Published in final edited form as:

Nat Rev Immunol. 2017 June ; 17(6): 391–400. doi:10.1038/nri.2017.34.

Antigen-inexperienced memory CD8⁺ T cells: where they come from and why we need them

Jason T. White,

Department of Microbiology and Immunology, The Peter Doherty Institute, University of Melbourne, 792 Elizabeth Street, Melbourne, Victoria 3000, Australia

Eric W. Cross, and

Department of Immunology and Microbiology, University of Colorado Denver at Anschutz Medical Campus, School of Medicine, Mail Stop 8333, Room P18-8115, 12800 East 19th Avenue, Aurora, Colorado 80045-2537, USA

Ross M. Kedi

Department of Immunology and Microbiology, University of Colorado Denver at Anschutz Medical Campus, School of Medicine, Mail Stop 8333, Room P18-8115, 12800 East 19th Avenue, Aurora, Colorado 80045-2537, USA

Abstract

Memory-phenotype CD8⁺ T cells exist in substantial numbers within hosts that have not been exposed to either foreign antigen or overt lymphopenia. These antigen-inexperienced memory-phenotype T cells can be divided into two major subsets: ‘innate memory’ T cells and ‘virtual memory’ T cells. Although these two subsets are nearly indistinguishable by surface markers alone, notable developmental and functional differences exist between the two subsets, which suggests that they represent distinct populations. In this Opinion article, we review the available literature on each subset, highlighting the key differences between these populations. Furthermore, we suggest a unifying model for the categorization of antigen-inexperienced memory-phenotype CD8⁺ T cells.

Lymphocytes within the peripheral T cell repertoire are classified as either naive or memory cells on the basis of their expression (or lack thereof) of various cell-surface markers and receptors. The expression of these surface markers correlates with various traits and functions that are specific to different T cell populations. For example, naive and central memory T (T_{CM}) cells express both CC-chemokine receptor 7 (CCR7) and CD62 ligand (CD62L; also known as L-selectin), which facilitate their surveillance of secondary lymphoid tissues. By contrast, other populations of memory T cells show increased expression of CD122 (also known as IL-2R β), CXC-chemokine receptor 3 (CXCR3) and the adhesion receptors CD44, CD11a and CD49d, all of which facilitate (among other things) their access to and responses within inflamed peripheral tissues. As these phenotypical

Correspondence to R.M.K. ross.kedi@ucdenver.edu.

Competing interests statement

The authors declare no competing interests.

changes occur in response to productive T cell receptor (TCR) signalling, the expression of these markers is classically viewed as a window into the history of a cell's encounter with antigen in the periphery. However, although the majority of CD8⁺ T cells in an unmanipulated host (that is, an animal that has not been challenged with antigen) display a naive phenotype, there also exists a substantial population of CD8⁺ T cells (15–20% of total circulating CD8⁺ T cells) that express phenotypical markers of immunological memory. This has been known for some time^{1,2}, but it was generally assumed to be the result of T cell responses to gut microbiota and/or exposure to unrelated pathogens. Although this is certainly true for some of the memory-phenotype T cells, present evidence indicates that the vast majority of these cells are antigen inexperienced, instead arising as a result of cytokine stimulation³.

Observations from lymphopenic animal models were crucial for establishing the settings in which these antigen-inexperienced memory cells form. CD8⁺ T cells transferred into a host deficient in T cells (genetically or as a result of irradiation) will undergo substantial rounds of proliferation^{4–7}. This homeostatic proliferation is dependent on cytokines such as interleukin-7 (IL-7), as well as on the expression of other cytokines, most of which signal through the common γ -chain (also known as CD132)^{8–15}. Although MHC molecules are required for naive T cells to undergo lymphopenia-induced homeostatic proliferation^{4,6,16}, their role is mainly to provide a tonic stimulus through the TCR rather than an actual antigenic stimulus. Although a precise definition of a 'tonic stimulus' has never been fully clarified, it can be loosely defined as an interaction that does not lead to overt T cell activation but results in signalling that is necessary for the T cell to maintain responsiveness to subsequent activation^{17–19}. Memory-phenotype cells that arise as a result of this form of homeostatic proliferation show consistently low expression of the $\alpha 4$ integrin CD49d; this is in sharp contrast to antigen-experienced memory T cells, which express high levels of CD49d^{5,6,20}. A close examination of the memory-phenotype T cells present in lymphoreplete antigen-inexperienced hosts revealed their universal low expression of CD49d³. Not surprisingly then, these cells were subsequently found to be abundant in hosts containing progressively fewer foreign antigens; for example, pathogen-free mice, germ-free mice and even mice fed an elemental diet free of potential food antigens (REF. 21 and C. Surh, personal communication) all have robust populations of memory-phenotype CD49d^{low}CD8⁺ T cells³. From these data, we can conclude that the normal lymphoreplete host can support the development of memory-phenotype CD8⁺ T cells in the complete absence of overt antigen recognition.

