CD4+ T cell memory

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Abstract

Specialized subpopulations of CD4+ T cells survey major histocompatibility complex class II–peptide complexes to control phagosomal infections, help B cells, regulate tissue homeostasis and repair or perform immune regulation. Memory CD4+ T cells are positioned throughout the body and not only protect the tissues from reinfection and cancer, but also participate in allergy, autoimmunity, graft rejection and chronic inflammation. Here we provide updates on our understanding of the longevity, functional heterogeneity, differentiation, plasticity, migration and human immunodeficiency virus reservoirs as well as key technological advances that are facilitating the characterization of memory CD4+ T cell biology.

CD4+ T cells play diverse roles in the immune system. Vaccinologists often emphasize their ability to promote class switching, somatic hypermutation and memory differentiation among B cells[1,2]. CD4+ T cells also provide help to CD8+ T cells by supporting their expansion and differentiation into functional memory T cells[3]. However, CD4+ T cells are also important effectors, killers and potent communicators, they regulate tissue homeostasis and wound healing and sound the alarm upon microbial invasion[4–7]. CD4+ T cells also have an essential role in restraining inflammation and constraining adaptive immune responses[8]. These diverse roles are accomplished by developmental specification events that result in unique CD4+ T cell lineages and differentiation states, which are considered as distinct subsets. The destruction of CD4+ T cells, as observed in untreated human immunodeficiency virus (HIV) infection, highlights the critical role of CD4+ T cells in maintaining functional immunity.

CD4+ T cells principally mediate surveillance through their T cell antigen receptor (TCR) and are restricted to major histocompatibility complex class II (MHC-II) molecules that present peptides of extracellular or phagosomal origin. MHC-II is constitutively expressed by professional antigen-presenting cells (APCs) including dendritic cells, macrophages and B cells. Upon receiving three signals from APCs (cognate antigen through the TCR, co-stimulation through co-stimulatory receptors and cytokines) for full activation, naive

CD4^{+} T cells undergo a proliferative burst and differentiate into various effector subsets that each are tailored to different types of immune mechanisms or infections[9–11] (Fig. 1). Most effector cells are short-lived, but a small fraction of T cells form long-lived memory cells that persist long beyond pathogen clearance. Memory CD4^{+} T cells mount an anamnestic response to reinfections that is quicker and of a higher magnitude than primary responses and can contribute to protective immunity[12]. We define CD4^{+} memory T cells as an antigen-experienced population that persists after antigen is presumably absent from the organism. This Review focuses on memory CD4^{+} T cells that persist after acute stimulations, such as infections or vaccines, with limited discussion of chronic stimulation contexts (tumors, autoimmunity) or memory regulatory T (T_{reg}) cells.

Memory CD4^{+} T cell longevity

In humans, memory CD4^{+} T cells longevity is best documented after viral infections or replicating vaccines. The most informative scenarios are those in which the pathogen in question is unlikely to be encountered naturally, so that memory T cells would not have an opportunity to be boosted. In a defining study, blood samples were assessed for interferon-γ (IFNγ)-producing CD4^{+} T cells upon in vitro restimulation with 'smallpox vaccine'-derived overlapping peptides using an ELISpot assay[13,14]. Memory CD4^{+} T cells could be detected 75 years after vaccination with an estimated $t_{1/2}$ of 8–12 years. Memory CD4^{+} T cells can be detected for at least 34 years after measles vaccination, and measles is rarely encountered in societies with high vaccination rates that achieve herd immunity[15]. Total mumps-specific CD4^{+} T cell immunity to measles, mumps and rubella (MMR) vaccination lasts at least 21 years[16]. More recently, memory CD4^{+} T cells were reported to persist for at least 11 years after severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) infections, 2 years after Zika virus, and several months after SARS-CoV-2 infection or mRNA vaccination[17–20].

Even with these landmark reports, we still have limited clinical information on memory CD4^{+} T cell longevity, despite the importance of the question. Complicating a general consensus, mouse studies suggest that CD4^{+} T cell memory may be less stable over time compared to CD8^{+} T cell memory[21]. Challenges in defining memory CD4^{+} T cell longevity may relate to the historic difficulty in quantifying antigen-specific T cells, the need for longitudinal or cross-sectional samples, the time it takes to acquire longitudinal samples and, in humans, the need to focus on infections that are not often reencountered. This latter issue arises, for example, when assessing the longevity of pertussis-vaccine specific immunity. While CD4^{+} T cell memory appears to persist for at least 5 years, periodic environmental exposures are likely[22]. Of note, all the above-mentioned longevity studies rely on cytokine secretion from peripheral blood mononuclear cells restimulated with peptides or virus to quantify the pathogen-specific memory CD4^{+} T cells. When considering the nature of certain memory CD4^{+} T cell subsets, this approach has its limitations and can only detect a small fraction of the pathogen-specific CD4^{+} T cell population. However, recent technological advances broaden the available tools to generate a more complete picture of the memory CD4^{+} T cell response.

Tools to study memory CD4+ T cells

Measuring pathogen-specific antibody titers has been feasible for about a century[23]. Quantifying antigen-specific T cell immunity is a recent innovation and rendered much more difficult due to MHC-restricted mechanisms of antigen detection, breadth of peptide epitopes that vary by MHC-II polymorphism and relevance of lymph nodes and tissue-localized T cells that are not represented within more easily sampled blood.

The development of the ELISpot assay in the 1980s allowed the detection of pathogen-specific restimulated memory CD4+ T cells[24]. However, human memory CD4+ T cell subsets, which are limited cytokine producers, are poorly detected with this technique. Moreover, analyses are usually restricted to blood samples, which may underrepresent important subsets. Sampling of lymph nodes through fine-needle aspiration or tissues through biopsies has become increasingly common. In combination with a recently developed cytokine-independent approach to identify pathogen-specific memory CD4+ T cells, these advances facilitate the detection of a broader variety of CD4+ memory subsets[25]. The 'activation-induced marker' (AIM) assay takes advantage of the fact that OX40 and CD25 are specifically upregulated upon antigen stimulation and is especially useful in situations where the exact CD4+ T cell epitope is unknown. In such cases, for example, during the emergence of SARS-CoV-2, T cells can be restimulated with peptide megapools covering the whole breadth of possible epitopes[26]. A disadvantage of the AIM assay is that reactivation of memory CD4+ T cells induces transcriptional and phenotypic changes and thus impairs the characterization of long-lived memory CD4+ T cells in a quiescent state.

TCR-transgenic mice, in which monoclonal T cells express a TCR specific for an epitope of interest, have served as a valuable tool. Adoptive cell transfer of naive TCR-transgenic T cells allows tracking of pathogen-specific CD4+ T cell responses without the need for restimulation[27]. Curiously however, TCR-transgenic CD4+ T cells often fail to model important aspects of 'normal' polyclonal endogenous responses and do not recapitulate the breadth and heterogeneity of effector and memory CD4+ T cells[28–30]. Specific TCR-transgenic cells may exhibit intrinsic subset differentiation biases (OT-II and SM1 cells may be biased toward follicular helper T (T_{FH}) cells, whereas TEa and SMARTA cells may be biased toward the T_H1 subset of helper T cells) or differ from polyclonal endogenous populations with respect to longevity.

