Human circulating and tissue-resident memory CD8$^+$ T cells

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ABSTRACT

Our current knowledge of human memory $CD8^+$ T cells is derived largely from studies of the intravascular space. However, emerging data are starting to challenge some of the dogmas based on this work, suggesting that a conceptual revision may be necessary. In this review, we provide a brief history of the field and summarize the biology of circulating and tissue-resident memory $CD8^+$ T cells, which are ultimately responsible for effective immune surveillance. We also incorporate recent findings into a biologically integrated model of human memory $CD8^+$ T cell differentiation. Finally, we address how future innovative human studies could improve our understanding of anatomically localized $CD8^+$ T cells to inform the development of more effective immunotherapies and vaccines, the need for which has been emphasized by the global struggle to contain SARS-CoV-2.

The human body is continually assailed by infectious agents and mutagenic events that require constant and pervasive immune surveillance. This role is fulfilled primarily by T cells, which are classically divided into two subsets based on coreceptor expression, namely "helper" $CD4^+$ T cells, which orchestrate different arms of the immune system, and "cytotoxic" $CD8^+$ T cells, which directly eliminate infected or malignant cells *in situ*. These processes occur in an anatomically defined manner by necessity, but we know relatively little about the spatial control of immune surveillance, largely because most studies of human lymphocyte subsets have been confined to analyses of the vascular circulation. As a consequence, we have an exquisite understanding of how immune effector functions are regulated in the periphery, especially within the $CD8^+$ T cell lineage[1], but a comparatively poor understanding of how these effector functions are deployed logistically at organ-specific sites of infection or malignancy. It is also important to recognize that the immune system operates primarily within tissue microenvironments under physiological and pathological conditions and that only a small fraction of all $CD8^+$ T cells circulate in the peripheral blood at any given time[2]. These considerations have galvanized the scientific community to better characterize the full spectrum of memory $CD8^+$ T cells in the human body, an effort that has clear implications for clinical translation.

Memory $CD8^+$ T cells derive from the clonal expansion of naive precursors activated by exposure to cognate antigenic peptides presented in the context of major histocompatibility complex (MHC) class I molecules[3]. This process is meticulously regulated by various cell types, including $CD4^+$ T cells, which help establish optimal niches for the development and maintenance of effective $CD8^+$ T cell immunity[4]. After priming and extensive rounds of proliferation[5,6], effector $CD8^+$ T cells emigrate from the site of primary antigen encounter in the relevant draining lymph nodes (LNs) via the efferent lymphatics and blood circulatory system (vasculature) to the site of challenge/infection, where subsequent antigen encounter triggers the release of immune weaponry. The clonal expansion process generates a broad array of functionally and phenotypically distinct effector $CD8^+$ T cells with specialized roles that collectively enable rapid antigen detection and threat eradication throughout the body[7]. Although our knowledge of these early events is based predominantly on infection models in mice, elegant studies of humans vaccinated against smallpox or yellow fever have revealed that acutely generated effector $CD8^+$ T cells are typically highly cytotoxic, widely distributed throughout the periphery, and poorly equipped to survive[8,9,10]. Many of these characteristics have also been observed during acute infections with other viruses, such as coronaviruses, flaviviruses, hepatitis viruses, herpesviruses, and retroviruses[11,12,13,14,15], suggesting common signatures that distinguish acute effector $CD8^+$ T cells from memory or exhausted $CD8^+$ T

cells, which have been reviewed in detail elsewhere[16,17]. A vast majority of these acute effector $CD8^+$ T cells are subsequently lost following antigen clearance, leaving a residual pool of diverse memory $CD8^+$ T cells comprising approximately 5–10% of the original antigen-specific population[7].

Heterogeneity has long been recognized as a feature of the $CD4^+$ T cell lineage, and over the course of the past decade, it has become clear that many distinct subsets also constitute the $CD8^+$ T cell lineage. Subset fate is likely determined by a combination of the initial antigenic stimulus that drives clonal expansion, integrated via the clonotypically expressed T cell receptor (TCR), and various costimulatory signals and microenvironmental cues, which collectively dictate the cell-intrinsic epigenetic program. However, we still lack a complete picture of the true complexity that exists with the human $CD8^+$ T cell lineage, especially in hard-to-access tissue sites, and we do not fully understand how currently defined subsets interrelate dynamically and ontogenetically. These knowledge gaps are critical obstacles in our quest to decipher the cellular mechanisms that underlie effective immune surveillance. In this review, we summarize the current status of the field in terms of what is known, what is not known, and what can be done to generate a holistic understanding of $CD8^+$ T cell-mediated immunity throughout the human body.

