Published in final edited form as:

Nat Rev Immunol. 2014 January ; 14(1): 24–35. doi:10.1038/nri3567.

Human memory T cells: generation, compartmentalization and homeostasis

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Abstract

Memory T cells comprise the most abundant lymphocyte population in the body for the majority of one's lifetime; however, our understanding of memory T cell generation, function and maintenance mainly derives from mouse studies, which cannot recapitulate the decades-long exposure to multiple pathogens that occurs in humans. Here, we review studies focused on human memory T cells that reveal key properties including subset heterogeneity and diverse tissue residence in multiple mucosal and lymphoid tissue sites. We also discuss how the function and adaptability of human memory T cells depend on spatial and temporal compartmentalization.

Introduction

The acquisition of antigen experience is marked by the generation and persistence of memory T cells which can provide life-long protection against pathogens. Studies of mouse models have demonstrated the robust generation of memory T cells in response to diverse pathogens, and their efficacious protective responses upon reinfection; however, the role of memory T cells in protecting and maintaining long-term health in humans is less clear.

In mouse models, it is well-documented that antigen-specific naive CD4⁺ or CD8⁺ T cells become activated upon antigen exposure, and subsequently undergo proliferative expansion and differentiation into effector T cells. It is generally thought that these activated, effector T cell populations contain the precursors of antigen-specific long-lived memory T cells, which persist *in vivo* as heterogeneous populations in multiple sites, and can coordinate protective immune responses upon pathogen re-exposure. Mouse memory CD8⁺ T cell-mediated protection has been demonstrated in the well-characterized lymphocytic choriomeningitis virus (LCMV) infection model and for additional mouse pathogens (for reviews, see^{1,2}). Memory CD4⁺ T cells can similarly mediate protective immune responses in mice to influenza virus^{3–5}, *Mycobacterium tuberculosis*⁶ and parasite⁷ infections.

While most of our current understanding of memory T cell generation, function and maintenance is based on results from mouse models, studies in mice cannot recapitulate the exposure to multiple pathogens that occurs in humans over decades. The duration of a mouse memory study is typically several months, with the lifespan of laboratory mice lasting 1–2 years, which constitutes only a small fraction of the decades-long duration of immunological memory in humans with an average lifespan of 75 years or longer. Additionally, humans are exposed to diverse pathogens through the aerodigestive tract, genital mucosa and skin, which are sites of extensive colonization with >2,000 species of commensal microorganisms⁸. By contrast, most experimental mice are maintained in highly stringent, pathogen-free conditions, thus limiting the breadth of microbial exposure. Therefore, the generation and maintenance of human memory T cells should be considered within the context of the unique human exposure to pathogenic and non-pathogenic microorganisms, and not only relative to mouse models in controlled *in vivo* settings.

Human T cell studies are generally limited in two respects: first, most studies sample only peripheral blood, though the vast majority of memory T cells reside in tissue sites, including lymphoid tissues, intestines, lungs and skin (see later). Second, most studies on human memory T cells use samples obtained from young- or middle-aged adults, although the majority of memory T cell responses are formed during childhood from primary infections. Recent conceptual and technological breakthroughs, however, are now enabling novel explorations of T cell responses in humans. In this Review, we integrate these new studies with previous findings on T cells from healthy and diseased patients for an analysis of the current knowledge of human memory T cells. We describe recent studies that are beginning to assess how memory is organized in human tissue sites, including their functional capacities and antigen specificities, and discuss their implications for promoting *in situ* immunity in response to vaccines through targeted therapies. We also discuss the accumulation of memory T cells over a lifetime and how compartmentalization and specificity of memory T cells is maintained through homeostasis.

Memory T cell accumulation throughout life

The frequency of memory T cells undergoes dynamic changes throughout an individual's lifetime that can be divided into three phases: memory generation, memory homeostasis, and immunosenescence (Fig. 1). At birth, all T cells in peripheral blood are naïve, and memory T cells develop over time in response to diverse antigen exposure. A dramatic increase in the proportion of circulating memory T cells occurs in the first decade of life, and memory T cells comprise up to 35% of circulating T cells by the end of the second decade⁹. During this initial memory generation phase, particularly during infancy and early childhood, individuals exhibit the highest susceptibility to pathogens as measured by infectious disease hospitalization rates¹⁰.

The second phase, termed memory homeostasis, occurs after age 20–25, when circulating memory T cell frequencies reach a plateau and remain stable throughout adulthood^{11,12} (Fig. 1). Thymic output gradually diminishes during this phase and T cell numbers are largely maintained through homeostatic cell turnover¹³. Individuals in these middle years are less susceptible to pathogens, as evidenced by the low hospitalization rate of infectious

diseases¹⁰, and the immune response trends to homeostasis. After decades of stable frequencies, the proportion and functionality of memory T cells becomes altered during immunosenescence, starting at 65–70 years of age^{9,12,14}. Immunosenescence also marks an increased susceptibility to pathogens due, in part, to age-associated immune dysregulation and non-immune-related physiological decline. As immunosenescence has recently been reviewed elsewhere^{14,15}, we focus here on the first two phases: memory generation and homeostasis.