MHC-II-restricted tetramers enable the direct detection of endogenous polyclonal CD4+ T cells, without the need for restimulation and functional assays[27,31]. Tetramer reagents are composed of four monomeric biotinylated MHC-II molecules, which are oligomerized using fluorescent streptavidin and loaded with a peptide of interest. Of importance is the avidity afforded by the polyvalency of the reagent, as monomeric MHC-II–peptide complexes fail to identify epitope-specific T cells[32]. In contrast to MHC-I-restricted tetramers for CD8+ T cells, MHC-II-restricted tetramers have low sensitivity, detecting only 5–30% of total responding CD4+ T cells to a given antigen[33]. Possible explanations include decreased peptide–TCR affinity of CD4+ T cells compared to CD8+ T cells and/or decreased affinity of the co-receptor CD4+ to the MHC-II protein in comparison to the CD8–MHC-I interaction. The detection of low-affinity CD4+ T cells can be improved by further increasing the

valency, as demonstrated by dodecamers[34]. Recently developed tetramers contain MHC-II molecules engineered for enhanced CD4 binding, allowing up to a fourfold increase in sensitivity of detecting antigen-specific $CD4^+$ T cells[35]. An important advantage of tetramers over AIM assays is that tetramer-binding $CD4^+$ T cells can be enriched for using magnetic beads, which facilitates detection of rare cell populations[27].

With the rise of next-generation sequencing, new possibilities to identify epitope-specific $CD4^+$ T cells will emerge. Algorithms for predicting dominant MHC-II-restricted epitopes contained with protein antigens, such as the Immune Epitope Database (IEDB; https://www.iedb.org/), have improved enough to be reasonably accurate. New approaches attempt to go even further by predicting epitope specificity based solely on TCR sequencing. This is based on structural predictions and may also leverage sequencing similarity of previously characterized clones[36]. As the databases of known epitopes and TCRs expand, the technology should only get more powerful. We anticipate that these newly developed tools will further enable research on $CD4^+$ T cells.

Differentiation and memory

Following activation of naive $CD4^+$ T cells, differentiation is informed by the nature of the pathogen and associated innate alarm signals[9,11]. Type 1 immune responses are elicited by intracellular infections that induce the expression of the transcription factor T-bet in $CD4^+$ T cells, and subsequent T_H1 cell differentiation through interleukin (IL)-12-mediated and IFN-γ-mediated STAT1 and STAT4 signaling (Fig. 2). T_H1 cells are characterized by their capacity to secrete IFNγ. Type 2 immune responses are triggered by extracellular parasites, certain allergens, or weak immunogens that promote Gata3-expressing T_H2 cells that are functionally defined by their ability to synthesize IL-4, IL-5 and IL-13 through IL-4-induced STAT6. Type 3 immune responses develop in response to fungal and extracellular bacterial infections and are driven by induction of the transcription factor RORγt, which promotes T_H17 differentiation. T_H17 cells secrete IL-17 through STAT3 signaling triggered by IL-6, IL-23, IL-1β or transforming growth factor-β. Type 1 cytokines promote $CD8^+$ T cell and macrophage responses, type 2 cytokines attract mast cells, basophils and eosinophils, and type 3 responses preferentially induce the influx of neutrophils to the focus of inflammation (Fig. 2)[9,11].

Type 1, 2 and 3 responses are all associated with parallel differentiation of Bcl6-expressing T_{FH} cells that help B cells and fine-tune humoral effector mechanisms (Fig. 2)[2]. T_{FH} cells are induced in a two-step differentiation process[37]. Firstly, dendritic cells induce expression of CXCR5 on activated $CD4^+$ T cells, allowing 'pre-T_{FH}' cells to sense CXCL13 and to migrate to the T cell–B cell border of secondary lymphoid organs (SLOs). The signals that initiate the T_{FH} cell program are still under investigation, but they include STAT3 signaling likely induced through IL-6, IL-21 and the co-stimulatory receptor inducible T cell co-stimulator (ICOS)[1]. Secondly, further stimulation by B cells reinforces the T_{FH} program and enables their migration into germinal centers located in B cell follicles. Here, T_{FH} cells select pathogen-reactive B cell clones to promote plasma cell and memory B cell differentiation, and long-lived antibody responses[1]. Germinal center B cells and their progeny with the highest affinity for antigen receive the most sustained T_{FH} cell help, thus

T_{FH} cells promote affinity maturation[1]. T_{FH} cells provide help to B cells through IL-21 secretion and cell-contact-dependent co-stimulation through CD40L, which interacts with CD40 on B cells and in return receive help through ICOS, which interacts with ICOSL on B cells[10]. Like non-T_{FH} cells, T_{FH} cells take on flavored characteristics based on the nature of the pathogenic insult and associated innate response mechanisms[38]. For this reason, a revised nomenclature has been proposed to refer to the three flavors of T_{FH} cells as the $T_{FH}1$, $T_{FH}2$ or $T_{FH}17$ subset[38]. All three T_{FH} flavours promote some IgG. However, while $T_{FH}1$ cells predominantly promote the generation of IgG responses, $T_{FH}2$ cells can induce isotype class switching toward IgE and $T_{FH}17$ cells are associated with antibody-skewing toward an IgA isotype[38]. This nomenclature is not rigid and may be influenced by the environment. For example, in response to lung influenza infection, $T_{FH}1$ cells may also promote local IgA through IL-21 secretion to local B cells[39,40].

The intrinsic factors or extrinsic cues that guide differentiation into T_{FH} versus non-T_{FH} effector cells have not been fully elucidated. Data suggest that this decision is initiated within the first two cell divisions[37]. Some findings have indicated a link between T_H1 versus T_{FH} cell differentiation and cumulative TCR signal strength[29,41–50]. Early in vitro work showed that TCR-transgenic cells stimulated with low antigen doses preferentially produce IL-4, which was associated with a T_H2 response[51–53]. High antigen doses triggered IFNγ production, and thus T_H1 differentiation. Yet, very high doses induced IL-4 production[51,52]. Several in vivo studies reported that cells receiving the strongest TCR signal preferentially differentiate into T_{FH} cells[29,43,46]. However, others found that T_{FH} cells can be induced by weak TCR signals, and that strong TCR stimulation preferentially induces T_H1 cell differentiation[41,42,44,45,47,48]. The variable results might be explained by the use of different experimental models, antigen doses and persistence, and myriad other variables, so a clear picture has not yet emerged. Cues may be multifactorial, including exposure to distinct cytokine microenvironments or dendritic cell subsets, although a recent study found no evidence for the latter[44]. There may also be intrinsic regulation of diversity (for example, asymmetric division) contributing to the T_{FH} and non-T_{FH} effector fate[54]. A recent model suggests that the decision is centered around IL-2 signaling[55].

