



Published in final edited form as:

Nat Rev Immunol. 2023 December ; 23(12): 807–823. doi:10.1038/s41577-023-00884-8.

Localization, tissue biology, and T cell state — implications for cancer immunotherapy

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Abstract

Tissue localization is a critical determinant of T cell immunity. CD8⁺ T cells are contact-dependent killers, which requires them to physically be within the tissue of interest to kill peptide–MHC class I-bearing target cells. Following their migration and extravasation into tissues, T cells receive many extrinsic cues from the local microenvironment, and these signals shape T cell differentiation, fate and function. Because major organ systems are variable in their functions and compositions, they apply disparate pressures on T cells to adapt to the local microenvironment. Additional complexity arises in the context of malignant lesions (either primary or metastatic), and this has made understanding the factors that dictate T cell function and longevity in tumours challenging. Moreover, T cell differentiation state influences how cues from the microenvironment are interpreted by tissue-infiltrating T cells, highlighting the importance of T cell state in the context of tissue biology. Here, we review the intertwined nature of T cell differentiation state, location, survival and function, and explain how dysfunctional T cell populations can adopt features of tissue-resident memory T cells to persist in tumours. Finally, we discuss how these factors have shaped responses to cancer immunotherapy.

TOC blurb:

Here, Schenkel and Pauken consider how specific patterns of T cell trafficking and localization in tissue microenvironments shape their immune functions in acute infection and cancer settings. They further consider the relevance of this for the efficacy of immune checkpoint blockade therapy in the clinic.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing Interests

The authors declare that they have no competing interests.

Peer review information

Nature Reviews Immunology thanks C. Pritzl, E. Teixeira-Pernas and the other, anonymous, reviewer for their contribution to the peer review of this work.

Introduction

Cancer immunotherapy [G] using checkpoint inhibitors [G] has truly been transformative for clinical cancer care. Inhibitors of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) were approved for use in patients with metastatic melanoma in 2011 and there are now seven FDA-approved inhibitors of the PD-1 pathway for use in more than 25 different late-stage cancer indications^{1–6}. Despite these successes, the majority of patients still do not have long-term durable responses following immune checkpoint blockade and response rates vary greatly depending on cancer type^{1–6}. As such, significant efforts are focused on making these therapies more effective.

A key goal has been to understand how immunotherapy directly impacts immune cell populations present within the tumour microenvironment. However, adaptive immune responses are not initiated or propagated in these tissue sites. Rather, naive T cells [G] are anatomically restricted to secondary lymphoid organs (SLOs) and blood, and must become activated in SLOs prior to going through a tightly regulated, heavily orchestrated process of migration to get into tissues. Consequently, in order to optimize immunotherapy for the treatment of cancer, it is imperative to consider the anatomy of the immune response, how T cell differentiation states can vary from location to location, and how the tissue microenvironment and/or tumour tissue impact the functional status and longevity of T cell populations in these locations.

Decades of work in models of acute infection have honed our understanding of the mechanistic basis of CD8⁺ T cell responses to pathogens in nonlymphoid tissues (see Box 1). Briefly, following activation, naive T cells give rise to different effector and memory T cell populations that eliminate the initial infection and provide long-term immunity to subsequent infections with the same pathogen. One memory T cell subset of particular relevance due to its emerging roles in cancer responses is the tissue-resident memory (T_{RM}) [G] cell subset, which is permanently retained within tissues^{7,8}. T_{RM} cells are stably maintained in many different locations, as demonstrated in both mice and humans^{9–12}, and upon antigen reactivation they provide superior secondary immunity within the tissue site, driving rapid clearance of re-infecting pathogens^{13–15}.

If antigen fails to be cleared, which is common during chronic infection and cancer, T cells go through an altered developmental trajectory that leads to T cell exhaustion^{16,17}. Early work characterizing exhausted T (T_{EX}) cells [G] showed that on a population level, T_{EX} progressively lose effector functions (for example, the ability to proliferate, produce effector cytokines, and kill target cells), upregulate exhaustion-associated transcription factors such as TOX, and express high and sustained levels of multiple inhibitory receptors (including PD-1, T cell immunoglobulin mucin 3 (TIM3), lymphocyte activation gene 3 (LAG3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT))^{16,18}. However, despite the notable erosion of effector functions, T_{EX} cells are not functionally inert. Rather, T_{EX} cells retain some residual functionality that contributes to immune responses against chronic pathogens and tumours^{19,20}. Furthermore, this residual effector activity can be functionally boosted using immunotherapy interventions including PD-1 blockade. It should be noted, however, that not all T_{EX} cells respond to PD-1 inhibitors in the same way (see Box 2)^{21–23}. One

subset, termed progenitor T_{EX} (T_{PEX}) cells [G], appears to be less dysfunctional, less epigenetically constrained, and more responsive in terms of proliferation to PD-1 inhibitors than other T_{EX} cell subsets^{22–30}. Consequently, intense efforts are currently focused on understanding the ontogeny of T_{EX} cells, how heterogeneity in T_{EX} cell populations relates to their ability to be therapeutically harnessed in chronic disease, and strategies to optimally engage T_{EX} cells to treat chronic diseases (see Box 2).

Once within a tissue, it is imperative that T cells undergo adaptations in response to local cues within the microenvironment^{8,31}. T cells must have a certain degree of plasticity to be able to adapt to these very different tissue microenvironments. The major nonlymphoid organs and organ systems differ substantially from the SLOs where naive T cells are maintained during homeostasis^{8,31}. Each organ system contains unique environmental pressures that are essential for their physiological functions (for example, nutrient intake and digestion in the alimentary tract, conducting electrical signals for pumping blood in the cardiovascular system). Extending this concept to neoplasia, tumours do not exist in a vacuum. Tumours exist, grow, and evolve surrounded by normal tissue cells and stroma, so all of the unique physiological attributes of each host tissue are also present in malignant lesions³². Moreover, the cancer context adds additional challenges such as chronic antigen exposure, chronic inflammation (or lack of inflammation), abnormal vasculature, immunosuppressive cytokines [G] and immunosuppressive leukocyte populations [G], fibrosis [G], nutrient deprivation and hypoxia [G]³². Ultimately, T cells have to cope with all of these environmental barriers to perform their effector functions and there is a pressing need to understand how to overcome these obstacles to maximize the potential of T cell-based therapies for cancer.

In this Review, we will discuss how CD8⁺ T cell developmental trajectories converge with tissue biology, and how factors such as migration to, localization within, and the ability to sense different types of microenvironments shape T cell responses. We highlight shared principles underlying CD8⁺ T cell migration and adaptation to tissue microenvironments in the settings of acute infection and cancer, as well as the unique challenges that arise in the cancer setting. Lastly, we consider the relevance of these concepts in terms of responsiveness to checkpoint inhibitors in the clinic. The primary focus of the Review will be on CD8⁺ T cells, which have been shown to be key drivers of anti-tumour immunity. Although some of the same concepts may extend to CD4⁺ T cells (for example, activation in lymph nodes and migration to tissues), other concepts may be less generalizable (for instance, issues pertaining to the formation and maintenance of CD4⁺ T_{RM} cell and CD4⁺ T_{EX} cell populations), and we refer the reader to other reviews on these topics^{16,31}.

Where it starts — lymphoid tissue

One of the most critical properties underlying the ability of T cells to execute any type of immune response is their migration to where they are needed and adaptation to their new environment. Lymphocyte trafficking is a dynamic, tightly coordinated, and heavily orchestrated process allowing for purposeful cellular locomotion and infiltration into tissues^{33,34}. Successful migration is dependent on T cells having the right selectins [G], chemokine receptors [G], and integrins [G] that are required to enter a tissue, as well as on

the tissue expressing the corresponding selectin ligands, chemokines, and integrin ligands, respectively^{33,34}. The necessity for migration is rooted in the fact that naive T cells are anatomically restricted to SLOs and blood^{7,35}. In homeostasis, there is a balancing act that dictates T cell dwell time in SLOs. This process is dependent on signals via CC-chemokine receptor 7 (CCR7), which keeps T cells in the T cell zone, and sphingosine 1 phosphate receptor 1 (S1PR1) [G], which senses the bioactive lipid sphingosine-1 phosphate in efferent lymphatics. Desensitization of CCR7 and re-sensitization of S1PR1 enables T cell migration out of the T cell zone and into the downstream efferent lymphatics^{36,37}.

In order for T cells to leave SLOs and respond to perceived threats in a nonlymphoid tissue, they must undergo antigen-driven activation in SLOs. The process of T cell activation is accompanied by their upregulation of surface proteins (including chemokine receptors, such as CXCR3, CXCR6 and CCR5, and integrins, such as $\alpha 4\beta 7$ and $\alpha 4\beta 1$) that are necessary for trafficking to sites of inflammation, as well as their downregulation of receptors involved in keeping T cells in SLOs^{8,31}. Inflammation and antigen stimulation drive dramatic changes to the ordinary rhythms of lymphocyte trafficking into and out of lymph nodes. Inflammation fundamentally changes the lymph node ultrastructure and causes lymph nodes to swell, via a process that involves CLEC2- and podoplanin-mediated regulation of actinomyosin contractility in fibroblastic reticular cells^{38–40}. Newer studies have revealed that sensory neuronal input, which can also regulate lymphocyte trafficking and motility in a $\beta 2$ -adrenergic-dependent manner, increases with inflammatory changes^{41–44}. Migration into the lymph node also changes, with high endothelial venules expressing various inflammatory chemokines, including CCL2 and CXCL9^{45–47}. This process recruits numerous additional leukocytes, including inflammatory monocytes, which have recently been shown to be critical regulators of naive and effector T cell egress during inflammation as they become a dominant source of S1P^{48,49}. Many of these lessons likely apply to the tumour-draining lymph node, as lymph node hypertrophy (or swelling) is often seen in pre-clinical mouse models and primary human samples^{50–53}. Indeed, lymph draining from tumours contains cytokines, extracellular vesicles and metabolites, all of which flood into the draining lymphoid tissue and likely affect trafficking and cellular function^{51,53,54}.