Two major subtypes of memory-phenotype CD8⁺ T cell that have phenotypical similarities to the cells that undergo lymphopenia-induced homeostatic proliferation have been described in normal wild-type mice; these populations have been referred to as 'innate CD8⁺ T cells' (REFS 22–25) and 'virtual memory T cells' (T_{VM} cells)^{26–30}. Both populations seem to undergo relatively normal TCR rearrangement and thymic selection (with the exceptions noted below), and emerge with an unrestricted TCR repertoire. During or subsequent to their maturation into a naive single-positive CD8⁺ T cell, these cells receive the necessary signals, either within the thymus or in the periphery, to promote their differentiation into memory-phenotype cells. At first glance, they bear a striking resemblance to one another, which has led to some researchers dismissing the differences in

their development and considering them as a single population³¹. However, recent data highlight sufficiently different developmental pathways and functional capabilities that warrant the treatment of these cells as distinct subsets²⁸. It is worth clarifying that the antigen-inexperienced memory-phenotype cells that we discuss here are distinct from innate lymphoid cells (ILCs), natural killer T (NKT) cells and mucosa-associated invariant T (MAIT) cells; ILCs lack an antigen receptor^{32,33}, whereas NKT cells and MAIT cells have an invariant or restricted TCR repertoire. Intraepithelial lymphocytes somewhat straddle the lines of these definitions, but as they are reasonably easy to distinguish from the other memory-phenotype cells and they have been well reviewed elsewhere^{34,35}, we limit our discussion in this Opinion article to innate CD8⁺ T cells and T_{VM} cells.

Innate memory T cells

A unique population of memory-phenotype CD8⁺ T cells (originally referred to as “innate CD8⁺ T cells” (REF. 23)) was initially discovered in mice lacking the TEC kinase IL-2-inducible T cell kinase (ITK). In these mice, the majority of CD8⁺ T cells in the thymus possess a memory phenotype (that is, they are CD44^{hi}CD122^{hi})^{22,24}. As more knockout mouse strains were found to have a similar phenotype (for example, those deficient for inhibitor of DNA-binding 3 (ID3), those deficient for Krueppel-like factor 2 (KLF2) and those deficient for CREB-binding protein (CBP; also known as CREBBP)), a pattern was deduced: these molecules are all involved in the upregulation of IL-4 or in the development of NKT cells^{25,26,30,36,37}. Furthermore, the absence of these molecules favours either the production of IL-4 or the development of more NKT cells that express promyelocytic leukaemia zinc finger (PLZF; also known as ZBTB16) and produce large amounts of IL-4 (REFS 26,38). Subsequent studies revealed that the increase in the number of memory-phenotype cells is not CD8⁺ T cell intrinsic for these gene deficiencies, and is instead dependent on the excess IL-4 produced by NKT cells or PLZF⁺CD4⁺ T cells^{26,39}. Although normal C57BL/6 (B6) mice have relatively few of these thymic innate CD8⁺ T cells, these cells account for a substantial percentage of total CD8⁺ T cells in wild-type BALB/c mice and form a population that is proportional in size to their populations of thymic PLZF⁺ IL-4-producing cells^{24,26,30,40}. Although they have yet to be well described outside of the thymus in a wild-type mouse (BALB/c or B6), their presence in the periphery of hosts deficient in ITK or resting lymphocyte kinase (RLK; also known as CERK1) is IL-15 dependent²². In the convention of the nomenclature that has been used to describe other memory T cell subsets (for example, T_{CM} cells, effector memory T (T_{EM}) cells and resident memory T (T_{RM}) cells (BOX 1)), we refer to these IL-4-dependent thymically derived cells as ‘innate memory T (T_{IM}) cells’ for the remainder of this article (FIG. 1).

