The multifaceted role of CD4$^+$ T cells in CD8$^+$ T cell memory

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Abstract | Following infection, T cells differentiate into a heterogeneous population of effector T cells that can mediate pathogen clearance. A subset of these effector T cells possesses the ability to survive long term and mature into memory T cells that can provide long-term immunity. Understanding the signals that regulate the development of memory T cells is crucial to efforts to design vaccines capable of eliciting T cell-based immunity. CD4$^+$ T cells are essential in the formation of protective memory CD8$^+$ T cells following infection or immunization. However, until recently, the mechanisms by which CD4$^+$ T cells act to support memory CD8$^+$ T cell development following infection were unclear. Here, we discuss recent studies that provide insight into the multifaceted role of CD4$^+$ T cells in the regulation of memory CD8$^+$ T cell differentiation.

Memory CD8$^+$ T cells are a principal component of immunity against intracellular pathogens such as viruses. They are distinguished by their capacity to survive long term, undergo rapid and robust proliferation and acquire effector function upon antigen re-exposure[1]. Memory CD8$^+$ T cells can vary in their phenotype, localization and function, allowing them to protect the host against a broad array of potential insults. CD8$^+$ T cells can mediate the killing of infected cells through many mechanisms, including the expression of granzymes and perforin, and the secretion of cytokines such as interferon-γ (IFNγ) and tumour necrosis factor (TNF). Unlike neutralizing antibodies that largely recognize proteins expressed on pathogen surfaces, CD8$^+$ T cells respond to peptide sequences presented on the surface of antigen-presenting cells. This allows them to recognize internal proteins of the pathogen that are less subject to evolutionary pressure and thus tend to be more highly conserved between different pathogen variants than external pathogen proteins. Therefore, CD8$^+$ T cells have the potential to provide broadly reactive protection against viruses, such as influenza virus or HIV, that rapidly mutate their surface proteins[2].

Despite the use of memory CD8$^+$ T cells in protection against pathogens that rapidly mutate to elude neutralizing antibodies, the development of T cell-based vaccines has proved to be problematic[3]. This failure is largely due to an incomplete understanding of the signals and cell types that operate at different stages of the immune response to influence the quantity and quality of developing memory CD8$^+$ T cells. In addition, knowledge of how CD8$^+$ T cells are able to enter and maintain residence in mucosal tissues is of crucial importance as many of the infections to which effective vaccines have not been developed initially infect mucosal sites such as the lungs, reproductive tract and skin. To generate a protective memory CD8$^+$ T cell response, it is important to understand the signals that are needed to position the memory T cells at the initial site of infection, as well as to induce a circulating memory pool that can prevent outgrowth of the pathogen.

The T cell response to acute infection can typically be divided into three phases — priming and expansion, resolution and contraction, and memory. During the first phase, naive CD8$^+$ T cells divide and differentiate into effector T cells that acquire the ability to produce IFNγ, TNF and cytotoxic proteins such as granzymes and perforin[4]. Following viral clearance, the contraction and resolution phase ensues, in which the majority of the effector CD8$^+$ T cells die, with ~5–10% of cells surviving. These cells enter the third stage — the 'memory' phase — and are maintained long term by signals such as interleukin-7 (IL-7) and IL-15 (REF. 5). Although there is considerable heterogeneity among long-lived CD8$^+$ T cells, they are typically divided into tissue-resident memory (T_{RM}), effector memory (T_{EM}) and central memory (T_{CM}) T cells. Differences in their localization, recall ability and effector functions allow them to provide overlapping layers of protection against potential reinfection[6]. T_{RM} cells are located in mucosal sites, such as the lungs, skin and reproductive tract, and are unique among memory T cell populations in that they do not re-enter the circulation. They are characterized by high expression of CD69 and CD103 (also known as αE integrin) and act as sentinels to provide immediate protection upon local secondary infection through direct effector functions and the recruitment

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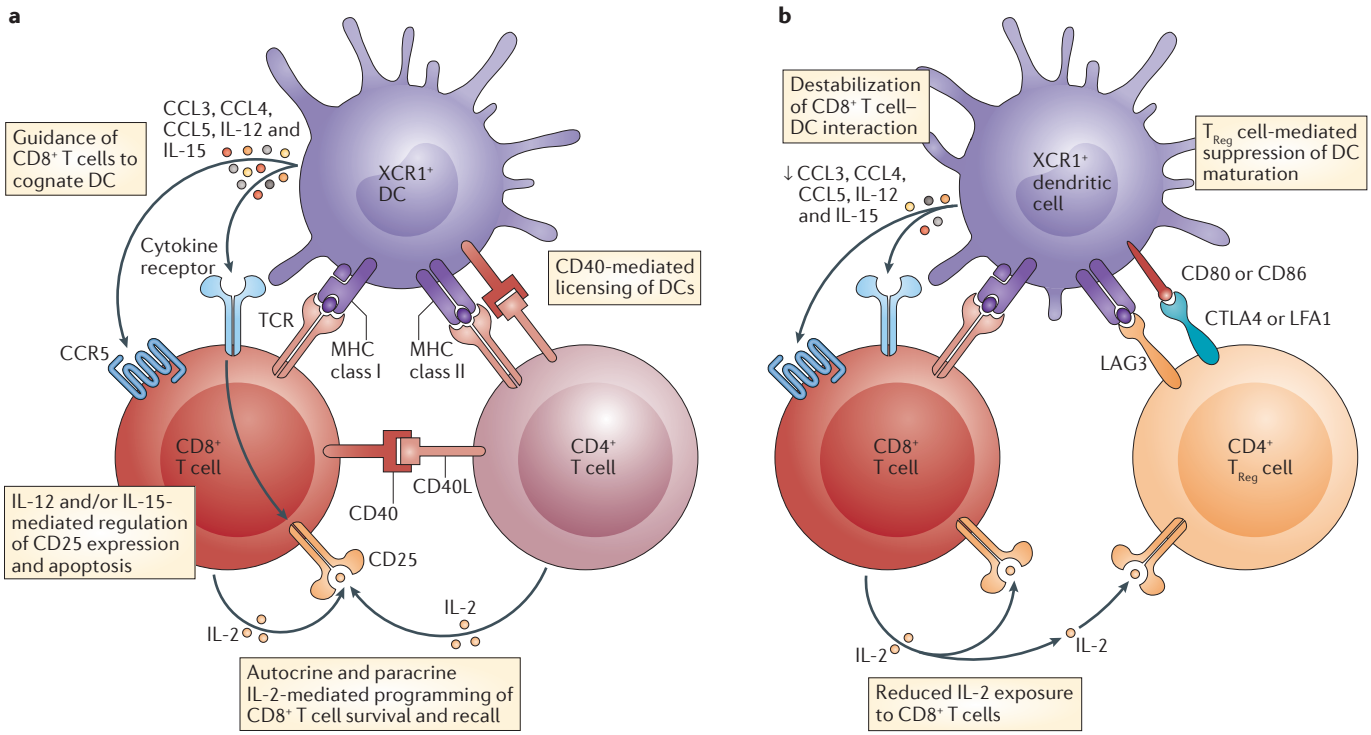