Circulating memory T cells

Lymphocytes were first recognized as long-lived immune cells in the 1950s. This discovery can be attributed to the pioneering work of James Gowans, who showed that lymphocytes recirculate from blood to tissues in a unidirectional manner and return to the vasculature via lymphatic vessels, which culminate in the right lymphatic duct and the thoracic duct[18]. In a series of innovative labeling experiments in adult rats, lymphocytes were found to undergo rapid antigen-specific proliferation in LNs, and transfer of these lymphocytes from the thoracic duct into inert recipients was found to confer immunological memory[18,19,20]. Although not defined at the time, most of these lymphocytes were T cells, and the seminal findings that emerged from this work underpinned the concept of immune surveillance.

Elegant studies followed in mice, rats, and sheep to extend this paradigm. Mackay and colleagues demonstrated that efferent lymph contains naive and antigen-experienced T cells, whereas antigen-experienced T cells are preferentially retained from afferent lymph[21,22,23,24]. As such, it was postulated that memory T cells recirculate continuously from tissues via lymphatic vessels and back again, presumably via the blood as a conduit. Later work showed that T cells use specific integrins (e.g., $\alpha4\beta7$), selectins (e.g., CD62L), and chemokine

receptors (e.g., CCR7 and CXCR3) to enter the parenchyma of tissues[25,26,27,28,29,30], setting the stage for the use of specific markers to identify memory CD8$^+$ T cell subsets in humans, aided by contemporaneous advances in flow cytometry[31,32]. Hamann and colleagues pioneered this process of classification, initially defining naive, memory, and effector CD8$^+$ T cells based on the expression of CD27 and CD45RA[33]. Sallusto and colleagues subsequently refined the categorization of memory CD8$^+$ T cells into two major subsets with distinct patterns of migration, namely central memory T (T_{CM}) cells, which express the secondary lymphoid organ (SLO)-homing receptors CCR7 and CD62L, and effector memory T (T_{EM}) cells, which lack CCR7 and CD62L and instead traffic to non-lymphoid tissues (NLTs)[34]. This fundamental study also revealed a clear functional dichotomy between T_{CM} cells, which are better able to proliferate, and T_{EM} cells, which are better equipped to mediate cytotoxicity. The advent of peptide-MHC tetramers[35] and intracellular cytokine staining technology[36] enabled further studies demonstrating the existence of virus-specific CD8$^+$ T_{EM} with revertant expression of CD45RA (T_{EMRA}) cells[37] as a highly differentiated subset in a pathway defined by the progressive loss of CD27 and CD28 (ref. [38]). Although the use of integrative single-cell technologies and multiparametric flow cytometry has revealed that memory CD8$^+$ T cells form a continuous landscape[39,40], there are certainly distinct nodes that define anatomically and functionally distinct memory CD8$^+$ T cells within this spectrum. However, it remains unclear precisely how these memory subsets are formed in relation to antigen-driven differentiation, with current evidence unable to distinguish reliably between asymmetric and linear models in humans or mice[41,42]. It is also notable here that functional and phenotypic diversity can extend to individual clonotypes specific for the same inciting antigen[43,44], indicating a need to understand subset fate beyond the attributes conferred by the expressed TCR.

Central memory T (T_{CM}) and stem cell-like memory T (T_{SCM}) cells

T_{CM} cells exhibit a propensity for survival, proliferate robustly upon recurrent antigen exposure, and home to SLOs. Like naive T cells, T_{CM} cells express CCR7 and CD62L, granting access to LNs via high endothelial venules, but unlike naive T cells, T_{CM} cells express CD45RO instead of CD45RA. T_{CM} cells also express high levels of the interleukin receptors IL-7R (CD127) and IL-15R (CD122), which integrate signals from the corresponding ligands to aid survival and facilitate intermittent homeostatic proliferation[1], and as a consequence, antigen-specific T_{CM} cells tend to accumulate with age[45]. In addition, T_{CM} cells produce large amounts of IL-2 *ex vivo* and express the coreceptors CD27 and CD28 alongside various transcription factors, such as TCF-1, BCL-6, ID3, and STAT3[34,41,46]. Heterogeneous expression patterns have nonetheless been described for some chemokine receptors, such as CCR4, CCR5, CXCR3, and CXCR5[43,46], and also for various inhibitory receptors, such as PD-1 and TIGIT,

likely reflecting differential antigen exposure and concomitant reactivation[47]. In line with this notion, the T_{CM} phenotype, which is classically associated with long-term memory after the resolution of acute infections, often characterizes $CD8^+$ T cell populations specific for persistent viral proteins that are encountered rarely, such as those with latent expression programs (e.g., Epstein-Barr virus) or those expressed in the context of low-level viral replication (e.g., elite control of human immunodeficiency virus type 1)[46,48,49,50].