Recent work has demonstrated that tumour-specific CD8⁺ T cells are readily identifiable in the tumour-draining lymph nodes^{55–59}. These tumour-specific CD8⁺ T cells generally display a T_{PEX} phenotype — for example, they express high levels of TCF1 and retain a high degree of functionality^{55–59}. By contrast, tumour-specific CD8⁺ T cells in the tumour itself generally display terminally exhausted-like states, including the expression of multiple inhibitory receptors and decreased proliferative potential.^{55–58,60,61} Importantly, the proliferative cell burst that occurs following PD-1 blockade largely occurs in the T_{PEX} cell population, and not in the terminally-exhausted T_{EX} cell [G] population^{22,23,60,62}. Consequently, the anatomical distribution of T_{PEX} versus terminally-exhausted T_{EX} cells is critically important to consider in the context of cancer immunotherapy, and highlights the notion that the tumour-draining lymph node may serve as a critical reservoir of CD8⁺ T cells that are capable of producing a more durable effector response following PD-1 blockade. Consistent with this concept, preclinical studies have highlighted the importance of the tumour-draining lymph node for responses to checkpoint blockade^{57,63}. Similarly, it

is speculated that the tumour-draining lymph node is important for protective anti-tumour immune responses when checkpoint inhibitors are given in the neoadjuvant setting (see Box 3), since delivering checkpoint inhibitors before primary tumour removal could enhance tumour cell killing and delivery of tumour antigens to the tumour-draining lymph node. However, there is evidence in preclinical models suggesting that the T_{PEX} cell population is sequestered in the tumour-draining lymph node, and is not efficiently recruited into the tumour^{59,61}. These tumour-specific CD8⁺ T cells persistently express high levels of CD69, although whether this is driven entirely by antigen or by some level of inflammation is unclear^{59,61}. If such T cells are sequestered in the tumour-draining lymph node, developing approaches to release these T cells from the lymph node so that they can be recruited into the tumour may be an effective approach for boosting anti-tumour immunity, particularly since the tumour-specific CD8⁺ T cells in the lymph node generally appear to be more functional and less exhausted than their corresponding counterparts in the tumour.

Re-positioning T cells to tissues

Following activation in the tissue-draining lymph node, T cells downregulate CD69, upregulate S1PR1, and exit the lymph node⁶⁴ (Figure 1). From there, T cells are tasked with disseminating to sites of antigen and inflammation^{33,34}. Activated T cells in draining lymphoid tissue can be imprinted with specific homing molecules that allow for preferential migration to the respective upstream organ (for example, $\alpha 4\beta 7$ and CCR9 regulate T cell migration into the gut, and E-selectin, P-selectin, CCR4 and CCR10 are involved in T cell trafficking in skin)^{65–69}. However, while specific homing molecules have been described for the small intestine and skin, lymphocyte migration does not appear to be driven by organ-specific migratory cues for other tissues. Rather, several integrins and chemokine receptors have been shown to be important for migration towards and into sites of inflammation, including the integrins $\alpha L\beta 2$ (which binds ICAMs) and $\alpha 4\beta 1$ (which binds VCAM1), and the chemokine receptors CXCR3 (the receptor for CXCL9, CXCL10 and CXCL11) and CCR5 (the receptor for CCL3, CCL4 and CCL5)^{7,33,34}. However, it should be noted that upregulation of the requisite receptors for trafficking is not sufficient to get T cells into a tissue of interest. In order for T cells to successfully migrate into a nonlymphoid organ, that tissue must also produce the requisite chemokines and integrin ligands to attract those T cells^{70,71}. The requirement for T cells to have the right receptors and the nonlymphoid organ to have the right chemokines and/or receptor ligands ensures that T cells do not end up at the wrong place at the wrong time.

T cell migration into the tumour microenvironment is required for productive responses in cancer (Figure 1). If T cells cannot infiltrate into tumours, they cannot kill cancer cells. While many of the same inflammatory mechanisms, including the secretion of chemokines (such as CXCL9, CXCL10, CCL4 and CCL5) and expression of integrin ligands (ICAMs and VCAM1) can drive T cell recruitment to tumours, numerous barriers exist that often prevent proper T cell accumulation in tumours^{72–77}. Mechanistically, these barriers can be T cell intrinsic (for example, T cells not expressing the right trafficking receptors), or extrinsic (for example, vascular endothelial cells and/or tumour cells not expressing the right ligands and/or chemokines) (Figure 1). One of the key steps for tissue entry is tethering to vascular endothelial cells permeating tissues and extravasating through the endothelial

cell layer into the tissue parenchyma. In the setting of cancer, angiogenesis — which is the de novo formation of blood vessels — is common due to the increasing metabolic demands of tumour cells⁷⁸. New vessel formation and growth are driven by multiple factors, including the production of vascular endothelial growth factor (VEGF) and hypoxia^{79–82}. However, tumoural angiogenesis forms maladapted blood vessels, which are not pruned appropriately, tend to be leaky and suffer from an inability to upregulate inflammatory chemokines and integrin ligands (termed vascular anergy [G]) due to continual VEGF stimulation^{79–82}. These atypical vasculature structures can present a number of challenges for T cell migration, representing a physical barrier against entry (Figure 1).

While the vasculature is a critical gatekeeper for entry into tumours, cancer cells are also able to secrete inflammatory chemokines, including CCL5, CXCL9 and CXCL10, to precipitate leukocyte recruitment into the tumour microenvironment^{72,76,83}. However, tumours can turn off chemokine expression directly through multiple mechanisms, including epigenetic silencing^{76,84,85} (Figure 1). Moreover, tumour cells can halt T cell trafficking by inhibiting inflammatory pathways (such as type I and type II interferon signalling) that drive chemokine production^{86–88}. Finally, tumour cells can also inhibit secretion of inflammatory chemokines from tumour-infiltrating leukocytes, as has been observed in the context of ovarian cancer, where macrophage production of CXCL9 is blocked by tumor cells⁷⁶. Collectively, these barriers represent a significant hurdle that must be overcome for T cell infiltration to occur during an anti-tumour immune response (Figure 1). The consequences of these different mechanisms that inhibit T cell migration can lead to immune-excluded or immunologically ‘cold’ microenvironments, which are often associated with poor responses to immunotherapy. Indeed, tumours with positive responses to checkpoint blockade therapy have increased levels of inflammatory chemokines^{72,89,90}. When considering these factors, it is important to be mindful of the dynamic nature of the immune response — early stage disease may exhibit a more productive inflammatory state, better T cell infiltration and a lower degree of T cell exhaustion^{55,56}. Indeed, recent work in autochthonous mouse models of lung adenocarcinoma has demonstrated the progressive loss of T cell recruitment into tumour lesions, and thus the decline of intratumoural T cells with advanced disease^{55,56}. While definitive human studies in multiple cancer types are lacking in this regard, data in pancreatic cancer supports these findings — T cell infiltration is highest in pre-malignant lesions, and decreases as a function of tumour progression⁹¹. These alterations are likely due to a multitude of factors, including decreasing levels of T cell stimulation in the tumour draining lymph node, inefficient recruitment and/or extravasation of T cells into tumours, and increasingly unfavorable (that is, immunosuppressive) tumour microenvironments. Additional work will be necessary to define factors that differentiate early and late stage disease.

Adapting is key to survival in tissues

After extravasation [G] into peripheral tissue sites, T cells undergo a broad series of changes that enable them to stay in the new tissue, including an adaptive process that drives numerous transcriptional and epigenetic alterations in order to acclimate to the local microenvironment^{8,31} (Figure 1). These alterations include the downregulation of cellular machinery that drives tissue egress (including downregulation of S1PR1 and upregulation

of CD69)^{92–95}, and the upregulation of adhesion receptors necessary to interact with the local microenvironment (including CD103)^{94,95}. Once within a nonlymphoid tissue, T cells experience numerous cues from their external environment that contribute to shaping their phenotype and function^{94,95}. However, each tissue is different in terms of the environmental constraints placed on residing T cells^{96–98}, and tissues containing malignancies have significantly greater intrinsic barriers for anti-tumour T cell responses. In this section, we will discuss basic principles of T cell retention within tissues and T cell plasticity to adapt to certain microenvironments, drawing parallels between T_{RM} cells and T_{EX} cells in terms of how each of these subsets handles these pressures.

Lessons from T_{RM} cell tissue adaptability

T_{RM} cells that form during acute infection represent an ideal model for studying the T cell adaptations that allow for durable persistence in nonlymphoid tissues and for rapid effector functions upon antigen reactivation^{94,95}. After sensing cognate antigen, CD8⁺ T_{RM} cells are able to rapidly divide, kill target cells and orchestrate robust and diverse innate and adaptive immune responses in peripheral tissue sites by producing the polyfunctional cytokines IFN γ , TNF and IL-2, collectively resulting in a local and potent anti-pathogen state^{13,14,99–101} (Box 4).