Figure 1 | **CD4⁺ T cell help to CD8⁺ T cells during immunization. a** | Following immunization, naive CD4⁺ T cells become activated and then interact with and license cognate XC-chemokine receptor 1 (XCR1)⁺ dendritic cells (DCs) through a CD40-dependent process. Licensed DCs express higher levels of MHC and co-stimulatory molecules and can recruit CD8⁺ T cells to their cognate DC through the secretion of CC-chemokine ligand 3 (CCL3), CCL4 and CCL5. Licensed DCs also secrete interleukin-12 (IL-12) and IL-15, which increase the expression of CD25 on CD8⁺ T cells and promote cell survival, respectively. Enhanced expression of CD25 facilitates the response of CD8⁺ T cells to IL-2 and promotes CD8⁺ T cell survival and their ability to proliferate on secondary antigen encounter. Both CD4⁺ T cells and CD8⁺ T cells act as sources of IL-2 and, in response to cellular antigens, are also capable of directly interacting through CD40–CD40 ligand (CD40L). **b** | Regulatory T (T_{Reg}) cells can modulate the CD8⁺ T cell response following immunization by suppressing the maturation state of DCs, thereby limiting their ability to stimulate CD8⁺ T cells. By limiting chemokine secretion by DCs, T_{Reg} cells can destabilize CD8⁺ T cell–DC interactions and accordingly promote the induction of high-affinity effector and memory CD8⁺ T cells. T_{Reg} cells also limit the sensing of IL-2 by CD8⁺ T cells by competing for available IL-2 and limiting the expression of CD25 by CD8⁺ T cells through the control of DC secretion of IL-12. CCR5, CC-chemokine receptor 5; CTLA4, cytotoxic T lymphocyte antigen 4; LAG3, lymphocyte activation gene 3 protein; LFA1, lymphocyte function-associated antigen 1; TCR, T cell receptor.

and reactivation of immune cells[7–12]. The developmental pathway and function of T_{RM} cells are reviewed elsewhere in this issue (see REF. 13). T_{EM} cells can migrate between tissues and secondary lymphoid organs and provide immune surveillance. T_{EM} cells have constitutive expression of some effector functions and lack expression of CD62L (also known as L-selectin) and CC-chemokine receptor 7 (CCR7)[1,5]. T_{CM} cells reside in secondary lymphoid organs and are typically characterized by expression of CD62L and CCR7, which are needed for entry therein. They possess the greatest proliferative potential among the memory T cell subsets and can rapidly expand and differentiate following re-challenge.

Considerable work over the past decade has sought to elucidate the signalling and transcriptional networks governing CD8⁺ T cell fate decisions, and these studies have been reviewed recently (see REF. 5). This Review focuses on the crucial roles of CD4⁺ T cells in the development of effector and memory CD8⁺ T cells[14–20]. We discuss recent studies that provide mechanistic insight into the process underlying the requirement for CD4⁺ T cells in memory CD8⁺ T cell maturation and function in different settings. This Review is divided into four main sections that discuss the involvement of CD4⁺ T cells in CD8⁺ T cell immunity following immunization or infection with pathogens that cause systemic, mucosal or chronic infections.

CD4⁺ T cell help following immunization

Primary response. Following immunization, CD4⁺ T cells are necessary for the induction of a robust primary CD8⁺ T cell response in terms of both cell number and functionality[21–28] (FIG. 1a). In addition, CD8⁺ T cells that differentiate in the absence of CD4⁺ T cell help are impaired in their long-term survival and display poor proliferative ability following secondary challenge[14,17,29,30]. Considerable work has sought to understand the processes underlying these defects.

A primary mechanism by which CD4⁺ T cells support the CD8⁺ T cell response following immunization is through the 'licensing' of dendritic cells (DCs). CD4⁺ T cell help is required for DCs to increase their

antigen-presenting and co-stimulatory capacities to levels that are sufficient to induce a robust effector CD8$^+$ T cell response, with both the CD4$^+$ T cells and CD8$^+$ T cells recognizing antigen presented by the same DC[7–12,23]. This licensing is mediated through CD40 (also known as TNFRSF5)–CD40 ligand (CD40L) interactions between the DC and cognate CD4$^+$ T cell that allow for the functional maturation of DCs[21,22]. The licensed DCs can subsequently interact with CD8$^+$ T cells and induce a strong primary response[24]. CD40–CD40L interactions can also occur directly between CD8$^+$ T cells and CD4$^+$ T cells in response to cellular antigen[17], although the functional importance of this interaction in other contexts appears to be minimal[31,32].

CD4$^+$ T cells can also support the primary CD8$^+$ T cell response through facilitation of their interaction with DCs. Before antigen encounter, CD8$^+$ T cells in the draining lymph node upregulate the chemokine receptor CCR5. These cells can then migrate towards the site of antigen-specific DC–CD4$^+$ T cell interactions owing to the production of chemokines such as CC-chemokine ligand 3 (CCL3) and CCL4 by the licensed DCs[33]. This guidance of naive CD8$^+$ T cells provides an explanation for how they can rapidly find their cognate antigen-presenting cell following immunization, despite the low frequency of both of these populations. Licensing of DCs by CD4$^+$ T cells can also facilitate the entry of naive CD8$^+$ T cells into the draining lymph node through expansion of the arteriole feeding the draining lymph node, as well as through enlargement of the draining lymph node itself, although the precise mechanism by which CD4$^+$ T cells regulate this process remains to be determined[34].