Once pathogens are eliminated, most effector CD4$^+$ T cells undergo cell death. However, ~5–10% of antigen-specific CD4$^+$ T cells persist and form memory cells. In contrast to naive CD4$^+$ T cells, memory CD4$^+$ T cells do not require stimulation through MHC-II for survival, are more abundant for a given antigen and elicit an anamnestic response that is quicker and higher in magnitude[12,56]. Several mouse studies implied that memory CD4$^+$ T cell numbers might be less stable over time compared to memory CD8$^+$ T cells, or that certain subsets within the CD4$^+$ T cell memory compartment are less stable[21,57,58]. However, this might depend on the nature of the pathogen in question rather than a generalizable phenomenon, as studies in humans indicate that while memory CD4$^+$ T cells are less stable than their CD8$^+$ T cell counterparts in response to measles vaccination, the opposite is true for smallpox vaccination[13–15].

Memory CD4+ T cells in secondary lymphoid organs

In line with previously identified memory CD8+ T cell subsets, the CD4+ T cell memory compartment has traditionally been subdivided into CD62L+CCR7+ central memory T (T_{CM}) cells and CD62L−CCR7− effector memory T (T_{EM}) cells[59]. T_{EM} cells are further partitioned into T_H1, T_H2 or T_H17 memory cells and have been well described in mice and humans[14,57,60–64]. These populations are poised to secrete effector cytokines rapidly upon reactivation and foster enhanced secondary responses to reinfections. T_{CM} cells are functionally defined by their ability to synthesize IL-2 and have less potential for rapid IFNγ or IL-4 secretion[59]. T_{CM} cells also exhibit enhanced proliferation potential and provide a less differentiated backup reservoir for T_{EM} cells. It has been debated whether T_{FH} cells persist as a distinct memory cell subset, and what role memory T_{FH} cells might play in humoral immunity[65].

Addressing T_{FH} cell memory has been hindered by phenotypic commonalities between T_{FH} cells and T_{CM} cells, including expression of TCF1, ICOS, Stat3 and ID3, and the apparent gradual waning in expression of the T_{FH} cell hallmark proteins CXCR5 and PD-1 (refs. 57,66–69). Reactivation of primary memory CD4+ T cells results in accelerated antibody responses compared to naive mice and the formation of secondary T_{FH} cells[70]. This suggested that T_{FH} effector cells could feed into the T_{CM} cell pool and, upon reactivation, differentiate into secondary T_{FH} effector cells. Several differentiation schemes feature either T_{CM} or T_{FH} cell memory but often do not include both populations within the same model as two distinct subsets[66,71,72] (Fig. 3). Increasing evidence supports the concept of memory T_{FH} cells as a distinct memory population, and recent studies in mice identified the simultaneous presence of both T_{CM} and T_{FH} memory cell populations with distinct transcriptional programs (Fig. 3)[28,68,69,73–75]. Using a nanobody that prevents NAD-induced cell death during isolation from tissues, T_{FH} cells were shown to persist for >400 d after an acute lymphocytic choriomeningitis (LCMV) infection, well after antigen is putatively cleared[28]. Here, long-lived T_{FH} cells maintained elevated expression of CXCR5 and PD-1 compared to non-T_{FH} cells (but lower than T_{FH} effector cells) and high expression of a receptor that is also expressed during the effector phase, the folate receptor FR4 (ref. 76). FR4^{hi} memory T_{FH} cells are also generated upon *Listeria monocytogenes* infection or mRNA vaccination, but for unclear reasons are absent in TCR-transgenic memory CD4+ T cell populations[28,77].

Nevertheless, the relationship between precursor T_{CM} cells, T_{CM} cells with T_{FH} potential, T_{FH} effector cells and memory T_{FH} cells is far from clear. During CD4-dependent extrafollicular B cell responses initiated at the T cell–B cell border, CD4+ T cells that express intermediate levels of CXCR5 during an effector response have been referred to as T_{FH} cells[29,78]. However, after LCMV infection, CXCR5+ cells include precursor CCR7+ T_{CM} cells that depend on the transcription factor Thpok and are transcriptionally distinct, but closer to T_H1 cells than T_{FH} cells[69]. At a memory timepoint, CCR7+CD4+ T cells often express CXCR5, but in certain contexts can also be found within the CXCR5− fraction[79]. Transfer of CXCR5^{int}CCR7+ cells 10 d after infection into naive mice revealed preferential homing to T cell zones, persistence, retention of a T_{CM} cell phenotype and IL-2 production upon reactivation, thus meeting previous definitions of T_{CM} cells, despite

expression of CXCR5 (ref. 57). CXCR5 is a target of Bcl6, and Bcl6-deficient CD4^{+} T cells fail to generate CXCR5-expressing effectors at the peak of the response[80]. Bcl6 acts as a transcriptional suppressor and competes with Blimp1 for the induction or suppression of T_{FH} and non-T_{FH} programs[81,82]. That said, initiation of CXCR5 expression early in the immune response can be independent of Bcl6, and CD4^{+} T cells deficient for both Bcl6 and Blimp1 were unexpectedly able to form CXCR5^{+} T cells; however, they did otherwise lack much of the stereotypic T_{FH} cell gene expression program[73,80,83]. Human circulating CXCR5^{+} memory $T_{FH}1$ cells, but not circulating memory $T_{FH}2$ or $T_{FH}17$ cells were shown to be poor promoters of B cell responses[84,85]. Thus, CXCR5 expression is not the sole defining criterion for functional T_{FH} cells, but resolving phenotypic and ontogenetic relationships remain challenges for the field.

Serum antibody is maintained by long-lived plasma cells in the bone marrow, which persist independently of CD4^{+} T cell help[86]. Therefore, a legitimate question is what are the memory T_{FH} cells needed for. Evidence suggests that memory T_{FH} cells might provide survival signals to long-lived plasma cells that localize in organs other than the bone marrow under homeostatic conditions[28]. Or upon reinfection, memory T_{FH} cells might accelerate differentiation of memory B cells into plasma cells or promote secondary germinal center reactions[70,87,88]. Better characterization of where memory T_{FH} cells localize and which cell subsets are in close proximity might better inform function. Speculatively, high expression of CXCR5 in combination with absence of CCR7 expression might allow some memory T_{FH} cells to stay positioned within the B cell follicle or colocalize with memory B cells in the subcapsular sinus[89,90]. The existence of antigen-specific memory T_{FH} cells in human SLOs has not been reported yet. While germinal centers in humans can persist for up to a year, the same microstructure is generally shorter-lived in mice, or persisting germinal centers seemingly change their antigen specificity over time[91]. This makes it more difficult to discriminate late effector T_{FH} cells from bona fide memory T_{FH} cells in the human setting. Perhaps sequencing of measles-specific and smallpox-specific memory CD4^{+} T cells from vaccine-draining lymph nodes will allow assessment of the persistence of human antigen-specific long-lived T_{FH} cells in the likely absence of natural boosting, as has recently been executed for smallpox-specific memory B cells[92]. Lastly, it should be noted that the distinction of T_{EM}, T_{CM} and memory T_{FH} cells underrepresents the heterogeneity of the CD4^{+} memory compartment in SLOs, as these three major subsets can be further subdivided. We discussed heterogeneity solely through the prism of functional and phenotypic attributes, but heterogeneity can be further expanded to different axes including localization, migration properties, proliferation potential and developmental plasticity (Fig. 3).