Concerted efforts to reveal the factors responsible for establishing tissue residency have demonstrated that a variety of cues are critical for the changes that occur in CD8⁺ T cells. CD69 and CD103 are markers often associated with CD8⁺ T_{RM} cells during acute infection, and both molecules can play critical roles in retaining these cells in tissues^{15,94–96,102,103}. CD69 antagonizes S1PR1, preventing CD8⁺ T cells from sensing S1P gradients in the lymphatic vessels draining the tissue that would drive egress¹⁰⁴. CD103 is an integrin (also known as α E β 7) that binds the intercellular adhesion molecule E-cadherin, which is particularly important for maintenance of CD8⁺ T cells in epithelial layers^{96,102,103,105,106}. CD69 and CD103 are not completely faithful markers of CD8⁺ T_{RM} cells — it is well documented that there can be CD8⁺ T_{RM} cells lacking surface expression of CD69 and/or CD103, and CD8⁺ T_{RM} cells can still form in the genetic absence of either CD69 or CD103^{9,96,107}. However, both CD69 and CD103 can play an important role in the retention of cells within certain microenvironments of some tissues, including epithelial layers in the small intestine and skin epidermis^{96,102,107}.

Cues from the tissue can impact expression of both CD103 and CD69 by CD8⁺ T cells. Studies of CD8⁺ T_{RM} cells have highlighted a role for the cytokine tumour growth factor β (TGF β) in regulating CD103 expression^{92,96,98,103,105,108}. TGF β is synthesized intracellularly and deposited in the extracellular matrix, where it remains in an inactive state unless cleaved^{109,110}. Different molecules and signals can induce cleavage of TGF β , including proteases, the integrins α v β 6 or α v β 8, reactive oxygen species, thrombospondin-1, or even changes in pH^{109,110}. Moreover, TGF β signalling can impact a large and broad number of critical host processes, including bone remodelling, wound healing, angiogenesis and fibroblast activation^{109,110}. From a leukocyte perspective, TGF β is a potent anti-inflammatory cytokine, acting to quell local immune responses^{109,110}. Despite the anti-inflammatory properties of TGF β , recent work has revealed the critical role

it plays in CD8⁺ T_{RM} cell maintenance in epithelial tissues^{92,96,98,103,105,108,111}. Elegant work where TGFβ_{RII} or CD103 was knocked out in anti-viral CD8⁺ T cells demonstrated a loss of T_{RM} cells in the skin epidermis and epithelium of the small intestine, while T_{RM} cells in other compartments were spared^{96,98,103,105,108}. Notably, constitutive TGFβ signalling appears to be important, at least in the small intestine epithelium, as blocking active TGFβ after CD8⁺ T_{RM} cells are established resulted in a loss of T_{RM} as well¹⁰⁸. Whether this was due to apoptosis, sloughing off with the epithelium, or migration out of the compartment remains to be determined.

Other cytokines, including IL-33 and type I interferons, have been implicated in establishing CD8⁺ T_{RM} cells in different peripheral tissue sites. Both cytokines have been shown to regulate the C-type lectin CD69 independently of antigen^{92,96,112,113}. Upregulation of CD69 is a common feature in CD8⁺ T_{RM} cells. However, CD69 expression is not required for the formation of CD8⁺ T_{RM} cells, as T_{RM} cells lacking CD69 expression have been documented and CD69-deficient CD8⁺ T cells are able to establish tissue residency in most peripheral tissues, with the exception of the skin and kidney^{9,102,107}. Additional non-cytokine based cues, including signalling via the aryl hydrocarbon receptor (AhR) [G] and hypoxia, have all been shown to induce phenotypical and transcriptional changes associated with CD8⁺ T_{RM} cells^{114,115}. Moreover, numerous transcription factors (including HOBIT, BLIMP1, Eomes, T-bet, and RUNX3) have been implicated as being important for the establishment and maintenance of tissue residency^{111,116,117}. However, the respective roles and functions of these transcription factors appears to be somewhat variable. For example, prior work has shown that while downregulation of Eomes and T-bet leads to enhanced enlodgement of T_{RM} cells in the skin epidermis, total knockout results in impaired long-term maintenance due to downregulation of CD122 and diminished IL-15 sensing¹¹¹. Notably, over expression of either T-bet or Eomes resulted in impaired CD103 expression and reduced T_{RM} cell formation¹¹¹. On the other hand, expression of HOBIT, BLIMP1, and RUNX3 was required for the formation and maintenance of T_{RM} cells in multiple tissues, including the skin, small intestine, and kidney^{116–118}. Significantly less is known about transcription factor requirements for CD4⁺ T_{RM} cells. Notably, RUNX3 does not appear to be important for CD4⁺ T_{RM} cells, demonstrating that there are still significant gaps in our understanding of the molecular drivers responsible for T_{RM} cell formation and longevity, and that these requirements may vary significantly depending on context and cell type¹¹⁸.

While it has become increasingly apparent that CD8⁺ T_{RM} cells play a dominant role in host protection in nonlymphoid tissues, recent work has highlighted the epigenetic and transcriptional diversity that CD8⁺ T_{RM} cells adopt across multiple tissues^{97,102,119,120}. However, despite this diversity, CD8⁺ T_{RM} cells remain relatively plastic, maintaining a poised epigenetic state similar to that seen in T_{CM} cells, which are a population thought to retain the capacity to differentiate into multiple effector T cells subsets in a similar manner to activated naive T cells¹²¹. Consistent with these findings, adoptive transfer of CD8⁺ T_{RM} cells into naive mice and re-stimulation demonstrates that T_{RM} cells are able to differentiate into effector T cells and to form not only T_{RM} cells, but also T_{CM} and T_{EM} cells, similarly to what is seen with reactivated T_{CM} cells^{121,122}. These findings have been extended in a HOBIT-reporter mouse model, nicely demonstrating CD8⁺ T_{RM} cell egress

and differentiation into functional circulating memory CD8⁺ T cells¹²³. Some tissues may drive less plastic T_{RM} cells, as may be the case for skin T_{RM} cells that develop after herpes simplex virus infection⁹⁸. Therefore, despite the numerous tissue-specific alterations that CD8⁺ T_{RM} cells undergo to adapt to their new environment, these cells remain functional and retain a high degree of plasticity in terms of reprogramming potential. Telologically, T_{RM} cell plasticity may be key with respect to tissue maintenance and functionality. Indeed, tissues are not static, and undergo multiple changes associated with time and environmental factors. Thus, the ability of T_{RM} cells to adapt to changes in the local microenvironment may be dependent on this inherent plasticity. However, this area remains underdeveloped, and additional work is needed to clarify the importance of T_{RM} cell plasticity.

Reprogramming potential of T_{EX} cells

In contrast to CD8⁺ T_{RM} cells, most CD8⁺ T_{EX} subsets have an inflexible epigenetic landscape [G] that limits their reprogramming potential and ability to persist in some types of environments (Box 4). T_{EX} cells follow a different developmental trajectory than memory T cells, with the epigenetic landscape of CD8⁺ T cells that go on to become full fledged T_{EX} cells diverging extremely early from CD8⁺ T cells that go on to become memory T cells^{124–129}. Indeed, recent work in lymphocytic choriomeningitis virus (LCMV) infection models in mice has demonstrated that significant transcriptional and epigenetic differences emerge as early as the first cell division¹³⁰. The one T_{EX} cell subset that seems to retain the highest amount of epigenetic plasticity is the T_{PEX} subset^{21,131}. Importantly, some memory properties can be maintained in CD8⁺ T_{PEX} cells^{21,125,131,132}. Early work demonstrated that if CD8⁺ T cells were isolated one week after activation in models of chronic infection or tumours and adoptively transferred into antigen-free mice, then they could persist, albeit to a lesser extent than effector CD8⁺ T cell isolated from acute infection hosts^{125,126}. Moreover, if CD8⁺ T_{EX} cells are maintained in the context of chronic antigen stimulation for roughly four weeks, T_{EX} cells can no longer persist or adopt functional features associated with bona fide memory T cells^{125,126}. More recent work has demonstrated that CD8⁺ T_{PEX} cells are more adept at antigen-independent persistence^{21,131,132}. However, while T_{PEX} cells may have more plasticity than other T_{EX} cell subsets, T_{PEX} cells have been shown to adopt a constricted epigenetic posture in chronic infection, even as early as day 5, which appears to limit their differentiation potential^{21,131}. This epigenetic scar persists even after CD8⁺ T_{PEX} cell transfer into antigen-free hosts, suggesting these changes are permanent. This limited potential to adopt canonical memory-like features is likely multifactorial, but would include a decreased sensitivity to the homeostatic cytokines IL-7 and IL-15^{128,133,134}.

While T_{EX} cells do appear to have a more constrained epigenetic profile, they are not inert, and can be therapeutically leveraged for anti-viral and anti-tumour immunity using several modalities including checkpoint blockade and cytokine stimulation. Although anti-PD-1 monotherapy is able to drive a proliferative burst and effector differentiation in CD8⁺ T_{PEX} cells in chronic infection and tumours^{23–25,135,136}, it does not fundamentally alter the CD8⁺ T_{PEX} cell epigenetic state^{21,22}. Thus the efficacy and durability of PD-1-induced functional reinvigoration is limited^{22,128,137,138}. However, more recent work combining anti-PD-1 with IL-2-based therapies have demonstrated improved quantitative and qualitative CD8⁺ T cell responses in chronic infection and cancer^{139,140}. Moreover, these changes

were associated with T_{EX} cell epigenetic alterations, restoring some, but not all epigenetic features associated with effector and memory CD8⁺ T cells¹³⁹. These findings have important ramifications for therapeutic modalities aimed at harnessing CD8⁺ T_{EX} cells to eliminate cancer, suggesting that the boost in anti-tumour immunity will likely be limited if only severely exhausted T_{EX} cells can be targeted.