Further insight was provided by two recent studies evaluating the spatio-temporal interactions of DCs and cognate T cells[35,36]. One found that CD4$^+$ T cell activation precedes that of CD8$^+$ T cells and occurs through interactions with migratory DCs[35]. Activated CD4$^+$ T cells upregulate CD40L expression and subsequently license XC-chemokine receptor 1 (XCR1)$^+$ DCs, which specialize in cross-presentation and therefore can engage and activate antigen-specific CD8$^+$ T cells[35]. A separate study found that both CD4$^+$ T cells and CD8$^+$ T cells are activated by distinct DC subsets (non-infected and infected DCs, respectively) at separate anatomical locations[36]. These activated T cells then interact with cognate XCR1$^+$ DCs in clusters, which act as the platform by which CD4$^+$ T cell help is provided to the CD8$^+$ T cells[36]. In the future, it will be important to clarify the differences between these and previous works regarding whether CD8$^+$ T cells are activated by distinct DCs before engagement with XCR1$^+$ DCs and whether initial engagement of CD8$^+$ T cells with XCR1$^+$ DCs precedes licensing of these DCs by CD40L-expressing CD4$^+$ T cells or whether these interactions occur simultaneously.

Secondary response. In addition to their role in the primary response, CD4$^+$ T cells are crucial for regulating the size, phenotype and functionality of the memory CD8$^+$ T cell population[14,17,27,29,30,37,38]. One suggested mechanism for this requirement is through the regulation of TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10)[39,40]. 'Helpless' CD8$^+$ T cells rapidly induce TRAIL expression upon restimulation and undergo activation-induced cell death, although TRAIL deficiency does not seem to rescue secondary responsiveness of helpless cells in all settings[30]. The ability of CD8$^+$ T cells to respond following secondary challenge seems to be imprinted during their initial priming phase[14,15], although the provision of IL-15 during vaccination is sufficient to rescue the secondary cytotoxic T lymphocyte response in the absence of CD4$^+$ T cells; this is probably due to IL-15-mediated suppression of the pro-apoptotic molecule BCL-2-associated X protein (BAX) and induction of the anti-apoptotic molecule BCL-XL (also known as BCL2L1)[28,39]. IL-15 production by antigen-presenting DCs is necessary even in the presence of CD4$^+$ T cells to allow for the secondary responsiveness of CD8$^+$ T cells[28]. These studies suggest a model in which DCs gain the ability to secrete IL-15 following CD4$^+$ T cell-mediated licensing.

IL-2 is also important during priming to imprint the secondary responsiveness of CD8$^+$ T cells[41,42]. CD4$^+$ T cells have been suggested as a key source of IL-2 (REFS 26,27,43,44), although other studies have found that CD8$^+$ T cells produce the IL-2 that is necessary for their own development[45], with licensed DCs enabling such autocrine secretion[44]. Autocrine IL-2 allows for the induction of NGFI-A-binding protein 2 (NAB2) expression by CD8$^+$ T cells, which mediates the suppression of TRAIL expression and allows for the secondary expansion of these cells[44]. Licensed DCs also produce IL-12p70 that acts directly on antigen-specific CD8$^+$ T cells to increase their expression of CD25 (also known as IL-2Rα), rendering these cells more responsive to IL-2 (REFS 43,46).

Regulatory CD4$^+$ T (T_{Reg}) cells can act to modulate IL-2 exposure to effector CD8$^+$ T cells during the priming phase and are necessary for the generation of functional memory CD8$^+$ T cells following immunization[47–50] (FIG. 1b). For example, competitive consumption of IL-2 by T_{Reg} cells can limit the availability of IL-2 to early activated CD8$^+$ T cells, thereby suppressing CD8$^+$ T cell clonal expansion but enhancing the functional responsiveness of memory CD8$^+$ T cells following secondary challenge[47]. Although autocrine IL-2 expression is necessary for the generation of functional memory CD8$^+$ T cells[45], prolonged exposure to IL-2 early after immunization promotes their terminal differentiation[51,52]. T_{Reg} cells also modulate the maturation state of DCs through lymphocyte function-associated antigen 1 (LFA1; also known as αL integrin)- and cytotoxic T lymphocyte antigen 4 (CTLA4)-mediated regulation of CD80 and CD86 expression, which may influence the ability of DCs to stimulate CD8$^+$ T cells during priming[53,54]. In addition, T_{Reg} cells regulate the stability of interactions between DCs and CD8$^+$ T cells through suppression of chemokine production by DCs and are crucial for the development of high-avidity effector and memory CD8$^+$ T cells[55–59]. Additional mechanisms by which T_{Reg} cells can regulate memory formation following infection are discussed in the following section.

Cross-presentation
The ability of certain antigen-presenting cells (primarily dendritic cells) to redirect exogenous antigens to the MHC class I pathway, allowing for the stimulation of naive CD8$^+$ T cells by these cells. This process is important for the induction of immune responses against most tumours and viruses that do not typically infect antigen-presenting cells.

'Helpless' CD8$^+$ T cells
CD8$^+$ T cells that have undergone activation and differentiation in the absence of CD4$^+$ T cell-dependent stimulation ('help').

Activation-induced cell death
A process by which fully activated T cells undergo programmed cell death following binding of the T cell receptor by antigen or mitogen. Cell death occurs through the engagement of death receptors (such as FAS or the tumour-necrosis factor family receptors) or the production of reactive oxygen species.

CD4+ T cell help following systemic infection

Primary response. Although CD4+ T cells are necessary for the development of functional memory CD8+ T cells in settings of both immunization and pathogen infection, their requirement for the induction of a primary CD8+ T cell response following infection is less clear, and conflicting results have been reported in some cases for the same pathogen[15,18,20,43,60–66]. The finding that Toll-like receptor 3 (TLR3) ligands and TLR9 stimulation can bypass the requirement for CD4+ T cell help following immunization, with similar results being found following vaccinia virus infection, helps to partially reconcile these contradictory results[25,67]. This work suggests a model in which TLR ligation induces DC licensing and the secretion of type I IFNs, which subsequently act directly on CD8+ T cells to induce CD25 upregulation and IL-2 production. Thus, infections that induce a strong type I IFN response, such as lymphocytic choriomeningitis virus (LCMV), bypass the requirement for CD4+ T cell help in the primary response, whereas infections such as vaccinia virus that induce a more IL-12-driven response may require CD4+ T cell help[60]. CD4+ T cell help is dispensable for the primary CD8+ T cell response to influenza virus only when T_{Reg} cells are also absent, which suggests that the method of CD4+ T cell depletion could help to explain the contradictory findings. Conflicting results reported for the same pathogen may also be due to differences in viral inoculum, virulence of recombinant strains or whether T cell receptor-transgenic T cells were transferred before infection[60]. Further work is necessary to fully understand the mechanisms underlying differences in the published literature.