T_{SCM} cells also express CCR7 and CD62L, but in contrast to classical T_{CM} cells, maintain a naive-like phenotype characterized by the expression of CD45RA. This relatively rare subset can be distinguished within the conventionally defined naive pool by evidence of antigen-specific clonal expansion and memory formation, including the upregulation of CD95, CD122, and CXCR3[51]. T_{SCM} cells display limited effector functionality but can proliferate extensively in response to antigen and self-renew[51]. Moreover, isotope labeling studies have revealed a dichotomy in the dynamics of T_{SCM} cell division, with a smaller subpopulation making a dominant contribution to the long composite half-life within the bulk phenotype[52,53]. The detection of antigen-specific T_{SCM}-like cells long after infection or vaccination is further compatible with the concept of durable quiescent memory[54,55]. It remains unclear to what extent the long-term survival of T_{SCM} cells can be promoted in niches beyond SLOs. However, it is clear that memory $CD8^+$ T cell subsets defined by the expression of CCR7 are clonotypically similar in blood and thoracic duct lymph[43], indicating uniform recirculation between tissues and the vasculature.

Effector memory T (T_{EM}) cells

In contrast to T_{CM} and T_{SCM} cells, which are relatively well circumscribed in terms of function and phenotype, T_{EM} cells are more heterogeneous and linked by an ability to traffic through lymphoid organs and NLTs. As the name suggests, T_{EM} cells are equipped to deploy an array of effector functions, including cytokines (e.g., IFN-γ and TNF), β-chemokines (e.g., CCL3, CCL4, and CCL5), pore-forming proteins (e.g., granulysin and perforin), and serine proteases (e.g., granzymes A, B, and H)[56]. At the molecular level, T_{EM} cells can also be defined by the expression of various transcription factors, including T-bet, TOX, Zeb2, ID2, and STAT4[41,46]. It is established that T_{EM} cells originate from the naive pool in response to antigenic stimulation, but the signals that drive heterogeneity within this loosely defined subset remain obscure.

For many years, researchers have categorized human effector memory subsets into T_{EM} and T_{EMRA} cells, based on the expression of CD45RO or CD45RA, respectively, with the latter

considered to be an end-stage phenotype. However, longitudinal tracking studies of human virus-specific $CD8^+$ T cells elicited by infection or vaccination have begun to challenge this view, revealing that T_{EMRA} cells can persist for a long time after the initial encounter with antigen[44,57]. In addition, isotope labeling studies combined with analyses of proliferation and telomere length have shown that T_{EMRA}-like cells defined by the expression of CD57 can self-renew *in vivo*[58]. It has also been shown recently that highly cytotoxic (granzyme B^+ $perforin^+$) T_{EM}/T_{EMRA} cells expressing the chemokine receptor CX3CR1 are largely confined to the vasculature, whereas noncytotoxic T_{EM}/T_{EMRA} subsets expressing CD27 and CD127 retain the capacity to proliferate and recirculate throughout the body[43].

Recirculating T_{EM} cells. Gerlach and colleagues identified a memory $CD8^+$ T cell population in the efferent lymphatics of mice that expressed intermediate levels of CX3CR1[59]. These cells were thought to represent a peripheral memory subset akin to early differentiated T_{EM} cells in humans based on the expression of CD27 and CXCR3 and demonstrable recirculation through NLTs and SLOs. They also found that $CX3CR1^{int}$ cells could regenerate and give rise to both T_{CM}-like and T_{EM}-like $CD8^+$ T cells. Parallel studies in humans confirmed the presence of classically defined T_{EM} and T_{EMRA} cells in thoracic duct lymph[43]. However, these cells were found to exhibit stem-like characteristics, including a robust ability to proliferate and produce IL-2. In line with these observations, recirculating T_{EM} cells were identified as functionally and transcriptionally equivalent to T_{CM} cells, with a chimeric effector/stem-like phenotype characterized by the expression of CD27, CD127, Eomes, and TCF-1, but notably lacking CX3CR1. In addition, recirculating T_{EM} and T_{EMRA} cells were shown to express negligible amounts of perforin and T-bet, indicating constraints on effector functionality (Figure 1).

Vascular T_{EM} cells. Gerlach and colleagues further identified a memory $CD8^+$ T cell population in the vasculature that expressed high levels of CX3CR1[59]. These cells were rarely found in tissues and were shown to be highly cytotoxic and poorly able to produce IL-2. Similar findings were reported in another study[60]. Subsequently, it became clear that human tissue-emigrant memory $CD8^+$ T cells, irrespective of phenotype, rarely express granzyme B or perforin[43]. In the same study, highly cytotoxic $CX3CR1^+$ T_{EM} cells were found to be restricted to the vasculature, a finding confirmed in rhesus macaques via the administration of fingolimod to block tissue egress, and clonotypically skewed compared with donor-matched noncytotoxic $CX3CR1^-$ T_{EM} cells in blood and efferent lymph, indicating ontogenetic divergence (Figure 1).