T_{EX} cells can adopt some T_{RM}-like features

While CD8⁺ T_{RM} and T_{EX} display significant differences in terms of multipotency, there is increasing evidence, particularly in the context of tumours, that T_{EX} cells may share features of T_{RM} cells that allow them to persist in tissues (Box 4). Indeed, RNA-seq analysis of tumour-infiltrating CD8⁺ T cells has revealed that CD8⁺ T_{EX} cells share some transcriptional features observed in CD8⁺ T_{RM} cells^{117,141–144}. The integrin CD103, which is required to maintain CD8⁺ T_{RM} cells in the small intestine epithelium and epidermis, is expressed by CD8⁺ T_{EX} cells in multiple cancer types^{117,141–146}. Moreover, CD103 expression on tumour-infiltrating CD8⁺ T cells has been shown to be prognostically favourable, igniting an interest in CD103⁺ T_{RM}-like cells as not only a biomarker for favourable responses in tumours, but also as a possible avenue to explore for immunotherapy^{117,141–144,147}. However, as discussed above, CD103 is regulated by TGFβ, an anti-inflammatory cytokine that is abundantly expressed in the tumour microenvironment^{148,149}. Consequently, it is unclear whether CD103 expression by CD8⁺ T cells in tumours is simply reflective of a TGFβ-rich microenvironment, or if there is some functional significance to the expression of this marker. Interestingly, different CD8⁺ T cell populations respond to TGFβ signalling very differently, so the persistence of CD103⁺ CD8⁺ T cells in tumours may at least in part reflect the type of T cells capable of surviving in the microenvironment. While TGFβ is able to drive CD103 expression, it only does so in a subset of IL-7R⁺CD8⁺ effector T cells; naive or memory CD8⁺ T cells do not upregulate CD103 expression in response to TGFβ, and KLRG1⁺ CD8⁺ effector T cells undergo apoptosis after exposure to TGFβ, demonstrating the importance of T cell differentiation in adapting to different signals^{61,102,103,150}. Recent work in acute infection has demonstrated that TGFβ is able to downregulate the transcription factor TCF1 in the lung, which appeared critical for the formation of T_{RM} cells¹⁵¹. Additionally, work in chronic infection and tumours has demonstrated that TGFβ can play important roles in modulating T_{PEX} cells, including in driving tissue residency within the tumour-draining lymph node, and in modulating mTOR activity and metabolism^{61,152,153}. Taken together, the CD103⁺ T_{RM}-like population of T_{EX} cells may represent former TCF1⁺ T_{EX} cells that were capable of responding to TGFβ to adapt to the tissue, rather than undergoing apoptosis. With this perspective in mind, the improved prognosis seen in patients with CD103⁺ T_{RM}-like T_{EX} cells may be associated with a larger TCF1⁺ T_{EX} cell response. Indeed, the latter population is known to be important for maintaining the native immune response to tumours and for propagating the immune response in checkpoint blockade^{22,23,55–57,60,154}. Future work developing ways to enable T_{EX} cells to better adapt to their tissue microenvironment in a similar manner to T_{RM} cells may be extremely useful for improving the longevity of T cell responses to tumours.

The tumour–immune microenvironment

Fundamentally, tumours are derived from self. They originate from a wide spectrum of cell types throughout the body. Concordantly, disease manifestation is markedly variable with respect to tumour progression and evolution, the resultant microenvironment, and concomitant immune response. The developmental and evolutionary processes that occur in tumours are markedly complex, but follow a stereotypical progression¹⁵⁵. For non-inherited cancers, a transformation event (for example, carcinogen exposure or ageing) occurs that generally produces a genetic alteration, resulting in a driver mutation^{156,157}. Some transformed cells will progress to pre-malignant lesions, which continue to accrue mutations and dysplastic features^{155–157}. After some time, pre-malignant lesions can evolve into primary malignant disease, whereby tumours are restricted to their tissues of origin but invade into new subanatomic compartments¹⁵⁵. Finally, after sufficient tumour evolution, some cancers are able to metastasize from their tissue of origin to new tissues, using either the lymph and/or blood as a conduit^{158–160} (Figure 2). Because of the diverse transcriptional changes that occur over the course of tumour evolution, the immune response, and especially the T cell response, changes considerably over the course of tumour progression^{155–157}. In this section, we will discuss how signals within the tumour microenvironment can shape T cell fate and function. Additionally, we will discuss emerging evidence in the field of metastasis, and how forming a metastatic lesion within certain tissues can lead to poor prognosis in the context of checkpoint blockade.

Primary tumour environmental challenges

In order to combat the tumour, T cells have to become activated in the tumour-draining lymph node, migrate to the tissue, and adapt to the unique tissue microenvironment containing the tumour (Figure 1). As discussed previously, there are significant hurdles at each of these steps that T cells have to overcome to get into the tumour microenvironment in the first place (Figure 1). Once within the tumour microenvironment, there are a number of additional challenges that T cells have to surmount, including chronic antigen exposure, chronic inflammation (or lack of inflammation), aberrant vasculature, immunosuppressive cytokines, immunosuppressive leukocytes, fibrosis, metabolic constraints and/or nutrient deprivation, and hypoxia³², some of which will be discussed here.

Chronic antigen exposure within the tumour microenvironment is of particular relevance to anti-tumour immunity because of the well described role it plays in the formation of T_{EX} cells. As stated above, once CD8⁺ T cells progress towards a T_{EX} cell differentiation state, it is incredibly difficult to recover CD8⁺ T cell function and plasticity^{125–128,161}. Moreover, the development of T cell exhaustion may make dealing with the other factors in the tumour microenvironment more challenging. A significant determinant for how T cell receptor stimulation is perceived by CD8⁺ T cells is based on the amount of stimulation received and the cell type presenting peptide–MHC class I. Undoubtedly, antigen presentation by tumour cells can drive T cell dysfunction¹⁸. However, the amount of antigen presented can have significant effects on T cell differentiation — even low levels of antigen expression can drive T cell dysfunction¹⁶². While tumours present antigens on MHC class I molecules via the endogenous pathway of antigen processing, other leukocyte populations, namely

macrophages and dendritic cells (DCs), are able to cross-present tumour-derived antigens on MHC class I. Macrophages are tasked with maintaining host homeostasis and responding to tissue disruption. In the context of the tumour microenvironment, macrophages often play a role in suppressing the CD8⁺ T cell response¹⁶³. In fact, recent work has demonstrated that macrophage–CD8⁺ T cell interactions tend to be long lived and prevent infiltration and elimination of tumour cells¹⁶⁴. This was demonstrated to be due to tumour antigen presentation by macrophages, and it was shown that macrophage antigen presentation to T cells is a strong inducer of T cell dysfunction¹⁶⁵. On the other hand, DCs, although far sparser in the tumour, are more likely to promote productive T cell activation in tumours^{166,167}.

Beyond chronic antigen, numerous other factors can dictate the efficacy of the T cell immune response. A substantial barrier to CD8⁺ T cell immunity is the stroma within tumours. Cancer-associated fibroblasts (CAFs) are a heterogeneous group of stromal cells with many significant roles in tumours, but are especially responsible for shaping the local ultrastructure of the tumour. CAFs are derived from local fibroblast precursors^{168,169}, and occupy a variety of cell states within tumours^{170–172}. The fibroblast composition and density of extracellular matrix can be important factors limiting T cell responses^{173,174}. Indeed, in pancreatic cancer in particular, local fibrosis markedly blunts T cell motility within the tumour microenvironment¹⁷⁵. Moreover, recent work has highlighted that myofibroblasts alter the extracellular matrix in pancreatic cancer¹⁷⁶. Here, it was demonstrated that myofibroblasts secrete a type I collagen homotrimer, consisting of three $\alpha 1$ subunits¹⁷⁶. This altered microenvironment changed the local composition of the microbiome and inflammatory chemokines, and preventing formation of type I collagen homotrimers resulted in improved CD8⁺ T cell responses against tumours¹⁷⁶.

Aside from physical constraints in the tumour microenvironment, numerous cytokines are elevated in tumours, including TGF β , IL-33 and type I IFN. As discussed above, TGF β and IL-33 can be important for driving a T_{RM}-like phenotype in CD8⁺ T cells^{148,149,177}. However these cytokines do more than drive direct changes in CD8⁺ T cell phenotype. TGF β also modulates CAFs to suppress productive immune responses^{178,179}. Additionally, TGF β can drive epithelial-to-mesenchymal transition [E] in tumour cells, promoting metastasis^{180–183}. These shifts in tumour cell state matter, as evolution into a mesenchymal or mesenchymal-like state can cause the upregulation of numerous inhibitory receptor ligands (such as PD-L1)¹⁸⁴, chemokines and cytokines¹⁸⁵ and changes in metabolism¹⁸⁶. IL-33, much like TGF β , can have intrinsic effects on tumour cell state and on CAFs¹⁸⁷. It also drives changes in numerous innate and adaptive leukocytes, including mast cells, eosinophils, macrophages, innate lymphoid cells and regulatory T cells^{188–190}. Importantly, eliminating IL-33 or TGF β results in improved CD8⁺ T cell responses in multiple different pre-clinical models^{189,191–193} despite the role of these cytokines in contributing to the establishment of residency and tissue-specific adaptations in CD8⁺ T cells. These data suggest that the potential benefits of IL-33 and TGF β on tumour-specific CD8⁺ T cells may be outweighed by the detrimental effects in terms of modulating immunosuppressive populations and tumour cell biology. Lastly, type I (IFN α /IFN β) and type II (IFN γ) IFNs have also been shown to be elevated in tumours, though the precise role of these

cytokines both in endogenous anti-tumour responses as well as in responses to checkpoint blockade has been somewhat controversial. While acute IFN signalling can have protective anti-tumour effects, chronic IFN signalling can be extremely immunosuppressive, which has been extensively reviewed elsewhere^{194,195}. Determining how to best modulate these cytokines for therapeutic gain remains an active area of investigation, and will likely be heavily context-dependent depending on disease type and stage.

