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Conversations that count: Cellular interactions that drive T cell fate

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Abstract

The relationship between the extrinsic environment and the internal transcriptional network is circular. Naive T cells first engage with antigen-presenting cells to set transcriptional differentiation networks in motion. In turn, this regulates specific chemokine receptors that direct migration into distinct lymph node niches. Movement into these regions brings newly activated T cells into contact with accessory cells and cytokines that reinforce the differentiation programming to specify T cell function. We and others have observed similarities in the transcriptional networks that specify both CD4+ T follicular helper (T_{FH}) cells and CD8+ central memory stem-like (T_{SCM}) cells. Here, we compare and contrast the current knowledge for these shared differentiation programs, compared to their effector counterparts, CD4+ T-helper 1 (T_{H1}) and CD8+ short-lived effector (T_{SLEC}) cells. Understanding the interplay between cellular interactions and transcriptional programming is essential to harness T cell differentiation that is fit for purpose; to stimulate potent T cell effector function for the elimination of chronic infection and cancer; or to amplify the formation of humoral immunity and longevity of cellular memory to prevent infectious diseases.

KEYWORDS
cellular interactions, lymphoid niche, migration, stem-like memory, T cell differentiation, T follicular helper, transcriptional regulation

1 | INTRODUCTION

The activation and differentiation of T cells play an essential role in orchestrating adaptive immune responses. These interactions determine how responses are tailored toward the pathogen and the success of T cell responses; whether pathogens or cancer cells are eliminated by effector cells, and if immunological memory is generated for long-lasting protection. The paths to each of these outcomes are determined by the cells that T cells interact with during their activation.

A key feature of immune cells is their motility. T cell migration is primarily driven by chemokines and their chemokine receptors, which co-ordinate cell migration into specific sites and promote T cell interactions.[1,2] Naive T cells that are single positive for either CD4 or CD8 enter circulation following their maturation in the thymus.[3] Upon their arrival in the secondary lymphoid organs, such as the spleen and lymph nodes, T cells seek out interactions with dendritic cells to search for the presentation of their cognate antigen.[4-6] Work from our group, and others has demonstrated that where these

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interactions occur, and the dendritic cell subsets involved, plays a critical role in establishing the gene networks that determine effector and memory differentiation for both CD4+ and CD8+ T cells.[1,7-15]

Following activation, CD4+ T cells can differentiate into a diverse set of effector helper cells. Each helper lineage is specified by distinct transcriptional regulators and cytokines that orchestrate a coordinated attack for the eradication of a specific type of pathogen.[16,17] T follicular helper (T_{FH}) cells are a distinct subset that promotes B cell maturation into high-affinity plasma and memory cells.[18-20] Unlike the other CD4+ T cell effectors, T_{FH} differentiation occurs irrespective of the type of pathogen to support germinal center formation, and thus, these cells are an essential link between the cellular and humoral responses.

In contrast to CD4+ T cell differentiation, the diversity in CD8+ T cells has only been more recently appreciated. In addition to the varied effector responses, CD8+ T cell differentiation leads to a heterogeneous pool of memory subsets. CD8+ T cell memory can be broadly grouped into either central memory or effector memory populations, which were originally defined by their distinct homing potential.[21,22] Central memory cells continually recirculate in blood and lymph and are found in secondary lymphoid organs, while effector memory cells can recirculate through, or reside within peripheral tissues. Each of these pools contains specialized memory populations. Recently, a subpopulation of central memory cells, termed stem-like memory cells (T_{SCM}), has been described in humans and mice.[23] T_{SCM} are programmed to promote self-renewal and repress terminal differentiation.[24-26] By seeding the effector cell pool, T_{SCM} mediate long-term immunity to chronic infection, provide superior recall responses to secondary infection, and control tumor growth following immunotherapy.[23]

From the initial description of CD8+ T_{SCM} cells, comparisons have been made concerning their relatedness to CD4+ T_{FH} cells.[24,27] These similarities are not only reflected in the cell-surface markers that define these subsets, but also the transcriptional regulators that determine their specific differentiation. Understanding these similarities may highlight the cellular interactions, cytokines, and lymphoid niches that promote these differentiation pathways. Investigating this potential could reveal new avenues to harness CD4+ T_{FH} and CD8+ T_{SCM} cells and steer vaccine design to focus on the T cell responses that would provide the greatest humoral response combined with long-lived, robust cellular memory. In this review, we compare and contrast our current knowledge for the differentiation of CD4+ T_{FH} and CD8+ T_{SCM} cells and their effector counterparts T_{H1} and T_{SLEC} cells. We discuss how the details of one system may inform the other, and the emerging technologies that may help address remaining questions. Combined with recent discoveries, future work which aims to understand the environmental and cellular interactions that support one or both of these T cell differentiation fates will drive the formulation of target vaccines that establish reliable, protective, and long-lived immunological memory. In addition, as both T_{FH} and T_{SCM} responses have been shown to promote the clearance of tumors following checkpoint blockade, this knowledge has the potential to inform and optimize the revival of T cell responses during chronic infection or for cancer immunotherapy.[25,28,29]

2 | DIVERSITY OF CD4+ AND CD8+ T CELL DIFFERENTIATION

2.1 | Pathogen-guided tailoring of CD4+ T cell differentiation

Following infection, $CD4^+$ T cells differentiate into distinct T-helper subsets that mediate clearance of and protection against diverse pathogens.[17,30] This dynamic T cell differentiation permits an exceptional flexibility to tailor responses for the clearance of distinct pathogens and protection from reinfection. T_{H1} cells secrete interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) and promote cellular immune responses mainly to intracellular pathogens, such as viruses and tumors. T_{H2} cells are formed during helminth and allergy challenges and secrete interleukin (IL)-4, IL-5, and IL-13. T_{H17} cells are identified through their production of IL-17A and IL-17F and are increased in the context of extracellular bacteria or fungi challenges.[16,17] It is important to note that not every immune response contains a single lineage, but rather there is a rich and diverse continuum of CD4+ T cell effectors. In addition, considerable plasticity exists between T_H subsets to fine-tune responses. However, as the differentiation of these lineage pathways is determined by the cytokine milieu present during infection, CD4+ T cell responses are skewed toward a T_H population that is tuned to respond to a distinct class of pathogen. Thus, flexibility within T_H functions insures immune responses are context-appropriate to facilitate host protection against a wide range of pathogens.

2.2 | Diversity within the T_{FH} population

Another essential role for CD4+ cells is to support and instruct the germinal center response, leading to the production of high-affinity antibody-producing cells and memory B cells. This task is performed by a distinct population known as T_{FH} cells. The differentiation of T_{FH} is distinct from the other T_H lineages, as this population is required to differentiate alongside each of the effector T_H subsets irrespective of the class of pathogen challenge. Still, there exists critical cytokine and transcriptional tipping points that can lead to a preference for differentiation toward either T_{FH} and CD4+ T cell effectors.[9] Functional heterogeneity within T_{FH} populations is a newly established concept. During B cell interactions within the germinal center, T_{FH} cells can secrete multiple cytokines, IL-21, IL-4, IL-2, IL-9, IL-10, IL-13, and IFN-γ.[19,31-34] The potential to secrete a specific cytokine combination represents functionally distinct T_{FH} subpopulations not only between distinct infectious and pathogenic settings but also over the course of an infection.[32,35,36] In particular, T_{FH} subpopulations defined by their distinct cytokine expression have been shown to influence the production of pathogenic antibodies in allergy and asthma in both humans and mice.[32,36]

Heterogeneity within T_{FH} populations was first appreciated in human studies, through the investigation of circulating peripheral blood T_{FH} subsets.[37,38] While these cells exhibit lower expression of