Secondary response. Despite it being appreciated that CD4+ T cells are necessary for CD8+ T cell memory maturation following acute pathogen infection, the mechanism (or mechanisms) by which this help is mediated and which CD4+ T cells are involved are still being clarified[15,20,62,68–70]. One study showed that CD4+ T cells are required after the priming and expansion phase and that this help can be antigen non-specific[70]. CD4+ T cell help following LCMV infection is independent of CD40-mediated licensing of DCs or CD8+ T cells, and passive transfer of LCMV-specific antibodies is sufficient to restore a protective memory CD8+ T cell response in the absence of CD40–CD40L interactions through the facilitation of viral clearance[31,71]. Helpless CD8+ T cells displayed increased expression of T-bet (also known as TBX21), a transcription factor involved in fine-tuning CD8+ effector and memory T cell differentiation, with reduction in T-bet expression restoring the functionality of these cells[72,73]. In addition, CD4+ T cell help influences the epigenetic state of memory CD8+ T cells, with helpless CD8+ T cells displaying reduced acetylation at the *IFNG* locus and increased methylation at the *IL2* promoter resulting in reduced responsiveness following restimulation[74]. Treatment with a histone deacetylase inhibitor is sufficient to partially restore the responsiveness of helpless CD8+ T cells[75].

Effector CD4+ T cells induced following acute pathogen infection display considerable phenotypic and functional heterogeneity that can influence their ability to provide help (BOX 1). Insight into the specific mechanism (or mechanisms) that underlie CD4+ T cell help was provided by the finding that IL-10, which is normally thought of as an immunosuppressive cytokine, is important for memory CD8+ T cell development. Although normal numbers of memory CD8+ T cells form in the absence of IL-10, the maturation of these cells was impaired, particularly the development of T_{CM} cells[76–78]. The cellular source of IL-10 that was needed to promote the phenotypic and functional qualities of the memory CD8+ T cells was unclear from these studies. Several cell types including myeloid cells, DCs and T cells can secrete IL-10 following LCMV infection[79,80]. Interestingly, and somewhat counter-intuitively, we found that T_{Reg} cells act as the relevant source of IL-10 required for memory CD8+ T cell maturation following LCMV infection. In line with the kinetics of CD4+ T cell help identified earlier[70], T_{Reg} cell-derived IL-10 primarily acted during the contraction and resolution phase to promote memory CD8+ T cell maturation through the suppression of pro-inflammatory cytokine production by DCs[49] (FIG. 2). A related study found that T_{Reg} cells influence CD8+ T cell memory maturation through CTLA4-mediated suppression of effector and proliferation programmes in effector CD8+ T cells[50]. So, T_{Reg} cells can promote functional memory CD8+ T cell development and function through many mechanisms.

In addition to this function, T_{Reg} cells can act during the priming phase to suppress the magnitude of the CD8+ T cell response, thereby restricting the number of cells that survive into the memory phase[81–83]. However, type I IFNs generated early after viral infection can directly inhibit T_{Reg} cell activation and proliferation, facilitating the generation of a robust effector T cell response[84]. As the levels of type I IFNs wane, T_{Reg} cell expansion occurs; the newly populating T_{Reg} cells display an

Box 1 | Effector CD4+ T cell differentiation

Following antigen recognition, naive CD4+ T cells can differentiate into distinct effector T helper (T_H) cell lineages capable of differentially regulating the immune response. The inflammatory environment in which recently activated CD4+ T cells develop drives their polarization and allows T cells to match their effector function to the pathogen encountered. Viral infection induces high levels of pro-inflammatory cytokines such as interleukin-12 (IL-12), interferon-γ (IFNγ) and type I IFNs that promote the induction of T_H1 cells. Conversely, IL-4 produced following helminth infection or allergic responses drives T_H2 cell differentiation whereas fungal infection induces transforming growth factor-β (TGFβ) and IL-6 secretion that promote T_H17 cell differentiation[130]. T follicular helper cell differentiation is regulated by signal transducer and activator of transcription 3 (STAT3)-signalling cytokines such as IL-6 and IL-21 and is dependent on iterative interactions with cognate dendritic cells and B cells[137–141]. CD4+ T cell subsets can be identified through the expression of cell surface markers, such as LY6C, P-selectin glycoprotein ligand 1 (PSGL1), CXC-chemokine receptor 5 (CXCR5) and programmed cell death protein 1 (PD1), and of canonical transcriptions factors T-bet, GATA-binding protein 3 (GATA3), retinoic acid receptor-related orphan receptor-γt (RORγt) and BCL-6, and through the secretion of cytokines such as IFNγ, tumour necrosis factor, IL-4, IL-5, IL-13, IL-17 and IL-21. The considerable plasticity among effector CD4+ T cell subsets *in vivo* helps to facilitate the generation of a diverse immune response capable of mediating pathogen clearance, promoting the humoral response and restraining immunopathology[130].

Functional exhaustion
A state of non-responsiveness of T cells resulting from chronic exposure to high levels of antigen marked by high expression of inhibitory receptors. Exhausted cells are impaired in their ability to proliferate and secrete cytokines, compromising their ability to control pathogen load.

activated phenotype with more robust IL-10 expression relative to T_{Reg} cells present in the steady state[49,85] (BOX 2). IL-10-competent T_{Reg} cells are mainly located in the white pulp of the spleen, near to DCs as well as memory precursor $CD8^+$ T cells, which is an optimal position to suppress the activation state of DCs and thus protect $CD8^+$ T cells from excess bystander inflammation and preserve their state as memory precursors[49,73,86–88].