Tumours are exceptional in their ability to metabolically dominate their local microenvironment, stealing resources to grow, proliferate and evolve¹⁹⁶. Because of this, the concentration of local metabolites is shifted in the tumour microenvironment as compared to in normal, non-neoplastic tissue [G]¹⁹⁶. Moreover, the specific resources used by tumours can be highly dependent on the types of mutations each tumour has (for example, *KEAP1* mutation in lung cancer drives a distinct metabolic state)¹⁹⁷ and on the tissue the tumours are located in, even where the tumours are of the same origin (for example, pancreatic tumours in the pancreas and those in the subcutaneous space use completely different metabolic pathways)¹⁹⁸. These changes inherently matter for T cells. Different T cell subsets all have discrete metabolic needs and requirements. For example, effector T cells are highly dependent on glycolysis, and in the context of a low glucose environment, T cell functionality declines^{199,200}. Moreover, metabolism can divert T cell differentiation state, and can either improve or compound alterations seen in T cell dysfunction^{201–204}. Collectively, tumour metabolism can have significant direct and indirect repercussions for anti-tumour immunity.

Metastasis – the recipient tissue matters

Metastasis is the process whereby cancer cells migrate away from the primary tumour site, intravasate into either blood or lymphatic vessels, and enter a different tissue to form a new neoplastic lesion^{158–160} (Figure 2). Metastasis can occur regionally in downstream lymph nodes and adjacent tissues, or can be distal, with tumour cells ending up in a location far away from the original primary tumour¹⁵⁸ (Figure 2). Patients with metastatic disease have a significantly worse clinical prognosis, and as such there have been intensive efforts to understand the biology behind metastatic spread^{158,205,206}.

Fundamentally, the biological processes engaged in metastatic versus primary tumours are distinct. In the context of primary tumours, the original transformed cells are derived from the tissue they are initiated in. However, with metastatic spread, one cell type may end up in a tissue lacking that cell type altogether. While metastasis profoundly changes the local microenvironment inhabited by the tumour cells, organ-specific factors are likely to play an important role in shaping the T cell response. Indeed, recent work examining T cells in 21 different tumour types found that T cell states were varied in each tumour type²⁰⁷. However, examining different tumour types that metastasized to the brain demonstrated a much more uniform cell state for T cells across tumour type in the same location, suggesting that the location of the metastatic lesion, rather than the tissue of origin, may be a critical regulator of the T cell response²⁰⁸. Consistent with this concept, when prostate cancer cells were transplanted subcutaneously into the flank or intraosseously into the bone marrow, the resultant T cell response was altered²⁰⁹. Mechanistically, local TGFβ levels were much

higher in the bone marrow than in the flank²⁰⁹. Increased TGF β was sufficient to change the CD4⁺ T cell response (skewing from a Th1 cell response to a Th17 cell response), and critically, this altered the response to checkpoint blockade therapy²⁰⁹.

A metastatic site that carries additional significance is the lymph node (Figure 2). The lymph node, as alluded to above, represents a safe-haven for tumour-specific CD8⁺ T cells, and is an important site for lymphocyte homeostasis^{55–57}. By virtue of being downstream of the primary tumour, the tumour-draining lymph node receives a variety of cues including cytokines, metabolites and other factors that can impact lymphatic and endothelial vessel structures, and, by extension, impact T cell recruitment^{53,210–215}. Metastasizing to the lymph node represents a significant immunological problem, as it may disrupt the reservoir of functional tumour-specific CD8⁺ T cells that are important for maintaining the anti-tumour immune response in the primary tumour lesion^{210,216}. Future studies examining how CD8⁺ T cell responses in metastatic lesions in diverse tissues differ from one another, and how the tissue site of the metastatic lesion shapes the immune response will be critical.

The impact of metastatic disease on immune responses to checkpoint blockade is currently an active area of investigation. Considering the diversity of potential combinations of factors that are likely to influence response to checkpoint blockade, this is an area that will take significant time and clinical data to disentangle. While it remains unclear how the location of the metastatic lesion contributes to increased resistance to checkpoint blockade therapy, there are common themes that have emerged. For example, the presence of metastatic lesions in the liver is often associated with poor response to checkpoint blockade²¹⁷. This has been seen in a number of primary cancer types²¹⁸, including melanoma, non-small cell lung cancer (NSCLC), microsatellite stable (MSS) metastatic colorectal cancer, advanced gastric cancer and advanced colorectal cancer²¹⁸. Mechanistically, it is speculated that the reason for this poor prognosis is the fact that the liver microenvironment can delete or broadly induce a state of immunological tolerance in liver-infiltrating T cells²¹⁷. The ability of the liver to induce tolerance has been observed extensively in the transplant setting, where liver transplant patients require less immunosuppression than patients receiving other types of tissue transplants^{217,219}. Recent work in a pre-clinical model utilized a two-tumour approach (one in the flank or lung and one in the liver) to interrogate the impact of tumours in the liver on systemic anti-tumour immunity and on outcomes to PD-1 immunotherapy²²⁰. Here, tumour antigens in the liver led to systemic suppression of anti-tumour immunity and reduced efficacy to PD-1 inhibitors. This immune suppression was dependent on regulatory T cells, and reducing regulatory T cell activity using either anti-CTLA4 or an EZH2 inhibitor could promote responsiveness to PD-1 blockade²²⁰. Future work extending these findings and further interrogating how metastases to certain organs impacts systemic immunity during PD-1 blockade are warranted to identify potential combination therapies that improve the efficacy of immunotherapy in patients with metastasized tumours.

Concluding remarks

CD8⁺ T cells are contact-dependent killers. Because the naive T cell repertoire is anatomically restricted to the lymph nodes, spleen and blood, migration is a requisite for all T cell-based immune responses occurring in nonlymphoid sites^{7,8}. Consequently, the ability

to localize and adapt to tissue microenvironments is essential for productive CD8⁺ T cell responses^{8,31}. Once within a tissue, T cells are profoundly shaped by local tissue-derived cues, a process that is important for persistence within the unique physiological constraints of that microenvironment^{94,95}. In cancer, each major step of the T cell response — from productive T cell activation in the tumour-draining lymph node, to migration to the tumour, infiltration of the tumour and adaptation to survive in the tumour microenvironment — represents an opportunity for the tumour to evade host immunity, but also a therapeutic opportunity that can be leveraged clinically (Figure 1). This complexity is amplified in the context of metastasis due to the combinations of primary tumour origin, site of metastatic lesion, current state of the T cell response, and the necessary cues/signals to get T cells to the metastatic lesion, collectively making metastatic disease more difficult to treat clinically (Figure 2).

While immunotherapy has come a long way in terms of curing cancer, additional progress must be made²²¹. Deficiencies in T cell migration and tissue-specific adaptations to thrive in the new microenvironment represent major therapeutic opportunities to augment the efficacy of immunotherapy. Starting from the initiation or generation of a response, better approaches to stimulate CD8⁺ T cells within the lymph node are needed. These modalities could include: one, newer vaccination-based methodologies, including lipid nanoparticles^{222–224}; two, approaches to release functional tumour-specific CD8⁺ T cells from the tumour-draining lymph node; and three, approaches that increase migratory DC numbers and their stimulatory capacity within the tumour-draining lymph node, as has been done in pre-clinical models with FLT3L and agonistic CD40 antibody^{55,225,226}. With respect to migration, approaches to surmount or fix vascular anergy may allow for optimal migration of effector T cells into the tumour microenvironment. Significant efforts should also be placed on understanding which T cell differentiation state is best suited to infiltrating and adapting to tumours, and how can T cells be manipulated (for example, through genetic manipulation) to best conform to this cell state. Moreover, efforts to help T cells better adapt and survive within the challenging tumour microenvironment may significantly improve outcomes following immunotherapy. For example, relieving metabolic constraints, nutrient deprivation, and/or hypoxia may improve T cell survival. Additionally, recent work has identified tertiary lymphoid structures (TLS) [G] within tumours, and the presence of these structures is associated with better prognosis and better responses to checkpoint blockade (Box 5). Finally, significant progress to disentangle basic mechanisms of immunological dysfunction in the setting of metastasis, and how to best utilize immunotherapy approaches to overcome these obstacles, will be necessary for future clinical success. Metastatic lesions remain a substantial and often intractable clinical disease, and this area could significantly benefit from reverse translation [G], an approach that takes clinical observations back to the preclinical setting to probe deep mechanism²²¹. Understanding how metastatic lesion location affects the overarching immune response, how to best get T cells to infiltrate metastatic lesions, and what strategies can be utilized to provoke the best immune response possible are all areas needing additional efforts.

A better understanding of the fundamentals of cancer immunology will be key to drive the next generation of immunotherapies. Applying lessons learned from acute infection and pre-clinical models will light the way, and hopefully help to turn the page on cancer fatality.

Acknowledgements

We apologize to colleagues whose work was not cited in our Review due to space constraints. This work was supported by a grant from the US National Institutes of Health (NCI, K08-CA256044 – J.M.S.).

Glossary Terms:

Adjuvant setting

Refers to therapies that are given after the primary cancer treatment (for example, after major surgery to remove the tumour) and that are intended to keep the cancer from returning.

Aryl hydrocarbon receptor (AhR)

A ligand-activated transcription factor that integrates environmental, dietary, metabolic and microbial cues within a cell to modulate immune responses in settings of both health and disease. This receptor acts in a ligand-specific, cell-type specific and context-specific manner.