Memory T cell frequency in blood is a vast underestimate of the total frequency and numbers of memory T cells in the whole body. Estimates of the number of T cells in human tissues are 2×10^{10} in the skin^{16,17}, 1×10^{10} in lungs¹⁸, 3×10^{10} in the intestines¹⁹, and 20×10^{10} in lymphoid tissues (that is, the spleen, lymph nodes and bone marrow)¹⁹. Therefore, peripheral blood T cells ($5\text{--}10 \times 10^9$ in human blood) represent only 2–2.5% of the total T cell complement in the body¹⁹, and memory T cells represent the predominant T cell subset in mucosal sites, skin, spleen and bone marrow²⁰. Early in infancy, T cells are observed to populate the intestines²¹, and lungs²², with 20% of these cells in the intestines exhibiting a memory phenotype in newborns²¹, perhaps due to antigens encountered *in utero* (see later). Recent studies in human tissues (described below) have demonstrated that by the end of puberty, lymphoid tissues, mucosal sites and the skin are populated predominantly by memory T cells, which persist through adult life and represent the most abundant lymphocyte population throughout the body^{11,23}.

Memory T cell subsets and heterogeneity

Heterogeneity in peripheral blood

Memory T cells in humans are classically distinguished by the expression of the CD45RO isoform and by lack of CD45RA isoform expression ($\text{CD45RO}^+\text{CD45RA}^-$)^{24,25}. $\text{CD45RO}^+\text{CD45RA}^-$ T cells are now known to comprise heterogeneous populations of memory T cell subsets. Nearly 15 years ago, Lanzavecchia and colleagues first identified this heterogeneity in human peripheral blood based on the expression of the lymph node homing CC-chemokine receptor CCR7²⁶. Naïve T cells uniformly express CCR7, reflecting their predominant residence in lymphoid tissue, whereas memory T cells are subdivided into $\text{CD45RA}^-\text{CCR7}^+$ central memory T (T_{CM}) cells, which traffic to lymphoid tissues, and $\text{CD45RA}^+\text{CCR7}^-$ effector memory T (T_{EM}) cells, which can migrate to multiple peripheral tissue sites. They further showed that T_{CM} cells produced more interleukin-2 (IL-2) than T_{EM} cells, which produced more effector cytokines, and they proposed a differentiation model with T_{CM} cells being an intermediate stage in development of naive T cells into T_{EM} cells in peripheral tissue sites²⁷. The existence of T_{CM} and T_{EM} subsets in lymphoid and peripheral tissue sites was confirmed in mouse models^{28,29}. The original model of functional memory T cell subsets has since been revised to include a further characterization of human peripheral blood memory T cell subsets in health and disease. Functionally, an effector capacity is not confined to T_{EM} cells, as both T_{CM} and T_{EM} cell subsets produce effector cytokines in response to viruses, antigens and other stimuli^{30–33}, although T_{CM} cells exhibit a higher proliferative capacity^{30,34}.

Recently, the expression of additional surface markers, including the death receptor CD95 (also known as FAS) and the memory-associated marker CD122 (also known as IL-2R β) in the context of a naïve-appearing T cells were found to delineate a new memory T cell subset in humans³⁵ and mice³⁶, designated T memory stem (T_{SCM}) cells, which exhibit superior survival potential and efficiently engraft in allogeneic transplant models. In humans, T_{SCM} cells resemble naïve T cells in that they are CD45RA⁺CD45RO⁻ and they express high levels of the co-stimulatory receptors CD27 and CD28, IL-7 receptor α -chain (IL-7R α), CD62L and CCR7. T_{SCM} cells have high proliferative capacity and are both self-renewing and ‘multipotent’, in that they can further differentiate into other subsets including T_{CM} and T_{EM} cells^{35,37}. These properties define their ‘stem cell-like’ capacities^{35,38}.

Elucidating the differentiation pathways for heterogeneous memory T cell subset development following naïve T cell activation has been an active area of debate and investigation. Although the lineage relationship of human T cell subsets is difficult to assign, a progressive differentiation pathway based on signal strength and/or extent of activation, places naïve, T_{SCM}, T_{CM} and T_{EM} cells in a differentiation hierarchy, serving as precursors for effector T cells^{38–40} (Fig. 2). Another model proposes the T_{EM} and T_{CM} cell subsets derive from effector T cells with T_{EM} cells giving rise to T_{CM} cells, and a third model posits a divergent generation of effector, T_{CM} and T_{EM} cell subsets (reviewed in reference⁴¹). Recent studies of the fate of individual T cells in mice during infection provides evidence that a single naïve T cell can give rise to heterogeneous memory T cell populations following activation^{42–44}, and supports a progressive differentiation model (Fig. 2). Moreover, these individual fates in mouse T cells can be determined at early times after activation due to asymmetrical division⁴⁵. The mechanisms and timing for human effector and memory T cell fate determination remain undefined.

The heterogeneity of memory T cell subsets in peripheral blood reveals only a small fraction of the total complexity of memory T cell distribution throughout the body. Seminal mouse studies showed that antigen-specific memory CD4⁺ and CD8⁺ T cells can populate and persist in multiple tissue sites long after virus or antigen was cleared^{28,29}. Early studies of surgical explants in humans showed that CCR7⁻ T_{EM} cells were the predominant memory T cell subset found in intestine and lungs, whereas tonsils contained both T_{CM} and T_{EM} cells⁴⁶. This diverse tissue distribution of memory T cells raised the question of whether they had just circulated through these tissues, or had become resident at these sites as a consequence of further differentiation.

In the last several years, mouse studies have established the existence of a new ‘tissue-resident’ memory T cell (T_{RM} cell) subset as a non-circulating subset that resides in peripheral tissue sites and, in some cases, elicits rapid *in situ* protective responses. Mouse CD4⁺ T_{RM} cells can be generated in the lungs from adoptive transfer of activated (effector) T cells⁴ or following respiratory virus infection⁴⁷, and are distinguished from splenic and circulating memory T cells by their upregulation of the early activation marker CD69, their tissue-specific retention in niches of the lung⁴⁷, and their enhanced ability to mediate protection to influenza virus infection compared to circulating memory CD4⁺ T cells⁴. An analogous non-circulating CD4⁺ T_{RM} cell subset has been identified in the bone marrow of mice following systemic virus infection that exhibits enhanced helper functions⁴⁸.