$CD4^+$ T cell help following mucosal infection

$CD8^+$ T_{RM} cells have a crucial role in guarding mucosal surfaces against pathogen challenge[89]. Although $CD4^+$ T cells are not needed to initiate a virus-specific primary response following mucosal infection by pathogens such as influenza virus, they are necessary for the optimal development of a memory $CD8^+$ T cell population capable of mediating protective immunity[19,83]. However, until recently the role of $CD4^+$ T cells in mediating tissue-specific $CD8^+$ T cell memory was unclear. Insight into this issue was provided by work showing that $CD4^+$ T cell help is needed for entry into the female reproductive tract following mucosal viral infection[90]. $CD4^+$ T cells indirectly mediated the entry of $CD8^+$ T cells into the tissue through IFNγ-dependent induction of chemokines by locally infected cells[90] (FIG. 3a). Although $CD4^+$ T cells do not seem necessary for $CD8^+$ T cell entry into the skin following viral infection, skin $CD4^+$ T_{RM} cells can facilitate the recruitment of circulating $CD8^+$ T cells in a CXC-chemokine receptor 3 (CXCR3)-dependent manner following challenge with *Leishmania major*[91,92].

We extended these studies to find that $CD4^+$ T cells are important for the development of airway-homing $CD8^+$ T_{RM} cells following influenza virus infection[93]. Although $CD8^+$ T cells entered the lung in the absence of $CD4^+$ T cell help, they failed to properly localize to the lung airways and had a reduced ability to recruit $CD8^+$ T cells from the circulation and mediate protective immunity following heterosubtypic challenge. Helpless $CD8^+$ T cells also displayed enhanced expression of T-bet, which rendered these cells less responsive to transforming growth factor-β (TGFβ)-mediated induction of CD103, an integrin that is essential for T_{RM} cell maintenance in the tissue[93]. Together, these studies provided a model in which IFNγ-producing $CD4^+$ T cells directed effector $CD8^+$ T cell migration into particular areas of certain mucosal tissues that then facilitated their exposure to signals, such as TGFβ, necessary for their continued maturation into $CD103^+$ T_{RM} cells (FIG. 3b).

T_{Reg} cells also may have a role in the formation of $CD8^+$ T_{RM} cells. In the absence of T_{Reg} cells, reduced numbers of $CD8^+$ T_{RM} cells were retained in the central nervous system (CNS) following infection with West Nile virus. The reduction in T_{RM} cell numbers is associated with decreased amounts of TGFβ in the CNS, suggesting that T_{Reg} cell-dependent modulation of TGFβ levels may be important in driving $CD8^+$ T_{RM} cell formation and retention in mucosal tissues[94] (FIG. 3c). This finding further supports a new, perhaps paradoxical, role for T_{Reg} cells in providing helper functions as opposed to suppressive functions for the generation of long-term T cell immunity.

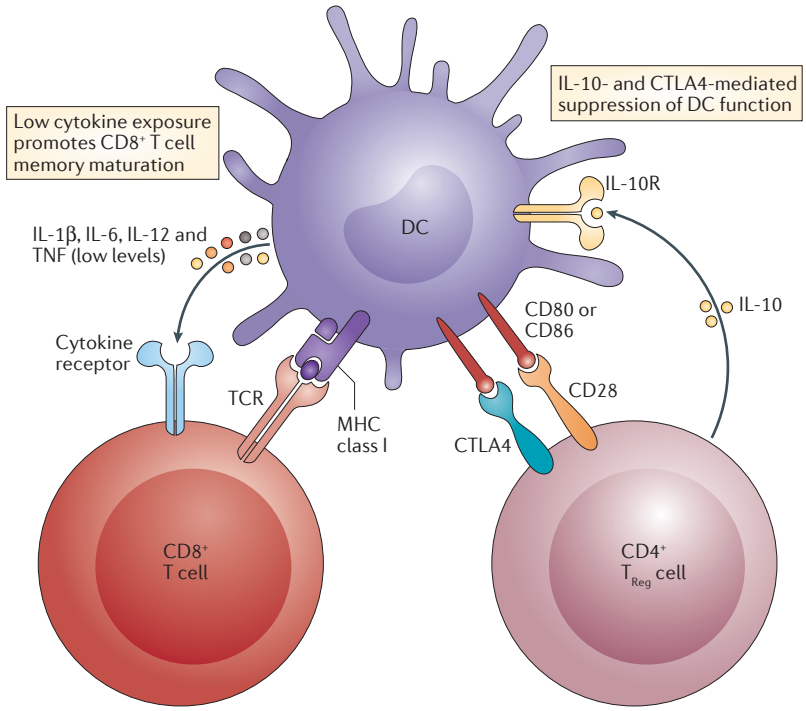


Figure 2 | **T_{Reg} cells promote memory $CD8^+$ T cell maturation during viral infection.** Following acute viral infection, regulatory T (T_{Reg}) cell expansion is initially suppressed owing to the presence of high levels of type I interferons (IFNs). As type I IFN levels wane, T_{Reg} cell numbers increase, and these cells adopt a more effector-like phenotype that is marked by increased expression of interleukin-10 (IL-10) and cytotoxic T lymphocyte antigen 4 (CTLA4). During the resolution phase of infection, T_{Reg} cell-derived IL-10 acts to suppress the maturation state of dendritic cells (DCs) and limit their production of pro-inflammatory cytokines. T_{Reg} cell expression of CTLA4 acts in a similar manner through modulation of the CD28–CD80 and/or CD28–CD86 signalling axis. Low levels of pro-inflammatory signals allow for the continued maturation of effector $CD8^+$ T cells into functional memory $CD8^+$ T cells. In the absence of T_{Reg} cell-derived IL-10 and/or CTLA4, DCs adopt a more mature phenotype and secrete higher levels of pro-inflammatory cytokines. The enhanced levels of pro-inflammatory cytokines are sensed by effector $CD8^+$ T cells and drive these cells to adopt a more terminally differentiated phenotype, limiting their ability to proliferate and mediate protective immunity upon re-encounter with the pathogen. IL-10R, IL-10 receptor; TCR, T cell receptor; TNF, tumour necrosis factor.

$CD4^+$ T cell help following chronic infection

$CD4^+$ T cells have crucial roles in the long-term maintenance of functional $CD8^+$ T cells in many models of chronic viral infection[95–99]. A central component of $CD4^+$ T cell help in this setting is the secretion of IL-21 (REFS 100,101). IL-21 sensing by $CD8^+$ T cells is necessary for the avoidance of clonal deletion and maintenance of effector activity, even in the presence of $CD4^+$ T cells, through induction of basic leucine zipper transcription factor ATF-like (BATF)[102,103]. IL-21 production by $CD4^+$ T cells in individuals infected with HIV-1 correlates with $CD8^+$ T cell functionality and viral control, suggesting that this pathway might be an important therapeutic target[104–106].