There are several reasons that might explain why cytotoxicity is a function limited to the intravascular space. One possibility is that many intracellular pathogens infect lymphocytes,

other haematopoietic cells, or vascular endothelial cells[61,62], the latter exemplified by cytomegalovirus (CMV)[63]. In line with this particular tropism, CMV-specific CD8$^+$ T cells often circulate at high frequencies in the vasculature[64], likely reflecting viral persistence in endothelial cells, and typically display a highly cytotoxic phenotype characterized by the abundant expression of granzyme B and perforin[65]. It is therefore tempting to speculate that cytotoxicity is a function that has evolved specifically to execute intravascular immune surveillance. Alternatively, vascular T_{EM} cells could act by docking to endothelial cells and engaging target cells in organs with fenestrated capillaries via cytoplasmic protrusions[66] and/or serve as a reservoir that, akin to neutrophils, could be mobilized immediately in response to infection, inflammation, or injury anywhere in the body. In line with this possibility, cytotoxic CD8$^+$ T cells tend to infiltrate inflamed tissue sites in many autoimmune diseases, cancers, and chronic infections[67,68,69,70,71]. However, tissue-resident features have also been ascribed to such disease-localized memory CD8$^+$ T cells[68,72], indicating a need for further studies to define the precise role of antigen-targeted cytotoxicity during active infection or inflammation.

Tissue-resident memory T (T_{RM}) cells

The early studies performed by Gowans and colleagues were fundamental to our current understanding of how lymphocytes respond to antigens and recirculate[18,19,20]. Many of the isotope dilution experiments and labeling methods used to track lymphocyte migration were nonetheless poorly suited to detect the possibility of tissue retention. The initial identification of human T_{CM} and T_{EM} cells was also confounded to some extent by limited sampling restricted to the vasculature[34]. At the time, it was recognized that a vast majority of memory CD8$^+$ T cells in NLTs, which were thought to represent recirculating T_{EM} cells, lacked expression of CCR7 and CD62L, but a growing literature soon emerged to suggest a different picture. Much of this later work was pioneered by Masopust and Lefrancois. They showed that T_{EM}-like cells were highly abundant in NLTs[73] and raised the concept of tissue residency[74], which was supported by studies of skin samples taken from humans and mice[75,76]. Definitive evidence for the phenomenon of tissue residency subsequently emerged from experiments in mice demonstrating the long-term retention of memory CD8$^+$ T cells after transplantation of grafted skin or intestinal tissue[77,78,79]. A variety of different approaches have since demonstrated that a vast majority of CD8$^+$ T cells in peripheral tissues are *bona fide* T_{RM} cells that do not recirculate but instead establish unique niches in almost every organ in mice[2], as reviewed elsewhere[80].

Human T_{RM} cell identification. Studies of T_{RM} cells in humans are complicated by the inevitable limitations of tissue access, but through the use of innovative sampling methods and analytical

techniques, we are starting to appreciate the fact that most human tissues harbor memory $CD8^+$ T cells with characteristics of residency. A key early study demonstrated that classical psoriatic lesions could be elicited in a T cell-dependent manner via the transfer of nonlesional skin grafts from patients with psoriasis to immunodeficient mice[75]. It was subsequently recognized that normal human skin contains T_{EM}-like cells expressing skin-homing receptors, such as CLA, CCR4, and CCR6[76,81]. This work was followed by other studies demonstrating the existence of functionally diverse T_{RM}-like cells in human bone marrow, lung and gastrointestinal tissue, and LNs, with distinct antigen targeting profiles in some cases indicating a lack of active recruitment from the vasculature[72,82,83,84]. A further study demonstrated that prolonged treatment of cutaneous T-cell lymphoma (L-CTCL) patients with alemtuzumab, an anti-CD52 antibody, resulted in the depletion of all circulating $CD8^+$ T cells without eliminating a population of T_{EM}-like cells in the skin[85]. Importantly, these residual cells were found to express the early activation marker CD69 (ref. [86]), which blocks lymphocyte egress from the tissues by downregulating sphingosine 1-phosphate receptor-1 (S1PR1)[87].

Subsequent work in other clinical settings reinforced the concept of tissue residency. A number of elegant studies demonstrated that donor-derived T cells with a T_{RM}-like phenotype can persist for years in recipients after human leukocyte antigen (HLA)-mismatched solid organ transplantation[88,89,90,91]. Similarly, allogeneic hematopoietic stem cell transplant recipients can retain host-derived T cells with a T_{RM}-like phenotype for at least 10 years in the skin, despite complete reconstitution of the circulating immune system[92,93]. Farber and colleagues further showed that $CD8^+$ T_{RM} cells defined by the expression of CD69 are present in almost every single human organ[94,95]. Moreover, these cells can be found during infancy, albeit with reduced expression of markers associated with tissue residency, indicating that extrinsic factors confined to the adult tissue microenvironment may be required for the acquisition of a fully mature T_{RM}-like phenotype[96]. Akin to innate immune cells[97], T_{RM} cells also exhibit organ-specific adaptations[98], likely representing mechanisms employed to ensure survival and maintain effective local immune surveillance.

