approach would promote a sustained increase in functional intratumoral effector cells.

An interesting alternative is to promote development of an immune response inside the tumor, circumventing the limitations of dendritic cell (DC) trafficking from tumor to lymph node (LN) and of effector cells trafficking in the reverse direction. This idea originated in studies from the early 2000's in which tumors were engineered to support naïve T-cell infiltration (11, 12). However, it has recently gained additional importance with the observation that many tumors are associated with tertiary lymphoid structures (TLS). TLS, which resemble LN, were initially described in conjunction with chronic and pathogen driven immune responses. However, they are now recognized as a common feature in juxtaposition to tumors, and are often associated with a positive prognosis in patients. Here we summarize the current state of knowledge about the significance of tumor-associated TLS. We place them in the context of immune infiltration into tumors more generally, and in the context of what is known about the development of conventional LN and inflammation-associated TLS. Finally, we point to the issues that still need to be addressed to harness them for therapeutic purposes.

Tumor-associated vasculature and control of T-cell infiltration into tumors

Infiltration of tumors by exogenously activated effectors

The entry of leukocytes, including T- and B-cells, into lymphoid and non-lymphoid tissues is controlled by sequential engagement of homing receptors (HR) (selectins, chemokine receptors, and integrins) that act with corresponding ligands on vascular endothelial cells to enable capture, rolling, firm adhesion, and extravasation (13–16). During differentiation, effector T-cells acquire the ability to enter peripheral tissues, including tumors, by upregulating HR that bind to cognate ligands expressed on inflamed vasculature. HR expression on activated CD8 T-cells is determined by the secondary lymphoid organ (SLO) in which priming occurs (17–21). Tissue-specific and inflammation-induced expression of different HR ligands, in conjunction with the patterns of HR expressed by T-cells, determines which tissues are infiltrated.

While the requirements for entry of effector T-cells and other leukocytes into inflamed peripheral tissues, particularly skin and gut, have been well-established, the requirements for entry into tumors remain inadequately defined. Several studies have unambiguously identified individual HR that mediate T-cell infiltration into some tumors (22–27), while others have shown correlations between individual HR or HR ligands and T-cell infiltrates (28–34). We recently completed a comprehensive analysis of the HR that mediate entry of CD8 T-cell effectors into B16 melanoma and Lewis lung carcinoma, and demonstrated that HR ligand expression on tumor-associated vasculature varies with anatomical location of the tumor (35). This also determines the ability of T cells activated in different SLO to enter tumors growing in different locations. Consistent with other work (25, 29, 32, 33, 36–41), we also found that HR ligand expression on tumor vasculature is often low. This is consistent with the low infiltration of adoptively transferred effector T-cells observed in several studies (42–45). Thus, one opportunity to improve cancer immunotherapy is to identify and manipulate the expression of HR and HR ligands to enhance infiltration of CD8

T-cell effectors into tumors. This approach has been explored by transducing adoptively transferred T cells to express various chemokine receptors, and has resulted in enhanced infiltration and tumor control (46–49).

Naïve T-cells enter tumors through LN-like vasculature and enhance tumor control

Naïve T-cells enter LN based on their expression of L-selectin and CCR7, which bind to peripheral node addressin (PNAd) and the chemokines CCL19/CCL21, respectively. The latter are expressed on specialized LN blood vessels called high endothelial venules (HEV). Since naïve T-cells do not generally enter peripheral tissue, it had been assumed that all intratumoral lymphocytes are effectors that differentiate in tumor-draining LN and home to the tumor thereafter. However, naïve T-cells were shown to infiltrate tumors that had been genetically modified to secrete Lymphotoxin-α (LTα) (11, 50) or LIGHT (12). Tumors expressing LTα accumulated activated T cells in the absence of SLO (50). Similar results were obtained by intratumoral injection of CCL21 or CCL21 transduced DC (51–53). It was suggested that naïve T cells infiltrated through a LN-like vasculature based on expression of CCL21 (12), or expression of CCL21, PNAd, and the accumulation of $CD62L^+$ T cells (11), although this was not shown. In all of these models, the infiltration of naïve T-cells, in conjunction with their differentiation to become effectors, was shown to promote tumor destruction. These studies established that tumors could be engineered to serve as a surrogate site for naïve T cells recruitment and their activation to become mediators of improved anti-tumor immunity.

More recently, we showed that adoptively transferred naïve CD8 T-cells can enter unmanipulated tumors and subsequently differentiate into functional effectors (54, 55). This also occurs in the absence of SLO (54). We also showed directly that naïve CD8 T-cell entry into tumors was entirely dependent on L-selectin and CCR7, and on the development of tumor-associated blood vessels that express PNAd and CCL21 (55). PNAd and CCL21 were co-expressed on ~5% of blood vessels in tumors growing in multiple anatomical locations (55). The primary source of CCL21 was intratumoral $gp38^+$ fibroblasts, and to a lesser extent, $CD31^+$ blood endothelial cells. It was also shown that preventing this influx of naïve T-cells diminished tumor control, indicating that it represents an important component of the immune response in unmanipulated tumors.

Mechanisms driving PNAd and CCL21 expression on tumor-associated vasculature

In adult LN, HEV morphology and expression of genes required for biosynthesis of PNAd is maintained by continuous engagement of Lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) expressed on DC with the Lymphotoxin-β Receptor (LTβR) expressed on blood endothelial cells (56, 57). However, the genes for PNAd biosynthesis can also be induced in cultured endothelial cells and monocytes by TNFα (58, 59). LTβR signaling does not control expression of CCL21 in adult LN (56). Developmentally, CCL21 expression depends upon signals from $LT\alpha_1\beta_2$ or the soluble homotrimeric form of Lymphotoxin-α_3 ($LT\alpha_3$), which engages Tumor Necrosis Factor Receptors 1 and 2 (TNFR) but not LTβR (60–63); TNFα (64, 65); and another TNFR family member, CD30(66). However, it is not clear whether these signals regulate the expression of CCL21 directly, or the development of CCL21 expressing cells. Overall, these results point to the central importance of $LT\alpha_1\beta_2$ - LTβR engagement in driving

development and maintenance of PNAd expression on blood endothelial cells, while control of CCL21 expression remains uncertain.

In tumors, the density of intratumoral DC (67) and regulatory T cell (Treg) depletion (68) were correlated with development of $PNAd^+$ $CCL21^+$ vasculature, but a cause and effect relationship was not established. Indeed, in murine models of melanoma and lung carcinoma, development of $PNAd^+$ $CCL21^+$ vasculature was not induced by either DC or LTβR signaling (55). Instead, induction of both molecules depended on CD8 T-cell effectors that had infiltrated tumors at an earlier point. In intraperitoneal (IP) tumors, PNAd expression depended on CD8 T-cell effectors secreting $LT\alpha_3$, which acted through TNFR expressed on endothelial cells and fibroblasts. CCL21, but not PNAd, also depended on IFNγ secreted by CD8 T-cell effectors, which acted through IFNγ receptors on fibroblasts and endothelial cells. Control of PNAd and CCL21 in subcutaneous (SC) tumors was more complex, in that both CD8 T-cell effectors and NK cells could independently induce expression of these molecules. While expression depends on signaling through TNFR, IFNγ seems to act redundantly with another as yet unidentified modulator to induce CCL21. These results demonstrate that the mechanisms controlling development of $PNAd^+$ $CCL21^+$ vasculature in tumors are distinct from those identified in SLO, and also differ between SC and IP tumors. It remains to be determined whether these or additional mechanisms operate in human tumors growing in different anatomical locations.

Tertiary lymphoid structures

TLS are lymphoid aggregates that frequently develop at sites of chronic infection, autoimmune disease, and allograft rejection (69–78). These structures have considerable morphological, cellular, and molecular similarity to SLO, particularly LN. Inflammation-associated TLS are associated with blood vascular endothelial cells that express PNAd and CCL21, similar to that of the tumors described above, and often have a characteristic “puffy” morphology. TLS contain B-cells, T-cells, and DC, which are typically organized into distinct functional compartments: a B-cell follicle mainly composed of naïve B cells, surrounding a germinal center composed of highly proliferative B cells; and a T-cell area mainly composed of T-cells and DC (77, 79). In SLO, this microarchitecture is orchestrated by the homeostatic chemokines CCL19, CCL21, CXCL13, and CXCL12 (80–83). These chemokines are also found in TLS (84–88). Cells resembling fibroblastic reticular cells (FRC) and follicular dendritic cells (FDC) have also been reported in well-developed TLS (63, 75, 84, 85, 87), suggesting that they may be the source of these chemokines and function as organizers of TLS. However, this remains to be directly demonstrated.

Identification and categorization of tumor-associated TLS

Well-organized TLS have been described in association with several different tumor types in humans, and two recent reviews have provided comprehensive summaries of this information (89, 90). The initial description in non-small cell lung cancers (91) documented large peritumoral aggregates of immune cells containing distinct T/DC and B/FDC zones, the latter of which also contained germinal centers. This group also documented the preferential expression of several homeostatic chemokines and HR ligands in these TLS

(92), which correlated with the presence of larger numbers of $CD4^+CD62L^+$ T cells (92, 93). Subsequent studies have largely replicated these findings in a number of different tumor types in humans, although the range of cellular and molecular markers examined has been variable (67, 94–113). We and others have also identified TLS in murine tumors (55, 114, 115).

One significant consideration is how tumor-associated TLS are distinguished from otherwise localized immune cell infiltrates. In addition to the histological documentation of lymphoid aggregates, which are typically located peritumorally, most human studies have relied largely or exclusively on the presence of only some TLS-associated elements, including $PNAd^+$ HEV (105, 106), mature DC (67, 91–93), B cells (99, 106), or T_{FH} cells (102). A gene signature that includes CCL19, CCL21, and CXCL13, the homeostatic chemokines that likely organize TLS, also has been used (96, 109, 106, 67, 111, 102). While it seems likely that the structures identified by all groups are indeed TLS, this points to the possibility of significant unappreciated heterogeneity in structure or composition that may arise in connection with different tumor types, anatomical locations, and primary or metastatic lesions.