Persistence of high antigen levels is a key driver of $CD8^+$ T cell functional exhaustion during chronic viral infection[107]. Therefore, an additional indirect role of $CD4^+$ T cell-derived IL-21 in bolstering the $CD8^+$ T cell response

Box 2 | **Regulatory CD4⁺ T cell differentiation**

CD4⁺ regulatory T (T_{Reg}) cells express the transcription factor forkhead box P3 (FOXP3) and are crucial for the prevention of excess immunopathology or autoimmunity through many mechanisms[126]. T_{Reg} cells have considerable functional and phenotypic heterogeneity. Central or naive T_{Reg} cells express CD62L and are predominantly found in the circulation and secondary lymphoid tissues. Following exposure to antigen and/or interleukin-2 (IL-2), T_{Reg} cells adopt a more effector-like state and downregulate CD62L expression and progressively upregulate expression of CD69 and killer cell lectin-like receptor subfamily G, member 1 (KLRG1). Acquisition of a more effector-like phenotype is accompanied by enhanced expression of suppressive molecules, such as IL-10 and cytotoxic T lymphocyte antigen 4 (CTLA4), and KLRG1⁺ T_{Reg} cells represent a terminally differentiated population[49,85].

Effector T_{Reg} cells seem to co-opt the transcriptional network of effector CD4⁺ T cells to match their suppressive function to their present environment. T-bet⁺ T_{Reg} cells express the chemokine receptors CXC-chemokine receptor 3 (CXCR3) and CC-chemokine receptor 4 (CCR4) and regulate skin and lung inflammation. Signal transducer and activator of transcription 3 (STAT3)-expressing T_{Reg} cells upregulate CCR6 and regulate gut homeostasis, whereas BCL-6-expressing T_{Reg} cells display high levels of CXCR5 and regulate the germinal centre response. T_{Reg} cells also accumulate in adipose tissue, are distinguished by the expression of peroxisome proliferator-activated receptor-γ (PPARγ) and are important for regulating adipose tissue metabolism[142]. T_{Reg} cells have recently been identified in muscle, where they promote muscle repair through secretion of the growth factor amphiregulin[143]. Amphiregulin-producing T_{Reg} cells also promote tissue repair and maintenance in the lungs following infectious lung injury[144].

during chronic infection may be through promoting viral control. Several effector CD4⁺ T cell subsets can secrete IL-21 following viral infection, including follicular helper T (T_{FH}) and T helper 1 (T_H1) cells[108]. Viral persistence promotes the differentiation of T_{FH} cells, which are necessary for the maintenance of the germinal centre response and the continued production of virus-specific antibodies in the face of prolonged high levels of virus replication and immunosuppression[109,110]. T_{FH} cell-derived IL-21 is also crucial for the germinal centre response, and deficiency in IL-21 results in impaired maintenance of the germinal centre, which is associated with reduced B cell affinity maturation and isotype class switching[111–113]. Together, these studies suggest that T_{FH}-cell derived IL-21 is needed to promote the virus-specific humoral response, thus allowing for the continued control of viraemia and the prevention of terminal exhaustion.

IL-2 treatment also can enhance CD8⁺ T cell responses during chronic infection and allows for enhanced control of viral burden[42,114]. The beneficial effect of such treatment is limited in the absence of CD4⁺ T cells, which can serve as a source of IL-2 during chronic infection[115]. IL-2 therapy also results in an increase in the number of T_{Reg} cells[115]. T_{Reg} cells adopt an activated phenotype during chronic viral infection and have enhanced expression of molecules related to their suppressive activity, including CTLA4, CD39 (also known as NTPDase 1) and IL-10 (REFS 79,116). Depletion of T_{Reg} cells during chronic infection resulted in a marked expansion of functional CD8⁺ T cells through a process dependent on CD4⁺ T cells and the expression of co-stimulatory molecules on DCs[116]. T_{Reg} cell depletion alone was not sufficient to reduce viral burden but did lead to a significant reduction in viral titres when combined with antibody-mediated blockade of programmed cell death 1 ligand 1 (PDL1)[116]. Therefore, in contrast to acute infection, suppression of the maturation state of DCs by T_{Reg} cells during chronic infection serves to dampen long-term effector CD8⁺ T cell responses. It is important to investigate whether the beneficial effect of IL-2 therapy is enhanced in the absence of T_{Reg} cells.

Similar to chronic infection, the immune response to cancer is marked by the persistence of antigen that can lead to a loss of T cell function[117]. Accordingly, CD4⁺ T cell help can promote, whereas T_{Reg} cells suppress, the antitumour CD8⁺ T cell response[118–121]. CD4⁺ T cells can enhance the recruitment, proliferation and effector function of CD8⁺ T cells within the tumour[121,122]. The secretion of IFNγ induces the expression of chemokines that are necessary to recruit CD8⁺ T cells to the tumour, whereas the production of IL-2 by tumour-specific CD4⁺ T cells can promote CD8⁺ T cell proliferation and expression of granzyme B[121]. CD4⁺ T cells can also directly suppress tumour growth through IFNγ and cytotoxic functions[123]. Conversely, T_{Reg} cells within the tumour can interact with DCs and suppress the expression of CD80 and CD86, leading to the induction of a dysfunctional state within CD8⁺ T cells that is marked by high expression of inhibitory receptors and poor effector function[119]. Given that T cell effector functions are highly dependent on glucose and glutamine, T cell-nutrient deprivation by T_{Reg} cells may be another component of immunosuppression observed in the tumour microenvironment[124–126]. Currently, many therapies are aimed at delivering CD4⁺ T cell help to tumour sites, including vaccinations and adoptive T cell transfer[127–129]. It is important to learn how CD4⁺ T cells are involved in the durable responses observed in some patients following immunotherapy treatment.