Memory CD8⁺ T cells were initially found to persist as circulating and resident populations in lymphoid tissues, lungs and mucosal tissues in mice⁴⁹. CD8⁺ T_{RM} cells generated following infection have subsequently been identified in multiple mouse tissues including the skin^{50,51}, vaginal mucosa^{52,53}, intestine^{49,54,55}, lungs^{47,56} and even brain⁵⁷. CD8⁺ T_{RM} cells are collectively distinguished from splenic and circulating memory CD8⁺ T cells by their increased expression of CD69 (similar to CD4⁺ T_{RM} cells), and by expression of the epithelial cell-binding integrin α E β 7 (also known as CD103)^{20,52,58–60}. Protection by CD8⁺ T_{RM} cells has been demonstrated in the skin of mice in response to intradermal herpes simplex virus 2 (HSV2) infection^{61–63} and to HSV infection at the vaginal mucosa^{52,53}. These mouse studies indicate that T cell-mediated memory responses, and in particular those with high protective capacity, are highly compartmentalized in tissue sites, necessitating the study of memory T cell populations in anatomic sites other than the blood.

Although T_{RM} cells are less well characterized in humans than in mice, recent studies are beginning to define these populations by sampling tissues from organ donors¹¹ and from surgical explants^{18,50}. A whole body analysis of T cells in multiple lymphoid and mucosal sites revealed that memory CD4⁺ T cells predominate throughout the body, and persist as CCR7⁺ or CCR7[–] subsets localized to lymphoid tissues and mucosal sites, respectively, whereas memory CD8⁺ T cells persist as mainly CCR7[–] subsets in all sites, with low numbers of CD8⁺ T_{CM} cells in lymphoid tissues and negligible numbers of these cells in other sites¹¹. Importantly, most memory T cells in human mucosal, lymphoid and peripheral tissue sites such as skin express the putative T_{RM} cell marker CD69^{14–16,20,58}, whereas circulating blood memory T cells uniformly lack CD69 expression¹¹. Together, these studies suggest that T_{RM} cells expressing CD69 are present as majority populations in human mucosal and peripheral tissue sites, similar to their counterparts in mice. Unlike mouse lymphoid memory T cells, a significant proportion of T_{EM} cells in human lymph nodes and spleen also express CD69¹¹, suggesting that T_{RM} may also be present in lymphoid tissue.

The pathway(s) and mechanisms for the generation of T_{RM} cells remain unknown and is an important area for future studies. Mouse studies suggest that T_{RM} cell generation occurs within tissue sites from either activated effector T cells and/or T_{EM} cell precursors that migrate to tissue sites^{47,64} (Fig. 2). We propose that the pathway to human T_{RM} cell development involves both migration and tissue-specific factors, as suggested by the examination of T_{RM} cell distribution and properties^{11,16,18} (Fig. 3). Blood-borne memory T_{CM} and T_{EM} cell subsets can enter certain tissue sites such as spleen, lungs, lymph nodes and bone marrow at a low frequency, but are not significantly represented in the skin and intestine. (T_{SCM} cells appear in lymph nodes in non-human primates⁶⁵ but their distribution in humans is not known). CD4⁺ T_{RM} cells (CCR7[–] CD69⁺) and CD8⁺ T_{RM} cells expressing CD103 are the predominant T cell subsets in the lungs, intestine, skin and bone marrow^{11,20,66}. Lymphoid tissues contain T_{CM} cells and T_{EM} cells, some of which also express CD69 but not CD103¹¹. It is not known whether additional markers define lymphoid T_{RM} and/or whether CD69⁺ T_{EM} cells in lymphoid tissue are true resident memory cells.

Human T_{RM} cells also exhibit tissue-specific properties (Fig. 3), suggesting *in situ* influences. T_{RM} cells in skin express cutaneous lymphocyte antigen (CLA) and the skin-homing-associated chemokine receptors CCR4 and CCR10^{16,67,68}; memory T cells in the

small intestine and colon express the gut-homing receptor CCR9⁶⁹ and the integrin $\alpha 4\beta 7$ ⁷⁰, and memory T cells in the lungs upregulate CCR6 expression¹⁸. There is also evidence for crosstalk between mucosal sites such as lung and intestines⁷¹. Migration of T_{EM} cells to the bone marrow in mice requires expression of integrin $\alpha 2\beta 1$ (also known as VLA2)⁶⁴, but it is not known whether human bone marrow T_{RM} cells have a similar requirement. This differential chemokine receptor and/or integrin expression may derive from activated or effector populations that enter the site^{72,73}, or alternately be upregulated during homeostasis of naive or memory T cells⁷⁴. This identification of human T_{RM} cells with tissue-specific signatures suggests anatomic control of memory T cell generation in humans.