In this regard, it was shown that the colorectal metastases to the lung were associated with well-developed TLS in humans, while renal cell carcinomas metastases to the same organ were associated with poorly developed TLS (110). In human melanoma, it was found that TLS frequently developed in metastatic lesions, yet were largely absent from primary tumors, despite the presence of a $PNAd^+$ vasculature (95). Similarly, in the transplantable murine models that we have studied, TLS form around $PNAd^+$ vasculature in all IP tumors, but not SC tumors, despite the presence of comparable LN-like vasculature (55, 116) (Fig. 1). The TLS found in these murine IP tumors also lack discreet B- and T-cell areas. Instead, T-cells and DC are distributed within large B-cell aggregates that are in turn organized within a reticular network of $podoplanin^+$ fibroblasts (55, 116). In these IP tumor-associated TLS, it appears that the same $podoplanin^+$ fibroblasts make both CCL21 and CXCL13, which may explain the lack of discreet B- and T-cell areas. How this difference in organization relative to more conventional SLO and TLS affects the nature of the immune response remains to be determined.

A second categorization of tumor-associated TLS is their anatomical relationship to the tumor mass. Almost all studies in humans have identified peritumoral TLS (summarized in (89)), and TLS in this location have also been described in a genetically engineered mouse model (115). A smaller number of human studies have identified tumors with intratumoral TLS, usually as a subset of the tumors with peritumoral structures, although a largely intratumoral representation has also been reported in germ cell tumors (117) (summarized in (89)). The TLS in the transplantable murine models that we have used are exclusively intratumoral (55, 116). In studies of human melanoma metastases using 7-color immunofluorescence, we have identified TLS in 38% of metastatic melanomas (Mauldin, et al, unpublished). They appear in 3 locations: stroma surrounding tumor nests [extratumoral]; at the periphery of the tumor nests [peritumoral] and intratumoral. In general, TLS are more organized with discreet B-cell and DC/T-cell zones in peritumoral and extratumoral locations (Fig. 2A–D) than within the tumor nests (Fig. 2E–F). However, some peritumoral

TLS also show an intermixing of T-cells within B-cell aggregates (Fig. 2C). FoxP3$^+$ putative Tregs are prevalent in the T-cell area. We have also detected intratumoral PNAd$^+$ vasculature that is not associated with well-organized aggregates, although there are diffuse distributions of lymphocytes and DC in the general vicinity (Fig. 2F). An important set of questions is whether these levels of TLS organization in different locations are formed by distinct mechanisms, have distinct immunological properties, or have distinct prognostic values in relation to patient survival and response to immunotherapy.

Prognostic significance and immunological impact of tumor-associated TLS

TLS are often found in lungs in response to acute infection (77, 78), where they lead to enhanced local anti-viral immunity through activation of naïve T-cells (71, 72). In keeping with this, tumor-associated TLS are usually a favorable prognostic indicator for patient survival (reviewed in (89, 90), although there are exceptions (110, 118). Several studies have pointed to the relationship between the densities of HEV, CD4 or T_{FH} cells, B cells, and mature DC in defining TLS, and to a relationship between the densities of TLS, levels of intratumoral T and B cells, and a T_H1/cytotoxic immune profile among tumor infiltrating lymphocytes (TIL) (92, 93, 96, 97, 99, 112, 119). These observations are most often interpreted to mean that tumor-associated TLS are sites for generating useful antitumor immune responses in newly entering naïve T and B-cells.

An alternative possibility is that TLS are simply proxies for more robust intratumoral CD8 T-cell effector activity. Since intratumoral CD8 T-cells have been implicated most often as positive prognostic factors for cancer patient survival, the prognostic value of TLS needs to be evaluated through multivariate analysis capable of distinguishing independent effects. One recent study concluded that TLS did have independent value after controlling for specific TIL variables (97). Another established that a subset of individuals with low TLS-associated DC density were at great risk of death, despite the presence of a high density of CD8 effectors (93). Interestingly, the presence of TLS in triple negative breast cancer added to the positive prognostic value of moderate levels of TIL, but had no impact if there were high levels of TIL present (105). At the other extreme, TLS had positive prognostic value in Merkel cell carcinoma, while T cell infiltration and several other immunological parameters did not (119). Similarly, neither CD8 or T_H17 TIL were associated with positive prognosis in gastric cancer patients; however, Tbet$^+$ cells, CD20 B cells organized into TLS, and coordinate expression of T_H1 and B cell genes were (103). Curiously however, DC-Lamp$^+$ cell density, another TLS marker, did not have prognostic value.

Conversely, a limited number of studies have shown that tumor-associated TLS are a negative prognostic factor for cancer patient survival. The presence of TLS in colorectal cancer has been associated with more advanced disease (118), while TLS in breast cancer was associated with a higher tumor grade, low representation of intratumoral immune cells, and a high frequency of LN metastasis (120). These studies are seemingly at odds with studies suggesting positive associations with patient survival in each of these diseases (67, 97, 102, 105–107, 110, 112), and remain to be fully reconciled. An intriguing study showed a negative impact of DC-Lamp and CD8 markers on survival of patient with renal cell carcinoma metastases to lung, but a positive impact on patients with colorectal cancer

metastases to the same organ (110). In murine lung adenocarcinoma, it was shown that TLS serve as a site for recruitment of Treg that restrain antitumor immune responses (115). It was also shown that TLS can function as niches for malignant hepatocellular progenitor cells (98). Interestingly, in human lung transplants, TLS have been associated with a lower probability of graft rejection, whereas some other organ transplants are more likely to be rejected when TLS are identified (121–123). Thus, both in cancers and in transplanted organs, immune rejection may be enhanced or reduced by the presence of TLS. This raises the possibility that some TLS are dominated by regulatory T cells and support tolerance, whereas others support immune rejection. It will be important to understand details of the composition and location of TLS and which are most relevant for immune rejection.

A characteristic feature of all tumor-associated TLS in both humans and mice is the presence of a large number of B-lymphocytes, often accompanied by T_{FH} cells. In recent years the consensus of the field has become that CD8 T-cells are the most important determinant of anti-tumor immunity; however, these observations suggest that intratumoral B cells may play an important role(s). On the positive side, several studies have suggested that intratumoral B-cells can capture and present tumor antigens to T cells (124–126). Perhaps even more heretical (in the current environment) have been suggestions that intratumoral antibody responses can be an effective component of anti-tumor immunity (127, 128). On the negative side, many studies have shown that B cells can play a suppressive role in anti-tumor immunity, and Breg cells have been found to accumulate in several tumor models (129–132). The differences in prognostic significance of tumor-associated TLS may lie, at least in part, to distinct functional attributes of the B-cell compartment in different tumors. However, the ways in which tumors may regulate these functional attributes remains to be discovered.

Collectively, these studies point to a complex interplay in which the prognostic value of TIL and TLS depends at the very least on tumor type. It seems likely that different tumor types may drive qualitatively distinct immune responses, distinguishable by their effector and regulatory mechanisms. Such responses may also evolve over time. In some tumors, or at certain times, these may be determined largely within conventional tumor-draining SLO, while in other circumstances, they may be determined within tumor-associated TLS. Evidence demonstrating such a temporal evolution has recently been reported (94). This suggests that TLS “quality” might also confer, or result from, distinct kinds of immunity or immune regulation. Genetically engineered mouse models (115) provide a valuable opportunity to evaluate these variables systematically.

Mechanisms that control the development of TLS

The development of conventional LN depends on interaction between $CD4^+CD3^{neg}$ lymphoid tissue inducer (LTi) cells expressing $LT\alpha_1\beta_2$ and RANKL (now known to be ILC3 cells (133)), and mesenchymal lymphoid tissue organizer (LTo) cells expressing LTβR (134–139). This leads to expression of CCL19, CCL21, and CXCL13, which attract additional LTi cells, and the recruitment and positioning of T- and B-cells (81, 140–142). In keeping with this, mice deficient in LTα, LTβ, or LTβR fail to develop all or most LN (143–146). The formation of FDC networks and germinal centers as an element of LN development depends

on engagement of TNFR as well as LTβR (147). LTβR signaling does not control expression of CCL21 in adult LN (56), although it does maintain CXCL13 expression in adult spleen (148) presumably through interaction of $LT\alpha_1\beta_2$-expressing B-cells with $LT\beta R^+$ FDC (149, 150).

A key question is whether the formation of TLS in general, and tumor–associated TLS in particular, occurs through pathways analogous to those controlling conventional LN development. Transgenic expression of LTα or LTβ in various organs leads to the formation of organized lymphoid aggregates typically identified as TLS, although they are not driven by an immune response (60–63). Transgenic expression of CCL21 also leads to formation of such structures, although PNAd expression still depends on LTβR signaling (151, 152), and this operates through mature CD4 T cells rather than LTi cells (153). Importantly, blockade of LTβR signaling prevents immune response-driven TLS formation in thyroid, lung, salivary gland, aorta, and heart (154–156, 71, 88, 157). These studies suggest that development of LN and TLS are mechanistically similar.

On the other hand, TLS have been shown to depend on TNFα in aorta, fat, and intestine (158–160), IL22 in salivary gland (84), and IL17A in lung and meninges (161, 88, 86). IL6 and IL23 also contribute to TLS development through IL17A dependent and independent pathways (162–164). While the exact mechanisms of action remain to be fully defined, TNFα induces CCL21 (64, 65), CXCL13 (148) and enzymes necessary for PNAd synthesis (58, 59), while IL22 and IL17A induce CXCL12 and CXCL13 in TLS-associated stromal cells (165, 166, 85, 88, 84). These cytokines sometimes induce TLS independent of LTβR signaling (158, 160), but in other instances act cooperatively, either by inducing distinct aspects of TLS structure (84, 86), or by acting at either the TLS induction or maintenance phases (88). Importantly, different pathogens can induce lung-associated TLS by distinct cellular and molecular pathways (85). The involvement of additional cytokines in formation and/or maintenance of many LTβR-dependent TLS remains to be evaluated.