T_{FH} identifying proteins CXCR5 and PD-1, they still resemble those found within the tonsil and lymph nodes.[39-41] Interestingly, circulating T_{FH} cells are defined by their expression of the chemokine receptors CCR6 and CXCR3. Studies assessing circulating T_{FH} in patients with inborn errors of immunity have shown that these populations are independently transcriptionally regulated.[42] In these patients, STAT1 deficiency leads to a loss in the CCR6+ population and a reciprocal accumulation of the CXCR3+ circulating T_{FH} cells. Several studies have investigated the relationship between T_{FH} subpopulations and the development of vaccine response or immune protection following infection. While some indicate that CXCR3+ circulating T_{FH} cells can be used as a biomarker of immune protection,[43,44] others demonstrate that protection of humoral responses is negatively correlated with CXCR3+ subpopulations.[38,42,45,46] Still in other settings, the relationship between T_{FH} heterogeneity and humoral response is less clear. This is the case in convalescent COVID-19 patients, where CCR6+ T_{FH} cells were negatively correlated with SARS-CoV-2 neutralizing antibodies, while the remaining CCR6-CXCR3−/+ population correlated with antibody levels.[47] It is currently unknown if the heterogeneity of human T_{FH} changes over time; however, it is more likely that this is imprinted by their developmental path and determined by the infection or vaccination environment. In animal models, we have demonstrated that diversity exists in the formation of T_{FH} cells between different viral infections.[8] This diversity is transcriptionally regulated. Specifically, we have identified a T-bet-dependent and T-bet-independent process for T_{FH} differentiation using the experimental systems of acute lymphocytic choriomeningitis virus (LCMV) and influenza.[8]

2.3 | Diversity within CD8+ effector T cell differentiation

Until recently, the diversity within CD8+ T cell differentiation was restricted to a bifurcation between effector and memory. However, it is now appreciated that substantial heterogeneity exits in each of these broad paths.[22,48]

The most common definition of CD8+ T cell effectors is found following viral infections and resemble CD4+ T_{H1} cells, both in terms of their transcriptional requirements and effector molecule production. These cells are identified via their surface expression of killer cell lectin-like receptor subfamily G, member 1 (KLRG1). CD8+ T cell effector cells are critical for the clearance of viral-infected cells due to their high production of effector molecules (such as IFN-γ and granzyme B).[22,49] This population of effector cells undergoes considerable contraction following pathogen clearance and are therefore referred to as short-lived effector T cells (T_{SLEC}).

As T_{SLEC} represent the CD8+ T cell equivalent of T_{H1} CD4+ T cells, so too do the less common T_{C2} and T_{C17} cells relate to T_{H2} and T_{H17} cells, respectively. However, both T_{C2} differentiation and T_{C17} differentiation are more strongly associated with aberrant and pathogenic immune responses than targeting responses toward diverse pathogenic infections.[50] Indeed, differentiation toward IL-5-producing-T_{C2} cells is associated with the development of severe asthma following infection.[50] In contrast, the population of IL-17-producing T_{C17} cells were originally suspected to be an abnormality of genetic modifications in mice. T_{C17} cells are enriched with the compound deletion of the T-box transcription factors T-bet and EOMES or T-bet and Blimp, or the deletion of TCF-1.[51-53] However, in recent years, knowledge of this subset has increased to define its role in protection against fungal infection and microbial colonization of the skin.[51-53] In addition, T_{C17} cells have also been found associated with gastrointestinal cancers and responses during influenza viral infection, making them novel targets for immunotherapy.[54]

In recent years, a population of CXCR5+ follicular cytotoxic T cells was identified during chronic infection.[37,55] Many of the key cell-surface markers and characteristic properties of these cells resemble that of a newly described T_{SCM} memory population. Follicular cytotoxic T cells were observed within B cell follicles, while T_{SCM} accumulate in the T cell zone. The literature has coalesced behind the T_{SCM} nomenclature and this population will be discussed in greater detail in the following sections.[24]

2.4 | Diversity within CD8+ memory T cell differentiation

CD8+ T cell memory cells are critical mediators of long-lived immunity and provide dynamic protection against intracellular pathogens and cancer. While these memory cells differentiate during the initial exposure to a pathogen, they do not undergo the same contraction seen in T_{SLEC} cells and are instead maintained long term, in an antigen-independent manner. Distinct from T_{SLEC} cells, memory can be distinguished during infection by the lack of KLRG1 expression and expression of the IL-7 receptor subunit-α, CD127, and CD27.[22]

The central memory population is identified via the expression of the CCR7+ CD44+, CD127+, and CD62L+. The expression of both CCR7+ and CD62L+ enforces their homing to secondary lymphoid organs and bone marrow. Within the central memory population, a particularly potent memory cell has been identified. Termed stem-like memory cells, T_{SCM} cells are marked by their expression of PD-1, Slamf6, SCA-1, CXCR5, ICOS, and the transcription factor TCF-1, although the expression of these may vary between acute and chronic settings.[56] T_{SCM} cells provide a greater proliferative boost, compared to other central memory cells, during recall responses and following PD-1 checkpoint blockade in chronic infection.[24,37] Further, T_{SCM} exhibit enhanced renewal capabilities and are maintained long-term.[25,27,57] While the mechanisms that underlie the enhanced multipotency of T_{SCM} are undefined, this population is of exceptional interest for its therapeutic potential.[23,58]

The effector memory population contains both CD127hi and CD127lo populations with low CD62L expression. These markers, along with the absence of CCR7, allow these cells to enter and circulate through peripheral tissues.[22] Previously, this confounded the identification of resident memory T cells, which are permanently retained in the periphery.[59] Furthermore, a recent report has transcriptionally

dissected the CD8+ T effector memory population to identify distinct, terminally differentiated effector memory cells, termed terminal-T_{EM} in both humans and mice.[60] Despite their persistence for over 70 days postinfection, these cells display some hallmarks of T_{SLEC} cells, including KLRG1 expression. Importantly, this study showed T_{EM} exhibit potent cytotoxicity, but have limited recall potential. This population was depleted after 3-5 months postinfection. As the T_{EM} population has been previously conflated with the effector memory, this has redefined the classical characteristic attributed to this memory population.

In addition to effector memory cells that are found recirculating in both lymphoid and non-lymphoid tissue, a tissue resident memory (T_{RM}) population also exists that is restrained within peripheral tissue, local to the site of initial infection.[59] In these sites, T_{RM} cells dominate the local response upon re-challenge infection.[61,62] In addition, the scanning behavior of T_{RM} cells in the skin can prevent the outgrowth of melanoma and is associated with a better prognosis in breast cancer patients.[63,64] The diversity in cell-surface markers and transcriptional signatures observed in CD8+ T cell T_{RM} populations, located in distinct anatomical sites, is imprinted by the pathogen and the tissue environment.[65]

Unlike the pathogen-driven differentiation of CD4+ T cells, for CD8+ T cells it is less clear what the determinants of memory heterogeneity are and what determines the predominance of one population over the other in central and effector memory populations. However, diversity in this regard does insure durable protection at multiple sites, allowing flexibility of responses upon recalled pathogen encounters.

3 | SHARED TRANSCRIPTIONAL REGULATION BETWEEN CD4+ AND CD8+ T CELL SIGNATURES

In addition to the correlation between cell-surface markers, there exist common attributes consistent within the transcriptional networks that are established between various subsets of CD4+ and CD8+ T cells. These networks act in concert with the extrinsic environment; first to move cells into specific niches, then to re-enforce differentiation based on the cytokines present in these regions (Figure 1). Here, we explore some of the central nodes of the transcriptional programs that are shared between CD4+ T_{H1} and CD8+ T_{SLEC} effector cells and alternatively, those that specify differentiation for CD4+ T_{FH} and CD8+ central memory cells and T_{SCM} cells. Within each of these CD4+ and CD8+ T cell branches, these regulators act to establish cell ontogeny and define functional outcomes. Understanding these shared transcriptional pathways may reveal shared environmental cues, such as cytokines and cellular interactions, which promote T cell differentiation. Here, we focus on central transcriptional nodes with implications for cell location and position.