Functional capacity of heterogeneous subsets

The extensive phenotypic and tissue complexity among heterogeneous memory T cell subsets suggests a corresponding functional heterogeneity. Mouse and human CD4⁺ T cells of a non-regulatory lineage are subdivided into functional subsets — the most prevalent being Th1 cells that produce interferon- γ (IFN γ), IL-2 and tumour necrosis factor (TNF), Th2 cells secreting IL-4, IL-5, IL-10 and IL-13, and Th17 cells producing IL-17. CD8⁺ T cells are not typically subdivided into functional subsets, and generally produce IFN γ and TNF, and express cytolytic markers such as perforin and CD107. In the peripheral blood of healthy individuals, most circulating memory CD4⁺ and CD8⁺ T cells produce IFN γ , IL-2 and/or TNF following shortterm stimulation, and only low numbers of IL-4-, IL-10- and IL-17-producing memory CD4⁺ T cells are observed^{35,75}. Human peripheral blood T_{EM}, T_{CM} and T_{SCM} subsets differ in the relative proportion of cells producing IL-2, IFN- γ and/or TNF: T_{EM} cells have the highest proportion of IFN γ - and TNF-producers and lowest proportion of IL-2-producing cells, T_{SCM} cells have the lowest proportion of IFN γ -producers, with more IL-2-producing cells compared to T_{EM}, while T_{CM} cells have the highest frequency of IL-2-producers and frequencies of IFN- γ and TNF intermediate between T_{SCM} and T_{EM} cells^{35,38} (Fig. 3). Variations in expression of chemokine receptors have also been associated with different functional capacities of human effector T cells. For example, *in vitro* polarized Th1 effector cells were found to express CXC receptor 3, CCR5 and CCR2, while Th2-polarized effector cells expressed CCR4 and CCR3⁷⁶. However, expression of these chemokine receptors can be transient, depend on the microenvironment⁷⁷, and does not consistently delineate functional subsets among resting memory T cells⁷⁸.

The functional capacities of human T_{RM} cells are beginning to be defined. Overall, memory CD4⁺ and CD8⁺ T cells in lymphoid and mucosal tissues obtained from healthy individuals exhibit rapid IL-2 and IFN γ production, respectively, when activated non-specifically with PMA/ionomycin¹¹. It was previously determined that individual circulating memory T cells can produce multiple cytokines, and the presence of these multifunctional or polyfunctional memory T cells correlated with superior recall and protective responses⁷⁹. There is emerging evidence that T_{RM} can be multifunctional, and also exhibit qualitative functional differences. Human bone marrow T_{RM} cells are polyfunctional for effector cytokines and cytolytic molecules^{48,80}, a substantial fraction of human lung T_{RM} cells produce multiple pro-inflammatory cytokines¹⁸, and human intestinal T_{RM} cells are also multifunctional¹¹. Other functions, however, appear to be confined to specific subsets and/or tissue sites. IL-17

is produced by a subset of CD4⁺ T_{RM} cells in mucosal sites, particularly in intestines in healthy individuals¹¹, by CCR6⁺ memory T cells in peripheral blood^{81,82}, and also by a subset of CD161⁺ T cells in inflamed tissue, such as the skin of patients with psoriasis⁸³. A subset of skin memory CD4⁺ T cells can produce IL-22 (and not IL-17) when cloned and expanded *ex vivo*⁸⁴, and IL-22-producing T cells have also been identified in inflamed skin in psoriasis⁸⁵. Thus, while predominant memory T cell functions, such as IFN γ production, are broadly distributed among multiple memory T cell subsets and tissues, T_{RM} cells in tissue sites can adopt multiple or distinct functional attributes which may also depend on tissue-specific inflammation.

Diverse antigen specificity of human memory T cells

Specificity in the adaptive immune response is intricately linked to the establishment and persistence of memory T cells that record previous antigen experiences via specific T cell receptors (TCRs). In mice, the development of memory T cells in response to viral pathogens is marked by extensive clonal expansion of virus-specific T cells, followed by the contraction and death of >90% of activated or effector T cells, and long-term persistence of virus-specific memory T cells at variable frequencies (<1 to >10% of the total number of T cells) depending on the virus^{2,86}. These mouse models generally examine memory T cell development and responses to one type of virus in otherwise sterile conditions. By contrast, in humans, antigen-specific memory T cells are generated and maintained in the face of thousands of different pathogens introduced at various life stages in the context of a dynamically maintained, heterogeneous T cell population. Assessing the physiological significance of antigen-specific memory T cells in humans has therefore proved challenging.

Pathogen-specific memory generation and maintenance

Our ability to measure the frequency of human antigen-specific memory T cells has increased in accuracy and sensitivity from classical functional recall assays to cytometry-based methods (Box 1). The assessment of human antigen-specific memory T cells has mostly occurred in the context of virus infections that are ubiquitous in healthy humans, including acute infections with influenza virus and chronic infections with viruses such as cytomegalovirus (CMV) and Epstein–Barr virus (EBV). Memory T cells responses to these viruses are generated as a result of a productive immune response that effectively controls the virus. Human T cell responses in chronic HIV infection have been extensively studied; however, mechanisms for their generation and maintenance are more complex, as memory T cells are the targets for chronic infection and virus persistence^{87–89} and HIV is not cleared by the immune system in most individuals (reviewed elsewhere^{90–92}).

Cross-sectional studies of memory T cell frequency in different age groups demonstrate that most virus-specific memory T cells are generated early in life. Congenital CMV infection was demonstrated to generate virus-specific memory CD8 T cells *in utero*⁹³. Furthermore, in a large cohort study of children and adults, CMV-specific CD8⁺ T cells were detected in the blood of infants, and their frequency remained stable throughout early childhood and young adulthood⁹⁴. Memory T cells specific for adenovirus are detected in early childhood and progressively increase in frequency until age 10, when they remain at a constant level throughout adulthood³¹. Influenza virus-specific T cells response are also detected in

children at levels comparable to those observed in adults^{95,96}. Together, these results suggest that despite the overall increase in circulating memory T cells over a lifetime (Fig. 1), memory T cells generated to ubiquitous pathogens exhibit early increases in frequency and stable maintenance throughout adulthood.