Aside from the involvement of distinct molecular signaling pathways in TLS formation, a variety of different inducing cells, in lieu of LTi cells, have been identified. This includes DC (71, 72, 167) and naïve B-cells (168), both expressing $LT\alpha_1\beta_2$; macrophages (158–160), T_H17 (161, 88, 86), NKT (158), γδ T-cells (85) and multiple populations of IL22-secreting adaptive and innate lymphoid cells (84). Overall, it should be expected that distinct molecular signals, conveyed by immune cells with pleiotropic regulatory signatures, will induce TLS with important differences in the immune responses that they support.

In this context, the mechanisms driving TLS formation in tumors remain almost entirely unknown. Analogous to the overexpression models described above, transgenic expression of LTα or LIGHT in tumors (11, 169) or intratumoral administration of CCL21 (52, 53) establishes that these pathways can lead to TLS formation. In transplantable murine tumors, TLS develop in IP but not SC tumors (55, 116), suggesting that the mechanisms controlling their development depend on anatomical environmental factors, and providing an opportunity to gain insight into these mechanisms through comparative analysis. Since $PNAd^+CCL21^+$ vasculature develops comparably in tumors growing in both locations, this

also points to distinct mechanisms controlling these two features, and to this vasculature in providing a nucleation site for TLS formation.

Compared to SC tumors, IP tumors showed a 10-fold increase in the number of B-cells relative to $CD31^+$ vascular endothelial cells, while the numbers of T cells and DC were similar (116). Interestingly, IP tumors also showed a 5-fold relative increase of $podoplanin^+$ fibroblasts, and these cells formed a reticular network surrounding the $PNAd^+CCL21^+$ vasculature, which was co-extensive with the B-cells (55, 116). While perivascular $podoplanin^+$ cells were evident in SC tumors, they had not expanded to form reticular networks. Also, $podoplanin^+$ cells in IP tumors expressed significantly higher levels of CXCL13 and the B-cell survival factors BAFF and APRIL (Rodriguez et al, in preparation), which are important for the development and survival of B-cells (170–172) and maintaining the integrity of germinal centers (173, 174). This suggests that elements of the IP tumor microenvironment induce both proliferation and differentiation of $podoplanin^+$ cells, which act as scaffolding for TLS formation. While our work establishes that CCL21 recruits naïve T-cells into IP and SC tumors (55), it remains to be directly determined whether and how these remaining molecules impact B-cell recruitment and the formation of TLS in IP tumors, and what is responsible for changes in their expression in tumor association $podoplanin^+$ fibroblasts (Fig. 3).

CONCLUSIONS

Tumor-associated TLS have been frequently correlated with improved patient survival, and in murine models, have generally been associated with the development of improved immune-mediated tumor control. However, tumor-associated TLS have also been identified as a locus of Treg accumulation, and they characteristically contain large numbers of B-cells, which exert a regulatory role in several tumor models. Much remains to be done to fully understand the immune mechanisms that are activated within these structures, and whether this varies based on the origin of the tumor, the oncogenic drivers leading to its formation, or its anatomical location as a primary tumor or metastatic lesion. To do so will require application of the full range of technologies that have been applied in previous studies, together with a more comprehensive analysis of molecules that mark various immune mechanisms. Advances in multi-spectral imaging of single sections, and entire tumors after tissue clearing, and advances in small scale RNAseq combined with laser capture dissection of TLS create new opportunities for comprehensive assessment of cellular composition and functional state of individual TLS in a variety of contexts. This work is also important as a means to more clearly establish the prognostic value of TLS, independent of other immune factors.

The pathways that drive the formation of $PNAD^+CCL21^+$ vasculature have been uncovered in a limited number of murine tumor models, but the factors that drive formation and/or maintenance of tumor-associated TLS remain almost completely unknown. Studies in a variety of different inflammation-driven TLS models have pointed to a number of molecular and cellular mediators that work in collaboration with or in lieu of more classic mechanisms that underlie the formation of SLO. This heterogeneity has been shown to reflect, at least in part, the nature of the inflammatory stimulus. One might also imagine a role for anatomical

location, reflecting the extent to which peripheral tissues are invested with different subsets of myeloid derived cells, innate lymphoid cells, or tissue resident memory cells. The impact of the pre-existing or early stage microenvironment in proximity to tumors on TLS formation is clear, but the exact drivers, and how they vary based on tumor origin or location, remain to be determined. These drivers are likely to also influence the composition and function of TLS, as mentioned above. Understanding these driver mechanisms will rely heavily on appropriate transplantable and genetically-induced mouse models, where they can be directly tested through knockout or blockade approaches. Their validity in human settings will be more difficult to establish, as it will depend largely on correlative studies to establish the presence or absence of various cells or signaling molecules. However, as argued above, incisive multiparameter correlative studies are key to understand the prognostic significance of TLS in humans. Together with work in mouse models, they will provide the basis for moving forward to manipulate TLS incidence, location, composition, and function.

Based on the preponderance of evidence, it seems highly desirable in most settings to increase the number of TLS as a way to promote an ongoing immune response in immediate proximity to tumor cells, avoiding the need for two-way cellular traffic between tumor and tumor-draining SLO. One open question is whether this is best achieved via intratumoral or peritumoral TLS. Related to this is the question of whether regulatory mechanisms that often occur within the tumor microenvironment are more prevalent within, or in proximity to, TLS. A second set of questions concerns TLS functionality. It remains to be established how TLS and tumor-draining SLO compare as sites for development of tumor specific CD8 T-cells, either numerically or over time. The presence of large numbers of B-cells in TLS has brought renewed focus on the positive and negative roles that B-cells might play in anti-tumor immunity, but this has yet to be clearly tied to the activity of B-cells within TLS. Finally, but more immediately in the current clinical environment, there are open questions about whether the presence of TLS, either pre- or post-treatment, has prognostic value in responses to checkpoint blockade therapy. Does checkpoint blockade enhance TLS formation, or enhance TLS function as a site for de novo anti-tumor immune responses? As new immune therapeutic modalities (IDO inhibitors, myeloid cell depletion, etc) come into the clinical landscape, these questions will only get broader.

Overall, the work accomplished to date clearly establishes the importance of tumor-associated TLS as an aspect of immunological control of tumors. However, a substantial amount of work remains to be done to fully understand how they are important, and how to begin to use them as an element of our developing armamentarium of immunological cancer control strategies.

References

1. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005; 353:2654–2666. [PubMed: 16371631]
2. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès

F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006; 313:1960–1964. [PubMed: 17008531]

3. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ, Odunsi K. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005; 102:18538–43. [PubMed: 16344461]
4. Erdag G, Schaefer JT, Smolkin ME, Deacon DH, Shea SM, Dengel LT, Patterson JW, Slingluff CL Jr. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer Res. 2012; 72:1070–1080. [PubMed: 22266112]
5. Angell H, Galon J. From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer. Curr Opin Immunol. 2013; 25:261–267. [PubMed: 23579076]
6. Gajewski TF, Louahed J, Brichard VG. Gene signature in melanoma associated with clinical activity. Cancer J. 2010; 16:399–403. [PubMed: 20693853]
7. Ulloa-Montoya F, Louahed J, Dizier B, Gruselle O, Spiessens B, Lehmann FF, Suciu S, Kruit WHJ, Eggermont AMM, Vansteenkiste J, Brichard VG. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol. 2013; 31:2388–2395. [PubMed: 23715562]
8. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, Alaparthy S, Berman D, Jure-Kunkel M, Siemers NO, Jackson JR, Shahabi V. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother. 2012; 61:1019–1031. [PubMed: 22146893]
9. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HEK, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014; 515:563–567. [PubMed: 25428504]
10. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013; 14:1014–1022. [PubMed: 24048123]
11. Schrama D, thor Straten P, Fischer WH, McLellan AD, Bröcker E-B, Reisfeld RA, Becker JC. Targeting of Lymphotoxin-α to the tumor elicits an efficient immune response associated with induction of peripheral lymphoid-like tissue. Immunity. 2001; 14:111–121. [PubMed: 11239444]
12. Yu P, Lee Y, Liu W, Chin RK, Wang J, Wang Y, Schietinger A, Philip M, Schreiber H, Fu YX. Priming of naive T cells inside tumors leads to eradication of established tumors. Nat Immunol. 2004; 5:141–149. [PubMed: 14704792]
13. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science. 1996; 272:60–66. [PubMed: 8600538]
14. von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. N Engl J Med. 2000; 343:1020–1034. [PubMed: 11018170]
15. Sackstein R. The lymphocyte homing receptors: gatekeepers of the multistep paradigm. Curr Opin Hematol. 2005; 12:444–450. [PubMed: 16217160]
16. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007; 7:678–689. [PubMed: 17717539]
17. Mora JR, Bono MR, Manjunath N, Weninger W, Cavanagh LL, Rosemblatt M, von Andrian UH. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature. 2003; 424:88–93. [PubMed: 12840763]
18. Mora JR, Cheng G, Picarella D, Briskin M, Buchanan N, von Andrian UH. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. J Exp Med. 2005; 201:303–316. [PubMed: 15642741]
19. Johansson-Lindbom B, Agace WW. Generation of gut-homing T cells and their localization to the small intestinal mucosa. Immunol Rev. 2007; 215:226–242. [PubMed: 17291292]
20. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. Nat Immunol. 2008; 9:981–987. [PubMed: 18711435]