Concluding remarks and perspective

A temporal model for the function of CD4⁺ T cells in regulating CD8⁺ T cell maturation into distinct subsets that can mediate protective immunity following infection and immunization has begun to emerge (FIG. 4). During the priming phase, CD4⁺ T cells, through IFNγ-mediated chemokine induction, license CD8⁺ T cell entry into mucosal tissues and facilitate their migration into a tissue microenvironment where they can sense the signals necessary for their long-term residence[90,93]. Effector CD4⁺ T cells also help to facilitate viral clearance through the induction of an antiviral humoral response, as well as directly through the secretion of effector molecules and cytokines[130]. Rapid viral clearance is important for restricting the degree of exposure of CD8⁺ T cells to antigen and inflammation, thus preventing functional exhaustion. High levels of type I IFNs present during this stage of infection also restrict T_{Reg} cell expansion and allow for a robust effector T cell response[84]. During immunization and certain infections, CD4⁺ T cells are also necessary to guide CD8⁺ T cells towards cognate XCR1⁺ DCs and to license these cells to provide the signals that enable autocrine secretion of IL-2, thereby promoting robust primary and secondary responses.

During the resolution phase, as the levels of type I IFNs wane, there is an expansion of activated T_{Reg} cell populations. IL-10 secretion by activated T_{Reg} cells can suppress the maturation state of DCs and limit their secretion of

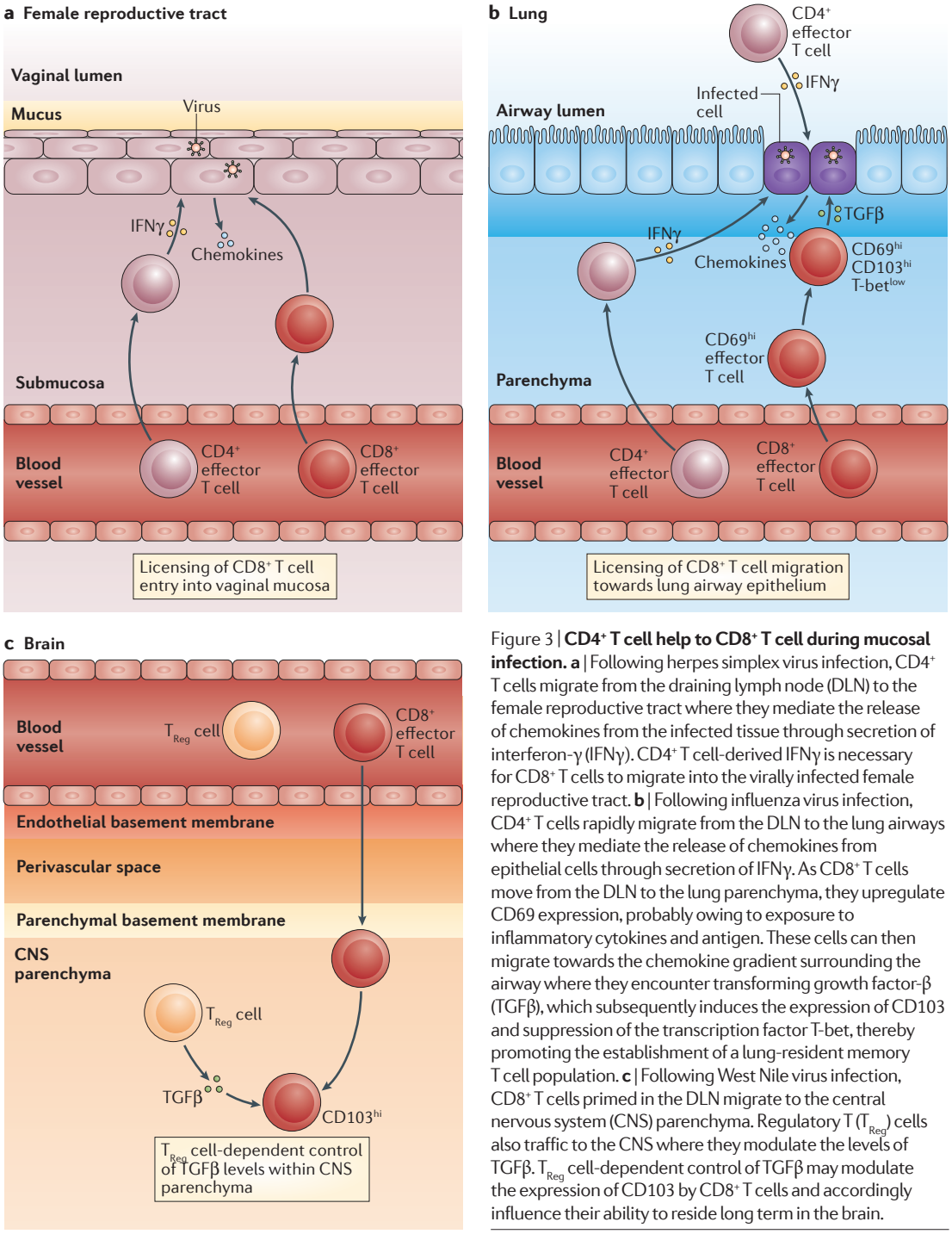
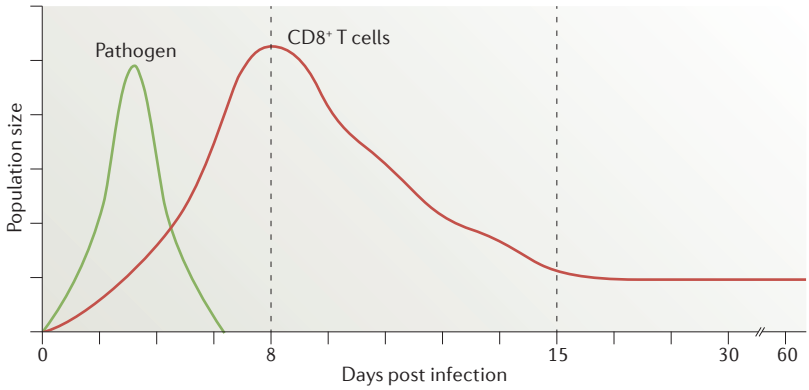