The ability to observe pathogen-specific human memory T cell development through the process of clonal expansion and contraction is not readily accomplished; however, prospective infection and vaccine studies have provided novel glimpses into this process. Circulating T cells specific for EBV or for influenza virus underwent clonal expansion, contraction and persistence as memory T cells following acute infection^{97–99}. Similarly, the administration of yellow fever and smallpox vaccines, which both consist of live viruses, stimulates robust CD8⁺ T cell clonal expansion, contraction and memory formation that is measurable in peripheral blood¹⁰⁰. However, there was no significant increase in the frequency of circulating, virus-specific T cells following acute infection with rotavirus¹⁰¹, which is a gastrointestinal virus, or with the lung pathogen respiratory syncytial virus (RSV)^{102,103}. This variability in observing memory T cell development in human blood may be due to inefficient mobilization of responses during infection¹⁰⁴, and/or compartmentalization of pathogen-specific responses at the infected site.

Several studies have demonstrated biased generation and maintenance of virus-specific effector and memory T cells in tissues versus circulation. Intradermal immunization with purified protein derivative (PPD) of *M. tuberculosis* resulted in the proliferation of antigen-specific T cells in skin but not in the blood¹⁰⁵, and similarly, cutaneous challenge with varicella zoster virus results in memory T cell accumulation in skin¹⁰⁶, suggesting that the formation of memory may occur at distinct sites. Lung tissue was found to contain an increased frequency of influenza virus-specific memory CD8⁺ T cells compared to the blood^{107,108} and spleen⁴⁷, and influenza virus-specific cells in human lungs exhibit a T_{RM} cell phenotype (that is, CD69⁺CD103⁺)^{47,109}. CD8⁺ T cells specific for HSV2 were found to persist in genital skin, but not in skin from other body regions¹¹⁰, suggesting local maintenance of skin T_{RM} cells. Similarly, memory CD4⁺ T cells specific for astrovirus, a common enteropathogenic virus, were detected in the small intestines¹¹¹. Together, these results suggest compartmentalization of pathogen-specific memory T cell responses that are preferentially maintained at the sites of initial effector T cell recruitment. These findings also indicate that accurate assessment of pathogen-specific memory T cell responses requires sampling of the initial infection site.

Memory T cell cross-reactivity

Despite their specificity, human memory T cells exhibit cross-reactivity to antigenic epitopes not previously encountered, which is possibly due to intrinsic properties of TCR recognition¹¹² and to the range and breadth of human antigenic experience. Memory CD4⁺ and CD8⁺ T cells specific for unique epitopes of avian influenza strain H5N1 were detected in healthy individuals that were not exposed to H5N1 infection assessed by serology^{113,114}. In addition, HIV-specific memory T cells have been identified in HIV-negative individuals¹¹⁵. Virus-specific memory T cells also show cross-reactivity to alloantigens, autoantigens and unrelated pathogens^{116,117}: EBV-specific human memory T cells

generated in HLA-B8 individuals exhibit allogeneic cross-reactivity to HLA-B44¹¹⁸, and influenza virus- and HIV-specific memory CD4⁺ T cells recognize epitopes from unrelated microbial pathogens¹¹⁵. Furthermore, T cells specific for the autoantigen myelin basic protein (MBP) recognized multiple epitopes from viral and bacterial pathogens^{117,119}. This cross-reactivity may enable memory T cells to mediate protection without initial disease — a phenomenon known as heterologous immunity¹²⁰. Heterologous immunity has been demonstrated in humans where EBV infection expanded clones of influenza virus-specific T cells¹²¹. The role of T cell cross-reactivity in determining how T cell subsets may be compartmentalized in tissue sites is not known, but could be an important mechanism for their homeostatic maintenance (See below).

Role of microbiome in generating memory T cells

Mucosal sites and the skin harbour resident bacteria and viruses, collectively referred to as the microbiome. Humans are exposed to >2,000 microbial species⁸, of which only a small fraction are pathogenic. It has been suggested that the very purpose of immune memory in vertebrates is to preserve proper immune homeostasis with commensals⁸. Studies in mouse models show that the presence and the composition of the microbiome are critical in promoting appropriate immune responses to pathogens and maintaining proper immune homeostasis (for a review, see¹²²). Whether the species of commensals present in certain sites influence the type of memory T cells that reside there is not known. In a limited study examining the reactivity to endogenous bacterial flora of T cells expanded from blood and intestinal biopsy samples of two patients, more bacteria-reactive T cells clones were isolated from intestines than from the blood¹²³, suggesting that intestinal T cells may exhibit biased cross-reactivity to intestinal flora. Although memory T cells specific for commensal bacteria have been recently detected in mice¹²⁴, they are associated with pathogenic infection of the intestines and could be a consequence of dysregulated immunity in inflammatory bowel disease. In humans, memory T cells survive far longer and are exposed to more antigens during their lifetime, and therefore commensal-specific responses may be part of the healthy immune balance between the microflora and the indigenous T cells.

Human memory T cell homeostasis

Specific clones of memory T cells expressing a unique TCR can persist for decades *in vivo*. Human memory T cell longevity is clearly demonstrated by studies showing that memory T cells specific for vaccinia virus — the etiologic agent of smallpox eradicated four decades ago — persisted in individuals vaccinated 25–70 years previously¹²⁵. However, mechanisms for memory T cell maintenance in humans remain unclear. In mouse models, the long-term requirements for T cell maintenance and homeostasis have been defined using mice deficient for various cytokines and cytokine receptors, for MHC molecules and/or for components of the TCR signalling cascade (for a review, see¹²⁶). These studies established that virus-specific memory CD8⁺ T cells do not require antigen or MHC for their maintenance, but rely on IL-15 for homeostasis and IL-7 for survival, whereas memory CD4⁺ T cells require TCR signalling and/or MHC class II molecules for their functional maintenance and homeostasis^{127–129}.