Box 1

Nomenclature for memory CD8⁺ T cell subsets

- Central memory T (T_{CM}) cells: a population of memory T cells that express CD127 (also known as IL-7R α), CD62 ligand (CD62L) and CC-chemokine receptor 7 (CCR7). T_{CM} cells recirculate through both secondary lymphoid tissues and peripheral tissues; display self-renewing proliferative capacity;

and both respond to and produce interleukin-2 (IL-2). This memory T cell subset is involved in protection against systemic infections.

- Effector memory T (T_{EM}) cells: a population of memory T cells characterized by moderate expression of CD127 and low levels of CD62L and CCR7. T_{EM} cells recirculate mostly through peripheral tissues, and they display reduced proliferative capacity but rapid effector function (cytotoxicity and cytokine production). These cells protect peripheral tissues during infectious challenges.
- Resident memory T (T_{RM}) cells: these memory cells typically express high levels of CD69 and CD103, and low levels of CD122. As the name implies, they are non-recirculating memory cells, instead maintaining residence within specific tissue sites. This exclusive tissue-residence property makes them highly protective against repeated local infectious challenges.

T_{VM} cells

Outside the thymus, memory-phenotype $CD8^+$ T cells have been noted in the secondary lymphoid organs of unmanipulated, specific-pathogen-free hosts for some time. These cells were often referred to simply as ‘endogenous memory’ T cells⁴¹ and were assumed to be the result of T cell responses to environmental antigens of one kind or another. Using tetramer pulldown assays (which use MHC tetramer staining and a magnetic bead enrichment method^{3,42}) to isolate the nominal antigen-specific repertoire, we and our collaborators found memory-phenotype cells within the pre-immune repertoire of T cells specific for antigens that had never before been encountered by the host³. These cells had the same phenotype as those that have undergone lymphopenia-induced homeostatic proliferation (that is, they were $CD44^{hi}CD122^{hi}CD49d^{low}$), which suggested that their development was dependent on cytokine stimuli and not on antigen. The subsequent identification of these cells in hosts free of microbiota, pathogens and even food-related antigens solidifies this conclusion (REF. 21 and C. Surh, personal communication). Since the original description of T_{VM} cells, we and others have determined that they are present in IL-4-deficient hosts^{27,43}, but are absent in mice deficient for either IL-15 or the transcription factor eomesodermin (EOMES)²⁷. As these cells were phenotypically similar to those undergoing ‘space filling’ homeostatic proliferation in a lymphopenic environment, the term ‘virtual memory’ seemed an appropriate, if somewhat ‘tongue-in-cheek’, name for this particular subset given the use of this term in computing to describe a form of memory that is based on an alternative use of disc space (FIG. 1).

A distinction with a difference

It is reasonable at this point to question whether a T_{IM} – T_{VM} cell paradigm propagates a distinction without a difference. Although some differences will already be evident, the two types of cell are highly similar in terms of their phenotype and their capacity to protect a host against infectious challenge^{27,30,44}. Indeed, although T_{IM} cells within the thymus express increased levels of CD49d relative to the T_{VM} cells found in the periphery²⁷, it

becomes nearly impossible to differentiate between the two subsets using this marker once the cells are outside the thymus. In addition, although T_{VM} cells are still present in IL-4-deficient mice, they express high levels of the IL-4 receptor²⁸, which suggests that even if IL-4 is unnecessary for their development, they are most probably still influenced by IL-4-induced signalling. Confusing things further, although T_{IM} cells are ablated in the absence of IL-4 regardless of the background mouse strain, the influence of IL-4 and IL-15 on T_{VM} cells in the periphery varies considerably depending on the strain of mice used^{45,46}. In an effort to make sense of these complexities and provide support for instigating a T_{IM}-T_{VM} cell paradigm, we present a comprehensive assessment of the differences between T_{IM} and T_{VM} cells (FIG. 2; TABLE 1), an overall and (hopefully) unifying model of the developmental interplay between the subsets (FIG. 1), and support for the translation of these differences beyond the mouse into the setting of human immunology (TABLE 1).