Developmental plasticity of memory CD4^{+} T cells

Epigenetic analyses revealed that memory $T_{H}1$ cells exhibit increased IFNγ, but not IL-4 promoter accessibility, whereas the opposite was found for memory $T_{H}2$ cells[93]. Although culturing in vitro differentiated $T_{H}2$ cells in $T_{H}1$ conditions can upregulate T-bet and IFNγ, ex vivo isolated effector memory $T_{H}2$ cells display little plasticity[93]. In contrast, $T_{H}17$ cells appear to have a less-fixed epigenetic state than memory $T_{H}1$ or $T_{H}2$ cells, potentially allowing differentiation into IFNγ-secreting $T_{H}1$ cells[94]. Indeed, memory $T_{H}17$

cells produce both IL-17 and IFNγ in nasal tissue upon reinfection with *Streptococcus pyogenes*[95]. Additionally, memory T_H17 cells express stem-associated proteins like CD27 and TCF1, implying a less differentiated state[61].

CD27 and TCF1 are also expressed on T_{CM} cells, albeit not as highly as on T_{FH} cells[28,67,69]. After LCMV infection, memory Ly6C−PSGL1+CD4+ T cells, which contain T_{CM} cells, show greater expansion upon reactivation than Ly6C+ T_{EM} cells, and generate both Ly6C− and Ly6C+ effector T cells[67]. When the Ly6C−PSGL1+CD4+ T cells and Ly6C−PSGL1− memory T_{FH} cells were adoptively transferred and raced against each other, both generated similar numbers of T_H1 cells and precursor T_{CM} cells, but memory T_{FH} cells were more efficient in generating secondary T_{FH} effectors[28]. Influenza-specific memory T_{FH} cells also exhibit exceptional plasticity[65]. In conclusion, memory T_{FH} cells appear to retain considerable developmental plasticity and enhanced potential to produce T_{FH} cell progeny.

Maintaining developmental plasticity within a response may be important. In the case of tuberculosis (TB), IFNγ-producing T_H1 cells are crucial for providing protection[96]. To improve the existing Bacillus Calmette–Guerin (BCG) vaccine, a modified vaccinia Ankara vector expressing a TB antigen was generated[97]. Despite the successful establishment of memory T_H1 cells, the clinical trial showed that the vaccine does not elicit any protection[98] and left unclear if this was a failure of concept or execution. A mouse study showed that memory-like T_H1 cells get efficiently reactivated but disappear quickly upon adoptive transfer and subsequent TB infection, indicative of a short-lived population[99]. In contrast, memory-like PD1+ICOS+CD4+ T cells seemed to persist longer, exhibited increased developmental plasticity and provided superior protection. One interpretation of these data is that an ideal TB vaccine would generate a balanced, heterogenous memory compartment consisting of terminally differentiated memory T_H1 cells and memory CD4+ T cells that maintain developmental plasticity and proliferative capacity to optimally respond to TB over a prolonged period.

Memory CD4+ T cells in nonlymphoid tissues

Memory CD4+ T cells can be segregated by their migration properties. Under homeostatic conditions, T_{CM} cells recirculate through blood, lymphatics and SLOs, whereas T_{EM} cells are mostly restricted to the blood and upon reactivation preferentially migrate to inflamed tissues[59]. Based on their transcriptional profile, the majority of antigen-specific memory T_{FH} cells within SLOs appear to be resident[28]. Nonlymphoid tissues (NLTs) are surveilled by abundant populations of memory CD4+ T cells, comprising largely tissue-resident memory T (T_{RM}) cells but also equilibrating populations[6,100–106] (Fig. 4). CD4+ T_{RM} cells share properties with CD8+ T_{RM} cells, including downregulation of the transcription factor KLF2 and the tissue egress molecules S1PR1 and CCR7, and upregulation of CD69 (refs. 6,107).

CD4+ T_{RM} cells in mice have been described in numerous tissues, including lung, skin, female reproductive tract, salivary glands, small intestine, large intestine, liver, kidneys, bone marrow and nasal tissue among others[6,101,108–110]. CD4+ T_{RM} cells are generated in numerous infection modalities such as viral, bacterial, fungal, helminth or parasitic infection, or vaccination settings such as live attenuated vaccines, inactivated vaccines,

acellular vaccines or mRNA vaccines, and can play protective roles[77,105],[109,111–120]. CD4+ T_{RM} cells are also engaged in pathogenic roles in autoimmune diseases such as asthma, inflammatory bowel disease or glomerulonephritis[121–123]. In humans, CD4+ T_{RM} cells have been shown to persist for years in the small intestine, lung and liver upon organ transplantation[124–126]. Identification of human T_{RM} cells in non-transplant situations is difficult and relies on surface markers, most prominently CD69. Of note, CD69 is an imperfect marker because in mice, parabiosis experiments revealed the existence of CD69⁻ T_{RM} cells and CD69 is also an early T cell activation marker[127–129]. Nevertheless, transcriptional profiling of human CD69+CD4+ T cells from tissues identified a partial overlap with mouse CD8+ T_{RM} cells[107]. Combined TCR and single-cell RNA sequencing revealed expanded CD4+ T cell clones uniquely present in NLTs, providing further opportunities to refine T_{RM} cell signatures[130].

In contrast to CD8+ T_{RM} cells, CD4+ T_{RM} cells often lack expression of CD103 (ref. 6). CD103 facilitates interaction with E-cadherin-expressing epithelial cells and promotes T cell adhesion in epithelial layers. Ectopic expression of Runx3 in CD4+ T cells increased CD103 expression and preferential localization to epithelial layers in the skin and small intestine[131]. Thus, CD4+ T_{RM} cells can adapt the Runx3-CD103 residency program, but why it is mainly restricted to CD8+ T_{RM} cells is unclear. Indeed, epithelial cells in various NLTs including lungs, small intestine or skin can express MHC-II[132–135]. In a *Streptococcus pneumoniae* infection model, absence of MHC-II expression in lung epithelial cells resulted in a redistribution of CD4+ T_{RM} cells and dysregulated barrier immunity upon reactivation[135].

CD4+ T_{RM} cells take on T_H1, T_H2 or T_H17 effector functions, recruit and support various innate and adaptive cells at sites of infection and perform sensing and alarming functions upon reactivation[6,7,30,79,105,109,111,113,117,121,136,137]. But they do more than that. For example, CD4+ T_{RM} cells can be involved in tissue remodeling via secretion of amphiregulin or can induce mucus metaplasia and airway hyperresponsiveness in an allergic asthma model[114,121,138]. However, compared to CD8+ T_{RM} cells, CD4+ T_{RM} cells are still understudied due to technical challenges.