3.1 | Transcriptional dichotomy of Bcl6 and Blimp1

Bcl6 (BTB-POZ; bric-a-bric, tramtrack, broad complex–poxvirus zinc finger) and Blimp1 (B lymphocyte-induced maturation protein 1; encoded by *Prdm1*) are a pair of reciprocal and antagonistic transcription factors which play a key role in determining memory and effector fate decisions in T cells.[66] Specifically, the transcriptional repressor Blimp1 drives terminal effector differentiation in both CD8+ and CD4+ T cells.[66,67] Conversely, Bcl6 is vital for the generation and maintenance of CD8+ T cell memory and also promotes T_{FH} development in CD4+ T cells.[27,68-71]

Bcl6 is the master regulator of T_{FH} cell fate. It is vital for humoral immunity, regulating B cell help and germinal center formation.[69,70] Early Bcl6 expression is induced by CD28 activation and cytokines such as IL-6, which activates STAT3 signaling to enhance Bcl6 expression and drive T_{FH} development. Bcl6 acts primarily to downregulate Blimp1 and to prevent effector T_H fates.[9,69,72] Bcl6 also represses Gata3, the T_{H2} master regulator, RORγt, required for

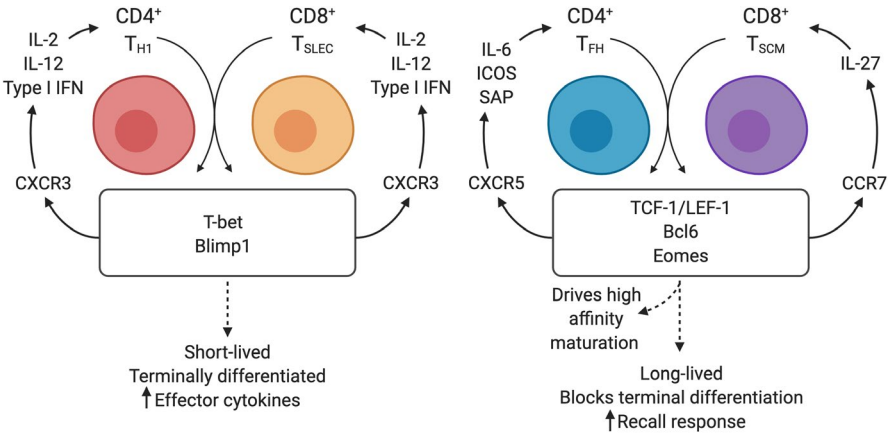

FIGURE 1 Circular relationship between transcriptional and environmental regulators of T cell differentiation. Following initial contact with dendritic cells, T cells begin to upregulate the transcription factors that regulate diverging fates. This leads to the upregulation or maintenance of chemokine regulators such as CXCR3, CXCR5, and CCR7. According to this expression, newly activated cells move into new regions and are exposed to a cytokine milieu that further feeds forward differentiation to re-enforce the transcriptional program and function of each cell subset

T_{H17} differentiation, and T-bet, the T_{H1} master transcription factor, to further limit alternate T_H fates.[19,69,71,73] As discussed in detail below, Bcl6 controls cell migration by silencing promoter regions of genes such as *Ccr7*, *Ccr6*, *S1pr1*, and *Psgl1* to promote retention within the lymph node and migration toward the T:B border.[74] Bcl6 further delays T cell egress by repressing KLF2, a transcription factor which initiates expression of S1PR1 and CD62L, revealing a similar mechanism observed in tissue resident CD8+ memory T cells to prevent lymphoid tissue homing.[75,76] Furthermore, overexpression of Bcl6 led to increased PD-1, CXCR5, CXCR4, and SAP which are critical for T_{FH} migration to the B cell follicles and interaction with B cells.[77-79] These interactions in turn reinforce Bcl6 expression as they progress into the B cell follicle and participate in germinal center reactions.

The role of Bcl6 in T_{FH} cells has informed studies investigating its role in the development and maintenance of CD8+ T cell memory. Here, as for CD4+ T cells, Bcl6 is inversely expressed to Blimp1, with higher levels of Bcl6 found in memory cells, particularly in T_{SCM} cells compared to effectors. As memory precursors mature into central memory cells, Bcl6 expression progressively accumulates while Blimp1 expression decreases.[80] Interestingly, in CD8+ T cells, this expression is dependent on IL-21, while in T_{FH} differentiation, the requirement for IL-21 remains controversial.[9] In memory precursors, Bcl6 binds directly to the *Tcf7* locus, driving TCF-1 expression, which is critical for memory differentiation.[27] Further, the forced expression of TCF-1 in Bcl6-deficient mice restores generation of memory precursors, demonstrating that Bcl6 functions upstream of TCF-1 as a critical regulator of memory formation during acute viral infection.[81]

In contrast to the expression of Bcl6, Blimp1 is highly expressed in CD8+ and CD4+ effector T cells. In CD8+ T cells, this expression drives terminal differentiation, migration to sites of inflammation and cytolytic T_{SLEC} function.[82,83] Blimp1 expression is induced by IL-2 and IL-12 and promotes the production of effector molecules IFN-γ and granzyme B.[84-87] Consistent with the counterbalancing roles of Bcl6 and Blimp1, central memory CD8+ T cells express low Blimp1 levels.[67] Blimp1 appears to repress transcriptional networks driving central memory formation, as loss of Blimp1 leads to the expansion of CD62L+ CD127+ memory cells during infection.[67] In line with the essential role in effector function, Blimp1-deficient mice fail to clear influenza infection due to the reduced migration of T_{SLEC} cells to the lung.[83] However, this function may be in concert with expression of T-bet, which directly regulates CXCR3.[52,88]

Unlike CD8+ effector T cells, early development of effector CD4+ T cells does not depend on Blimp1 expression.[89,90] Rather, Blimp1 is expressed later in CD4+ T cell differentiation and as such is associated with highly committed, cytokine-producing CD4+ T_H cell subsets.[87,90] This profile is similar to its expression profile in differentiated CD8+ effector cells and in plasma cells, both which have an increased secretion capacity.[66] CD4+ T cell effectors demonstrate reduced proliferation, again supporting the notion that $Blimp^{hi}$ CD4+ T cells may have similar transcriptional wiring to CD8+ effectors. Additionally, overexpression of Blimp1 in CD4+ T cells leads to hyperproliferation and consequent autoimmunity.[90] Combined, the dichotomy of Bcl6 and Blimp1 expression reveals a key branching point between CD4+ and CD8+ effectors and CD8+ memory and CD4+ T_{FH} fates.

3.2 | T-box factor regulation of T cell fates

T-box transcription factors, T-bet, and eomesodermin (Eomes) are essential in the differentiation and function of effector and memory T cells. Similar to Blimp1 and Bcl6, the relative expression of T-bet and Eomes acts to tip the balance between terminally differentiated effector and memory fates. In CD8+ T cells, increasing T-bet expression promotes effector lineages early in T cell development, while Eomes is required to promote and sustain memory fate.[49,91,92] Similarly, in the CD4+ lineage, T-bet is considered a master regulator of driving formation of T_{H1} cells, while restricting non-T_{H1} effector fates.[93]

In both precursor and committed cell subsets, T-bet plays a role repressing alternative T fates. In addition to direct repression, this is achieved by forming complexes with other lineage-defining transcription factors, such as RORgt, GATA3, and Bcl6, to block T_{H17}, T_{H2}, and T_{FH} differentiation, respectively.[9,94,95] Formation of these complexes sequesters these other lineage-defining factors away from their DNA binding sequences and thus blocks their action.[91,96] Additionally, T-bet cooperates with RUNX3 to antagonize GATA3 activity and also silences the *Il4* locus to repress IL-4 expression.[97,98] In CD8+ T cells, T-bet expression is graded according to the level of inflammation.[49] High levels of T-bet expression instruct a transcriptional program that is distinct from cells with moderate T-bet expression. In this setting, T-bet collaborates with the transcription factor ZEB2 to turn on the cytotoxic, terminally differentiated T_{SLEC} differentiation program.[99] Combined, these studies show that in both CD4+ and CD8+ T cells, T-bet is a master at driving effector differentiation through the co-operation and coercion of other transcriptional regulators.