Cancer immunotherapy

A type of treatment that targets the host immune system to fight cancer.

Central memory T (T_{CM}) cells

A population of memory T cells that is anatomically restricted to spleen, lymph nodes, and blood, and that uses the same trafficking molecules as naive T cells (namely CCR7, CD62L and LFA1) to circulate through these organs. T_{CM} cells are thought to retain the highest level of plasticity in terms of re-differentiating into other T cell subsets and possess the greatest degree of longevity of the memory subsets.

Checkpoint inhibitors

Refers to a type of immunotherapy where monoclonal antibodies are used to block major immunological ‘checkpoints’ for immune activation. These checkpoint molecules generally refer to inhibitory receptors expressed by T cells, including CTLA4, PD-1, LAG3, TIM3 and TIGIT, though in some cases the ligand for these receptors (for example, PD-L1) are the target instead of the receptor itself.

Chemokine receptors

A family of G-protein coupled receptors involved in cellular migration and activation.

Effector T cells

T cells that have recently encountered antigen and have fully differentiated into an activated state. This differentiation process includes proliferation and acquisition of effector functions, such as inflammatory cytokine production and cytotoxicity.

Effector memory T (T_{EM}) cells

A population of memory T cells that surveys non-lymphoid tissue and blood following antigen clearance. T_{EM} cells are thought to be a recirculating population, which differentiates them from canonical tissue resident memory T cells, which permanently establish residency in a tissue. T_{EM} cells are generally thought to be less long-lived and less plastic than central memory T cells.

Epigenetic landscape

Epigenetic regulation refers to the broad set of heritable changes in gene expression that occur independently of changes to the DNA sequence (for example, DNA methylation, histone modifications). The epigenetic landscape refers to the entire set of accessible chromatin regions in a cell, which dictates cell lineage, fate, and effector potential by controlling which genes can actually be expressed.

Epithelial-to-mesenchymal transition (EMT)

A complex, biological process that allows polarized epithelial cells that normally interact with a basement membrane to convert into a mesenchymal state, enabling enhanced migratory capacity, invasiveness, increased production of extracellular matrix components, and increased resistance to apoptosis. This allows the cell to detach from the basement membrane and migrate away from the epithelial layer. This process occurs during normal embryonic development, tissue generation, organ fibrosis, and wound healing. This process is notably exploited by cancer cells, and is a major pathway involved in tumour invasiveness and metastasis.

Exhausted T cells (T_{EX})

A type of T cell dysfunction that is common in chronic infection and cancer. Following activation and differentiation, chronic antigen exposure causes T_{EX} cells to progressively lose effector activity and effector potential, marked by decreased proliferation, cytokine production, and cytotoxicity. T_{EX} cells also express high levels of co-inhibitory receptors and the transcription factor TOX.

Extravasation

The process of cellular migration from the blood vessels into a tissue.

Fibrosis

The process by which fibrous connective tissue accumulates in response to tissue injury or damage

Hypoxia

Refers to a tissue environment where oxygen levels are low

Immunosuppressive cytokines

Broadly refers to a class of cytokines capable of suppressing or dampening host immune responses. These cytokines are often overexpressed in cancer, and can include IL-10 and TGFβ.

Immunosuppressive leukocytes

Refers to leukocyte populations that are capable of countering pro-inflammatory immune responses, and often lead to immunotherapy resistance in the context of cancer. These populations include regulatory T cells, myeloid-derived suppressor cells, and some populations of tumour-associated macrophages and neutrophils.

Integrins

A family of transmembrane receptors that is critical for facilitating cell-cell adhesion and/or cell-extracellular matrix adhesion. Integrins are heterodimers. In humans, there are at least 18 different alpha subunits and 8 different beta subunits, which can heterodimerize to form 24 heterodimers. Integrins bind ligands that are members of the immunoglobulin superfamily.

Memory T cells

Antigen-experienced T cells that persist long term after antigen clearance. There are multiple subtypes of memory T cells classified broadly based on location, including central memory T cells (restricted to secondary lymphoid organs), effector memory T cells (found recirculating through tissues), and tissue resident memory T cells (permanently retained within a tissue). Memory T cells can re-acquire effector properties upon antigen re-encounter more rapidly than naive T cells.

Naive T cells

T cells that have not yet become activated by cognate peptide–MHC presented by professional antigen presenting cells. Naive T cells are anatomically restricted to the spleen, lymph nodes, and blood, using the trafficking molecules CCR7 (chemokine receptor binding CCL19 and CCL21), CD62L (selectin binding 6-sulpho sialyl Lewis X oligosaccharides present on high endothelial venules), and LFA1 (an integrin that binds ICAM1) to mediate entry into these sites.

Neoadjuvant setting

Broadly refers to therapies that are given before the primary cancer treatment (for example, prior to major surgery to remove the tumour).

Non-neoplastic tissue

A tissue that has not transformed and/or does not contain a tumour

Progenitor T_{EX} (T_{PEX}) cells

A subset of T_{EX} cells that expresses high levels of the transcription factor TCF1, lower levels of co-inhibitory receptors, and retains higher proliferative capacity than other T_{EX} cell subsets. The T_{PEX} cell subset contains stem-like properties, being able to divide to give rise to more T_{PEX} cells, as well as differentiate into other T_{EX} cell subset including the terminally-exhausted T_{EX} cell subset. This subset preferentially proliferates in response to PD-1 checkpoint blockade.

Resident memory T (T_{RM}) cells

A population of memory T cells that establishes residency within a given tissue (that is, once it enters, it does not leave). T_{RM} cells have been described in both lymphoid tissue and

non-lymphoid tissue. The surface markers CD69 and CD103 have both been associated with T_{RM} cells, though not all T_{RM} cells express these markers.

Reverse translation

An approach where observations are made from clinical samples that are hypothesis generating, and then those hypotheses are subsequently tested in preclinical mouse models where mechanism can be interrogated.

Selectins

A family of cell surface adhesion molecules that is important for leukocyte trafficking. Selectins are single chain, transmembrane glycoproteins that bind fucosylated, sialylated, or sulfated ligands.

Sphingosine-1-phosphate receptor 1 (S1PR1)

A G-protein coupled receptor that binds the phospholipid sphingosine 1-phosphate (S1P). S1PR1 regulates T cell migration between tissues and circulatory fluids. S1PR1 plays a critical role in T cell egress from lymph nodes and tissues by enabling T cells to sense high levels of S1P in efferent lymphatics and blood. S1PR1 is directly antagonized by CD69 at the cell surface, so if CD69 is expressed, T cells fail to up regulate S1PR1 and respond to S1P.

Terminally-exhausted T_{EX} cells.

A subset of T_{EX} cells that is terminally differentiated, expresses low to no TCF1, and high levels of co-inhibitory receptors including PD-1, TIM3, LAG3, and TIGIT. Terminally-exhausted T_{EX} have poorer proliferative capacity and inflammatory cytokine production than other T_{EX} cell subsets, but do retain a heightened ability to kill target cells. Terminally-exhausted T_{EX} cells cannot differentiate into other T_{EX} cell subsets, and are poorly proliferative in response to PD-1 checkpoint blockade.

Tertiary lymphoid structures (TLS)

Induced ectopic lymphoid structures that develop in non-lymphoid tissues and/or tumours. TLS are organized aggregates of immune cells that resemble secondary lymphoid organs, but are not encapsulated. TLS are generally associated with inflamed tissues, and have been documented in cancer, autoimmunity, and chronic inflammatory disorders.

Vascular anergy

A phenomenon that occurs when blood vessels receive continual VEGF stimulation, causing them to become unable to upregulate inflammatory chemokines and integrin ligands to permit leukocyte trafficking

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Key Points:

- Productive T cell-mediated immunity is dependent on the ability of T cells to traffic to the site where they are needed and adapt to the new tissue site.
- Not all T cells interpret cues from their tissue microenvironment in the same way — T cell differentiation state shapes the way external cues are sensed by the cell.
- While most exhausted CD8⁺ T cell subsets have an inflexible epigenetic framework that limits their adaptability to certain types of microenvironments, they can adopt some resident memory-like features to persist in tissues.
- Primary tumours represent aberrant versions of the original host tissue and place strains on T cells, including a high antigen load, an abnormal vasculature, hypoxia, nutrient deprivation and an abnormal extracellular matrix.
- Metastasis involves tumour cells from one tissue invading and establishing residence in another tissue, and the physiology of the destination tissue can shape both the new tumour microenvironment and the immune response.
- T cell migration and adaptability to different types of microenvironments represent therapeutic opportunities to improve outcomes during cancer immunotherapy.

Box 1:**T cell differentiation following priming of naive T cells**

Under optimal priming conditions to foreign antigens, naive T cells become activated and undergo a differentiation process where they develop into highly functional effector T cells [G] capable of migrating to sites of antigen and inflammation to eliminate antigen-bearing target cells^{7,8}. CD8⁺ T cells kill target cells in a contact-dependent manner, requiring them to migrate to peripheral sites of pathogen entry and replication to perform their effector functions. Because of the necessity to function in diverse tissue microenvironments, effector T cells evolved to efficiently adapt to different types of tissues^{7,8}. If antigen is cleared, memory T cell [G] populations form that retain high recall potential, including the ability to re-acquire effector functions including rapid proliferation and production of effector molecules (including effector cytokines and cytotoxic molecules) upon antigen reencounter. Memory T cells are characterized into subsets including central memory T (T_{CM}) cells [G], effector memory T (T_{EM}) cells [G], and T_{RM} cells, which are broadly defined based on location, tissue residence, longevity, and plasticity^{7,8}. Because acute infection models drive robust and stable CD8⁺ T_{RM} cell responses that are highly capable of eliciting robust recall responses upon antigen reencounter, these model systems are instructive for identifying and understanding the optimal conditions for differentiation and effector potential. Moreover, they have been informative to establish the basal requirements for T_{RM} cells to thrive in different organ systems.