An elegant approach to probe human memory T cell turnover or longevity is to administer deuterated water or deuterated glucose to volunteers and subsequently examine the incorporation of deuterium by T cells *in vivo*^{130,131}. The calculated half-life of human T cells using this approach was found to vary according to the type of labelling, the limited sampling of peripheral blood, the timing of sampling and the mathematical algorithm¹³². On average, human naïve T cells have a longer half-life than memory T cells (1–8 years versus 1–12 months)¹³²; CD4⁺ T cells have a shorter half-life than CD8⁺ T cells and T_{EM} cells have a shorter half-life than T_{CM} cells¹³³. Compared with naïve T cells, human memory T cells also have shorter telomeres in individuals of all ages¹³⁴, which indicates their more extensive replicative history. These studies suggest that memory T cells in the circulation are maintained, in part, by continuous, homeostatic turnover. Turnover and replicative history of human memory T cells in tissue compartments remains completely unexplored and will be important to assess T_{RM} cell stability.

Transcriptome, epigenetic and deep sequencing analysis of human memory T cells provide new evidence into mechanisms for memory T cell longevity^{135,136}, and the potential role of TCR and cytokine signals in this process. Human memory CD4⁺ and CD8⁺ T cells exhibit transcriptional upregulation of genes encoding TCR-coupled activation markers compared with naïve T cells, including multiple MHC class II molecules, chemokine receptors, CD95 and effector molecules¹³⁵. Moreover, activation-induced epigenetic changes in the loci of effector cytokine genes are maintained in circulating human memory CD8⁺ T cells^{135,137}. Deep sequencing of TCR genes revealed conserved clonotypes and reduced diversity among human memory T cell subsets^{138,139}. Together, these findings implicate tonic TCR signalling in human memory CD4⁺ and CD8⁺ T cell maintenance, potentially due to cross-reactivity to self-antigens, environmental antigens and/or resident commensal organisms. Requirements for cytokine signals in this maintenance are not clearly defined, although individuals with mutations in the cytokine-induced transcription factor signal transducer and activation of transcription 3 (STAT3) exhibit reduced memory T cell frequency and responses¹⁴⁰. Whether requirements for T_{RM} cell maintenance differ according to the tissue site is not known. For example, memory T cells may be preferentially maintained by cross-reactive TCR-mediated interactions with microbial antigens at mucosal sites due to high antigen density, whereas those in blood, lymphoid tissue, spleen and bone marrow may be maintained preferentially through responses to homeostatic cytokines such as IL-7 or IL-15, which are abundantly expressed in these sites⁶⁴.

Antigen-specific memory T cells in space and time

A proposed schematic of how T cell subsets and specificities for ubiquitous microbial antigens are distributed in space (from circulation to peripheral tissue sites) and time (from infancy to old age) is presented in Fig. 4. T_{EM} and T_{RM} cell compartmentalization in mucosal and peripheral tissue sites is initiated during infancy and stably maintained throughout life. T_{RM} cells in these sites may be enriched in specificities for pathogenic and non-pathogenic microbial species that populate or infect these tissues. Dynamic changes occur in the circulation and in lymphoid tissue, including the reduction in naïve T cells and increase in T_{CM} and T_{EM} cell subsets with increasing age. Circulating T_{EM} cells may be enriched in specificities for chronic and systemic pathogens, but not for microbiota in

tissues. We propose that this sequestration of memory T cell specificities in distinct anatomic compartments could be a mechanism to stabilize and preserve pathogen-specific memory T cells, and maintain immune homeostasis in the body. Through additional tissue sampling and integration of large datasets with computation and statistical approaches, it will be possible in future studies to directly test this model (Fig. 4) and map the organization of T cell subsets and specificities within the human body throughout life.

Implications for Vaccines

The induction of memory T cells through vaccination has great potential to provide efficacious protective immunity to multiple types of pathogens, including viral, intracellular bacterial and parasitic infections, because of their broad specificity for internal and conserved pathogen epitopes, their residence in diverse sites of infection, and their longevity. Immune protection for existing vaccines is largely associated with the generation of neutralizing antibodies, whereas the potential of memory T cells to mediate protection has not been realized¹⁴¹. Several recent human challenge studies have demonstrated a protective correlate for memory T cells. One such study with influenza virus found a direct correlation between the presence of virus-specific memory CD4⁺ T cells and reduced illness to influenza virus challenge¹⁴². In addition, generation of circumsporozoite protein (CSP)-specific memory CD4⁺ T cells from a malaria vaccine was correlated with protective anti-parasite immunity¹⁴³. Multifunctional circulating memory CD4⁺ T cells are similarly associated with HIV-infected non-progressors⁷⁹. Therefore, while generation of CD8⁺ memory T cells is often the focus of mouse studies for protection, memory CD4⁺ T cells may also be a relevant subset to target in humans.

The consideration of anatomic location of vaccine administration appears critical in the design of vaccines to promote generation of pathogen-specific memory T cells at the right place and time. Administering a vaccine or attenuated pathogen at the site of infection in the skin, lung, rectal or genital mucosal surfaces could enhance generation of T_{RM} cells *in vivo*. Studies in mice have tested a “prime and pull” strategy in which pathogen-specific T cells are primed through systemic vaccines and then recruited specifically to a tissue site through the local administration of chemoattractants⁵³. In humans, the introduction of the smallpox vaccine through skin scarification promotes local T cell responses, and long-term persistence of vaccinia virus-specific memory CD4⁺ T cells^{23,125}. There are also promising results from HIV vaccine studies in non-human primate models using CMV-mediated vectors to promote polyclonal generation of T_{EM} cells to multiple epitopes¹⁴⁴. HIV subunit vaccination using these CMV vectors via multiple routes leads to T_{EM} cell generation and population in mucosal and lymphoid sites, and lasting protective immunity^{145,146}. These studies show that adjusting the route and mode of immunization can enable memory T cell-mediated protective immunity to intractable pathogens.