An FR4+CXCR5+ T_{FH}-like T_{RM} cell population that promotes local antibody and CD8+ T_{RM} cell responses through IL-21 secretion was identified in lungs of influenza-infected mice[30,137,139]. T_{FH}-like T_{RM} cells preferentially localize near B cells within inducible bronchus-associated lymphoid tissues, whereas T_H1-like T_{RM} cells are positioned at the outer boundaries of these structures[30]. Thus, the formation of T_{FH}-like T_{RM} cells in NLTs upon acute stimulations might require induction of tertiary lymphoid structures (TLSs). In humans, SARS-CoV-2-specific CD4+ T_{FH}-like cells have been identified in lungs; however, their stimulation history is unknown[140]. Circulating IgG antibodies exudate into tissues and mucosal compartments. Nevertheless, local memory T_{FH} cells might be important to induce rapid plasma cell differentiation upon reinfection to increase local antibody titers or fine-tune tissue-specific antibody responses, such as promoting dimeric IgA within mucosae[141,142]. Moreover, blood-borne antibodies poorly access some sites under homeostatic conditions, including olfactory tissues[143]. However, infections and vaccination with mucosal adjuvants can establish humoral protection of the olfactory epithelium through locally produced antibodies that are dependent on CD4+ T cell help.

Conceptually, the decentralization of cellular immunity from SLOs to NLTs has become well established[144–146]. Identification of T_{FH}-like T_{RM} cells and resident memory B (B_{RM}) cells in NLTs provides evidence that decentralization of immunity extends to the humoral arm as well.

Secondary lymphoid organ CD4⁺ T_{RM} cells

Memory CD4⁺ T cells also take up permanent residence in SLOs. Data from human lymph nodes revealed that a surprisingly large proportion (~70%) of CD4⁺ T cells express CD69 without downregulating IL-7R, indicative of a resting memory population[147]. Using photoconvertible proteins, it was shown that 7 d after photoconversion, a substantial fraction of antigen-experienced CD4⁺ T cells in lymph nodes and Peyer's patches retained the label, indicative of a resident population[148]. Tracking of antigen-specific memory CD4⁺ T cells using either a photoconvertible system or parabiosis further confirmed the existence of SLO T_{RM} cells[6,149,150]. Of note, the resident population is not restricted to a T_{FH} cell phenotype (T_{FH} cells make up ~7%) but exhibits substantial heterogeneity[148]. Differences in migration attributes might allow for a refined subdivision of T_{EM} cells. For example, Ly6C⁺ memory T_H1 cells might be mostly restricted to the blood. However, a Ly6C⁻ memory T_H1 counterpart has been described with decreased expression of Klf2 and S1pr1 mRNA. This subset might take up permanent residence in a lymph node after leaving NLTs[6,28]. At this point, little is known about the ontogeny of CD4⁺ SLO T_{RM} cells and their anatomic localization and function upon reactivation.

CD4⁺ T_{RM} cells as a reservoir for HIV

CD4⁺ T cells are the major reservoir of HIV. Antiretroviral therapy (ART) suppresses replication of HIV in CD4⁺ T cells from the blood to undetectable levels but does not eradicate the virus in infected cells. Thus, infected people undergo lifelong therapy. Subsequent viral rebound is thought to occur in a small fraction of latently infected long-lived memory CD4⁺ T cells[151]. A drug concentration-dependent spatial model argues that ongoing viral replication may occur in pharmacologic sanctuaries within lymphoid tissues because ART drug levels are below the threshold to efficiently suppress replication in non-activated CD4⁺ T cells[152]. Importantly, viral DNA molecules in CD4⁺ T cells were increased in the cervix, ileum and bronchoalveolar lavage compared to blood samples years after suppressive treatment, suggesting that NLTs might be sites of viral persistence too[153–155]. Analysis of cellular reservoirs in the cervix of aviremic women revealed that CD4⁺ T_{RM} cells express markers associated with susceptibility for HIV infection and make up >95% of infected CD4⁺ T cells[154,156]. Furthermore, the overall frequency of CD4⁺ T_{RM} cells decreased in ART-treated versus uninfected women. Studies in macaques have shown that initiating ART 3 d after infection is too late to prevent viral rebound after 24 weeks of continued therapy, suggesting that the viral reservoir is seeded in nonlymphoid tissues as early as 1–2 d after infection[157,158]. In addition, limited tissue penetration of antiretroviral drugs might lead to subtherapeutic concentrations at the sites of infection[159]. Quantitative studies in mice indicate that CD8⁺ T_{RM} cells constitute perhaps the most abundant subset of antigen-experienced CD8⁺ T cells, and the same may be true for CD4⁺ T cells[127]. Thus,

CD4$^+$ T$_{RM}$ cells may represent a quantitatively relevant HIV reservoir that will not be sampled in blood and could in theory provide sanctuaries for reemerging infection.

Memory T$_{reg}$ cells

There have been increasing investigations into the durability of T$_{reg}$ cells, many of which are specific for constitutively expressed self-antigens and non-self-antigens, and whether T$_{reg}$ cell maintenance exclusively depends on tonic TCR stimulation. Analyses of putative memory T$_{reg}$ cells are technically challenging due to limitations in defining antigen specificity, low abundance and lack of unstimulated memory-specific markers[160–162].

By using an elegant mouse model in which expression of a self-antigen in the skin was turned off, established T$_{reg}$ cells were shown to persist in the absence of antigen[163]. Subsequently, fetus-specific T$_{reg}$ cells were identified in the SLOs of pregnant mice, and these T$_{reg}$ cells persisted after delivery[164]. In a viral infection model, T$_{reg}$ cells were shown to persist in the lung, rapidly expand upon reinfection, and potently suppress effector CD4$^+$ T cells to mitigate tissue damage without negatively impacting viral clearance, providing further evidence for memory[165,166]. In contrast, inducible genetic tracing approaches indicated that inflammation-experienced T$_{reg}$ cells lack functional memory, potentially avoiding generalized host immunosuppression[167]. Thus, memory T$_{reg}$ cells may be contextual, although more investigations are needed.