Sites of development

One of the most salient (and easily distinguishable) differences between T_{IM} and T_{VM} cells is their distinct sites of origin: T_{IM} cells develop in the thymus, whereas T_{VM} cells develop in the periphery^{29,43} (FIG. 1; TABLE 1). As for the precursors of each cell type, it is unclear whether or not the conversion of a developing CD8⁺ T cell into a T_{IM} cell within the thymus is purely stochastic or based on an as-yet-unidentified property of the T cell. By contrast, we recently showed that the naive T cells with the highest levels of CD5 expression (that is, those with a CD44^{low}CD5^{hi} phenotype) are the most likely to convert into T_{VM} cells in the periphery²⁸. CD5 is a surface molecule for which the expression levels directly correlate with the strength of signal that a developing T cell receives through its TCR upon positive selection by self-peptide-MHC in the thymus. Thus, CD5 expression is an effective proxy for the T cells within the repertoire that have the greatest affinity for self-ligands. CD5 was previously shown to stratify the homeostatic proliferation potential of naive T cells within a lymphopenic environment, with the T cells that show the highest CD5 expression being the most proliferative. Thus, it was perhaps not surprising to find that CD5^{hi} naive T cells are also the most likely to convert into a T_{VM} cell phenotype within lymphoreplete hosts^{27,28} (FIG. 1). The differential responsiveness of naive CD8⁺ T cells based on their CD5 levels adds to a growing number of studies showing that thymic selection produces a far more heterogeneous naive T cell pool than was previously appreciated. By performing RNA sequencing, Stephen Jameson and colleagues⁴⁷ conclusively demonstrated this heterogeneity between CD5^{hi} and CD5^{low} T cells, with predominant differences being in the expression of factors that are involved in sensing and responding to the inflammatory environment (for example, receptors such as CXC-chemokine receptor 3 (CXCR3) and CD122, and the transcription factor EOMES)⁴⁷. Our data confirmed their initial results and extended this transcriptional analysis to include T_{VM} cells, showing that the expression of receptors sensitive to inflammation increases even further as cells enter the T_{VM} cell pool²⁷. It remains to be determined whether the transcriptional profiles of these subsets will be useful in establishing a definitive precursor of T_{IM} cells as well.

Cytokine dependency

In conjunction with these sites of origin, the story of T_{IM} cell and T_{VM} cell development is largely a tale of two cytokines: IL-4 and IL-15. It is fair to say that the major difference between these two cell types is in the part played by IL-4: T_{IM} cell development is abrogated in the absence of IL-4, whereas T_{VM} cell development is not. This requirement for IL-4 was demonstrated in the earliest publications, in which mice deficient in ITK, ID3 or RLK were found to produce increased numbers of T_{IM} cells compared with wild-type controls^{22,24}. In these strains, the loss of IL-4, PLZF or NKT cells resulted in the loss of thymic T_{IM} cells^{26,37}. The connection between T_{IM} cells and IL-4, PLZF and NKT cells is not limited to these mutant strains, as the size of the T_{IM} cell pool can also be accurately predicted from the amount of IL-4 produced by NKT cells in wild-type mouse strains^{38,40}. Thus, an obligate connection exists between intrathymic levels of IL-4 and T_{IM} cell development (FIG. 1).

By contrast, IL-4 deficiency has a more limited impact on the appearance of T_{VM} cells in the periphery. Whereas the loss of IL-4 produces a small, but reproducible and statistically significant, decrease in the frequency of T_{VM} cells, 80–90% of the T_{VM} cell population remains intact in the absence of IL-4 (REFS 27,43,48) (note that these studies were conducted in B6 mice; see the ‘Mouse strains’ section for strain considerations). However, cells with a T_{VM} cell phenotype are absent from the periphery of IL-15-deficient mice and are also not detected in mice with CD122-deficient T cells²⁷. Experiments that used an IL-15-reporter mouse developed by Philippa Marrack and colleagues showed that basic leucine zipper transcriptional factor ATF-like 3 (BATF3)-dependent dendritic cell (DC) subsets are the predominant producers of IL-15 in the steady state²⁷. By comparing T_{VM} cell development in BATF3-deficient mice on different background strains (B6/129 versus B6 background), we identified CD8α⁺ lymphoid-resident DCs as being the cells that are largely responsible for T_{VM} cell development in the periphery²⁷. Productive IL-15 signalling for T_{VM} cell development requires EOMES^{27,49}, and results in the increased expression of both EOMES and T-bet. Curiously, however, T-bet expression is not necessary for T_{VM} cell development, and this transcription factor may even inhibit their development, as T-bet-deficient mice often have elevated frequencies of T_{VM} cells (R.M.K., unpublished observations). Regardless, the loss of IL-15 expression (in B6 mice) has essentially the same impact on T_{VM} cell development in the periphery as does the loss of IL-4 on T_{IM} cell development in the thymus.