Human T_{RM} cell classification. No definitive marker exists to define *bona fide* T_{RM} cells. Many human studies have used CD69 in combination with a lack of CCR7, CD62L, and CX3CR1 to identify $CD8^+$ T_{RM} cells under conditions of steady state[80]. Although often used as a marker of activation, CD69 can be expressed constitutively by T_{RM} cells in the absence of antigen stimulation, without coincident upregulation of CD25 (refs. [72,99]). In addition, $CD69^+$ $CD8^+$ T cells in NLTs express the integrins CD49a and CD103, often in combination with tissue-specific chemokine receptors, such as CCR5, CXCR3, and CXCR6, and/or other homing

markers, such as CLA[72,80,95,99]. T_{RM} cells also express certain inhibitory receptors, such as CD101 and PD-1[80,99], which potentially act to limit inflammatory and fibrotic sequelae and prevent tissue injury[100]. Other inhibitory receptors are less commonly expressed by T_{RM} cells, however, including CD39, LAG-3, and TIM-3, which are typically associated with terminal exhaustion[68,101,102]. In fact, recent data suggest that exhausted $CD8^+$ T cells represent an epigenetically distinct lineage, programmed at least in part by the canonical transcription factor TOX[103,104,105,106,107]. Concordantly, T_{RM} cells tend to express relatively low amounts of TOX[108,109] and instead express other transcription factors, such as Blimp1, Hobit, Runx3, and Notch[90,99,110,111]. This core signature is nonetheless insufficient to define T_{RM} cells as a unique lineage. A better hallmark may be the downregulation of KLF2, which controls the expression of S1PR1 and the formation of resident memory in mice[112], although more work is required to determine whether human T_{RM} cells possess a clear transcriptional identity.

CD103 has also been used as a canonical marker for human T_{RM} cells. Alternatively known as integrin $\alpha E\beta 7$, CD103 binds to the epithelial cell-specific ligand E-cadherin, promoting the retention and survival of T_{RM} cells at barrier sites throughout the body[113,114,115]. Accordingly, the expression of CD103 differs considerably among T_{RM} cells in different organs as a function of epithelial cell proximity[95,98,116]. Differential expression of CD103 also occurs among T_{RM} cells located in anatomically distinct LNs. For example, T_{RM} cells in the mesenteric LNs, which drain much of the gastrointestinal tract, more commonly express CD103 than T_{RM} cells in the pulmonary or superficial LNs[94,95]. These differences could reflect variable infiltration by $CD8^+$ T cells migrating from adjacent NLTs[117]. Current evidence indicates that $CD69^+$ $CD103^+$ $CD8^+$ T cells are preferentially retained in the gut and lungs for many years after transplantation[88,90]. However, it still remains unclear to what extent human T_{RM} cells can be defined by the expression of CD103, especially in terms of tissue specificity and *in situ* stability (Figure 2).

Ex-T_{RM} cells. To complicate the overall picture, it has recently become apparent that not all T_{RM} cells reside permanently in a given tissue, contravening the classical view that a lack of migration is hallmark of tissue residency. In addition to the repopulation of SLOs by nascent T_{RM} cells derived from nearby NLTs[117], established T_{RM} cells can occasionally rejoin the circulation[118,119]. These ex-T_{RM} cells maintain epigenetic elements imprinted during residency but nonetheless downregulate CD69. It is notable here that T_{RM} cells have a relatively limited capacity to differentiate, which varies across organs but appears to be a particularly strict constraint among subsets that express CD103 (ref. [118]).

Studies in humans are beginning to shed light on the biology of ex-T_{RM} cells defined among circulating subsets according to the expression of CD103. One very early study demonstrated that CD103 could be used as a marker to define naive $CD8^+$ T cells with high levels of TCR rearrangement excision circles, indicative of recent thymic emigration[120]. However, most circulating $CD103^+$ $CD8^+$ T cells exhibit a mixed T_{CM}/T_{EM}-like phenotype, compatible with different tissue origins and an ability to access both SLOs and NLTs[43,72]. In thoracic duct lymph, approximately 20% of memory $CD8^+$ T cells express CD103, potentially representing ex-T_{RM} cells in transit from tissue sites to the vasculature[43]. Using a humanized skin transplant model in mice, another study found that $CD4^+$ T cells expressing both CD103 and CLA could downregulate CD69, exit the skin, join the circulation, and migrate to other sites in the skin[121]. Moreover, a study of patients undergoing myeloablative chemotherapy followed by allogeneic hematopoietic transplantation revealed that host-derived $CD103^+$ T cells in the skin can emerge into the circulation, concurrently maintaining a T_{RM}-like transcriptomic identity[122]. It is nonetheless important to note that surface expression of CD103 can be induced by immune activation and signaling via TGF-β[123]. In addition, donor T_{RM} cells can persist for more than a decade in the liver after transplantation without egressing into the hepatic circulation, indicating remarkable *in vivo* stability[124]. These collective observations highlight the need for additional studies to define the ontogeny and pertinence of ex-T_{RM} cells in the setting of human work.