A central role of T-bet is the direct activation of genes that are essential for effector T cell function. As such, T-bet is specifically binding directly to the *Ifng* locus to drive expression of the canonical T_{H1} cytokine IFN-γ.[93,100,101] This source of IFN-γ is an essential primer for the immune system and can subsequently induce T-bet expression in naive CD4 T cells, which do not express T-bet, in a STAT1-dependent manner.[102] Thus, T_{H1}-produced IFN-γ production functions as a positive feed-forward loop to drive further T_{H1} differentiation and induce IFN-inducible chemokines to form peripheral and lymph node inflammatory niches.[7,103] Further to this, T-bet directly binds and regulates the expression of the chemokine receptor, CXCR3. This expression further re-enforces the inflammatory loop generated by T-bet, as it moves cells into regions of inflammation via CXCR3. In these regions, T-bet via IFN-γ production induces local expression of the CXCR3 ligands, CXCL9, and CXCL10, leading to further recruitment to the site.[7,88]

Expression of T-bet is induced by multiple inflammatory cytokines. The most characterized of these is IL-12 through the

activation of STAT4.[30,49,94,95,101] For CD4+ T cells, multiple cytokines can tip the balance toward T-bet expression and T_{H1} differentiation including IL-2, type I IFN, and IL-12.[8,11,104] Alternatively, others such as IL-6 reduce T-bet expression and increase Bcl6 to promote T_{FH} development.[9]

In CD8+ T cells, T-bet and Eomes are expressed by distinct populations, representing reciprocal differentiation paths.[105] T-bet is highest early in CD8+ effectors but is downregulated in memory cells, while Eomes expression is low in early effectors but upregulated later as memory cells emerge.[106] As such, the phenotype, function, and persistence of memory and effector CD8+ T cells are sensitive to the relative expression of Eomes and T-bet.[49,105-107] T-bet and Eomes share significant homology and perform partially redundant roles in CD8+ T cells, including regulation of IFN-γ and granzyme B.[51,108] Initial T-bet expression in naive CD8+ T cells is induced by T cell receptor (TCR) and IFN signaling. As mentioned, this is further amplified in effector T cells by IL-12-mediated signals in addition to mTOR activity.[49,109,110] In contrast, Eomes is induced in early effectors in a Runx-dependent manner, enhanced by IL-2 signaling, but is limited by IL-12 and mTOR.[109-111] Eomes expression increases further in memory cells in response to WNT signaling and TCF-1.[26,85,106,112] In memory CD8+ T cells, Eomes plays a critical role in cell survival and homeostatic regulation by increasing expression of IL2RB (CD122) and IL-15 signaling to promote homeostatic proliferation.[49,85,105] Importantly, during secondary challenge, Eomes-deficient memory precursors elicit an impaired recall response, generate less central memory, and demonstrate reduced secondary expansion.[112] In chronic infection, both the T-bet+ and the distinct Eomes+ populations are required to keep viral load in check. The Eomes+ cells provide a reservoir which replenishes the terminally differentiated T-bet expression pool.[113-115] Thus, in this setting, the Eomes-expressing CD8+ population in chronic infection is marked by CXCR5 expression and falls into the T_{SCM} subset of central memory.[24]

3.3 | TCF-1 and LEF-1, the master regulators of stemness

T cell factor-1 (TCF-1, encoded by *Tcf7*) is a high mobility group box (HMG) family member, and transcription factor essential for T cell development and differentiation.[116,117] TCF-1, along with HMG family member lymphoid enhancer-binding factor-1 (LEF-1), is a key player in establishing the T cell lineage in thymocytes, specifying distinct CD4+ T cell lineages, and regulating CD8+ T cell fate decisions. Unlike the previously mentioned transcriptional nodes, TCF-1 expression is established in thymocytes and its expression is maintained in CD8+ central memory cells and CD4+ T_{FH} cells following activation.[54]

TCF-1 and LEF are also essential during early T_{FH} development. Overexpression of TCF-1 amplifies T_{FH} cell differentiation and increases expression of T_{FH} associated genes, such as CXCR5 and PD-1.[27,118,119] Mechanistically, TCF-1 promotes early T_{FH} differentiation in CD4+ T cells by binding directly to the *Bcl6* promoter to enhance BCL6 expression, as well as acting downstream of Bcl6 during T_{FH} differentiation. Further, TCF-1 directly restricts T_{H1} differentiation by binding to the *Prdm1* and *IL-2Ra* loci in T_{FH} cells to repress Blimp1 and IL2Ra expression. The blocking of IL-2 signaling promotes T_{FH} over T_{H1} cell differentiation.[69,119,120] In addition, TCF-1 is enriched at the IL-6 receptor gene locus, which suggests a role for TCF-1 in increasing IL-6 responsiveness, a vital cytokine for T_{FH} differentiation.[121] Indeed, loss of TCF-1 leads to a decrease in IL-6Ra and IL-6.[118] While the mechanism is unclear, TCF-1 deficiency also reduces the expression of Achaete-scute homologue-2 (ASCL2). ASCL2 is required to downregulate CCR7, allowing migration to the T:B border.[122] Additionally, TCF-1 directly targets costimulatory marker inducible T cell co-stimulator (ICOS), another signaling molecule important for T_{FH} cell commitment and growth.[118] Thus, TCF-1 acts in multiple ways to both initiate and reinforce T_{FH} differentiation.

Distinct from other T_H subsets, T_{FH} cells form a critical component of the memory pool, that repress exhaustion in chronic infection and maintain stem-like potential.[27,123,124] Upon re-exposure, this population re-initiated B cell helper function.[125] The generation of these memory cells was impaired by the loss of TCF-1, suggesting that TCF-1 plays an essential role for CD4+ T cell memory stemness, particularly in T_{FH} cells.[126]

In the last decade, many studies have revealed that TCF-1 plays a key role in the formation and persistence of memory CD8+ T cells.[26,127] The *Tcf7* gene is expressed highly in naive and memory CD8+ T cells, but is decreased in T_{SLEC} cells.[54] The rapid downregulation of TCF-1 during effector differentiation is mediated, in part, by IL-12.[128] Early studies proposed that memory CD8+ T cell differentiation required Wnt signaling and β-catenin binding to TCF-1 to establish cell fate; however, the role of Wnt ligands in vivo remains disputed.[26,127,129] In the absence of TCF-1 during infection, memory formation is restricted while effector-associated genes, including Blimp1, T-bet, ID2, are reciprocally upregulated.[26,127,128] In keeping with this, several studies have found that TCF-1 and the effector molecule granzyme B are reciprocally expressed.[56] Loss of TCF-1 further impairs the expansion of memory CD8+ T cells upon secondary challenge, indicating that TCF-1 is essential for both memory formation and secondary expansion.[26,127] Mechanistically, TCF-1 directly binds to the regulatory region of *Eomes* and increases its expression to drive development of CD8+ central memory precursors. Based on the similar expression of TCF-1 in CD8+ T_{SCM} and T_{FH} cell differentiation, the mechanisms TCF-1 utilizes to regulate each of these compartments are likely similar. This has been demonstrated by TCF-1-mediated repression of *Tbx21* and *Prdm1* transcription and induction of Bcl6 in CD8+ memory precursors. TCF-1 also establishes functional similarities between these cells, in particular the longevity of memory persistence is conserved in these TCF-1-expressing populations.[27,123,130] As such, TCF-1 has been identified as playing a critical role in repressing exhaustion and maintaining T cell stemness during chronic and acute infection.[23,24,27,57,131] High expression of TCF-1 in CD8+ T_{SCM} cells is essential in chronic LCMV

infection, as loss of TCF-1 leads to a failure to generate stem-like progenitor cells. This in turn results in impaired viral control and a rapid loss of CD8+ T cells.[23,24,131] Combined these studies suggest a shared role of TCF-1 between T_{FH} and central memory T_{SCM} cells, promoting long-term survival and proliferation which are essential in chronic infection and recall responses.

4 | THE ROOM WHERE IT HAPPENS

The majority of T cell priming occurs within secondary lymphoid organs, which are organized into specific niches (Figure 2). These regions promote T cell priming of naive and newly activated cells in contact with other cells that steer T cell differentiation in a manner tailored toward the infecting pathogen. While most of these processes occur in either draining lymph nodes or the spleen, peripheral sites such as the skin, liver, and lung can mediate T cell differentiation: either by priming naive cells, or tuning and enhancing T cell function and memory locally.