21. Ferguson AR V, Engelhard H. CD8 T cells activated in distinct lymphoid organs differentially express adhesion proteins and coexpress multiple chemokine receptors. J Immunol. 2010; 184:4079–86. [PubMed: 20212096]
22. Yamada M, Yanaba K, Hasegawa M, Matsushita Y, Horikawa M, Komura K, Matsushita T, Kawasuji A, Fujita T, Takehara K, Steeber DA, Tedder TF, Sato S. Regulation of local and metastatic host-mediated anti-tumour mechanisms by l-selectin and intercellular adhesion molecule-1. Clin Exp Immunol. 2006; 143:216–227. [PubMed: 16412045]
23. Sasaki K, Zhu X, Vasquez C, Nishimura F, Dusak JE, Huang J, Fujita M, Wesa A, Potter DM, Walker PR, Storkus WJ, Okada H. Preferential expression of very late antigen-4 on type 1 CTL cells plays a critical role in trafficking into central nervous system tumors. Cancer Res. 2007; 67:6451–6458. [PubMed: 17616706]
24. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, Balint K, Katsaros D, O'Brien-Jenkins A, Gimotty PA, Coukos G. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. Nat Med. 2008; 14:28–36. [PubMed: 18157142]
25. Fisher DT, Chen Q, Skitzki JJ, Muhitch JB, Zhou L, Appenheimer MM, Vardam TD, Weis EL, Passanese J, Wang WC, Gollnick SO, Dewhirst MW, Rose-John S, Repasky EA, Baumann H, Evans SS. IL-6 trans-signaling licenses mouse and human tumor microvascular gateways for trafficking of cytotoxic T cells. J Clin Invest. 2011; 121:3846–3859. [PubMed: 21926464]
26. Bose A, Taylor JL, Alber S, Watkins SC, Garcia JA, Rini BI, Ko JS, Cohen PA, Finke JH, Storkus WJ. Sunitinib facilitates the activation and recruitment of therapeutic anti-tumor immunity in concert with specific vaccination. Int J Cancer. 2011; 129:2158–2170. [PubMed: 21170961]
27. Mikucki ME, Fisher DT, Matsuzaki J, Skitzki JJ, Gaulin NB, Muhitch JB, Ku AW, Frelinger JG, Odunsi K, Gajewski TF, Luster AD, Evans SS. Non-redundant requirement for CXCR3 signaling during tumoricidal T-cell trafficking across tumour vascular checkpoints. Nat Commun. 2015; 6:7458. [PubMed: 26109379]
28. Kunz M, Toksoy A, Goebeler M, Engelhardt E, Bröcker E, Gillitzer R. Strong expression of the lymphoattractant C-X-C chemokine Mig is associated with heavy infiltration of T cells in human malignant melanoma. J Pathol. 1999; 189:552–558. [PubMed: 10629557]
29. Garbi N, Arnold B, Gordon S, Hämmerling GJ, Ganss R. CpG motifs as proinflammatory factors render autochthonous tumors permissive for infiltration and destruction. J Immunol. 2004; 172:5861–5869. [PubMed: 15128765]
30. Hensbergen PJ, Wijnands PG, Schreurs MW, Scheper RJ, Willemze R, Tensen CP. The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis. J Immunother. 2005; 28:343–351. [PubMed: 16000952]
31. Musha H, Ohtani H, Mizoi T, Kinouchi M, Nakayama T, Shiiba K, Miyagawa K, Nagura H, Yoshie O, Sasaki I. Selective infiltration of CCR5(+)CXCR3(+) T lymphocytes in human colorectal carcinoma. Int J Cancer. 2005; 116:949–956. [PubMed: 15856455]
32. Clark RA, Huang SJ, Murphy GF, Mollet IG, Hijnen D, Muthukuru M, Schanbacher CF, Edwards V, Miller DM, Kim JE, Lambert J, Kupper TS. Human squamous cell carcinomas evade the immune response by down-regulation of vascular E-selectin and recruitment of regulatory T cells. J Exp Med. 2008; 205:2221–2234. [PubMed: 18794336]
33. Quezada SA, Peggs KS, Simpson TR, Shen Y, Littman DR, Allison JP. Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. J Exp Med. 2008; 205:2125–2138. [PubMed: 18725522]
34. Lohr J, Ratliff T, Huppertz A, Ge Y, Dictus C, Ahmadi R, Grau S, Hiraoka N, Eckstein V, Ecker RC, Korff T, von Deimling A, Unterberg A, Beckhove P, Herold-Mende C. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-β. Clin Cancer Res. 2011; 17:4296–4308. [PubMed: 21478334]
35. Woods AN, Wilson AL, Srinivasan N, Zeng J, Dutta A, Peske JD, Tewalt EF, Gregg RK, Ferguson AR, Engelhard VH. Anatomical location and effector cytokines determine homing receptor ligand expression on tumor-associated vasculature and the entry of effector CD8 T-cells. Cancer Immunol Res. in press.

36. Weishaupt C, Munoz KN, Buzney E, Kupper TS, Fuhlbrigge RC. T-cell distribution and adhesion receptor expression in metastatic melanoma. Clin Cancer Res. 2007; 13:2549–2556. [PubMed: 17473183]
37. Dengel LT, Norrod AG, Gregory BL, Clancy-Thompson E, Burdick MD, Strieter RM, Slingluff CL Jr, Mullins DW. Interferons induce CXCR3-cognate chemokine production by human metastatic melanoma. J Immunother. 2010; 33:965–974. [PubMed: 20948440]
38. Enarsson K, Johnsson E, Lindholm C, Lundgren A, Pan-Hammarström Q, Strömberg E, Bergin P, Baunge EL, Svennerholm AM, Quiding-Järbrink M. Differential mechanisms for T lymphocyte recruitment in normal and neoplastic human gastric mucosa. Clin Immunol. 2006; 118:24–34. [PubMed: 16157508]
39. Dirkx AEM, oude Egbrink MGA, Kuijpers MJE, van der Niet ST, Heijnen VVT, Steege JCAB, Wagstaff J, Griffioen AW. Tumor angiogenesis modulates leukocyte-vessel wall interactions in vivo by reducing endothelial adhesion molecule expression. Cancer Res. 2003; 63:2322–2329. [PubMed: 12727857]
40. Yoong KF, McNab G, Hübscher SG, Adams DH. Vascular adhesion protein-1 and ICAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma. J Immunol. 1998; 160:3978–3988. [PubMed: 9558106]
41. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, McKee M, Gajewski TF. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res. 2009; 69:3077–3085. [PubMed: 19293190]
42. Griffith KD, Read EJ, Carrasquillo JA, Carter CS, Yang JC, Fisher B, Aebersold P, Packard BS, Yu MY, Rosenberg SA. In vivo distribution of adoptively transferred indium-111-labeled tumor infiltrating lymphocytes and peripheral blood lymphocytes in patients with metastatic melanoma. J Natl Cancer Inst. 1989; 81:1709–1717. [PubMed: 2810387]
43. Fisher B, Packard BS, Read EJ, Carrasquillo JA, Carter CS, Topalian SL, Yang JC, Yolles P, Larson SM, Rosenberg SA. Tumor localization of adoptively transferred indium-111 labeled tumor infiltrating lymphocytes in patients with metastatic melanoma. J Clin Oncol. 1989; 7:250–261. [PubMed: 2644399]
44. Economou JS, Belldegrun AS, Glaspy J, Toloza EM, Figlin R, Hobbs J, Meldon N, Kaboo R, Tso CL, Miller A, Lau R, McBride W, Moen RC. In vivo trafficking of adoptively transferred interleukin-2 expanded tumor-infiltrating lymphocytes and peripheral blood lymphocytes. Results of a double gene marking trial. J Clin Invest. 1996; 97:515–521. [PubMed: 8567975]
45. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006; 12:6106–6115. [PubMed: 17062687]
46. Craddock JA, Lu A, Bear A, Pule M, Brenner MK, Rooney CM, Foster AE. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J Immunother Hagerstown Md 1997. 2010; 33:780–788.
47. Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE, Heslop HE, Brenner MK, Dotti G, Savoldo B. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood. 2009; 113:6392. [PubMed: 19377047]
48. Peng W, Ye Y, Rabinovich BA, Liu C, Lou Y, Zhang M, Whittington M, Yang Y, Overwijk WW, Lizée G, Hwu P. Transduction of Tumor-Specific T Cells with CXCR2 Chemokine Receptor Improves Migration to Tumor and Antitumor Immune Responses. Clin Cancer Res. 2010; 16:5458. [PubMed: 20889916]
49. Moon EK, Carpenito C, Sun J, Wang LCS, Kapoor V, Predina J, Powell DJ, Riley JL, June CH, Albelda SM. Expression of a Functional CCR2 Receptor Enhances Tumor Localization and Tumor Eradication by Retargeted Human T cells Expressing a Mesothelin-Specific Chimeric Antibody Receptor. Clin Cancer Res. 2011; 17:4719. [PubMed: 21610146]
50. Schrama D, Voigt H, Eggert AO, Xiang R, Zhou H, Schumacher TNM, Andersen MH, thor Straten P, Reisfeld RA, Becker JC. Immunological tumor destruction in a murine melanoma model by targeted LTalpha independent of secondary lymphoid tissue. Cancer Immunol Immunother. 2008; 57:85–95. [PubMed: 17605009]

51. Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, Chamberlain JS, Giedlin M, Aukerman L, Mulé JJ. T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine augmentation of dendritic cell-based immunotherapy. Cancer Res. 2001; 61:2062–2070. [PubMed: 11280767]
52. Kirk CJ, Hartigan-O'Connor D, Mule JJ. The dynamics of the T-cell antitumor response: chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res. 2001; 61:8794–8802. [PubMed: 11751401]
53. Turnquist HR, Lin X, Ashour AE, Hollingsworth MA, Singh RK, Talmadge JE, Solheim JC. CCL21 induces extensive intratumoral immune cell infiltration and specific anti-tumor cellular immunity. Int J Oncol. 2007; 30:631–639. [PubMed: 17273764]
54. Thompson ED, Enriquez HL, Fu YX, Engelhard VH. Tumor masses support naive T cell infiltration, activation, and differentiation into effectors. J Exp Med. 2010; 207:1791–1804. [PubMed: 20660615]
55. Peske JD, Thompson ED, Gemta L, Baylis RA, Fu YX, Engelhard VH. Effector lymphocyte-induced lymph node-like vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. Nat Commun. 2015; 6:7114. [PubMed: 25968334]
56. Browning JL, Allaire N, Ngam-Ek A, Notidis E, Hunt J, Perrin S, Fava RA. Lymphotoxin-beta receptor signaling is required for the homeostatic control of HEV differentiation and function. Immunity. 2005; 23:539–550. [PubMed: 16286021]
57. Moussion C, Girard JP. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. Nature. 2011; 479:542–546. [PubMed: 22080953]
58. Pablos J, Santiago B, Tsay D, Singer M, Palao G, Galindo M, Rosen S. A HEV-restricted sulfotransferase is expressed in rheumatoid arthritis synovium and is induced by lymphotoxin-α/β and TNF-α in cultured endothelial cells. BMC Immunol. 2005; 6:6. [PubMed: 15752429]
59. Tjew SL, Brown KL, Kannagi R, Johnson P. Expression of N-acetylglucosamine 6-O-sulfotransferases (GlcNAc6STs)-1 and -4 in human monocytes: GlcNAc6ST-1 is implicated in the generation of the 6-sulfo N-acetyllactosamine/Lewis × epitope on CD44 and is induced by TNF-α. Glycobiology. 2005; 15:7C–13C.
60. Sacca R, Cuff CA, Lesslauer W, Ruddle NH. Differential Activities of Secreted Lymphotoxin-α3 and Membrane Lymphotoxin-α1β2 in Lymphotoxin-Induced Inflammation: Critical Role of TNF Receptor 1 Signaling. J Immunol. 1998; 160:485–491. [PubMed: 9552007]
61. Cuff CA, Schwartz J, Bergman CM, Russell KS, Bender JR, Ruddle NH. Lymphotoxin α3 Induces chemokines and adhesion molecules: insight into the role of LTα in inflammation and lymphoid organ development. J Immunol. 1998; 161:6853–6860. [PubMed: 9862717]
62. Cuff CA, Sacca R, Ruddle NH. Differential induction of adhesion molecule and chemokine expression by LTα3 and LTαβ in inflammation elucidates potential mechanisms of mesenteric and peripheral lymph node development. J Immunol. 1999; 162:5965–5972. [PubMed: 10229834]
63. Drayton DL, Ying X, Lee J, Lesslauer W, Ruddle NH. Ectopic LTαβ directs lymphoid organ neogenesis with concomitant expression of peripheral node addressin and a HEV-restricted sulfotransferase. J Exp Med. 2003; 197:1153–1163. [PubMed: 12732657]
64. Johnson LA, Jackson DG. Inflammation-induced secretion of CCL21 in lymphatic endothelium is a key regulator of integrin-mediated dendritic cell transmigration. Int Immunol. 2010; 22:839–849. [PubMed: 20739459]
65. Wiede F, Vana K, Sedger LM, Lechner A, Körner H. TNF-dependent overexpression of CCL21 is an underlying cause of progressive lymphoaccumulation in generalized lymphoproliferative disorder. Eur J Immunol. 2007; 37:351–357. [PubMed: 17236235]
66. Bekiaris V, Gaspal F, Kim MY, Withers DR, McConnell FM, Anderson G, Lane PJL. CD30 is required for CCL21 expression and CD4 T cell recruitment in the absence of lymphotoxin signals. J Immunol. 2009; 182:4771–4775. [PubMed: 19342654]
67. Martinet L, Filleron T, Guellec SL, Rochaix P, Garrido I, Girard JP. High endothelial venule blood vessels for tumor-infiltrating lymphocytes are associated with Lymphotoxin β–producing dendritic cells in human breast cancer. J Immunol. 2013; 191:2001–2008. [PubMed: 23825314]
68. Hindley JP, Jones E, Smart K, Bridgeman H, Lauder SN, Ondondo B, Cutting S, Ladell K, Wynn KK, Withers D, Price DA, Ager A, Godkin AJ, Gallimore AM. T-cell trafficking facilitated by

high endothelial venules is required for tumor control after regulatory T-cell depletion. Cancer Res. 2012; 72:5473–5482. [PubMed: 22962270]

69. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol. 2006; 7:344–353. [PubMed: 16550197]
70. Carragher DM, Rangel-Moreno J, Randall TD. Ectopic lymphoid tissues and local immunity. Semin Immunol. 2008; 20:26–42. [PubMed: 18243731]
71. GeurtsvanKessel CH, Willart MA, Bergen IM, van Rijt LS, Muskens F, Elewaut D, Osterhaus AD, Hendriks R, Rimmelzwaan GF, Lambrecht BN. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. J Exp Med. 2009; 206:2339–2349. [PubMed: 19808255]
72. Halle S, Dujardin HC, Bakocevic N, Fleige H, Danzer H, Willenzon S, Suezer Y, Hammerling G, Garbi N, Sutter G, Worbs T, Forster R. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. J Exp Med. 2009; 206:2593–601. [PubMed: 19917776]
73. Hayasaka H, Taniguchi K, Fukai S, Miyasaka M. Neogenesis and development of the high endothelial venules that mediate lymphocyte trafficking. Cancer Sci. 2010; 101:2302–2308. [PubMed: 20726857]
74. van de Pavert SA, Mebius RE. New insights into the development of lymphoid tissues. Nat Rev Immunol. 2010; 10:664–674. [PubMed: 20706277]
75. Link A, Hardie DL, Favre S, Britschgi MR, Adams DH, Sixt M, Cyster JG, Buckley CD, Luther SA. Association of T-zone reticular networks and conduits with cctopic lymphoid tissues in mice and humans. Am J Pathol. 2011; 178:1662–1675. [PubMed: 21435450]
76. Huang HY, Luther SA. Expression and function of interleukin-7 in secondary and tertiary lymphoid organs. Semin Immunol. 2012; 24:175–189. [PubMed: 22444422]
77. Neyt K, Perros F, GeurtsvanKessel CH, Hammad H, Lambrecht BN. Tertiary lymphoid organs in infection and autoimmunity. Trends Immunol. 2012; 33:297–305. [PubMed: 22622061]
78. Stranford S, Ruddle NH. Follicular dendritic cells, conduits, lymphatic vessels, and high endothelial venules in tertiary lymphoid organs: Parallels with lymph node stroma. Front Immunol. 2012; 3:350. [PubMed: 23230435]
79. Jones GW, Hill DG, Jones SA. Understanding Immune Cells in Tertiary Lymphoid Organ Development: It Is All Starting to Come Together. Front Immunol. 2016; 401
80. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B cell–attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. J Exp Med. 1998; 187:655–660. [PubMed: 9463416]
81. Ansel KM V, Ngo N, Hyman PL, Luther SA, Förster R, Sedgwick JD, Browning JL, Lipp M, Cyster JG. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature. 2000; 406:309–314. [PubMed: 10917533]
82. Luther SA, Tang HL, Hyman PL, Farr AG, Cyster JG. Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. Proc Natl Acad Sci. 2000; 97:12694–12699. [PubMed: 11070085]
83. Allen CDC, Ansel KM, Low C, Lesley R, Tamamura H, Fujii N, Cyster JG. Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. Nat Immunol. 2004; 5:943–952. [PubMed: 15300245]
84. Barone F, Nayar S, Campos J, Cloake T, Withers DR, Toellner K-M, Zhang Y, Fouser L, Fisher B, Bowman S, Rangel-Moreno J, de la M, Garcia-Hernandez L, Randall TD, Lucchesi D, Bombardieri M, Pitzalis C, Luther SA, Buckley CD. IL-22 regulates lymphoid chemokine production and assembly of tertiary lymphoid organs. Proc Natl Acad Sci. 2015; 112:11024–11029. [PubMed: 26286991]
85. Fleige H, Ravens S, Moschovakis GL, Bölter J, Willenzon S, Sutter G, Häussler S, Kalinke U, Prinz I, Förster R. IL-17–induced CXCL12 recruits B cells and induces follicle formation in BALT in the absence of differentiated FDCs. J Exp Med. 2014; 211:643–651. [PubMed: 24663215]
86. Pikor NB, Astarita JL, Summers-Deluca L, Galicia G, Qu J, Ward LA, Armstrong S, Dominguez CX, Malhotra D, Heiden B, Kay R, Castanov V, Touil H, Boon L, O'Connor P, Bar-Or A, Prat A, Ramaglia V, Ludwin S, Turley SJ, Gommerman JL. Integration of Th17- and lymphotoxin-derived

signals initiates meningeal-resident stromal cell remodeling to propagate neuroinflammation. Immunity. 2015; 43:1160–1173. [PubMed: 26682987]

87. Rangel-Moreno J, Moyron-Quiroz JE, Hartson L, Kusser K, Randall TD. Pulmonary expression of CXC chemokine ligand 13, CC chemokine ligand 19, and CC chemokine ligand 21 is essential for local immunity to influenza. Proc Natl Acad Sci. 2007; 104:10577–10582. [PubMed: 17563386]
88. Rangel-Moreno J, Carragher DM, de la L, Garcia-Hernandez M, Hwang JY, Kusser K, Hartson L, Kolls JK, Khader SA, Randall TD. The development of inducible bronchus-associated lymphoid tissue depends on IL-17. Nat Immunol. 2011; 12:639–646. [PubMed: 21666689]
89. Hiraoka N, Ino Y, Yamazaki-Itoh R. Tertiary Lymphoid Organs in Cancer Tissues. Front Immunol. 2016; 7:244. [PubMed: 27446075]
90. Sautès-Fridman C, Lawand M, Giraldo NA, Kaplon H, Germain C, Fridman WH, Dieu-Nosjean MC. Tertiary lymphoid structures in cancers: prognostic value, regulation, and manipulation for therapeutic intervention. Fontiers Immunol. 2016; 407
91. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, Rabbe N, Laurans L, Tartour E, de Chaisemartin L, Lebecque S, Fridman WH, Cadranel J. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol Off J Am Soc Clin Oncol. 2008; 26:4410–4417.
92. de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, Cremer I, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res. 2011; 71:6391–6399. [PubMed: 21900403]
93. Goc J, Germain C, Vo-Bourgais TKD, Lupo A, Klein C, Knockaert S, de Chaisemartin L, Ouakrim H, Becht E, Alifano M, Validire P, Remark R, Hammond SA, Cremer I, Damotte D, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res. 2014; 74:705–715. [PubMed: 24366885]
94. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Pagès F, Speicher MR, Trajanoski Z, Galon J. Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. Immunity. 2013; 39:782–795. [PubMed: 24138885]
95. Cipponi A, Mercier M, Seremet T, Baurain JF, Théate I, van den Oord J, Stas M, Boon T, Coulie PG, van Baren N. Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. Cancer Res. 2012; 72:3997–4007. [PubMed: 22850419]
96. Coppola D, Nebozhyn M, Khalil F, Dai H, Yeatman T, Loboda A, Mulé JJ. Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. Am J Pathol. 2011; 179:37–45. [PubMed: 21703392]
97. Di Caro G, Bergomas F, Grizzi F, Doni A, Bianchi P, Malesci A, Laghi L, Allavena P, Mantovani A, Marchesi F. Occurrence of tertiary lymphoid tissue is associated with T-cell infiltration and predicts better prognosis in early-stage colorectal cancers. Clin Cancer Res. 2014; 20:2147–2158. [PubMed: 24523438]
98. Finkin S, Yuan D, Stein I, Taniguchi K, Weber A, Unger K, Browning JL, Goossens N, Nakagawa S, Gunasekaran G, Schwartz ME, Kobayashi M, Kumada H, Berger M, Pappo O, Rajewsky K, Hoshida Y, Karin M, Heikenwalder M, Ben-Neriah Y, Pikarsky E. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. Nat Immunol advance online publication. 2015
99. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, Lepelley A, Becht E, Katsahian S, Bizouard G, Validire P, Damotte D, Alifano M, Magdeleinat P, Cremer I, Teillaud JL, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med. 2014; 189:832–844. [PubMed: 24484236]
100. Giraldo NA, Becht E, Pagès F, Skliris G, Verkarre V, Vano Y, Mejean A, Saint-Aubert N, Lacroix L, Natario I, Lupo A, Alifano M, Damotte D, Cazes A, Triebel F, Freeman GJ, Dieu-Nosjean MC, Oudard S, Fridman WH, Sautès-Fridman C. Orchestration and Prognostic Significance of Immune Checkpoints in the Microenvironment of Primary and Metastatic Renal Cell Cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2015; 21:3031–3040.