Integrated human classification model

The classification of human memory $CD8^+$ T cells is currently based on a simplistic linear differentiation model according to the expression patterns of CCR7 and CD45RA as follows: $CCR7^+$ $CD45RA^+$ (naive) → $CCR7^+$ $CD45RA^-$ (T_{CM}) → $CCR7^-$ $CD45RA^-$ (T_{EM}) → $CCR7^-$ $CD45RA^+$ (T_{EMRA}). This paradigm is supported in large part by studies of chromatin accessibility and DNA methylation[125,126]. However, the emerging data summarized above indicate that a revision is necessary, accounting in particular for the functional and migratory diversity of T_{EM} cells and the ability of T_{RM} cells to rejoin the vascular circulation.

Current evidence suggests that T_{CM} cells recirculate, localize centrally to SLOs, and proliferate rapidly in response to antigen. The functional, phenotypic, and transcriptomic profiles of T_{CM} cells also overlap considerably across individuals and tissues, irrespective of specificity. In contrast, T_{EM} and T_{EMRA} cells exhibit much greater heterogeneity, reflected by the various subset nomenclatures used in the existing literature[59,127,128]. Similarly, recent data have led to a reclassification of exhausted T cells as a distinct lineage based on the expression of TCF-1 and several inhibitory receptors, including PD-1, TIGIT, and TIM-3[47,104,105,106,107]. In line with

this revision, it seems rational to propose that human T_{EM} cells could be categorized more usefully into two distinct subsets based on the differential expression of CX3CR1 and TCF-1, namely TCF-1$^+$ CX3CR1$^-$ precursor T_{EM} (pT_{EM}) cells, which are able to proliferate vigorously, recirculate, and scan peripheral tissues for cognate antigens, and TCF-1$^-$ CX3CR1$^+$ terminal T_{EM} (tT_{EM}) cells, which are highly cytotoxic and confined to the vasculature under conditions of steady state. An analogous classification could also be used to distinguish TCF-1$^+$ T_{EMRA} (pT_{EMRA}) cells from the more common CX3CR1$^+$ T_{EMRA} (tT_{EMRA}) cell population. This approach is facilitated in practice by the existence of surface markers that parallel the expression of TCF-1, such as CD127 (ref. [46]). The situation is more complicated in the case of T_{RM} cells, which by definition reside in tissues and typically express low levels of CCR7 and CX3CR1. However, multiple subsets of T_{RM} cells can be identified across a spectrum of differentiation, at least in mice[118]. In addition, ex-T_{RM} cells do not usually express CD69, although epigenetic features of previous tissue residency may inform the future identification of a core phenotype[119]. Other memory subsets have not been included here for reasons of clarity, but a global classification should encompass a more nuanced view of T_{SCM} cells[51], refined to incorporate the dichotomy between exhaustion and functionality[47], and virtual memory cells[129] within a flexible and integrated structure. Current data nonetheless permit an immediate revision to the simplistic linear model that is biologically instructive and eminently tractable using basic flow cytometry (Figure 3).

Models to study human CD8$^+$ T cell recirculation and residency

It seems inevitable that rapid technological progress will lead to the identification of other functionally relevant immune subsets in the near future. In the following sections, we highlight a few models that might feasibly be used to enhance our understanding of human CD8$^+$ T cell biology, with a particular focus on tissue recirculation and tissue residency.

Organ donors. The use of donated organs has transformed the study of human immunology by providing researchers with tissues that were previously either available only in limited quantities or wholly inaccessible[130]. For example, human organ research underpinned the observation that CD8$^+$ T_{RM} cells populate most tissues in the body[130], and more recently, this approach has been used to provide a holistic view of virus-specific T cell immunity[64,131,132]. The emerging use of single-cell technologies and spatial transcriptomics[133,134] in this context further promises to map the cellular and molecular elements of immune surveillance across all major organ systems in the human body. It is also possible that the availability of intact human material could enable the development of improved organoid, explant, or even whole-organ transfusion models to conduct adoptive transfer experiments, investigate how different tissues

imprint the programmable functions of T_{CM}, T_{EM}, and T_{RM} cells, and visualize cellular interactions and patterns of migration *ex vivo*.