Chronic antigen stimulation and exhaustion

The persistence of antigen has a profound effect on CD4$^+$ T cell differentiation, although this varies by the type of infection, tumor or autoimmune context. Compared to mouse naive CD4$^+$ T cells, memory CD4$^+$ T cells that persist after clearance of acute LCMV Armstrong infection largely share patterns of gene expression with CD4$^+$ T cells exposed to chronic LCMV Cl13 (ref. 168). However, persistent LCMV antigen stimulation eventually induces CD4$^+$ T cell exhaustion, which is characterized by a lower magnitude of cells, decreased cytokine production and upregulation of inhibitory receptors including PD-1, Lag3 and CTLA-4. These inhibitory markers are also expressed by T$_{FH}$ cells, and CD4$^+$ T cell differentiation upon chronic LCMV infection is skewed toward a T$_{FH}$ phenotype[168–170]. However, exhaustion markers, such as TOX, are much higher on a per-cell basis in chronically stimulated T$_H$1 and T$_{FH}$ cells compared to acutely stimulated T$_{FH}$ effectors[42]. A memory-like TCF-1$^+$Bcl6^{lo} progenitor T cell population that shares similarities with T$_{CM}$ or T$_{FH}$ precursor cells was proposed to sustain both T$_{FH}$ and T$_H$1 effector responses during chronic viral infection[171]. However, a shift toward T$_{FH}$ cell differentiation is not a generalizable feature of chronic antigen stimulation. For instance, T$_H$1 cells become the dominant subset in response to chronic phagosomal infections, such as *Mycobacterium tuberculosis* or *Salmonella enterica*[172,173]. Tumor-infiltrating CD4$^+$ T cells kill cancer cells, destroy tumor vessels and sustain leukocyte responses mainly through the secretion of IFNγ, TNF and IL-2 (ref. 174). While most CD4$^+$ T cells within the tumor environment are T$_H$1-like cells, tumor-draining lymph nodes and tumor-associated TLSs also contain T$_{FH}$-like cells, reminiscent of the heterogeneity and spatial distribution of influenza-induced pulmonary CD4$^+$ T$_{RM}$ cells[175] (Fig. 4). However, in contrast to the well-established role of

T_{FH} cells in vaccination and infection, the role of T_{FH} in antitumor immunity is less clear, and might include contributions to forming TLS through CXCL13 secretion, promotion of CD8^{+} T cell and B cell responses through production of IL-21 (ref. 175).

CD4^{+} T cells participate in numerous autoimmune conditions including allergic asthma, inflammatory bowel disease, psoriasis, multiple sclerosis and rheumatoid arthritis[176,177]. Although T_H2-like cells are implicated in asthma, autoreactive CD4^{+} T cells often display a T_H17 or a T_H1–T_H17 hybrid phenotype[79,176,177]. In comparison to CD4^{+} T cells responding to foreign antigen, the affinity of self-reactive CD4^{+} T cells is typically lower, because high-affinity clones usually get deleted in development or differentiate into T_{reg} cells[178]. In an elegant mouse study, engineered CAR T cells eliminated specific autoimmune-reactive CD4^{+} T cells[179]. CAR T cells that only depleted high-affinity self-reactive CD4^{+} T cells failed to ameliorate established experimental autoimmune encephalomyelitis, a model of multiple sclerosis[179]. However, CAR T cells that also depleted low-affinity self-reactive CD4^{+} T cells reversed disease[179]. It seems likely that low-affinity CD4^{+} T cells may better maintain function in contexts of chronic antigen stimulation, whether it be derived from self, tumor or infection.

Conclusion

The diverse biology of memory CD4^{+} T cells, the sequestration of subsets in tissues outside of blood, the complexity of assays for their identification and scarcity of antigen-specific populations all provide challenges to their study. However, substantial strides have recently been made, resulting in increasingly sophisticated models that describe divisions of labor and ontogenetic relationships among highly specialized memory CD4^{+} T cell subsets. Issues that are critical to address in the future include a better description of memory CD4^{+} T cell heterogeneity, including transcriptional regulation, migration properties and spatial distribution of memory CD4^{+} T cells within lymphoid and nonlymphoid tissues. A better understanding of how memory CD4^{+} T cells communicate with various immune and non-immune cellular networks within distinct microenvironments could help inform strategies to manipulate harmful autoimmune-reactive memory CD4^{+} T cells, reprogram memory CD4^{+} T cells to fight cancer and to foster new vaccination strategies that induce the right subsets at the right location to efficiently harness the protective potential of memory CD4^{+} T cells. Thus, gaining a more complete grasp of memory CD4^{+} T cell biology should have broad implications.

Acknowledgements

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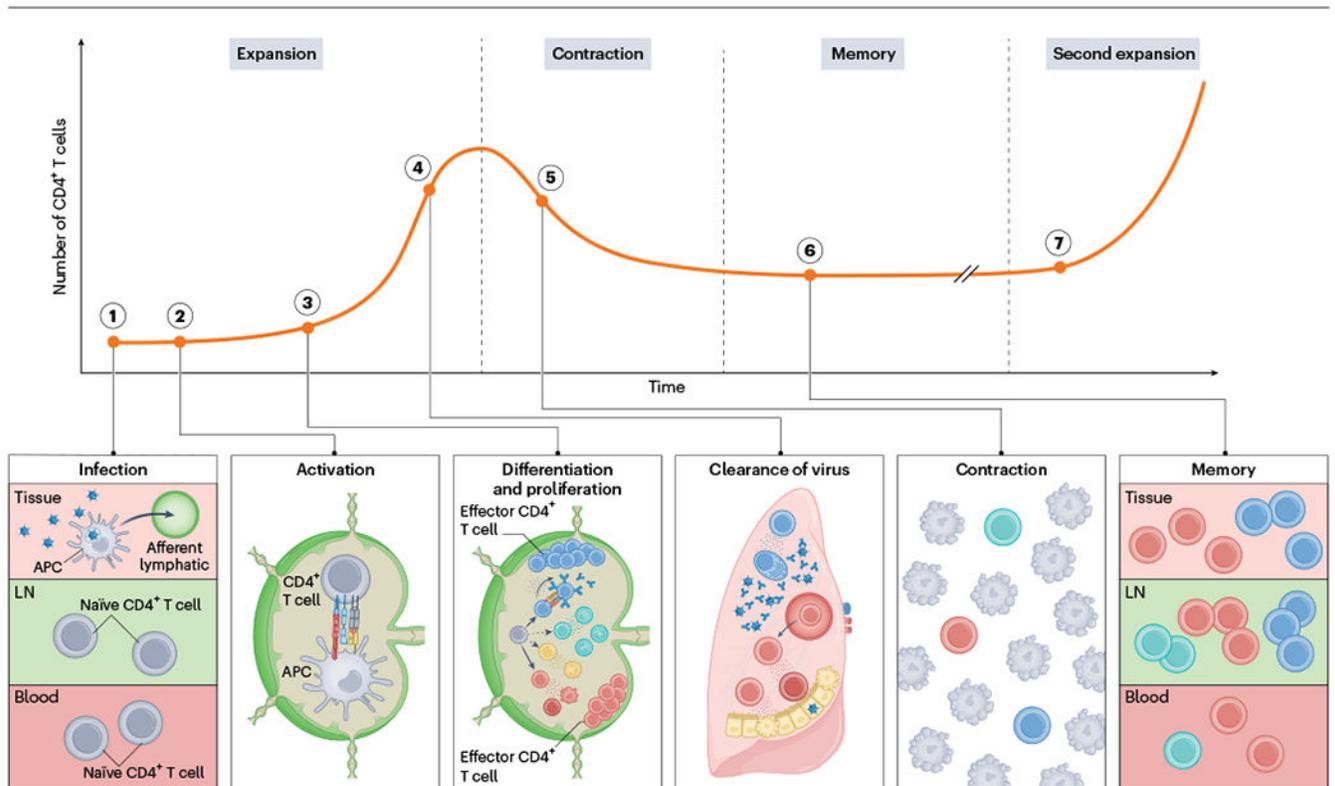
Fig. 1 |. Dynamics of a CD4^{+} T cell response.