Timing is another important consideration for memory T cell generation and vaccine design. Memory T cells generated early in life are maintained at a measurable frequency throughout a healthy adulthood, based on studies in peripheral blood^{31,94}, suggesting that priming for memory T cell generation at early life stages, when fewer memory T cell clones are present, enables them to establish a niche for long-term persistence. Peripheral tissues have

low numbers of memory T cells at birth, but become populated by memory T cells by early childhood (see above), indicating that site-specific vaccines could promote effective tissue targeting at early life stages. Other considerations, such as targeting of tissue dendritic cells for the development of protective immunity at specific sites⁷¹, and the co-administration of immunomodulators to enhance memory T cell generation¹⁴⁷, could be integrated into the design of vaccines for generating durable, *in situ* T cell immunity.

Conclusions and perspective

Improved and more sensitive methods to determine function and antigen specificity of memory T cells, combined with high throughput approaches, are enabling us to define the molecular landscape of human immune cells. When combined with human tissue and blood samples, we have an unprecedented opportunity to obtain a more comprehensive understanding of the immune system in the context of the human lifespan, which is not possible in animal models. Future studies examining site-specific immune responses should focus on understanding how memory T cells are generated in early life and on clarifying the role of the microbiome in these processes to identify novel strategies for promoting effective immunoregulation and pathogen-specific immunity.

Acknowledgments

The authors wish to thank Joseph Thome and Damian Turner for critical review of the manuscript. D.L.F. is supported by NIH grants AI106697, AI100119, and AI083022.

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Glossary terms

Memory homeostasis	The stable maintenance of memory T cell numbers through multiple mechanisms including continuous turnover, responses to homeostatic cytokines and non-cognate T cell receptor interactions.
immunosenescence	The decreased function of the immune system with age. In particular, the number of naive T cells decreases as thymic function decreases.
telomeres	Regions of highly repetitive DNA at the end of linear eukaryotic chromosomes. They protect the ends of the chromosome from shortening on replication.
ELISPOT	An antibody-capture-based method for enumerating specific CD4 ⁺ and CD8 ⁺ T cells that secrete a particular cytokine (often interferon- γ).
MHC tetramer	A method of visualizing antigen-specific T cells by flow cytometry. Typically, four MHC molecules with their associated peptides are held together by streptavidin, which has four binding sites for biotin, which is attached to the tail of the MHC molecule. These four-peptide–MHC complexes (tetramers) can bind peptide-specific T cell receptors. The streptavidin molecules are often labelled with a fluorochrome so that binding can be assessed by flow cytometry.

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Box 1 | Approaches for analyzing antigen-specific T cell memory

The techniques for examining antigen-specific T cell responses have increased in sensitivity and specificity in recent years. The classical limiting dilution assay requires *ex vivo* antigen-driven expansion of human T cell populations, but this approach is limited because non-proliferating T cell clones are under-represented. More recently, development of the ELISPOT assay provided a sensitive, yet robust functional method for detecting antigen-specific memory T cells by their rapid production of effector cytokines, and enabled their precise quantitation without the need for antigen-driven population expansion.

The development of MHC tetramer reagents¹⁴⁸ allowed for the visualization and quantitation of epitope-specific T cells based solely on TCR specificity, although this method lacks a functional readout. TCR staining with tetramers, pentamers or MHC multimers has enabled a more sensitive estimation of memory T cell specificity in humans compared with direct functional recall responses to antigens as measured by ELISPOT or limiting dilution assays^{149,150}. Moreover, new techniques for enrichment of antigen-specific T cells using labelled tetramer reagents and secondary binding reagents coated to magnetic beads¹¹⁵ enables detection of rare antigen-specific T cell populations, even among naïve T cells that have not undergone *in vivo* expansion.

The most recent technical advance in antigen-specific T cell detection is achieved through the use of cytometry by time-of-flight mass spectrometry (CyTOF) which employs the technique of mass cytometry by using antibodies labelled by different masses, enabling >50 parameters to be assayed on single cells¹⁵¹ without the problems of compensation that occurs in multiparameter flow cytometry. Coupling different mass labels to tetramer reagents allows for the combinatorial assessment of >50 TCR specificities in a single sample¹⁵². Use of these new technologies makes the assessment of T cell epitope specificities from small clinical biopsy samples possible, which will be essential for future diagnostic application of antigen-specificities in immune monitoring.

Key Points

- Most of our understanding of memory T cell generation, function, and maintenance is based on mouse studies, which cannot recapitulate the decades-long exposure to diverse antigens and microbiota that occurs in humans.
- Memory T cell frequency changes dynamically throughout the human lifetime that can be divided into three phases: generation, homeostasis, and immunosenescence.
- CD45RO⁺CD45RA⁻ T cells comprise diverse memory subsets, including central memory (T_{CM}), effector memory (T_{EM}), memory T cells (T_{SCM}) and resident memory (T_{RM}), that are heterogeneous in their generation, distribution, and function.
- Antigen-specific memory T cells to ubiquitous pathogens and possibly endogenous flora are generated early in life, and are preferentially compartmentalized at the sites of infection throughout adulthood.
- Human memory T cells in diverse tissue sites are homeostatically maintained potentially through tonic TCR signaling, can exhibit extensive cross-reactivity and persist for decades.
- Induction of memory CD8⁺ and CD4⁺ T cells through vaccination can enhance protection against pathogens, and may be improved by considering anatomic location and timing of administration during early life stages.