Transplantation. HLA-mismatched transplantation models have been used increasingly over the past few years to infer tissue residency[88,89,90,91]. A clear advantage of this approach is the ability to distinguish reliably between cells of donor and recipient origin. Solid organ transplantation models have allowed researchers to demonstrate that donor-derived $CD8^+$ T cells expressing CD69 and CD103 persist *in situ*[88,89,90,91] and investigate the role of T_{RM} cells in allograft rejection and tissue-specific immune protection[135,136]. Many of these studies were nonetheless conducted over relatively short periods of time. The fact that tissue biopsies are often collected longitudinally for routine monitoring purposes could provide a window of opportunity here to examine which phenotypic markers delineate short-term versus long-term tissue residency. Additional biopsy collections from other tissue sites may also be feasible to determine whether T_{RM} cells can migrate via the lymphatics and establish long-term residency elsewhere, as described in mice[118].

Treatment modalities. In some instances, the core features of human T_{EM} and T_{RM} cells have been defined by studying how certain drugs, such as alemtuzumab and fingolimod, affect the immune system[43,85]. The continual emergence of new drugs that directly or indirectly block or enhance T cell activity will almost certainly facilitate comparable discoveries in the near future. A key example is the development of checkpoint inhibitors for cancer immunotherapy[137]. These agents block interactions (e.g., PD-1/PD-L1) that suppress tumor-specific immune surveillance, which can involve T_{RM} cells *in situ*[68,138], and reinvigorate exhausted stem-like $CD8^+$ T cells displaying features of tissue residency in draining LNs[139,140,141]. Autoreactive stem-like progenitors have also been identified in murine pancreatic LNs[142], suggesting a mechanistic explanation for the link between checkpoint blockade and the development of autoimmunity. In parallel, the use of checkpoint inhibitors for coincident indications has enhanced our understanding of virus-specific adaptive immunity, both in the context of natural infection and in response to vaccination against influenza A virus (IAV)[143,144].

Immunosuppressive drugs have similarly illuminated our understanding of immunological memory[57,145,146,147,148,149]. A particularly exciting advance in recent times has been the clinical introduction of a new class of drugs that block chemokine receptors, such as CCR1, CCR5, CXCR1, CXCR2, CXCR4, and CXCR6 (ref. [150]), providing unprecedented opportunities to define the mechanisms that govern immune cell migration. Moreover, increasing numbers of patients with chronic diseases are being treated with agents that modulate the immune

system, potentially enabling long-term studies of how specific molecular targets control the differentiation, function, and migration of T cells in response to antigenic challenge and under conditions of steady state.

Vaccination. Vaccines represent one of the most successful interventions in the history of medicine. Of particular note, live-attenuated vaccines against smallpox and yellow fever virus have been used to study the dynamics of human antigen-specific $CD8^+$ T cell responses over time[151], critically informing our understanding of how memory subsets form[8,9,10], maintain clonotypic heterogeneity[44], and potentially redifferentiate[152]. It is now possible to acquire substantially more information from such studies using new technologies to characterize and track individual antigen-specific $CD8^+$ T cell clonotypes epigenetically, proteomically, and transcriptomically[153,154]. These approaches in combination with advanced bioinformatics will likely be required to decipher the biological principles that shape the ever-changing landscape of adaptive immunological memory.

The advent of mRNA vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spawned an explosion of reports in the literature charting the induction and maintenance of cellular immunity. In contrast to many other platforms, mRNA vaccines induce robust[57,155], polyfunctional[57], and durable T cell responses[156] against SARS-CoV-2. New clinical trial data on mRNA vaccines against other respiratory viruses may further enhance our understanding of these vaccine-induced immune responses in the near future[157,158]. Although most human vaccine studies to date have focused on the generation of immune responses in the vasculature, a few investigators have gone beyond the exclusive use of peripheral blood samples to assess memory B and $CD4^+$ T cell responses in biopsied LNs[159,160,161,162]. It is notable here that even less invasive approaches, such as fine-needle aspiration and the collection of buccal or nasopharyngeal swabs, can also be suitable for the study of T_{RM} cells induced by infection and/or vaccination[163,164]. Importantly, these sampling methods are generally acceptable among human volunteers on a recurrent basis, enabling longitudinal analyses of mucosal immunity.

Challenge studies. Human challenge studies are a type of clinical trial in which healthy volunteers are deliberately exposed to an antigen or pathogen under controlled conditions to study the mechanisms of disease[165]. After local or systemic delivery of the agent under investigation, the immune response is monitored closely over time, typically via frequent sampling of the peripheral blood and/or tissue sites, alongside clinical assessments of safety and tolerability[165]. This approach has been used to assess immune responses against many

pathogens, including IAV, rhinoviruses, SARS-CoV-2, and *Mycobacterium bovis*[165,166,167,168,169,170,171]. Challenge studies offer a unique opportunity to define the immune correlates of protection from disease. For example, the observation that $CD4^+$ T cell responses protect against IAV was uniquely enabled by this approach[171], which allows immune profiling immediately prior to an infectious event defined in terms of dose, route, and time. The use of minimally invasive tissue sampling techniques would further enhance the advantages of these studies for the purpose of characterizing the dynamics of tissue-recirculating and tissue-resident immunity.