That said, although the peripheral pool of T_{VM} cells is not IL-4 dependent, it is inarguably IL-4 sensitive. For one, it is likely that IL-4-dependent T_{IM} cells from the thymus seed the periphery at a rate that is yet to be determined. As cells derived from T_{IM} cells and T_{VM} cells seem to have the same phenotype once they are in the periphery (that is, a CD44^{hi}CD122^{hi}CD49d^{low} phenotype), the degree to which the number of cells with a T_{VM} phenotype decreases in IL-4-deficient hosts could mostly reflect the contribution of T_{IM} cells to the periphery (FIG. 1). In addition, and not exclusive of this, IL-4 could also more directly influence T_{VM} cell development. It is unclear at this point whether IL-4 is in some way developmentally necessary for a full complement of T_{VM} cells; whether IL-4 in the periphery can convert naive T cells into T_{VM} cells (analogously to the role of IL-15 in T_{VM}

cells); and whether IL-4 expands the existing T_{VM} cell population (FIG. 1). Evidence for each possible model has been published, although so far no data conclusively distinguish between these non-exclusive alternatives. First, it is conceivable that thymic IL-4 'programmes' a developing T cell in such a way that, once it becomes mature, the circulating naive T cell is more sensitive to the peripheral signals that convert naive CD8⁺ T cells into T_{VM} cells, potentially by tuning its responsiveness to IL-15. For example, IL-4 might help to induce increased expression of EOMES and/or CD122 in a developing T cell in the thymus, making the cell more sensitive to receiving IL-15 stimulation in the periphery. Indeed, type I interferon (IFN) has been shown to influence the size of the peripheral T_{VM} cell pool, most likely through just such a mechanism⁴⁹. Were this to be the case, however, one might expect the T_{VM} cells in IL-4-deficient mice to show reduced intracellular expression of EOMES, as is seen in T cells defective in type I IFN signalling⁴⁹. As the expression of EOMES is equivalent in T_{VM} cells from IL-4-deficient and those from wild-type mice (REF. 27 and R.M.K., unpublished observations), this seems unlikely.

Second, peripheral IL-4 may directly convert some naive T cells into T_{VM} cells independently of IL-15. Some data are consistent with this, including the fact that peripheral T_{VM} cells are always reduced in number in IL-4-deficient mice. In this model, IL-4 is required to drive the production of all T_{IM} cells in the thymus and a proportion of T_{VM} cells in the periphery of wild-type mice. The loss of IL-4 would then lead to the ablation of T_{IM} cell production in the thymus and a loss of only the IL-4-dependent T_{VM} cells in the periphery, which would leave behind IL-4-independent T_{VM} cells. Potentially supporting this model are data showing that an excess of IL-4 in the periphery (either in mice that overexpress IL-4 or in mice administered exogenous IL-4 complexes) induces the proliferation of naive T cells and their upregulation of some phenotypical markers of memory, most notably EOMES and CXCR3 (REFS 48,50). However, two points are noteworthy with regard to this possible role of IL-4 in T_{VM} cell development. First, the fact that T_{VM} cells are absent from IL-15-deficient mice, in which IL-4 expression is normal, calls into question the capacity of IL-4 alone to promote the development of T_{VM} cells in the periphery. Second, the stimulation of naive cells with excess IL-4 induced T cell proliferation but not the upregulation of established markers of antigen-inexperienced memory T cells such as CD122 or CD44 (REF. 51). Similarly, the transfer of naive CD8⁺ T cells into mice overexpressing IL-4 induces their acquisition of an EOMES^{hi}CD49d^{low} phenotype, but again their expression of CD122 remains low⁴⁸. Consistent with these results, *in vivo* administration of high doses of IL-4 in our laboratory induces the proliferation of both naive and memory-phenotype CD8⁺ T cells *in vivo*, but lowers their expression of both CD44 and CD122 (J.T.W., unpublished observations). This loss of CD44 and CD122 is in sharp contrast to the influence of excess IL-15 stimulation, which leads to the substantial upregulation of both of these markers^{27,28} (FIG. 2). This is not to dispute the fact that high-dose IL-4 stimulation of naive-phenotype CD8⁺ T cells in the periphery creates memory-phenotype cells of some sort, but their consistent lack of canonical T_{VM} cell markers, such as CD44 and CD122, makes them difficult to include in the T_{IM}-T_{VM} cell paradigm. Indeed, the importance of peripheral IL-4 was highlighted in independent reports from the laboratories of Steve Jameson and David Hildeman^{45,46}, in which the authors