Due to their accessibility for dynamic imaging, much of the work detailing the organization of T cell priming and differentiation has been performed in lymph nodes. Both CD4+ and CD8+ T cells have been shown to make multiple contacts with dendritic cells on their way through the differentiation process.[5,6,132] Upon early activation following the engagement of antigen presented in the context of MHC, T cells rapidly expand and upregulate key defining transcription factors that determine where, within secondary lymphoid organs, they will move to in order to encounter further polarizing differentiation signals.[1,74] Conventional dendritic cells (cDCs) are professional antigen-presenting cells and have been classified into two major branches.[133] Type 1 cDCs (cDC1) are considered efficient in presenting cell-associated antigens through cross-presentation, while type 2 (cDC2) dendritic cells can perform some presentation but are traditionally thought to be focused on activation of CD4+ T cells.[133] These distinct subsets are strategically located within the different lymphoid regions.[13,134] Thus, within these distinct niches or "rooms" T cells engage with dendritic cells and other accessory cells of influence to co-ordinate the efficient eradication of pathogens or cancer and to establish a pool of memory cells for long-lived protection.

4.1 | Effector differentiation in the outer limits of lymph nodes

The effector differentiation of both CD4+ and CD8+ cells occurs in parallel, with strong correlations in the transcription factors, cellular partners, and migration cues that co-ordinate this differentiation path.[1] CXCR3 is a chemokine receptor that is highly expressed on both CD4+ T_{H1} and CD8+ T_{SLEC} cells. Previously, it was thought that this chemokine moves fully differentiated cells out of secondary lymphoid organs and into peripheral sites of inflammation.[103] Following the observation that CXCR3 is upregulated well before CD4+ T cells leave the lymph node, we identified a counter intuitive role for this receptor in T_{H1} differentiation.[7] In this setting, newly activated cells moved from the T cell paracortex located in the center of lymph nodes, into the interfollicular regions (IFRs), between B cell follicles at the lymph node periphery (Figure 2). The IFR provides a unique lymph node niche that has a rich stromal cell network.[135] Here, antigen can be deposited, either during particulate

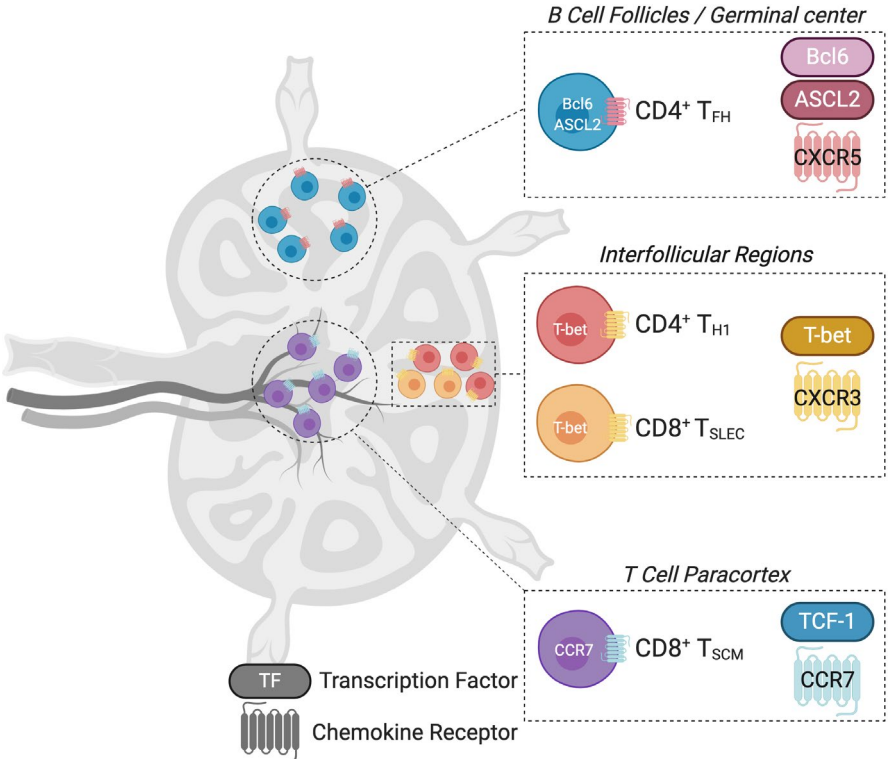

FIGURE 2 The lymph node compartments where T cell differentiation occurs. The specific transcriptional program of T_{H1} or T_{SLEC} effectors, T_{FH}, and T_{SCM} cells moves cells into spatially distinct regions of draining lymph nodes

viral infection or by using specific vaccination strategies that target antigen to this region.[1,136,137] Using a dual CXCR3 ligand reporter mouse, we demonstrated that CXCR3+ CD4+ T cell movement is directed by the expression of CXCL9 and CXCL10 in this region.[7] While this study did not reveal the precise dendritic cell or stromal cell population that move T cells into this area, we have now demonstrated that type 2 conventional dendritic cells (cDC2) express high levels CXCL10 in the IFR.[138] Similar to CD4+ T_{H1} differentiation, CD8+ effectors are also found in IFRs and this migration correlates with robust formation of effector function.[139] CD8+ T cells in IFRs form clusters around cDC2 cells consistent with their expression of CXCL10.[134] A potential splenic correlate of this niche is the marginal zone or red pulp, where CD8+ T_{SLEC} cells have also been shown to position in a CXCR3-dependent manner.[140,141]

Multiple studies have demonstrated that expression of CXCR3 is essential for CD8+ effector differentiation.[141-143] As outlined above, CXCR3 expression is principally regulated by the transcription factor T-bet.[103] Consistent with this relationship, T-bet deficiency resembles the loss of effector differentiation observed with loss of CXCR3.[49,143] We have shown the first observable expression of T-bet in CD8+ T cells is found within pockets of IFRs; however, it is likely that moderate T-bet induction is sufficient for CXCR3 expression and migration into this region.[138] While CD8+ T cells and cDC2s cluster in IFRs, it is unclear if this interaction is mediated through cDC2-derived CXCL10, or if stromal cell expression moves T cells into this region. We have shown that CD4+ T cells preferentially interact with CXCL10+ expressing DCs, but the specific location of these interactions and the DC subset was not defined in this study.[7] In addition, there are other accessory cells located in this region that may drive the differentiation toward T_{H1} and T_{SLEC} formation. While relatively scarce in secondary lymphoid organs, monocyte-derived dendritic cells (moDCs) can induce both T_{H1} and T_{H17} responses.[133,145,146] Further, moDC promotion of T_{H1} differentiation is associated with their location in IFRs following vaccination.[137] Recently, Bosteel et al provided an important advance to the dendritic cell literature, revealing that these cells are not capable of antigen processing; however, they can express high levels of IL-12 to polarize effector T cells.[147-149] In addition to moDCs, an inflammatory subpopulation of cDC2 cells, named infDCs, has recently been characterized.[145] Given the inflammatory milieu in IFRs following viral infection, this type I IFN-dependent population may be a critical regulator of T cell effector fate.[145]

The identity of the key cytokine or cytokines produced by dendritic cells or accessory cells in this region that promotes effector differentiation remains to be fully elucidated. IL-12 is a good candidate for this as it has been demonstrated to steer T cell differentiation toward both CD4+ T_{FH} and CD8+ T_{SLEC} cell formation and away from other fates, in a T-bet dependent manner.[128,146,150] Despite not being located within draining lymph node IFRs, cDC1 cells are commonly associated with T_{H1} responses due to their high production of IL-12.[16] Indeed, in ex vivo cultures, cDC1s promote greater formation of T_{H1} cells.[151] This suggests that within IFRs in vivo, it is other accessory cells, either infDCs or moDCs that are present that can produce the high levels of IL-12 needed to skew CD4+ and CD8+ T cell effector differentiation. In addition to IL-12, other cytokines such as IL-2, type I, and II IFNs have been demonstrated to promote T_{H1} and/or T_{SLEC} differentiation.[9] The neighbor cell producing these additional cytokines is not clear, but may include other T cells with opposing fates as well as specific dendritic cell populations.[11,104]

4.2 | T_{FH} differentiation: a stepwise progression to the follicle

The differentiation of T_{FH} cells is dynamically regulated through time and in spatially distinct regions of secondary lymphoid organs.[74] This process takes place in three distinct steps and each involves a unique set of interacting partners that imprint and reinforce differentiation into functional, germinal center resident T_{FH} cells.[16,152,153]