1. Naive CD4^{+} T cells quiescently recirculate through blood (dark red) and lymphoid (light green) tissues. Upon infection, for example by a respiratory pathogen, APCs migrate from infected barrier sites (magenta) to the draining lymph nodes through afferent lymphatics and present peptides from the pathogen on MHC-II molecules. 2. Recognition of the peptide–MHC-II complex through TCR in combination with co-stimulation and cytokine signals lead to the activation, differentiation and expansion of naive CD4^{+} lymph node T cells. 3. CD4^{+} T cells proliferate and differentiate into various effector subsets that each become poised to make specialized contributions to immunity. 4. Many proliferated T cells leave the lymph nodes and migrate to the infected tissue through blood to assist in pathogen control at sites of infection. 5. Once the infection is cleared, most pathogen-specific CD4^{+} T cells die resulting in contraction of the population. 6. However, a few survive to establish long-lived memory and stay widely distributed across the body. 7. Upon reinfection, memory CD4^{+} T cells can mount anamnestic responses that are quicker and of higher magnitude than a primary response.
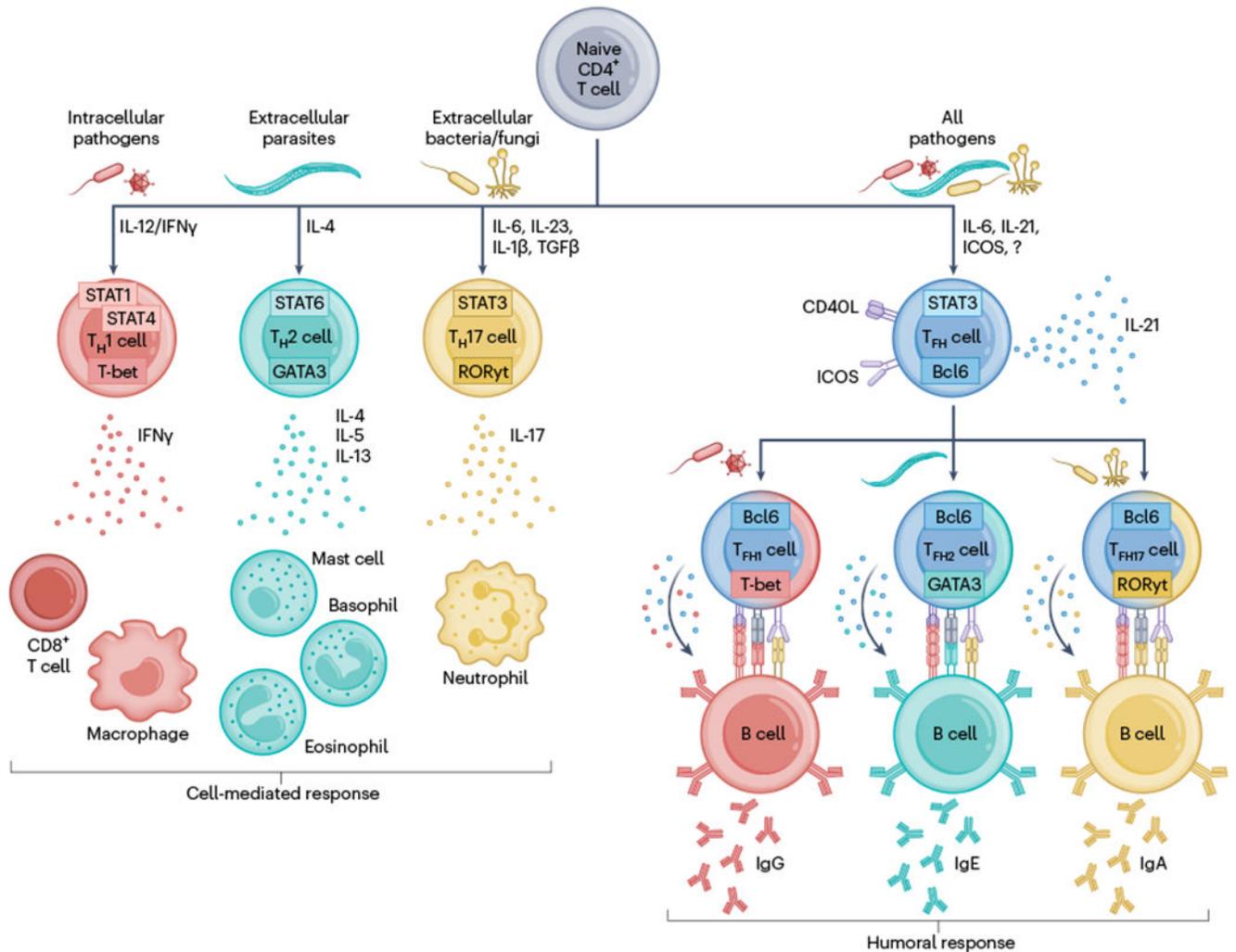
Fig. 2 |. CD4+ T cell differentiation is tailored to specific classes of immunogens.
Naive CD4+ T cells differentiate into specialized effector subsets that support cell-mediated or humoral responses. Intracellular pathogens induce T-bet-expressing T helper (T_H) 1 cells that secrete IFNγ to potentiate CD8+ T cell and macrophage responses. Extracellular parasites induce GATA3-expressing T_H2 cells that secrete IL-4, IL-5 and IL-13 to recruit and activate mast cells, eosinophils or basophils. Extracellular bacteria or fungi trigger the formation of RORγt-expressing T_H17 cells that produce IL-17 to trigger neutrophil responses. In parallel, immunogens induce Bcl6-expressing T_FH cells that receive help from B cells through ICOS and in turn promote humoral responses by providing CD40L and cytokines to B cells through direct cell-to-cell interaction, triggering isotype