convincingly showed that the absence of IL-4 has dramatic effects on the overall development of functional CD8⁺ T cell memory in response to infectious challenge.

Lastly, and in our view the most probable explanation, physiological levels of IL-4 (as opposed to the high doses of IL-4 used in the experiments described above) may exert effects on T_{VM} cells in the periphery by further expanding the pre-existing T_{VM} cell pool. The fact that T_{VM} cells have significantly elevated expression of IL-4 receptor subunit- α (IL-4R α ; compared with naive T cells) suggests that IL-4 can stimulate and expand those T_{VM} cells that have already formed in response to IL-15 (REFS 28,51,52). Given this, we suggest that the influence of IL-4 on peripheral T_{VM} cells in wild-type mice is most likely to be the result of IL-4-induced thymic T_{IM} cell transit to the periphery combined with some degree of IL-4-induced proliferation of established T_{VM} cells (FIGS 1,2). Whether the effect of IL-4 on T_{VM} cells is stochastic, or if the T_{VM} cell pool can be subdivided into cells that are more or less susceptible to proliferating in response to IL-4 (analogous to what is seen for CD5 levels and IL-15 sensitivity), remains to be determined.

It is worth mentioning that T_{IM} cells have also been shown to be IL-15 dependent (in B6 mice; see the 'Mouse strains' section below), further complicating the hierarchy of cytokine dependencies for this cell type. Although this property of T_{IM} cells has not been extensively explored, IL-15 may have important roles in the survival of T_{IM} cells and/or their transit from the thymus into the periphery²² (FIG. 1). The transcription factor KLF2 is required for T cells to egress from the thymus, as KLF2 regulates the expression of sphingosine 1-phosphate receptor 1 (S1P1)^{39,53}. KLF2 is also responsible for regulating S1P1 expression in activated CD8⁺ T cells and memory CD8⁺ T cells, specifically in response to IL-15 (REF. 54). Given these data, it is possible that T_{IM} cells require IL-15 stimulation to increase KLF2 and S1P1 expression in order to gain access to the thymic efferent lymphatics. IL-15 is also well known for its capacity to influence memory T cell survival⁵⁵, so although the egress of T_{IM} cells may well be intact in the absence of IL-15, their survival may not. Fortunately, these aspects of T_{IM} cell development are highly amenable to study and will certainly be ascertained in the near future.

Functions of T_{IM} and T_{VM} cells

Both T_{IM} and T_{VM} cell subsets are poised to produce IFN γ when they come into contact with their cognate antigen. As a result, both enact antigen-specific immune protection against infection far better than do naive CD8⁺ T cells^{27,28,44}. However, T_{VM} cells have two additional functions that are yet to be demonstrated in T_{IM} cells: namely, antigen-independent cytokine production and lytic activity (TABLE 1). Similarly to antigen-experienced memory CD8⁺ T cells, T_{VM} cells can produce IFN γ when stimulated with a combination of IL-12, IL-15 and IL-18. This occurs in the absence of any antigen encounter and facilitates a more innate-like role for T_{VM} cells in response to the inflammatory milieu alone. T_{IM} cells express EOMES at levels similar to those seen in T_{VM} cells^{22,25,26,38,39}, but they do not express T-bet^{26,38,39}, which may be a factor in determining whether or not they possess a similar capacity to produce IFN γ in response to stimulation with inflammatory cytokines alone. Regardless, it is highly unlikely that T_{IM} cells display the same bystander killing function as T_{VM} cells. T_{VM} cells express a range of molecules (for example,