Interacting dendritic cells are generally considered essential for directing the initial phases of T_{FH} differentiation.[154] Several studies have shown that depletion of cDC2s reduced the amount of T_{FH} differentiation, suggesting they are the prominent dendritic cell regulator of this lineage. Early contact between CD4+ T cells and dendritic cells leads to the upregulation of Bcl6; however, during early stages of differentiation, Bcl6 can be co-expressed with T-bet, the T_{H1} defining factor.[95,101] Thus, this initial activating event is not sufficient to define early fate bifurcation. These contacts do, however, result in the directed movement from the paracortex to the T:B cell border.[153] In addition to Bcl6, this migration is instructed by the ASCL2 helix-loop-helix transcription factor that acts in multiple ways to drive T_{FH} ontogeny.[122] Expression of ASCL2 leads to the upregulation of CXCR5 and downregulation of CCR7. It is worth noting that the initial location of pre-T_{FH} cells at the T:B border occurs independently of Bcl6 and CXCR5, suggesting the downregulation of CCR7 and loss of retention in the paracortex is critical for migration.[155,156] Instead early T_{FH} migration to the T:B border requires the G protein-coupled receptor, EBI2 (GPR183).[157] In these regards, the initial stage of pre-T_{FH} migration resembles that of T_{H1} cells, although it is clear that T_{FH} cells do not reach the outer limits of the IFRs, as shown for T_{H1} cells.[7,101,158]

The T:B border is a critical site where decisions between T_{H1} and T_{FH} differentiation are made.[11,74,152,153] Distinct infections can elicit a unique set of inflammatory signals and recruitment of interacting partners that can shift the balance of differentiation either toward or away from T_{FH} differentiation.[11] Viral infections that induce a high expression of type I IFNs lead to increased dendritic cell production of IL-6 and skew differentiation toward T_{H1} cells. Further at the T:B border, the upregulation of EBI2 promotes CD4+ T cell interactions with IL-2 quenching cDC2s, which favors T_{FH} differentiation.[157] Again, this work suggests that cDC2s are more permissive for T_{FH} differentiation than for T_{H1}, and that for the latter additional accessory cells are required to enter this site and direct effector polarization. Critically, in this location, pre-T_{FH} cells form long-lived contacts with B cells through ICOS:ICOS-ligand interactions, which leads to further upregulation of the canonical T_{FH} genes, *Bcl6*, *Cxcr5*,

and *Il21*.[77,78,153,156] Interestingly, these ICOS:ICOSL interactions are formed with bystander and not antigen-specific B cells at the T:B border.[156] Although the importance of engagements with both cDC2s and B cells is well established, it is unclear if the three cells are required to interact at the same time at the T:B border. Further, one study has demonstrated that, during Plasmodium infection, dendritic cell interactions were dispensable, and B cells were solely responsible for the generation of T_{FH} responses.[159] In contrast, augmenting antigen presentation can make T_{FH} cell generation independent of B cells, highlighting the context-dependent nature of this process.[160]

Increasing CXCR5 expression directs T_{FH} cells into the CXCL13-expressing B cell follicles, to take part in germinal center reactions.[78,155] Interestingly, once CXCR5 permits entry into the germinal center, it is no longer required for the maintenance of cells in this location.[161] This is the final step in the differentiation process and allows T_{FH} cells to accomplish their role to select high-affinity B cells.[74,77] Unlike B cell interactions at the T:B border, within the germinal center T:B contacts are short-lived, cognate interactions that maintain these microstructures within the B cell follicle via SAP:Ly108, CD40L:CD40, and ICOS:ICOSL mediated interactions.[23,78,79,154,162,163] Despite the multistep process for a T_{FH} cell to enter a germinal center, this location can be further influenced by decreased expression of Bcl6, PD-1, or increased CXCR3, which will move established CXCR3 cells out of the germinal center structure.[161,164]

4.3 | Differentiation of T_{SCM} cells in the center of lymph nodes

Despite the shared transcriptional regulation, there are significant distinctions in location of CD4+ T_{FH} cells and CD8+ T_{SCM} cells. The first description of T_{SCM} (formerly known as follicular cytotoxic) cells was found due to their expression of CXCR5. Some researchers found these cells positioned according to CXCR5 expression in the B cell follicles, in both models of chronic infection and in the spleens of HIV patients.[37,55] In contrast to this, others showed that they instead remained in the T cell paracortex of secondary lymphoid organs.[24] To clarify this discrepancy, we have shown that the T_{SCM} precursors are retained in the T cell zone following viral infection, and their differentiation is enhanced in the absence of CXCR3.[138] While the transcriptional regulation of T cell effector molecules is well established, less attention has been placed on the transcriptional regulators of T cell location.[17,30,53] Delving into this further, we showed that TCF-1, the key transcriptional regulator of T_{SCM} cells, binds to the *Ccr7* locus.[138] Supporting this, multiple studies have demonstrated that CCR7 is downregulated in the absence of TCF-1 or in T cell populations that lack TCF-1.[27,53,165] The retained expression of CCR7 on T_{SCM} is in contrast to T_{FH} cells, where this receptor is downregulated rapidly following the upregulation of Bcl6.[155] For T_{FH} cells, the expression of CCR7 acts as a counterbalance retention signal, such that when CCR7 expression is maintained, cells are not capable of entering the B cell follicle, even when CXCR5 is overexpressed.[155] Therefore, it is likely that as T_{SCM} cells co-express both CCR7 and CXCR5, the retention within the paracortex is maintained to block migration out of this region. As discussed above, the key transcriptional difference between these populations could be the expression of ASCL2 in T_{FH} cells, which orchestrates the upregulation of CXCR5 and downregulation of CCR7.[122] The role of ASCL2 in T_{SCM} has so far not been investigated, but, unlike CCR7 it has not been reported to be expressed in transcriptional characterization studies.[24,25,56,165,166]

Despite T_{SCM} being positioned in the lymph node paracortex or splenic T cell zone,[24] it remains unclear if these precursors require contact with B cells at the T:B border in a similar manner to that shown for T_{FH} cells. Initial characterization of these cells demonstrated they co-expressed T_{FH} cell-surface markers ICOS and Slamf6, both of which facilitate T_{FH} cell engagement with B cells.[55] A recent report showed transcriptional expression of EBI2, a molecule that is similarly expressed by pre-T_{FH} cells and directs them to the T:B border.[157,166] Again, it is unclear if in this region T_{SCM} cells contact B cells or the IL-2 quenching dendritic cells producing the EBI2 ligand.[157] Further, when T_{SCM} cells were transferred into B cell-deficient μMT recipients they were less capable of inhibiting viral replication than those transferred into wild-type mice.[37] These data suggest that while T_{SCM} cells may not need B cells to form, they potentially provide essential signals to function.

The specific dendritic cell subset that promotes differentiation toward T_{SCM} remains unknown. Conventional DC1 cells reside in the paracortex of lymph nodes, positioning themselves as interacting partners during T_{SCM} differentiation.[134] Dynamic imaging studies have shown that CD8+ T cells interact with cDC1 cells.[10,12] In these interactions, cDC1 cells act as conduits to facilitate CD8+ and CD4+ T cell interactions.[10,12] This three-cell interaction facilitates the formation of memory, although T_{SCM} differentiation was not assessed in either of these studies. While these cells highly express CXCL9, loss of CXCL9 does not appear to alter T_{SCM} differentiation in vivo.[138] Previous work has shown that these interactions are alternatively instructed by CCR5.[167] Unlike cDC2s, cDC1s are thought to be a more homogeneous population[133]; however, it could be that subsets of dendritic cells with a defined imprinting potential reside in the paracortex. In addition, T_{SCM} may be maintained in the T cell zone by directly contacting the gp38+ CCL21-expressing fibroblastic reticular cells (FRCs) located here.[140] IL-27 is one cytokine that has been described to specifically promote T_{SCM} expansion and coincidentally also promotes the expansion of T_{FH} cells.[168] Although there is more work to be done to identify other factors that specifically direct differentiation toward T_{SCM}, an alternative hypothesis is that positioning in the T cell paracortex instead protects these precursors away from other regions in lymph nodes where they may come into contact with inflammatory accessory cells or cytokines that promote effector and terminal differentiation. Supporting this hypothesis, it is the absence of inflammatory singles such as IL-12 and type I IFN that steer differentiation away from T_{SCM}.[27,128] We have recently shown that altering the location of CD8+ T cells by blocking CXCR3 responsiveness

restricts cells to the paracortex. This change in T cell position, from the IFRs to the center of the lymph node, coincides with a decrease in effector differentiation and increased T_{SCM} precursor formation.[138]