Figure 1. Memory T cell frequency, pathogen susceptibility and mortality throughout human life

Memory T cells pass through three distinct phases: memory generation, memory homeostasis and immunosenescence. Memory T cells are mostly generated following antigen exposure during infancy, youth and young adulthood (ages 0–20). Their levels subsequently plateau and are maintained through homeostasis throughout adulthood (ages 30–65), after which they enter the third stage and exhibit senescent changes (ages 65 and up). Previous studies have shown that there is an increase in the frequency of memory T cells in the blood (red line) over time^{9,12}. In the whole body, which includes the blood, intestines, lungs, skin, liver, brain and lymphoid tissues, the overall frequency of memory T cells (black line) also increases with age¹¹. The increase in memory T cell frequency throughout the body inversely correlates to a decrease pathogen susceptibility (dashed line) calculated from infectious disease hospitalization rates (per 10,000 people) recorded from 1998–2006 in the US based on 40,085,978 total hospitalizations¹⁰.

Figure 2. Model for the generation of human memory T cell subsets

A schematic model for differentiation of circulating and tissue-resident memory T cell subsets. Progressive differentiation of the three major circulating subsets — stem cell memory T cells (T_{SCM} cells), central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells) — from activated naïve T cells is shown relative to the extent of antigen exposure. Effector T cells (T_{Eff} cells) represent terminally differentiated cells, and death is one outcome of increased antigen exposure and proliferation. Naïve, T_{SCM} and T_{CM} cells circulate and migrate to lymphoid tissue, whereas T_{EM} and T_{Eff} cells are the subsets with the capacity to traffic to peripheral tissues. Tissue-resident memory T cells (T_{RM} cells) in peripheral tissue sites may derive from either T_{EM} or T_{Eff} cells that migrate to these sites via tissue-specific influences. It is possible that T_{CM} cells could develop into T_{RM} cells in lymphoid sites (dotted line). T_{RM} cells in peripheral compartments are likely terminally differentiated since they do not circulate or convert to other memory T cell subsets.

Figure 3. Schematic of memory T cell heterogeneity in peripheral blood and tissues
A Diagram shows the tissue distribution and migration patterns of the major human memory T cell subsets, including three major circulating populations —, stem cell memory T cells (T_{SCM} cells), central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells) — and T_{RM} cells in multiple sites, as well as CD8⁺ T_{RM} cells that are defined by expression of CD103 and are associated with mucosal sites and skin. The individual sites are defined in terms of circulation (red), lymphoid origin (grey) or peripheral tissues (yellow). Circulating T_{SCM}, T_{CM} and T_{EM} cell subsets migrate from the blood and circulate through the spleen

and lungs, where they can be primed to migrate to intestines⁷¹. They also migrate via the lymphatics and efferent vessels to lymph nodes. T_{RM} cells predominate in skin, lungs, bone marrow and intestines, but may also be present within the CD69⁺T_{EM} subsets in the spleen and lymph nodes. Mucosal sites and the skin also contain specific CD103⁺ T_{RM} cells. The expression of certain chemokine receptors and/or integrins is associated with T cell migration and/or residence in lymph nodes (CCR7), skin (CCR4, CLA and CCR10), intestines (CCR9 and integrin $\alpha 4\beta 7$), lungs (CCR6) and bone marrow (integrin $\alpha 2\beta 1$). **B** Key phenotypic and functional properties of circulating and resident subsets are shown, with CD45RA and CCR7 distinguishing circulating memory T cell subsets and CD69 (and CD103) expression delineating T_{RM} cells from circulating subsets. Memory subsets can produce similar types of recall cytokines, such as interleukin-2 (IL-2), interferon- γ (IFN γ) and tumour necrosis factor (TNF), but differ in the extent and quality of these responses.

Figure 4. Model for compartmentalization of antigen-specific memory T cell subsets in space and time

This schematic shows the relative naïve and memory T cell subset frequencies in the circulation and peripheral sites and at different life stages. The schematic also shows the biased specificity for microbial antigens derived from pathogens that has been observed in specific tissue sites, including cytomegalovirus (CMV) in the blood; Epstein-Barr virus (EBV) in the spleen; vaccinia virus in lymph nodes; influenza virus in the lungs; rotavirus in the intestine and herpes simplex virus (HSV) and varicella zoster virus (VZV) in the skin. In addition, the specificities of some memory T cells at mucosal sites (lungs, intestine and skin) are biased for antigens from the microflora. The relative frequencies of each T cell subset in each tissue site for youths through adults are compiled from Ref. 11, and are extrapolated for infants based on Refs. 21, 22. At birth, there is a preponderance of naïve T cells in the circulation, and an abundance of mucosal microbial antigens are encountered during infancy, resulting in seeding of mucosal sites with T_{EM} cells specific for mucosal pathogens, which could develop into T_{RM} cells *in situ*. Infant skin has few, if any T cells (R. Clark, personal communication) and therefore estimates for memory T cell content in skin begin during youth. During childhood, exposure to the ubiquitous pathogenic and non-pathogenic microbial species in each site occurs and new memory T cells are formed which are partitioned as T_{RM} cells in skin and mucosal sites, and as T_{CM} cells in lymphoid tissues. This basic partitioning of antigen-specific memory T cell subsets in tissues is maintained

during adulthood, with more T_{EM} and T_{CM} cell subsets gradually accumulating in the circulation and lymph nodes that could potentially replenish and/or convert to T_{RM} cells that are lost through attrition.