switching, affinity maturation and differentiation into memory or antibody-secreting cells. T_FH cells take on some characteristics of their cell-mediated response counterparts, expressing low levels of T-bet, GATA3 or RORγt, leading to biased secretion of IFNγ, IL-4 or IL-17 and skewing the antibody response toward specific isotypes.
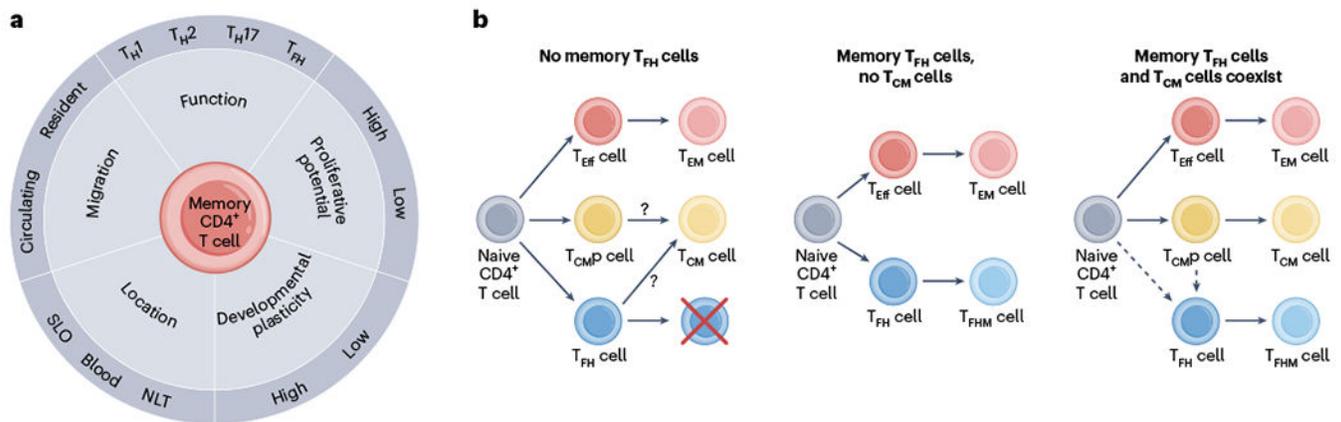
Fig. 3 |. Memory CD4+ T cell heterogeneity and ontogeny.
a, Memory CD4+ T cells exhibit heterogeneity in many parameters, including function, migration, location, developmental plasticity and proliferative potential. This makes categorization of defined subsets challenging. **b,** Existing memory CD4+ T cell differentiation schemes. Left, excludes memory T_{FH} cells. Middle, specifies memory T_{FH} cells, but excludes the concept of T_{CM} cells. Right, includes both memory T_{FH} cells and T_{CM} cells. T_{FH} cells might differentiate directly from activated naive CD4+ T cells, or come from a common T_{CM} cell precursor.
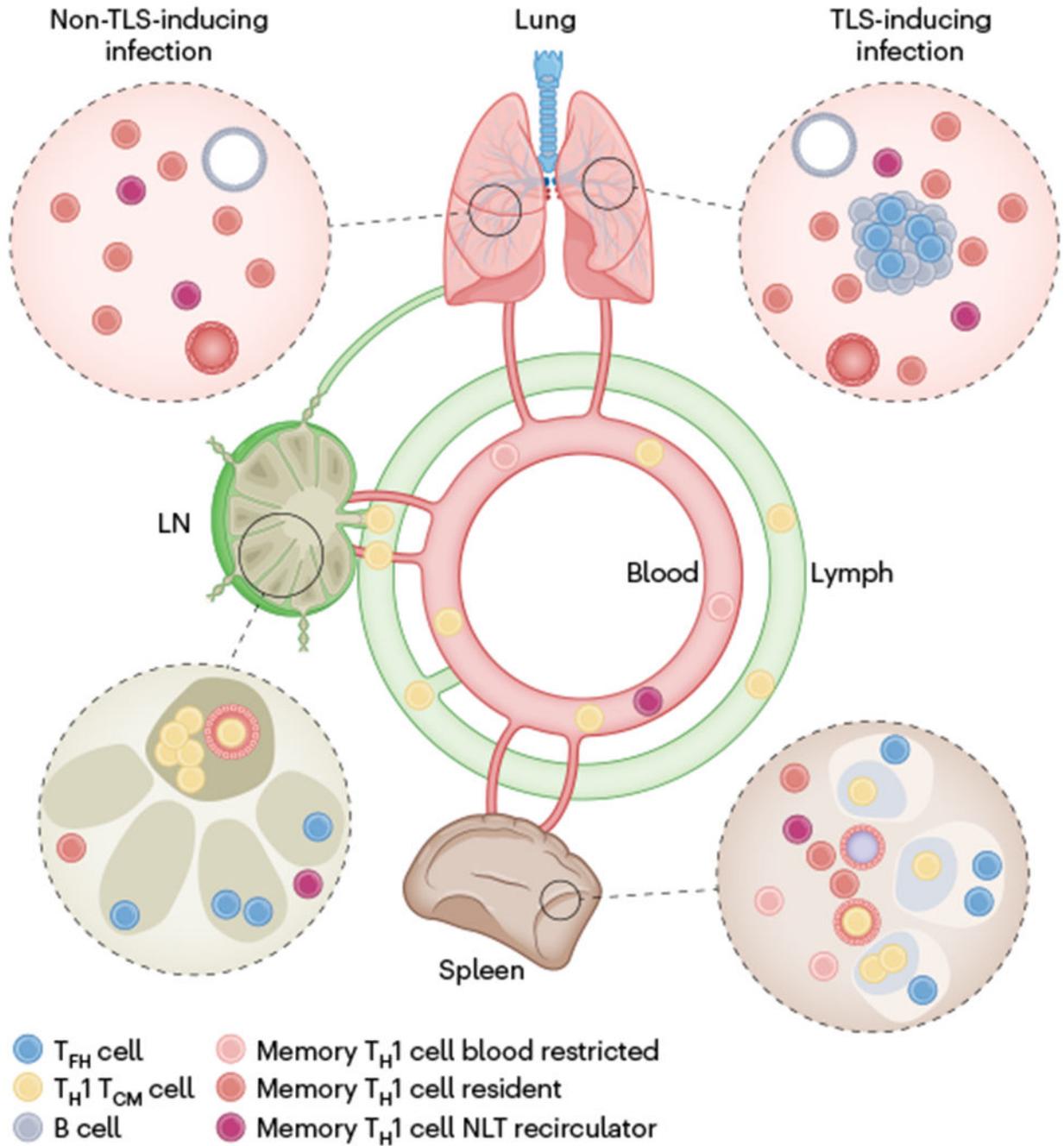
Fig. 4 |. Immunosurveillance by CD4^{+} T cells.

Memory CD4^{+} T cell immunosurveillance strategy after a respiratory viral infection that elicits a type I response. Although we focus on memory T_H1 cells, both memory T_H2 and memory T_H17 cells have been well documented to take up permanent residence in NLTs. In contrast to T_H1 T_{CM} cells, which express CD62L, many memory T_H1 cells cannot access lymph nodes (LN) through high endothelial venules. These memory T_H1 cells in lymph nodes might represent T_H1 cells that recirculate constitutively through NLTs and enter lymph nodes through afferent lymphatics ('backdoor entry') from upstream NLTs, or

SLO-resident memory T_H1 cells. Memory T_H1 cells in the blood comprise blood restricted cells, NLT recirculators and T_{CM} cells. FR4^{hi} memory T_{FH} cells populate SLOs and may reside at the outer boundaries of B cell follicles. T_{FH}-like CD4$^+$ T_{RM} cells have been identified in lungs of influenza-infected mice. In contrast to resident T_H1 cells, resident T_{FH} cells seem locally restricted to TLSs.