Combined, it is clear that the migration of CD4+ and CD8+ T cells is driven by the dynamic regulation of chemokine receptors. This is controlled at the transcriptional level where early T-bet expression leads to the upregulation of CXCR3 in T_{H1} and T_{SLEC} cells; TCF-1 insures the retention of T_{SCM} in the T cell paracortex via CCR7 expression; and T_{FH} cells localize in the B cell follicle due to expression of Bcl6 which instructs CXCR5 upregulation. Curiously, we have shown that early precursors of both T_{FH} cells and T_{SCM} cells can also upregulate CXCR3.[8,138] In these instances, it appears that the CCR7 and CXCL13 migration cues counterbalance CXCR3 ligands to override the movement of non-effector cells into the locations (paracortex and B cell follicles) where these cells further differentiate.

5 | CELLS OF INFLUENCE

As discussed, the location where CD4+ and CD8+ T cell differentiation occurs and been elucidated. Within these regions, several accessory cells are associated with skewing toward a particular cell type; however, the precise interactions remain poorly defined. Interactions that lead to T_{FH} differentiation are the most clearly defined to require staged interactions with cDC2 cells and B cells.[16,74,152] Effector T cell differentiation can also be associated with cDC2 engagement and in some conditions with the presence of inflammatory moDCs. In contrast, T_{SCM} cells likely require contact with cDC1 cells within the T cell paracortex. Additionally, simultaneous contact with CD4+ T cells and cDC1 cells is required for promotion of CD8+ T memory formation. The serial engagement of T cells with distinct immune populations, which has been dynamically studied by multi-photon microscopy, suggests distinct roles of dendritic cell subsets during T cell differentiation. Instead, it is likely that these events work together in an additive manner to guide the transcriptional programming of T cells and determine the balance between effector and memory subsets. In this regard, movement of T cells into specific regions facilitates additive interactions that promote a single fate.

5.1 | Stromal cells as non-immune cells of influence

In addition to DC, moDC and B cell interactions, non-immune cells are emerging as important modulators of T cell fate. Lymphoid stromal cells represent important cell organizers that enable T cell and B cell recruitment to secondary lymphoid tissues.[169] FRCs are found in close contact with T cells and act as guiding paths for dynamic scanning of naive T cells in search of their cognate antigen.[169,170] Previously, we have described the upregulation of CXCR3 ligands in the IFRs following vaccination.[7] FRCs express CXCL9, CXCL10, and CXCL11 within the paracortex and IFRs of resting mice and following infection.[171,172] These studies demonstrate that FRCs not only guide the location of cells in steady state, but can dynamically redistribute cells during an immune response. This role for stromal cells in facilitating interactions between immune cells is well established. As the stromal cell compartment becomes increasingly disorganized with age, dysfunction in both the dendritic cell and stromal cell compartment may both contribute to the dampened efficacy of vaccination with age.[173,174]

Emerging studies suggest that in addition to this structural role, stromal cells, in particular FRCs, may directly influence T cell differentiation. When added to human T cell activation cultures, FRCs led to decreased proliferation and memory cell formation of both CD4+ and CD8+ T cells.[175] Interestingly, this inhibition was relieved in combination with IL-6 and TGF-β and PD-L2 blockade, factors known to regulate either T_{FH} or T_{SCM} cell differentiation. Another study demonstrated that FRC co-culture limits the production of CD8+ T cell IFN-γ, but increased T cell production of IL-2.[176] Importantly, this was independent of the block in T cell proliferation that is mediated through FRC iNOS production. Consistent with the IL-2 production, FRCs altered the epigenome of T cells leading to heightened differentiation to memory and persistence in vivo.[176] A contrasting study demonstrated FRCs provided pro-survival and proliferation signals to T cells.[177] Compared to FRC from human tonsil, follicular lymphoma stromal cells expressed the adhesion molecule ICAM and several chemokine ligands including CCL2, CCL5, CCL11, and CXCL10. Further, recent work has demonstrated that lymph node stromal cells are a checkpoint for peripheral tolerance and present self-peptide to convert CD4+ T cells into T regulatory cells and restrict germinal center formation.[178]

Much remains to be understood about how the stromal compartment directly regulates T cell differentiation. As outlined, some studies have shown contrary effects, and this may be due to different sites of isolation of FRCs and potentially distinct isolation protocols. Overlapping adhesion molecules, chemokine profiles, and skewing cytokines between stromal and dendritic cells additionally complicate the role of each cell type in vivo. To separate these influences, most of the studies to date have revealed interactions between T cells and stromal cells in in vitro co-culture systems. Although genetic models of FRCs exist, these make it difficult to determine a direct role in T cell differentiation, as the cellular niches of secondary lymphoid organs are disrupted in these mice and the survival of naive T cells is severely impacted.[179,180] More work needs to be done to validate that the interactions defined with these genetic models are indeed critical during a normal immune response. To help overcome this issue, a novel human tissue in situ assay has been developed to dissect the regulators of T cell differentiation in live tissue explants.[175]

6 | HARNESSING T_{FH} AND STEM-LIKE MEMORY

Vaccines are one of public health's greatest achievements, preventing many millions of illnesses and deaths every year.[181] Traditional

vaccination strategies have focused on the overall magnitude of the immune response produced. However, we can now appreciate how to manipulate immune responses to promote the most valuable T cell protection for future infectious challenge. Indeed, a preference toward CD4+ T_{FH} cells to boost humoral responses and long-lived CD8+ T_{SCM} cell differentiation is preferred rather than exuberant effector response. While all current vaccine strategies provide protection via humoral immunity, many of the recalcitrant pathogens for which no vaccine is currently available [including human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, and *Plasmodium* species] are resistant to humoral immunity.[182] Strategies that specifically direct the generation of memory $CD8^+$ T cells could aid in eliminating these intracellular pathogens.[181-183] Furthermore, $CD8^+$ T cells have the potential to be utilized in the development of therapeutic vaccines to establish potent effector function against chronic infection and cancer.[181] In contrast, rapid proliferative responses with robust cytokine and effector molecule production of CD4+ T_{H1} and CD8+ T_{SLEC} populations may be preferred where strategies are focused on overcoming an immediate challenge such as these settings. Therefore, the type of T cells required in each of these vaccination contexts is distinct. Thus, there is potential to exploit the shared transcriptional and cellular interactions that determine CD4+ and CD8+ effector T cells, or those that determine T_{FH} and T_{SCM}. Some new vaccination methods may influence the location of T cells within secondary lymphoid organs, which could, in turn, generate T cells that are fit for purpose depending on the response required.