101. Goeppert B, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, Joehrens K, Warth A, Renner M, Mehrabi A, Hafezi M, Thelen A, Schirmacher P, Weichert W. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. Br J Cancer. 2013; 109:2665–2674. [PubMed: 24136146]
102. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, Ravoet M, Le Buanec H, Sibille C, Manfouo-Foutsop G, Veys I, Haibe-Kains B, Singhal SK, Michiels S, Rothé F, Salgado R, Duvillier H, Ignatiadis M, Desmedt C, Bron D, Larsimont D, Piccart M, Sotiriou C, Willard-Gallo K. CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest. 2013; 123:2873–2892. [PubMed: 23778140]
103. Hennequin A, Derangère V, Boidot R, Apetoh L, Vincent J, Orry D, Fraisse J, Causeret S, Martin F, Arnould L, Beltjens F, Ghiringhelli F, Ladoire S. Tumor infiltration by Tbet+ effector T cells and CD20+ B cells is associated with survival in gastric cancer patients. Oncoimmunology. 2016; 5:e1054598. [PubMed: 27057426]
104. Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer. 2015; 112:1782–1790. [PubMed: 25942397]
105. Lee HJ I, Park A, Song IH, Shin SJ, Kim JY, Yu JH, Gong G. Tertiary lymphoid structures: prognostic significance and relationship with tumour-infiltrating lymphocytes in triple-negative breast cancer. J Clin Pathol. 2015; 69:422–430. [PubMed: 26475777]
106. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie J-J, Rochaix P, Girard J-P. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. Cancer Res. 2011; 71:5678–5687. [PubMed: 21846823]
107. McMullen TPW, Lai R, Dabbagh L, Wallace TM, de Gara CJ. Survival in rectal cancer is predicted by T cell infiltration of tumour-associated lymphoid nodules. Clin Exp Immunol. 2010; 161:81–88. [PubMed: 20408858]
108. Meshcheryakova A, Tamandl D, Bajna E, Stift J, Mittlboeck M, Svoboda M, Heiden D, Stremitzer S, Jensen-Jarolim E, Grünberger T, Bergmann M, Mechtcheriakova D. B cells and ectopic follicular structures: novel players in anti-tumor programming with prognostic power for patients with metastatic colorectal cancer. PloS One. 2014; 9:e99008. [PubMed: 24905750]
109. Messina JL, Fenstermacher DA, Eschrich S, Qu X, Berglund AE, Lloyd MC, Schell MJ, Sondak VK, Weber JS, Mulé JJ. 12-Chemokine gene signature identifies lymph node-like structures in melanoma: potential for patient selection for immunotherapy? Sci Rep. 2012; 2:765. [PubMed: 23097687]
110. Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean MC, Riquet M, Crozet L, Ouakrim H, Goc J, Cazes A, Fléjou JF, Gibault L, Verkarre V, Régnard JF, Pagès ON, Oudard S, Mlecnik B, Sautès-Fridman C, Fridman WH, Damotte D. Characteristics and Clinical Impacts of the Immune Environments in Colorectal and Renal Cell Carcinoma Lung Metastases: Influence of Tumor Origin. Clin Cancer Res. 2013; 19:4079–4091. [PubMed: 23785047]
111. Sakai Y, Hoshino H, Kitazawa R, Kobayashi M. High endothelial venule-like vessels and lymphocyte recruitment in testicular seminoma. Andrology. 2014; 2:282–289. [PubMed: 24519996]
112. Väyrynen JP, Sajanti SA, Klintrup K, Mäkelä J, Herzig KH, Karttunen TJ, Tuomisto A, Mäkinen MJ. Characteristics and significance of colorectal cancer associated lymphoid reaction. Int J Cancer. 2014; 134:2126–2135. [PubMed: 24154855]
113. Wirsing AM, Rikardsen OG, Steigen SE, Uhlin-Hansen L, Hadler-Olsen E. Characterisation and prognostic value of tertiary lymphoid structures in oral squamous cell carcinoma. BMC Clin Pathol. 2014; 14:38. [PubMed: 25177210]
114. Bergomas F, Grizzi F, Doni A, Pesce S, Laghi L, Allavena P, Mantovani A, Marchesi F. Tertiary Intratumor Lymphoid Tissue in Colo-Rectal Cancer. Cancers. 2011; 4:1–10. [PubMed: 24213222]
115. Joshi NS, Akama-Garren EH, Lu Y, Lee DY, Chang GP, Li A, DuPage M, Tammela T, Kerper NR, Farago AF, Robbins R, Crowley DM, Bronson RT, Jacks T. Regulatory T cells in tumor-associated tertiary lymphoid structures suppress anti-tumor T cell responses. Immunity. 2015; 43:579–590. [PubMed: 26341400]

116. Rodriguez AB, Peske JD, Engelhard VH. Identification and characterization of tertiary lymphoid structures in murine melanoma. Tertiary Lymphoid Structures: Methods and Protocols Methods in Molecular Biology. in press.
117. Willis SN, Mallozzi SS, Rodig SJ, Cronk KM, McArdel SL, Caron T, Pinkus GS, Lovato L, Shampain KL, Anderson DE, Anderson RCE, Bruce JN, O'Connor KC. The microenvironment of germ cell tumors harbors a prominent antigen-driven humoral response. J Immunol Baltim Md 1950. 2009; 182:3310–3317.
118. Bento DC, Jones E, Junaid S, Tull J, Williams GT, Godkin A, Ager A, Gallimore A. High endothelial venules are rare in colorectal cancers but accumulate in extra-tumoral areas with disease progression. OncoImmunology. 2015; 4:e974374. [PubMed: 25949892]
119. Behr DS, Peitsch WK, Hametner C, Lasitschka F, Houben R, Schönhaar K, Michel J, Dollt C, Goebeler M, Marx A, Goerdt S, Schmieder A. Prognostic value of immune cell infiltration, tertiary lymphoid structures and PD-L1 expression in Merkel cell carcinomas. Int J Clin Exp Pathol. 2014; 7:7610–7621. [PubMed: 25550797]
120. Figenschau SL, Fismen S, Fenton KA, Fenton C, Mortensen ES. Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients. BMC Cancer. 2015; 15:101. [PubMed: 25884667]
121. Hsao HM, Li W, Gelman AE, Krupnick AS, Kreisel D. The Role of Lymphoid Neogenesis in Allografts. Am J Transplant. 2015 n/a-n/a.
122. Koenig A, Thaunat O. Lymphoid Neogenesis and Tertiary Lymphoid Organs in Transplanted Organs. Front Immunol. 2016; 7
123. Li W, Bribriesco AC, Nava RG, Brescia AA, Ibricevic A, Spahn JH, Brody SL, Ritter JH, Gelman AE, Krupnick AS, Miller MJ, Kreisel D. Lung transplant acceptance is facilitated by early events in the graft and is associated with lymphoid neogenesis. Mucosal Immunol. 2012; 5:544–554. [PubMed: 22549742]
124. Bruno TC, Ebner P, Moore B, Squalls O, Waugh K, Eruslanov EB, Singhal S, Mitchell J, Franklin W, Merrick D, McCarter M, Palmer BE, Kern J, Vignali D, Slansky JE. Antigen-presenting tumor B cells impact the phenotype of CD4 tumor infiltrating T cells in lung cancer patients. J Immunol. 2017; 198:130.26–130.26.
125. Coughlin CM, Vance BA, Grupp SA, Vonderheide RH. RNA-transfected CD40-activated B cells induce functional T-cell responses against viral and tumor antigen targets: implications for pediatric immunotherapy. Blood. 2004; 103:2046–2054. [PubMed: 14630810]
126. Yuseff MI, Pierobon P, Reversat A, Lennon-Duménil AM. How B cells capture, process and present antigens: a crucial role for cell polarity. Nat Rev Immunol. 2013; 13:475–486. [PubMed: 23797063]
127. Germain C, Gnjatic S, Dieu-Nosjean MC. Tertiary lymphoid structure-associated B cells are key players in anti-tumor immunity. Immunother Vaccines. 2015; 6:67.
128. Nelson BH. CD20+ B cells: the other tumor-infiltrating lymphocytes. J Immunol. 2010; 185:4977–4982. [PubMed: 20962266]
129. Affara NI, Ruffell B, Medler TR, Gunderson AJ, Johansson M, Bornstein S, Bergsland E, Steinhoff M, Li Y, Gong Q, Ma Y, Wiesen JF, Wong MH, Kulesz-Martin M, Irving B, Coussens LM. B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. Cancer Cell. 2014; 25:809–821. [PubMed: 24909985]
130. Schioppa T, Moore R, Thompson RG, Rosser EC, Kulbe H, Nedospasov S, Mauri C, Coussens LM, Balkwill FR. B regulatory cells and the tumor-promoting actions of TNF-α during squamous carcinogenesis. Proc Natl Acad Sci U S A. 2011; 108:10662–10667. [PubMed: 21670304]
131. Schwartz M, Zhang Y, Rosenblatt JD. B cell regulation of the anti-tumor response and role in carcinogenesis. J Immunother Cancer. 2016; 4:40. [PubMed: 27437104]
132. Shah S, Divekar AA, Hilchey SP, Cho HM, Newman CL, Shin SU, Nechustan H, Challita-Eid PM, Segal BM, Yi KH, Rosenblatt JD. Increased rejection of primary tumors in mice lacking B cells: inhibition of anti-tumor CTL and TH1 cytokine responses by B cells. Int J Cancer. 2005; 117:574–586. [PubMed: 15912532]

133. Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F, Santo JPD, Eberl G. Lineage Relationship Analysis of RORγt+ Innate Lymphoid Cells. Science. 2010; 330:665–669. [PubMed: 20929731]

134. Kim D, Mebius RE, MacMicking JD, Jung S, Cupedo T, Castellanos Y, Rho J, Wong BR, Josien R, Kim N, Rennert PD, Choi Y. Regulation of peripheral lymph node genesis by the tumor necrosis factor family member TRANCE. J Exp Med. 2000; 192:1467–1478. [PubMed: 11085748]

135. Mebius RE. Organogenesis of lymphoid tissues. Nat Rev Immunol. 2003; 3:292–303. [PubMed: 12669020]

136. Randall TD, Carragher DM, Rangel-Moreno J. Development of secondary lymphoid organs. Annu Rev Immunol. 2008; 26:627–650. [PubMed: 18370924]

137. Withers DR. Lymphoid tissue inducer cells. Curr Biol. 2011; 21:R381–R382. [PubMed: 21601793]

138. Lane PJL, Gaspal FM, McConnell FM, Withers DR, Anderson G. Lymphoid tissue inducer cells: pivotal cells in the evolution of CD4 immunity and tolerance? Front Immunol. 2012; 3:24. [PubMed: 22566908]

139. Fletcher AL, Acton SE, Knoblich K. Lymph node fibroblastic reticular cells in health and disease. Nat Rev Immunol. 2015; 15:350–361. [PubMed: 25998961]

140. Nakano H, Gunn MD. Gene duplications at the chemokine locus on mouse chromosome 4: multiple strain-specific haplotypes and the deletion of secondary lymphoid-organ chemokine and EBI-1 ligand chemokine genes in the plt mutation. J Immunol. 2001; 166:361–369. [PubMed: 11123313]

141. Ohl L, Henning G, Krautwald S, Lipp M, Hardtke S, Bernhardt G, Pabst O, Förster R. Cooperating mechanisms of CXCR5 and CCR7 in development and organization of secondary lymphoid organs. J Exp Med. 2003; 197:1199–1204. [PubMed: 12732661]

142. Luther SA, Ansel KM, Cyster JG. Overlapping roles of CXCL13, interleukin 7 receptor α, and CCR7 ligands in lymph node development. J Exp Med. 2003; 197:1191–1198. [PubMed: 12732660]

143. De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, Smith SC, Carlson R, Shornick LP, Strauss-Schoenberger J, Russell JH, Karr R, Chaplin DD. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science. 1994; 264:703–707. [PubMed: 8171322]

144. Alimzhanov MB, Kuprash DV, Kosco-Vilbois MH, Luz A, Turetskaya RL, Tarakhovsky A, Rajewsky K, Nedospasov SA, Pfeffer K. Abnormal development of secondary lymphoid tissues in lymphotoxin β-deficient mice. Proc Natl Acad Sci U S A. 1997; 94:9302–9307. [PubMed: 9256477]

145. Koni PA, Sacca R, Lawton P, Browning JL, Ruddle NH, Flavell RA. Distinct roles in lymphoid organogenesis for lymphotoxins alpha and beta revealed in lymphotoxin beta-deficient mice. Immunity. 1997; 6:491–500. [PubMed: 9133428]

146. Fütterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The lymphotoxin β receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity. 1998; 9:59–70. [PubMed: 9697836]

147. Kuprash DV, Alimzhanov MB, Tumanov AV, Anderson AO, Pfeffer K, Nedospasov SA. TNF and Lymphotoxin β Cooperate in the Maintenance of Secondary Lymphoid Tissue Microarchitecture But Not in the Development of Lymph Nodes. J Immunol. 1999; 163:6575–6580. [PubMed: 10586051]

148. Ngo VN, Korner H, Gunn MD, Schmidt KN, Riminton DS, Cooper MD, Browning JL, Sedgwick JD, Cyster JG. Lymphotoxin alpha/beta and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. J Exp Med. 1999; 189:403–412. [PubMed: 9892622]

149. Tumanov AV, Kuprash DV, Lagarkova MA, Grivennikov SI, Abe K, Shakhov AN, Drutskaya LN, Stewart CL, Chervonsky AV, Nedospasov SA. Distinct Role of Surface Lymphotoxin Expressed by B Cells in the Organization of Secondary Lymphoid Tissues. Immunity. 2002; 17:239–250. [PubMed: 12354378]

150. Tumanov AV, Grivennikov SI, Kruglov AA, Shebzukhov YV, Koroleva EP, Piao Y, Cui CY, Kuprash DV, Nedospasov SA. Cellular source and molecular form of TNF specify its distinct functions in organization of secondary lymphoid organs. Blood. 2010; 116:3456–64. [PubMed: 20634375]

151. Luther SA, Lopez T, Bai W, Hanahan D, Cyster JG. BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. Immunity. 2000; 12:471–481. [PubMed: 10843380]

152. Luther SA, Bidgol A, Hargreaves DC, Schmidt A, Xu Y, Paniyadi J, Matloubian M, Cyster JG. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. J Immunol. 2002; 169:424–433. [PubMed: 12077273]

153. Marinkovic T, Garin A, Yokota Y, Fu YX, Ruddle NH, Furtado GC, Lira SA. Interaction of mature CD3+ CD4+ T cells with dendritic cells triggers the development of tertiary lymphoid structures in the thyroid. J Clin Invest. 2006; 116:2622–2632. [PubMed: 16998590]

154. Furtado GC, Marinkovic T, Martin AP, Garin A, Hoch B, Hubner W, Chen BK, Genden E, Skobe M, Lira SA. Lymphotoxin beta receptor signaling is required for inflammatory lymphangiogenesis in the thyroid. Proc Natl Acad Sci U S A. 2007; 104:5026–5031. [PubMed: 17360402]

155. Gatumu MK, Skarstein K, Papandile A, Browning JL, Fava RA, Bolstad A. Blockade of lymphotoxin-beta receptor signaling reduces aspects of Sjögren syndrome in salivary glands of non-obese diabetic mice. Arthritis Res Ther. 2009; 11:R24. [PubMed: 19222863]

156. Gräbner R, Lötzer K, Döpping S, Hildner M, Radke D, Beer M, Spanbroek R, Lippert B, Reardon CA, Getz GS, Fu YX, Hehlgans T, Mebius RE, van der Wall M, Kruspe D, Englert C, Lovas A, Hu D, Randolph GJ, Weih F, Habenicht AJR. Lymphotoxin β receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE−/− mice. J Exp Med. 2009; 206:233–248. [PubMed: 19139167]

157. Motallebzadeh R, Rehakova S, Conlon TM, Win TS, Callaghan CJ, Goddard M, Bolton EM, Ruddle NH, Bradley JA, Pettigrew GJ. Blocking lymphotoxin signaling abrogates the development of ectopic lymphoid tissue within cardiac allografts and inhibits effector antibody responses. FASEB J. 2012; 26:51–62. [PubMed: 21926237]

158. Bénézech C, Luu NT, Walker JA, Kruglov AA, Loo Y, Nakamura K, Zhang Y, Nayar S, Jones LH, Flores-Langarica A, McIntosh A, Marshall J, Barone F, Besra G, Miles K, Allen JE, Gray M, Kollias G, Cunningham AF, Withers DR, Toellner KM, Jones ND, Veldhoen M, Nedospasov SA, McKenzie ANJ, Caamaño JH. Inflammation-induced formation of fat-associated lymphoid clusters. Nat Immunol. 2015; 16:819–828. [PubMed: 26147686]

159. Furtado GC, Pacer ME, Bongers G, Bénézech C, He Z, Chen L, Berin MC, Kollias G, Caamaño JH, Lira SA. TNFα-dependent development of lymphoid tissue in the absence of RORγt^{+} lymphoid tissue inducer cells. Mucosal Immunol. 2014; 7:602–614. [PubMed: 24129162]

160. Guedj K, Khallou-Laschet J, Clement M, Morvan M, Gaston AT, Fornasa G, Dai J, Gervais-Taurel M, Eberl G, Michel JB, Caligiuri G, Nicoletti A. M1 macrophages act as LTβR-independent lymphoid tissue inducer cells during atherosclerosis-related lymphoid neogenesis. Cardiovasc Res. 2014; 101:434–443. [PubMed: 24272771]

161. Peters A, Pitcher LA, Sullivan JM, Mitsdoerffer M, Acton SE, Franz B, Wucherpfennig K, Turley S, Carroll MC, Sobel RA, Bettelli E, Kuchroo VK. Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. Immunity. 2011; 35:986–996. [PubMed: 22177922]

162. Cañete JD, Celis R, Yeremenko N, Sanmartí R, van Duivenvoorde L, Ramírez J, Blijdorp I, García-Herrero CM, Pablos JL, Baeten DL. Ectopic lymphoid neogenesis is strongly associated with activation of the IL-23 pathway in rheumatoid synovitis. Arthritis Res Ther. 2015; 17:173. [PubMed: 26156866]

163. Khader SA, Guglani L, Rangel-Moreno J, Gopal R, Junecko BAF, Fountain JJ, Martino C, Pearl JE, Tighe M, Lin Y, Slight S, Kolls JK, Reinhart TA, Randall TD, Cooper AM. IL-23 is required for long-term control of Mycobacterium tuberculosis and B cell follicle formation in the infected lung. J Immunol Baltim Md 1950. 2011; 187:5402–5407.