Conclusions and future perspectives

In recent times, we have begun to appreciate that the intravascular space is a unique compartment that harbors a reservoir of highly cytotoxic immune cells, such as neutrophils, NK cells, and T_{EM}/T_{EMRA} cells, suggesting that current paradigms based on studies of peripheral blood samples conducted over many decades do not necessarily reflect the true diversity of $CD8^+$ T cells in the human body. Moreover, emerging data are starting to refine our understanding of how $CD8^+$ T cells localize to distinct niches and mediate pathogen-specific effector functions without incurring tissue damage, although further work is required to generate an anatomically complete picture. As sampling methods and single-cell technologies continue to evolve, this goal becomes eminently more achievable. Ultimately, these efforts hold the potential to reveal new insights into previously “hidden” facets of $CD8^+$ T cell immunity, with consequent implications for the development of better prophylactic and therapeutic immune interventions against cancer and infectious diseases, the urgent need for which has been highlighted recently in the context of SARS-CoV-2.

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AUTHOR CONTRIBUTIONS

All authors participated in the writing process and contributed intellectually.

COMPETING INTERESTS

M.B. is a consultant for Oxford Immunotec, Mabtech, BMS, and MSD. D.A.P. and L.K.M. have no competing interests to declare. M.R.B. is a consultant for Interius Biotherapeutics.

FIGURE LEGENDS

Figure 1. Heterogeneity of $CD8^+$ T_{EM} cells. Recirculating T_{EM} cells exhibit stem-like precursor characteristics with increased expression of CD27, CD28, and CD127, along with the inhibitory receptor PD-1 and various chemokine receptors, such as CXCR3. Precursor T_{EM} cells also produce IL-2, proliferate vigorously in response to antigen, and exhibit substantial plasticity in keeping with the expression of TCF-1. In contrast, vascular T_{EM} cells lack CD27, CD28, and CD127, and instead express the fractalkine receptor (CX3CR1) and the inhibitory receptor CD244, commonly alongside CD18 (LFA-1) and CD57. These cells are highly cytotoxic and exhibit features of terminal differentiation. Terminal T_{EM} cells are generally confined to the intravascular space and express granzyme B, perforin, and T-bet.

Figure 2. Characteristics of $CD8^+$ T_{RM} cells. T_{RM} cells are omnipresent and exhibit tissue-specific functions and phenotypes attuned to the local microenvironment. For example, many T_{RM} cells in the gastrointestinal tract express CD49a and CD103, unlike T_{RM} cells in LNs. Irrespective of location, T_{RM} cells typically express the memory markers CD27 and CD45RO, the inhibitory receptors CD101 and PD-1, and the chemokine receptors CXCR3 and CXCR6. These cells also generically downregulate various homing receptors, such as CCR7, CD62L, CX3CR1, and S1PR1, as well as the transcription factor KLF2. T_{RM} cells rapidly secrete proinflammatory chemokines and cytokines and can proliferate vigorously but lack cytotoxic activity, at least under conditions of steady state. Recent studies also suggest that T_{RM} cells can rejoin the circulation and/or migrate to other tissues after encountering antigen. The features of these ex-T_{RM} cells in humans remain largely unknown.

Figure 3. Classification of memory $CD8^+$ T cell subsets in humans. (**a**) Naive $CD8^+$ T cells are primed by antigen encounters in the LNs and differentiate into highly cytotoxic effector cells that undergo clonal expansion. Effector $CD8^+$ T cells migrate to peripheral sites to locate and kill cells flagged by presentation of the inciting antigen. After resolution of the initial threat, approximately 5–10% of these acutely mobilized effector $CD8^+$ T cells differentiate into distinct memory subsets, which exhibit various degrees of heterogeneity and potentially seed the T_{RM} compartment during migration[172]. (**b**) T_{SCM} and T_{CM} cells express CCR7 and migrate to LNs. These cells are *bona fide* recirculators and proliferate vigorously in response antigen. T_{EM} cells are more diverse and can be segregated into two biologically relevant subsets, namely $CD127^+$ $CX3CR1^-$ precursor T_{EM} (pT_{EM}) cells, which retain the ability to proliferate and recirculate, and $CD127^-$ $CX3CR1^+$ terminal T_{EM} (tT_{EM}) cells, which are highly cytotoxic and generally confined to the vasculature. T_{RM} cells express low levels of CCR7 and CX3CR1 and

high levels of CD69. These cells can respond promptly and recruit other immune subsets to the site of antigen challenge. Many T_{RM} cells, especially in epithelial tissues, also express CD103.

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