The structure and anatomy of lymph nodes, along with the distinct positioning of T cells within them, allow for the delivery of drugs or antigens directly into specific sites.[184] The ability of these platforms to target distinct lymph node niches relies on specific design factors. Several vaccine vehicles can be used to target delivery to lymph nodes; in doing so, this improves the efficacy of T cell responses.[185] A key approach for this is via the use of nanoparticles, such as silica and protein nanoparticles, for targeted lymph node delivery of subunit vaccines.[184-188] These platforms increase the size of antigen which assists in the uptake and deposition within lymph nodes. We and others have demonstrated that these systems favorably affect lymphatic uptake and preferentially lodge in the lymph node IFRs.[136,189,190] While this strategy enhanced germinal center formation, this was done in the absence of increased T_{FH} differentiation in both animal and non-human primates.[191] Consistent with the increased recruitment of T cells to the IFR being associated with effector differentiation, this system enhanced the efficacy of anti-cancer vaccines, compared to soluble vaccination.[186] A recent study has demonstrated that the route used to deliver nanoparticle vaccines dramatically alters T cell differentiation.[166] Specifically, intravenous delivery heightened differentiation toward T_{SCM}, while subcutaneous delivery resulted in more terminally differentiated effector cells. Therefore, it appears that the route of nanoparticle entry into secondary lymphoid organs may alter where antigens are deposited. This demonstrates that it is not the magnitude of response postvaccination that matters, but the specific path of T cell differentiation promoted. In this system, despite the lower amplification of antigen-specific CD8+ T cells following intravenous administration, the differentiation toward T_{SCM} differentiation was essential to clear tumors cells more rapidly following checkpoint blockade.[166] Compared to subcutaneous delivery where antigen landed in IFRs with extended up take of MoDCs, intravenous delivery in the spleen was very transiently presented by cDC1s and MoDCs and the expression of IL-12 and IFN-α again was transient compared to settings where T_{SLEC} cells were preferentially made.[166]

The increased efficacy and safety of these nanoparticle vaccines lie in the ability to recapitulate the deposition of viral particles in the IFR. In addition, the structure-based design of self-assembling protein nanoparticles allows multiple epitopes to be complexed in a highly immunogenic manner, allowing multivalent presentation of antigen. This system has been used to elicit potent neutralizing immunity using a dual-antigen self-assembling SARS-CoV-2 nanoparticle in mice and non-human primates.[192] Excitingly, these vaccines also provide a structure-based design for the generation of broadly reactive universal vaccine platforms that target complete classes of viral strains, such as coronavirus or influenza.[188,192]

An alternative strategy is the investigation of how different adjuvants deposit antigen into spatially distinct regions. This has recently been investigated for the promotion of T_{H1} cell differentiation in vivo.[137] This study compared soluble Toll-like receptor and antigen delivered in the mineral oil emulsion, incomplete Freund's adjuvant (IFA). The use of the oil emulsion formulation altered the antigen deposition, moving it to the IFRs, rather than the medulla of draining lymph nodes. In addition, this approach promoted the recruitment of inflammatory monocytes which expressed CXCL10 to drive antigen-specific CD4+ T cells into this region.[137] Although not tested, presumably this would have also been associated with increased CD8+ T cell effector differentiation, but come at a loss of T_{FH} and T_{SCM}. Other newer adjuvant formulations, such as GLA-SE, have been shown to heighten T_{FH} and memory T cell formation; however, as yet how these alter the deposition of antigen or inflammatory signals has not been demonstrated.[193]

A final approach that aims to exploit specific T cell:DC interactions is via the targeting of specific dendritic cell populations.[194] This strategy exploits our understanding of T:DC interactions to target antigen to restrict presentation to a particular dendritic subtype. The optimal way to do this is to generate a genetic fusion of the antigen to the Fc portion of the antibody heavy chain to target a dendritic cell-specific surface molecule. The two molecules that have shown the most potential for this strategy are using Fc fusions proteins for targeting CLEC-9A, which is specifically expressed on cDC1s or targeting to DEC-205 which is more broadly expressed dendritic cells and langerin cells.[194] Targeting DEC-205 with adjuvants results in potent effector responses for both CD4+ and CD8+ T cells with strong humoral responses. The addition of adjuvant in this system is critical as without it, there are minimal effector responses and tolerance mechanisms related to regulatory T cells are induced.[195] Similarly, without adjuvant, targeting CLEC-9A also results in the induction of CD8+ T cell tolerance. As discussed above, the choice of adjuvant can steer and tailor immune responses and this is true also,

in combination with CLEC-9A targeting.[195] However, in the absence of adjuvant, CLEC-9A drives potent CD4+ T_{FH} differentiation, germinal formation, and humoral responses, that exceed that seen with DEC-205 targeting.[197,198] Interestingly, targeting cDC1s via CLEC-9A positions these cells at the T:B border of lymph nodes and allows more contact with antigen-specific B cells and T_{FH} progenitors in this region.[199] Combined, these two targeting approaches hold promise to promote specific T cell engagement with both dendritic cells and/or antigen-specific B cells. While the focus of studies to date has investigated CD4+ and CD8+ effector T cell differentiation and function, it remains to be seen how these individual targeting strategies promote the generation of long-lived memory. Again, it is likely that adjuvant selection with each of these approaches will determine outcomes, highlighting the flexibility of these strategies.

7 | CONTACT-TRACING T CELL INTERACTIONS

Given the potential of harnessing both T_{FH} and T_{SCM} cells, the next questions in the field will be to more completely understand and identify the cellular contacts that promote formation of these subsets. In recent years, a host of new technologies and analysis pipelines have been developed to overcome some of the challenges for understanding the cellular interactions that govern transcriptional signatures of T cell fates. The most powerful of these incorporate advancements in imaging modalities combined with innovative transcriptional analysis.

One technology that is particularly suitable to determine cell interactions in a particular region is NICHE-seq.[200] This combines a photoactivatable fluorescent marker to color-in regions of interest using two-photon laser scanning microscopy. Following dissociation of tissues, cells are sorted to identify niche components which are then analyzed by single cell RNA-seq (scRNA-seq). This method has been used to exceptional effect to characterize the T:B border CD4+ T cell priming niche during viral infection.[11] This revealed increased proportions of monocytes and dendritic cells following LCMV infection, when T_{FH} differentiation is inhibited, compared to recombinant VSV where T_{FH} differentiation is promoted. As discussed above, gene signatures within the niche also demonstrated increased type I IFN, which was subsequently shown to control T_{FH} differentiation in this setting.[11] A further application of this technology would be to combine NICHE-seq with a comprehensive ligand:receptor bioinformatic analysis to broadly identify the cellular interactome within a specific region.[201] The current tool kit available to bioinformatically resolve cellular interactions has recently been reviewed.[202] These analyses could also be complemented assessing the physically interacting cells (PIC-seq) approach.[203] This method calibrates tissue dissociation protocols to insure interacting cell conjugates are preserved prior to scRNA-seq analysis. Following conjugate RNA-seq, the interacting partners are bioinformatically separated. Of interest, some of the receptor:ligand and downstream transcriptional events can be associated with specific interacting cells.[203] Combining these approaches within a specific lymphoid niche offers new opportunities to understand how inflammatory cues and directly interacting cells facilitate T cell differentiation.

More advanced methods have been developed to analyze specific T cell interactions. A proximity-dependent labeling system that crosses cell:cell interfaces known as LIPSTIC (Labelling Immune Cell Partnerships by SorTagging Intercellular Contacts) has been developed to investigate CD40:CD40L interactions.[204] In this system, ligands and receptors are genetically fused to the *Staphylococcus aureus* transpeptidase sortase A (SrtA), or a tag residue. When the ligand and receptors interact, Srt A catalyzes the transfer of substrate onto the tagged receptor, recording the history of interaction that can be detected by flow cytometry. This study revealed that initial vaccine-induced interactions occur between 24 and 72 hours following T cell transfer and these interactions were primarily with cDC2 as opposed to cDC1s.[204] This approach is highly adaptable to other receptor:ligand interactions and cell types of interest. An extension of this approach, termed FucolD, provides another option for interaction-dependent labeling of T cells using cell-surface enzymatic fucosyl-biotinylation.[205] In this system, antigen-specific T cells were labeled by their interacting dendritic cell partners within the tumor environment. Importantly, this approach is a proximity-based labeling system, meaning that, unlike LIPSTIC, the receptor mediating the interaction is not required to be known prior to experimentation.

8 | CONCLUSION

Given the transcriptional similarities between CD4+ and CD8+ effectors, as well as the similarities between T_{FH} and T_{SCM}, we have discussed in this review what is currently known for these subsets and contrasted the spatial location and environmental cues that promote specific differentiation toward each of these paths. While the secondary lymphoid niches that imprint specific T cell differentiation have been elucidated, there remains much left to understand regarding the specific interactions within these sites that direct cell fate. This information is key to understanding the ways to harness T cell differentiation for therapeutic and vaccine interventions and will allow the generation of T cells that are fit for purpose; either to stimulate potent T cell effector function for the elimination of chronic infection and cancer, or to amplify the formation of humoral immunity and longevity of cellular memory to prevent current and emerging infectious diseases.

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CONFLICT OF INTEREST

The author has no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

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