NKG2D, IL-18 receptor, signal transducer and activator of transcription 4 (STAT4) and granzyme B) that Martin Prlic and colleagues⁵⁶ showed can facilitate bystander (antigen-nonspecific) killing by CD8⁺ T cells. The expression of these effector molecules facilitates T_{VM} cell-mediated bystander protection against infectious agents²⁸. Underscoring what may be the most important difference between the T_{IM} cell and T_{VM} cell subsets, T_{IM} cells do not express NKG2D^{30,50}. Thus far, only T_{VM} cells are known to be capable of both cytokine production and target recognition and killing independently of antigen recognition. Although not associated with any specific effector function, T_{VM} cell populations also expand in the host during ageing and show preferential trafficking to the liver²⁸. Although antigen stimulation may help to facilitate the persistence of T_{VM} cells over time⁵⁷, both of these features of T_{VM} cells are also probably related to their responsiveness to IL-15. IL-15 induces the long-term survival of memory CD4⁺ and CD8⁺ T cells, and the elevated CD122 expression of T_{VM} cells will almost certainly provide them with a competitive advantage for responding to IL-15. Furthermore, IL-15 is highly expressed in the liver⁵⁸. Given the high frequency of T_{VM} cells in the liver, it probably serves as a site where T_{VM} cells can be most efficiently stimulated over time.

In addition to T_{VM} cells, NK cells, MAIT cells, $\gamma\delta$ T cells and NKT cells are also enriched within the liver compared with in the blood. Interestingly, all of these populations display forms of innate-like behaviour that are not displayed by conventional $\alpha\beta$ T cells^{59,60}. Part of the shared functionality of these cells is probably due to the physiology of the liver. The portal vein supplies 80% of the blood flow into the liver, bypassing the spleen (the remaining 20% is arterially supplied)⁶¹. Thus, the liver is a front-line immune surveillance organ in which dietary antigens and pathogens from the gastrointestinal tract are often first encountered. Chronic exposure to metabolites derived from the gut flora from this portal blood flow creates a tolerogenic environment in the liver, in part owing to the induction of IL-10 production^{61–63}. It has been postulated that the presence of so many innate-like lymphocytes, which have less strict requirements for inflammatory cytokine production than do conventional $\alpha\beta$ T cells, may help to overcome this tolerogenic milieu by promoting the maturation of antigen-presenting cells⁵⁹. For the purposes of host protection, the importance of the liver in controlling infection is shown by the increased morbidity and mortality seen in mice that lack Kupffer cells (liver-resident macrophages), and by the fact that this increase is not seen in splenectomized mice after *Borrelia burgdorferi* infection⁶⁴. Furthermore, many chronic viral infections and particular developmental stages of parasites reside within the liver. These pathogens are difficult to clear for myriad reasons, yet it seems likely that inflammatory cytokine production and bystander killing by T_{VM} cells, in the absence of antigenic stimulation, could help to mount a more productive response against these difficult-to-clear pathogens.

Aside from their capacity to produce IFN γ in response to inflammatory cytokines³⁰, considerably fewer details are known about the functions of T_{IM} cells. The reliance of T_{IM} cells on IL-4 for their development might suggest that this population may be useful in host defence during type 2 immune responses (for example, in immune responses against helminths) in which IL-4 has an important role; this argument is bolstered further by the induction of a memory-like phenotype in peripheral T cells after the injection of IL-4 (REFS 48,50,65). If T_{IM} cells do have a role in type 2 immune responses, there is a possibility that

T_{VM} cells and T_{IM} cells act in complementary ways to each other, forming a type 1–type 2 axis within the antigen-inexperienced $CD8^+$ T cell populations. That said, this is currently purely speculative, and firm conclusions about the functional properties and potential benefits of T_{IM} cells and IL-4-induced memory cells await further experimentation.