Figure 3 | **CD4⁺ T cell help to CD8⁺ T cell during mucosal infection. a** | Following herpes simplex virus infection, CD4⁺ T cells migrate from the draining lymph node (DLN) to the female reproductive tract where they mediate the release of chemokines from the infected tissue through secretion of interferon-γ (IFNγ). CD4⁺ T cell-derived IFNγ is necessary for CD8⁺ T cells to migrate into the virally infected female reproductive tract. **b** | Following influenza virus infection, CD4⁺ T cells rapidly migrate from the DLN to the lung airways where they mediate the release of chemokines from epithelial cells through secretion of IFNγ. As CD8⁺ T cells move from the DLN to the lung parenchyma, they upregulate CD69 expression, probably owing to exposure to inflammatory cytokines and antigen. These cells can then migrate towards the chemokine gradient surrounding the airway where they encounter transforming growth factor-β (TGFβ), which subsequently induces the expression of CD103 and suppression of the transcription factor T-bet, thereby promoting the establishment of a lung-resident memory T cell population. **c** | Following West Nile virus infection, CD8⁺ T cells primed in the DLN migrate to the central nervous system (CNS) parenchyma. Regulatory T (T_{Reg}) cells also traffic to the CNS where they modulate the levels of TGFβ. T_{Reg} cell-dependent control of TGFβ may modulate the expression of CD103 by CD8⁺ T cells and accordingly influence their ability to reside long term in the brain.

pro-inflammatory cytokines, thus allowing the preservation of less-differentiated effector CD8⁺ T cells[49]. T_{Reg} cell expression of CTLA4 acts in a similar manner by limiting CD80 and/or CD86 stimulation on DCs by CD28 (REF. 50). Effector CD8⁺ T cells are then able to continue to mature and develop into memory CD8⁺ T cells that can rapidly respond on pathogen re-encounter. T_{Reg} cells also act during the resolution phase to modulate TGFβ levels within mucosal tissues, thereby bolstering the induction of CD8⁺ T_{RM} cells[94]. Following the resolution phase, CD4⁺ T cells may contribute to the maintenance of memory CD8⁺ T cells through suppression of the outgrowth of viral reservoirs that could drive terminal exhaustion of CD8⁺ T cells.

In this Review, we have focused on the recent advances that provide insight into the role of CD4⁺ T cell help in promoting the maturation of memory CD8⁺ T cells following immunization and infection. Although much progress has been made, there remain several unresolved questions and challenges facing the field.

a



b

Priming phase	Resolution phase	Memory phase
• Dendritic cell licensing • IFNγ-mediated licensing of CD8+ T cell entry into mucosal tissue • Antiviral effector CD4+ T cell response • Promotion of humoral immune response	• T_{Reg} cell-mediated regulation of inflammatory environment through IL-10 • T_{Reg} cell-dependent promotion of quiescence through CTLA4 • T_{Reg} cell-dependent modulation of TGFβ levels within tissue	• Continued prevention of viral outgrowth

Figure 4 | **Temporal model of CD4+ T cell help during infection. a** | Following infection, antigen-specific CD8+ T cells rapidly proliferate during priming and differentiate into cytotoxic T lymphocytes that mediate viral clearance. Most of these cells die over the next several weeks during the resolution phase of the response. Only a small percentage of effector T cells (5–10%) survive and further mature into functional memory CD8+ T cells. **b** | CD4+ T cells have distinct roles during these phases to regulate the development of CD8+ T cell memory. During the priming phase in certain infection models, CD4+ T cells are required to license XC-chemokine receptor 1 (XCR1)+ dendritic cells, which can provide guidance cues to CD8+ T cells and enable autocrine secretion of interleukin-2 (IL-2). CD4+ T cells also license the entry of CD8+ T cell into mucosal tissues and promote viral clearance through the induction of a virus-specific effector CD4+ T cell response and a humoral response. Later in the resolution phase, regulatory T (T_{Reg}) cell-derived IL-10 facilitates the development of a mature memory CD8+ T cell population and can promote functional quiescence of memory T cells through expression of the inhibitory receptor cytotoxic T lymphocyte antigen 4 (CTLA4). T_{Reg} cells may also modulate transforming growth factor-β (TGFβ) levels in mucosal sites to promote CD103 expression and accordingly regulate tissue-resident memory T cell development. During the memory phase, the CD4+ T cell-dependent immune response allows for continued suppression of viral outgrowth. IFNγ, interferon-γ.

For example, our understanding of how CD8+ T cell migration into affected tissues and tumours is regulated by CD4+ T cells mainly relies on analysis that depends on tissue digestion or imaging of fixed samples. As technology improves, it will be important to use intravital imaging to dynamically monitor CD8+ T cell entry and migration within these sites to yield further insight into this process and open up new avenues of research. It is also unclear what role CD4+ T cell help might have in regulating the metabolic programming of CD8+ T cells. Switches in metabolic state have recently been found to be important drivers of CD8+ T cell differentiation and function, so it will be important to understand whether CD4+ T cells are directly or indirectly governing this process[124,125,131–134]. In addition, a comprehensive analysis of the transcriptional and epigenetic alterations in helpless CD8+ T cells that emerge over the course of infection has not been performed and could bolster our understanding of this process. In particular, this type of kinetic approach could inform our understanding of why there seems to be a specific window during infection during which CD4+ T cell help is most needed to promote a CD8+ T cell pool that can mediate protective immunity[49].

As our understanding of the mechanisms underlying CD4+ T cell help grows, it will be important to harness these findings therapeutically to boost vaccine efficacy. Considering the difficulties surrounding previous attempts at developing effective T cell-based vaccines, it may be necessary to modify existing vaccination approaches to more closely mirror the natural response to infection. This may be particularly relevant for vaccines against mucosal viruses, such as influenza virus and HIV, to which it is probably necessary to induce T_{CM}, T_{EM} and T_{RM} cell populations to optimally protect the host. Work is underway to develop vaccines that can drive CD8+ T cells into mucosal sites and expose them to the signals necessary for their continued maintenance in the tissue, similar to the function of CD4+ T cells during mucosal infection[9,135]. Therapeutic interventions that are designed to suppress inflammatory levels in individuals following vaccination through expansion of T_{Reg} cells or provision of IL-10 may also be a novel approach to bolstering systemic T cell immunity. Individuals suffering from chronic infection have impaired development of protective immunity following vaccination, potentially owing to high levels of bystander inflammation, and they would be an attractive target for this type of intervention[136]. It will also be important to explore the efficacy of combining existing immunotherapies used to treat individuals with chronic infection or cancer with therapies designed to modulate the quality or quantity of CD4+ T cell help.

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Competing interests statement
The authors declare no competing interests.