Box 2:**Exhausted T cell heterogeneity – lessons from infection**

Early work from the Wherry group demonstrated heterogeneity within exhausted T (T_{EX}) cell populations in chronic LCMV infection in mice^{26,27}, with critical ramifications for both responses to PD-1 checkpoint blockade²⁶ and longevity of the T_{EX} cell response²⁷. Seminal work in 2016^{23–25,227} further refined the conceptualization of two of the major T_{EX} cell subsets, showing that the transcription factor TCF1 was required for the formation of the precursors of exhausted T (T_{PEX}) cell population. Since these original papers, there has been tremendous efforts to understand the lineage relationships between the T_{PEX} and terminally-exhausted T_{EX} cell subsets, identify key molecular and cellular regulators of these major cell lineages, and define additional functional heterogeneity between these two polar extremes^{22,60,124,131,228–232}.

T_{PEX} cells are generally believed to be the least differentiated of the exhausted subsets, with the greatest reprogramming potential and responsiveness to checkpoint blockade^{21–23,139,140}. Additionally, T_{PEX} cells show different metabolic properties compared to other T_{EX} cell subsets^{152,233–235}, which may aid their reprogramming potential. In T_{PEX} cells, TCF1 is the critical lineage-defining transcription factor, driving expression of Eomes and MYB which reinforce this programme, and are critical for maintaining T_{PEX} cells^{124,232}. Moreover, additional work has demonstrated the critical need for BACH2 expression, and BATF repression to maintain T_{PEX} cells^{131,236}. TCF1-KLRG1⁺ effector-like T cells can form early during chronic LCMV infection, and appear to branch off early from the T_{PEX} cell population and do not follow the same differentiation trajectory as canonical T_{EX} cells¹²⁴. Along the differentiation trajectory that leads to terminally-exhausted T_{EX} cells, T_{PEX} cells give rise to intermediate-like or transitory T_{EX} cell populations^{135,136,237}. Different groups have given different names to each subset. One study described Tex^{prog1} , Tex^{prog2} , Tex^{int} and Tex^{term} , which followed a lineage trajectory²³⁷. In another study, a transitory population developed from T_{PEX} cells that expressed CX₃CR1 and TIM3, but retained higher levels of granzyme B and lacked the marker CD101^{135,136}. The most terminally-differentiated T_{EX} cell subset is generally thought to express CD101 and high levels of other inhibitory receptors, including TIM3^{135,136}. While there is evidence to suggest that similar subpopulations exist in some cancer types in both mice and humans^{22,55,56,60,166,216,238} (for example T_{PEX} cells in the lymph node, terminally-exhausted T_{EX} cells in immunologically hot tumours), additional work is needed to understand how much of this T_{EX} cell framework from chronic infection will extrapolate to cancer, and how to best therapeutically harness the T cell subsets present in diverse cancer types.

Box 3:**Neoadjuvant checkpoint blockade**

With the successes of checkpoint blockade in advanced, non-resectable disease^{1–6}, there is interest in using checkpoint inhibitors in earlier stage disease. Here, checkpoint blockade can be given in the adjuvant setting [G] (after surgery), or in the neoadjuvant setting [G] (prior to surgery). Perioperative anti-CTLA4 (ipilimumab) was first used in localized urothelial carcinoma, where safety was demonstrated²³⁹. Since this initial work, larger clinical studies have examined the efficacy of neoadjuvant checkpoint inhibitors (targeting PD-1, CTLA4, or both) in several cancer types^{3,221,240}. Studies in resectable early stage NSCLC^{241–244}, triple-negative breast cancer (TNBC)^{245–247}, and locally advanced melanoma^{248–253} in particular have shown tremendous promise. Neoadjuvant checkpoint-based immunotherapy is now FDA approved for specific indications in combination with chemotherapy, including pembrolizumab plus chemotherapy for clinical stage II or III TNBC (July 2021) and nivolumab plus chemotherapy for early stage NSCLC (March 2022). Considering successes in clinical trials, it is anticipated that this approach will become more common in the clinic.

Despite the clinical successes for neoadjuvant checkpoint blockade, we lack an understanding of why this approach is so effective^{3,221,240}. One proposed mechanism is that an early surge in tumour cell killing can release antigens from the primary tumour to the tumour-draining lymph node, improving T cell activation in the lymph node. This could lead to the generation of a more functional anti-tumour T cell response, and after surgical removal of the primary tumour, the T cells remaining in the patient could serve as a source of protection against either relapse or micrometastatic lesions. A major advantage of harnessing the adaptive immune system to eliminate cancer is the notion that T cells are long lived and can traffic to and survey nearly every tissue in the body if adequately primed. Neoadjuvant checkpoint blockade may provide an effective approach for eliciting a broad acting systemic T cell-mediated immune response^{3,221,240}.

Box 4:**Comparison of shared features between T_{RM} and T_{EX} cell states**

T_{RM} cells that form during acute infection are an ideal example of functional CD8⁺ T cells in tissues. T_{RM} cells are characterized by their superior ability to mediate host protection against pathogen reencounter, retaining high proliferative capacity, cytotoxicity, and the ability to produce inflammatory cytokines rapidly upon rechallenge (see Box Table)^{13–15,94,99}. These cells are also highly capable of adapting to their tissue microenvironment, are long lived, and retain a high degree of epigenetic plasticity, possessing the ability to re-differentiate into T_{CM} cells or T_{EM} cells upon antigen rechallenge^{31,94,121–123}. Notably, the longevity of T_{RM} cells varies depending on the tissue (for example, they are shorter lived in lung and longer lived in skin epidermis). Conversely, T_{EX} cells lose the ability to sustain proliferation, are poorly cytotoxic, and show impaired cytokine production compared to T_{RM} cells (see Box Table)¹⁶. T_{EX} cells comprise multiple subsets, with the T_{PEX} cell population retaining higher proliferative potential and showing less signs of dysfunction than the more terminally-exhausted T_{EX} cell subset^{19,20,23,27}. While T_{EX} cells are inferior in host protection compared to T_{RM} cells, they are not inert, and do possess some ability to kill, produce cytokines, and provide some level of host protection. Consequently, T_{EX} cells are important for the host in terms of generating some level of protection in these chronic settings, and provide a therapeutic opportunity to improve outcomes since some T_{EX} cells (for example, T_{PEX} cells) can be at least temporarily reinvigorated to function better^{22,23,60}. Similarly to T_{RM} cells, T_{EX} cells do retain some ability to adapt to their microenvironment. Like T_{EX} cells, T_{RM} cells do express higher levels of inhibitory receptors than other memory subsets.

Characteristic	Level of characteristic shown in T cell subset		
	Tissue resident memory T cells (T _{RM})	Precursors of exhausted T cells (T _{PEX})	Exhausted T cells (T _{EX})
Epigenetic plasticity	High	Medium	Low
Proliferative capacity	High	Medium	Low
Longevity	High	Medium	Low
Antigen-free maintenance	High	Medium	Low
Cytokine secretion	High	Medium	Low
Cytotoxicity	High	Low	Medium
Sensitivity to tissue-derived cues	High	Unknown	Unknown
Contribution to host protection	High	Medium	Low
Expression of inhibitory receptors	Medium	Medium	High
Antigen addiction	Low	Unknown	High

Box 5:**Tertiary lymphoid structures in cancer**

Emerging work has demonstrated that clusters of leukocytes can be identified in tumours in pre-clinical mouse models and primary human samples. The most common cluster identified is tertiary lymphoid structures (TLS), which are defined by the presence of high endothelial venules and can develop in or around tumours^{254–258}. The degree of organization for TLS is variable, ranging from disorganized (with a mixed B cell and T cell infiltrate) to structures with an established T cell zone, follicles and active germinal centres. TLS have been associated with better clinical prognosis and response to checkpoint blockade therapy^{255–259}. While there are many outstanding questions regarding TLS in cancer, a current hypothesis is that these structures represent areas where T cells can be primed or continuously activated by antigen presenting cells (APCs). Mouse models of viral infection and autoimmunity have demonstrated that a variety of cues result in the genesis of these structures, including lymphotoxin, CXCL12, CXCL13, type I interferon and IL-17^{260,261}. What factor or factors drive TLS formation in tumours is unknown, but recent work has demonstrated that checkpoint blockade may be sufficient to generate TLS²⁶². The other recent TLS-like structure that has been described in tumours consists of proliferative TCF1⁺CD8⁺ T cells and MHC class II⁺ APCs^{166,263}. This co-clustering of TCF1⁺CD8⁺ T cells and APCs is prognostically favourable. Patients lacking these co-clusters tend to have poorer T cell infiltration into tumours and progressive disease¹⁶⁶. How these structures form, and what their precise role is remains unclear.