164. Goya S, Matsuoka H, Mori M, Morishita H, Kida H, Kobashi Y, Kato T, Taguchi Y, Osaki T, Tachibana I, Nishimoto N, Yoshizaki K, Kawase I, Hayashi S. Sustained interleukin-6 signalling leads to the development of lymphoid organ-like structures in the lung. J Pathol. 2003; 200:82–87. [PubMed: 12692845]

165. Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, Yi J, Guentert T, Tousson A, Stanus AL, Le TL, Lorenz RG, Xu H, Kolls JK, Carter RH, Chaplin DD, Williams RW, Mountz JD. Interleukin 17–producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. Nat Immunol. 2008; 9:166–175. [PubMed: 18157131]

166. Gopal R, Rangel-Moreno J, Slight S, Lin Y, Nawar HF, Fallert Junecko BA, Reinhart TA, Kolls J, Randall TD, Connell TD, Khader SA. Interleukin-17-dependent CXCL13 mediates mucosal vaccine–induced immunity against tuberculosis. Mucosal Immunol. 2013; 6:972–984. [PubMed: 23299616]

167. Muniz LR, Pacer ME, Lira SA, Furtado GC. A Critical Role for Dendritic Cells in the Formation of Lymphatic Vessels within Tertiary Lymphoid Structures. J Immunol. 2011; 187:828–834. [PubMed: 21666055]

168. McDonald KG, McDonough JS, Newberry RD. Adaptive immune responses are dispensable for isolated lymphoid follicle formation: antigen-naive, lymphotoxin-sufficient B lymphocytes drive the formation of mature isolated lymphoid follicles. J Immunol. 2005; 174:5720–5728. [PubMed: 15843574]

169. Kim HJ, Kammertoens T, Janke M, Schmetzer O, Qin Z, Berek C, Blankenstein T. Establishment of early lymphoid organ infrastructure in transplanted tumors mediated by local production of Lymphotoxin α and in the combined absence of functional B and T cells. J Immunol. 2004; 172:4037–4047. [PubMed: 15034015]

170. Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: a tutorial on B cell survival. Annu Rev Immunol. 2003; 21:231–264. [PubMed: 12427767]

171. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science. 2001; 293:2111–2114. [PubMed: 11509691]

172. Varfolomeev E, Kischkel F, Martin F, Seshasayee D, Wang H, Lawrence D, Olsson C, Tom L, Erickson S, French D, Schow P, Grewal IS, Ashkenazi A. APRIL-deficient mice have normal immune system development. Mol Cell Biol. 2004; 24:997–1006. [PubMed: 14729948]

173. Kalled SL. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. Semin Immunol. 2006; 18:290–296. [PubMed: 16931038]

174. Vora KA, Wang LC, Rao SP, Liu ZY, Majeau GR, Cutler AH, Hochman PS, Scott ML, Kalled SL. Cutting Edge: Germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. J Immunol. 2003; 171:547–551. [PubMed: 12847217]

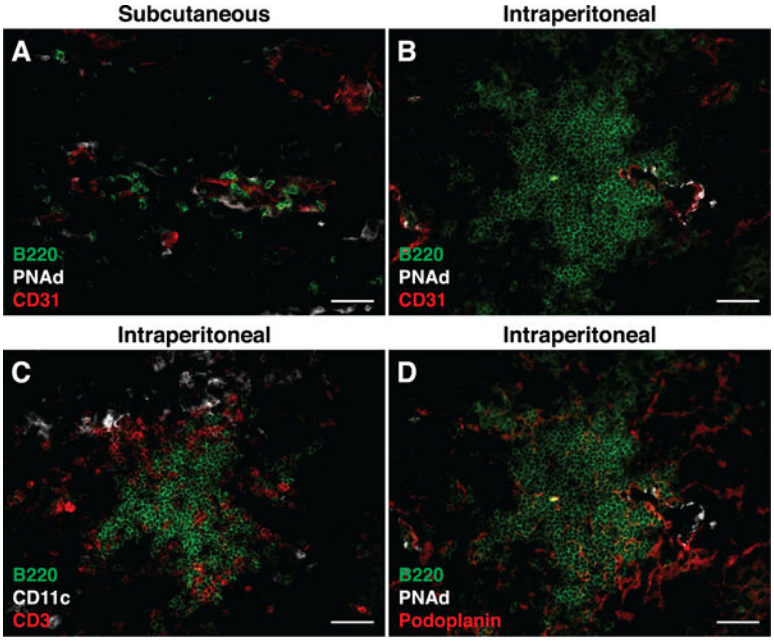

Figure 1.

Intratumoral TLS with a nonclassical organization are found in association with $PNAd^{+}$ vasculature in intraperitoneal but not subcutaneous murine B16-OVA melanoma.

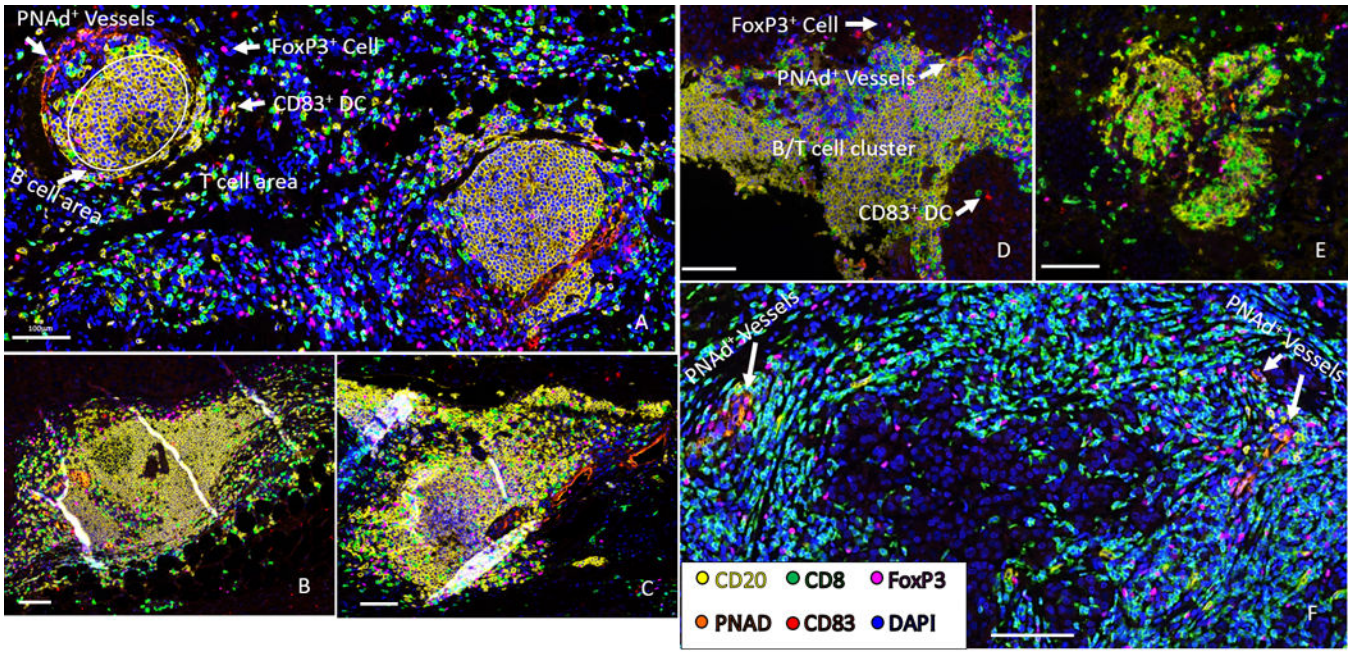

Figure 2.

Multispectral imaging of TLS in human melanoma metastases localized outside the tumor area (A); peritumoral, at the peripheral edge of tumor (B–D); or intratumorally (E–F). All scale bars are equal to 100 μm. Artefactual tissue creases due to sectioning can be seen on images B and C.

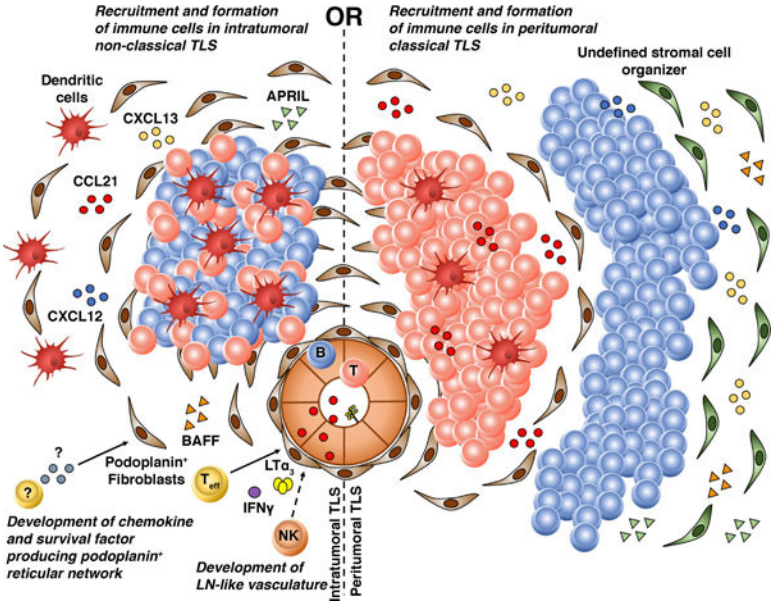

Figure 3.

Model for the formation of nonclassical and classical tumor associated TLS.