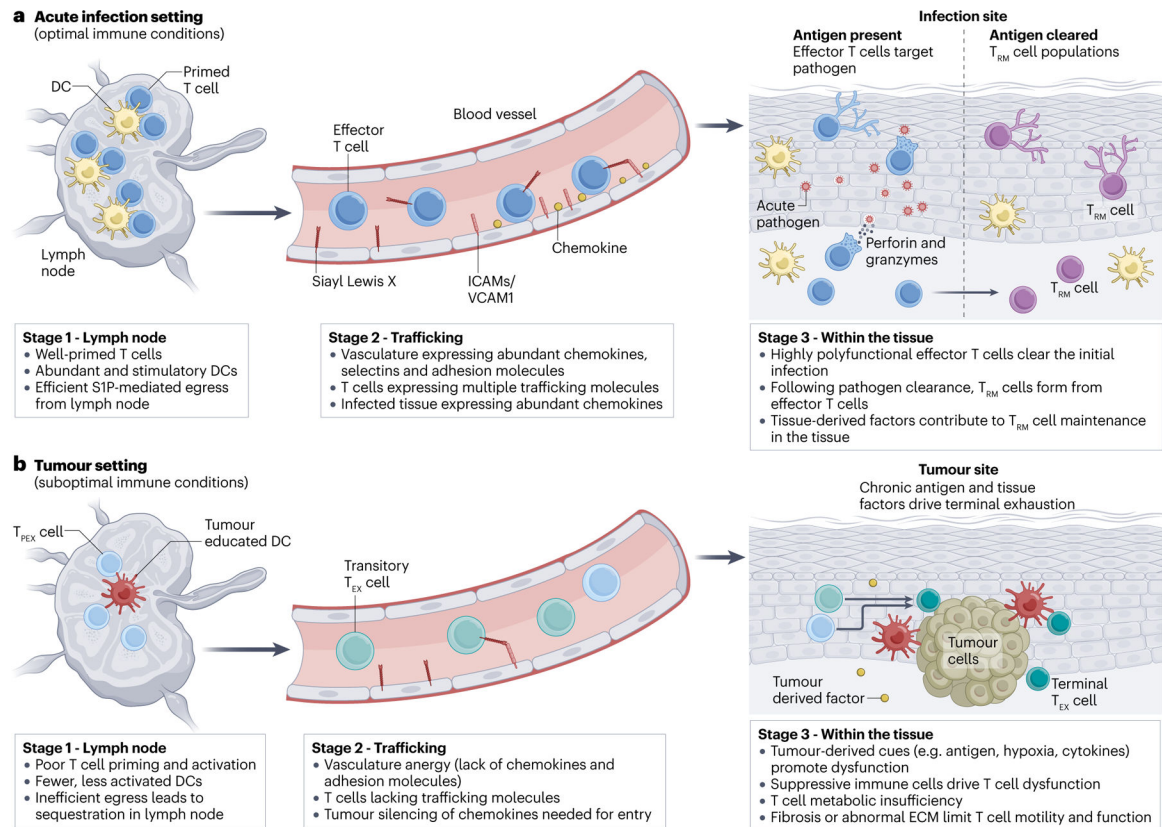


Figure 1: Comparison of the development of optimal and suboptimal T cell responses.

The figure compares the development of a T cell response in (a) an optimal setting (for example, in acute infection) and (b) in a suboptimal setting (for example, in cancer). The major stages of the $CD8^+$ T cell response are depicted, including: stage 1, T cell priming in the tissue-draining lymph node; stage 2, T cell trafficking through blood vessels and extravasation into tissues; and stage 3, T cell adaptation within the tissue microenvironment and their execution of effector functions in tissues. During acute infection (a), T cells are primed in tissue-draining lymph nodes with abundant, stimulatory dendritic cells (DCs), resulting in full effector T cell differentiation and they egress from the lymph node via S1PR1–S1P. Effector T cells enter the vasculature and bind abundant chemokines, selectin ligands, and integrin ligands present on the inflamed vasculature. These effector T cells then extravasate into tissues and are highly polyfunctional, producing inflammatory cytokines (for example, $IFN\gamma$, TNF and IL-2) and killing target cells. The concerted efforts of these highly effective, polyfunctional effector T cells results in clearance of the pathogen. Following pathogen clearance, a population of $CD8^+$ T_{RM} cells forms from the effector T cell population and establishes permanent residency within the tissue. T_{RM} cells use molecules such as CD69 and CD103 to stay within the tissue. In the cancer setting (b), $CD8^+$ T cells encounter barriers to successful anti-tumour immunity. In the tumour-draining lymph node, $CD8^+$ T cells can be poorly primed owing to fewer, less-stimulatory DCs. Moreover, some tumour-specific $CD8^+$ T cells become sequestered, failing to go through the normal S1PR1–S1P egress process. In the vasculature, tumour-specific $CD8^+$ T cells can lack the requisite trafficking molecules to efficiently migrate to sites of inflammation.

Alternatively, vascular anergy can occur, where blood vessels receive continual VEGF stimulation, causing them to become unable to upregulate inflammatory chemokines (such as CXCL9 and CXCL10) and integrin ligands (such as VCAM1 and ICAM1) that permit leukocyte trafficking and extravasation. Within the tumour, CD8⁺ T cells are exposed to chronic antigen stimulation, immunosuppressive cytokines and leukocyte populations, hypoxia and/or nutrient deprivation, and fibrosis and/or abnormal extracellular matrix deposition — this limits T cell motility and function. However, exhausted T cells (T_{EX}) cells are not inert. Though functionally not as effective as effector T cells, T_{EX} cells do continue to respond against the tumour, producing some inflammatory cytokines and killing some tumour cells. T_{EX} cell subsets show differential anatomical distribution. Precursors of T_{EX} cells (T_{PEX}) cells are preferentially enriched in the tumour-draining lymph node, while terminally-exhausted T_{EX} cells are more enriched in the tumour itself. While the state of the T_{EX} cells in the blood is still an active area of investigation, it is thought that the blood is enriched in more intermediate-like T_{EX} cell states (such as the CX₃CR1⁺ transitory T_{EX} cell state).

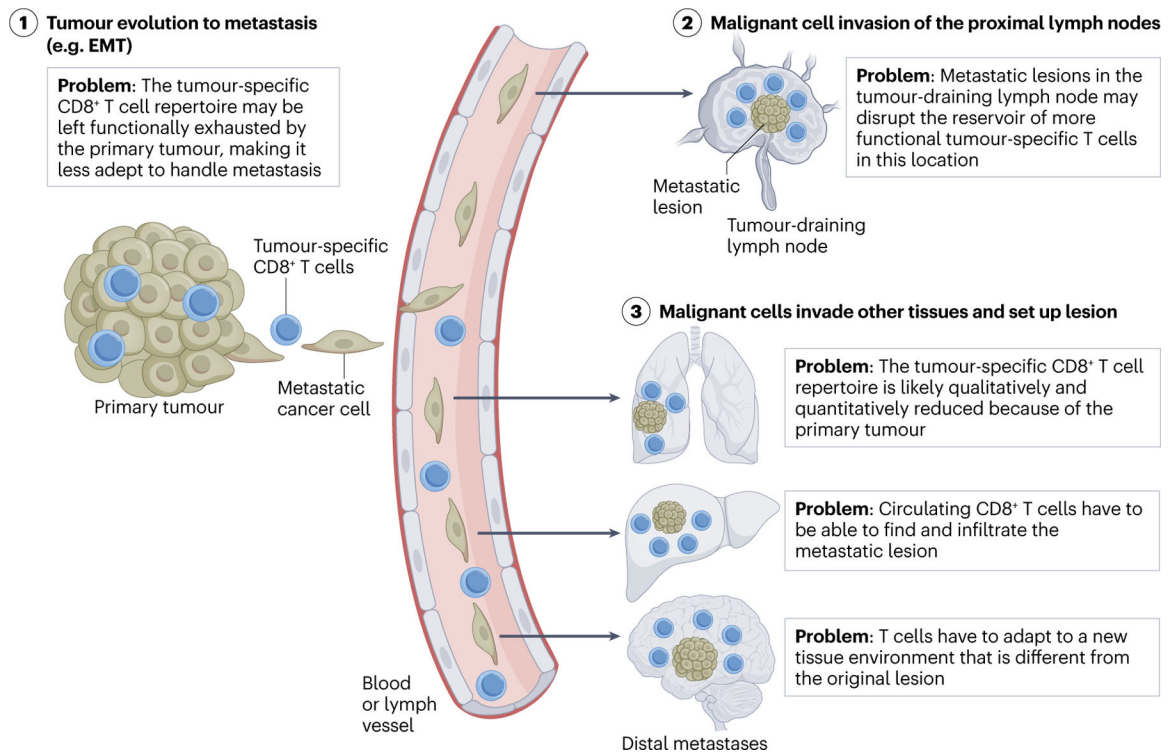


Figure 2: Immunological challenges associated with metastasis.

(1) Metastasis is a complex process whereby malignant cells from one location intravasate into blood or lymphatic vasculature, and migrate to another tissue (for example, epithelial to mesenchymal transition (EMT) shown here). A major immunological hurdle for tackling metastasis is that many of the CD8⁺ T cells in the primary tumour are already exhausted, and may be unable to exit the tissue to search out and destroy the metastatic lesion. (2) Metastases often first localize to the lymph nodes proximal to the primary tumour. The tumour-draining lymph node contains a functional reservoir of tumour-specific CD8⁺ T cells. How a metastatic lesion in the lymph node impacts this reservoir remains to be determined, but likely will impact the anti-tumour immune response. (3) Malignant cells can also invade other tissues, but must adapt to survive and establish a lesion. CD8⁺ T cells have to be able to find and infiltrate the metastatic lesion in order to execute effector functions. This is compounded by tumour-specific CD8⁺ T cell repertoire dysfunctionality, and likely due to sequestration of these cells in the primary tumour or tumour-draining lymph node. Thus, there must be circulating tumour-specific CD8⁺ T cells with the requisite trafficking molecules (on the T cell and vasculature), and if they do infiltrate, they must adapt to yet another tissue microenvironment. It is unclear how plastic or fit the circulating tumour-specific CD8⁺ T cells must be to overcome these barriers.