Lastly, regardless of their origin, the presence of antigen-inexperienced memory-phenotype $CD8^+$ T cells specific for nominal antigen necessitates some reorientation of our views of ‘primary’ T cell responses to pathogens. It is now clear that essentially every T cell response, regardless of prior antigen exposure, is composed of both naive and memory responders. Aside from adding to the expanding complexity and heterogeneity of the primary immune response^{28,47}, this has already been shown to have an impact on the formation of antigen-specific memory. Stephen Jameson and colleagues⁴⁴ showed that T_{VM} cells preferentially become T_{CM} cells (as opposed to T_{EM} cells) after the resolution of a primary immune challenge. More recently, the laboratories of Stephen Jameson and David Hildeman separately showed differences in the formation of $CD8^+$ T cell memory between wild-type and IL-4-deficient BALB/c mice^{45,46}. Importantly, they showed that the impact of IL-4 occurred before pathogen exposure, which indicates that the alteration in conventional memory T cell subsets was directly related to the participation of T_{IM} and T_{VM} cell subsets. T_{VM} cells behave in the same way to a secondary challenge as do cells that have followed the classical naive-to- T_{CM} cell pathway⁴⁴, so it is not clear how far beyond the development of ‘primary immune memory’ (that is, the memory population formed after a single antigen encounter) the influence of T_{IM} and T_{VM} cells extends. Regardless, the universal presence of these cells in almost all model systems (even in recombination-activating gene (RAG)-deficient TCR-transgenic mice^{3,27}) mandates that they are taken into account.

Mouse strains

It is important to note that the vast majority of studies examining both T_{IM} and T_{VM} cell development and function have been performed in B6 mice. Although little has been done in other strains, such as the BALB/c strain, early indications are that both similarities and differences exist. First, the relationship between PLZF, NKT cells and IL-4 and T_{IM} cell development that was originally identified in B6 mice seems to also hold true in the BALB/c strain. The BALB/c strain and other strains that have elevated thymic levels of PLZF, NKT cells and IL-4 have correspondingly increased populations of T_{IM} cells. Similarly to B6 mice, the loss of IL-4 in BALB/c mice also ablates T_{IM} cell development^{26,38}. Thus, although the magnitude of T_{IM} cell development is determined by the mouse strain, the mechanisms that drive T_{IM} cell development seem to be the same.

Second, in both B6 and BALB/c mice, the loss of T_{IM} cells in the thymus does not lead to a complete loss of T_{VM} cells in the periphery. IL-4 deficiency in BALB/c mice does produce a greater reduction in T_{VM} cells in the periphery than is seen in IL-4-deficient B6 mice; whereas 80–90% of the T_{VM} cell population in the periphery of B6 mice is intact, the numbers of T_{VM} cells in IL-4-deficient BALB/c mice are reduced to ~30–50% of the levels seen in wild-type BALB/c mice^{45,46}. Given the increased thymic levels of PLZF and IL-4 in BALB/c mice, T_{IM} cells would be expected to contribute more to the peripheral pool of memory-phenotype cells in the BALB/c strain than in the B6 strain. Furthermore, if the

increased levels of IL-4 in the thymus of BALB/c mice correlate with increased systemic IL-4 levels, this would cause more proliferation of T_{VM} cells in the periphery of BALB/c mice than in that of B6 mice. Thus, a greater influence of IL-4 on T_{VM} cell frequency in the BALB/c host than in the B6 host is perhaps not surprising. However, the T_{VM} cell frequency in an IL-4-deficient BALB/c is far from zero, which indicates that cytokines other than IL-4 are responsible for the development of cells with a T_{VM} phenotype in the periphery. The obvious candidate is of course IL-15, but the available data suggest that this cytokine does not have the same obligate role in T_{VM} cells in BALB/c mice as it does in B6 mice. A recent report from David Hildeman and colleagues⁴⁵ compared antigen-specific T_{VM} cells in the periphery of wild-type, *Il4*^{-/-}, *Il15*^{-/-} and combined *Il4*^{-/-}*Il15*^{-/-} mice on the BALB/c background. Surprisingly, although the percentage of bulk T_{VM} cells was somewhat reduced (D. Hildeman, personal communication), IL-15-deficient hosts showed little reduction in the number of T_{VM} cells specific for lymphocytic choriomeningitis virus nucleoprotein.

Although more analysis of these hosts is necessary, at the very least these data indicate far less of a reliance on IL-15 for T_{VM} cell development in the BALB/c strain than in the B6 strain. Curiously, this result is in line with the expression of CD122 in the two strains; CD122 is expressed at much higher levels in B6 mice than in the BALB/c strain (S. Jameson and D. Hildeman, personal communication). Given the reduced expression of this IL-15 receptor subunit in BALB/c mice, it is perhaps not surprising that the presence or absence of T_{VM} cells (which in the BALB/c mouse are CD44^{hi}CD122^{hi}CXCR3^{hi} (REF. 46)) is less dependent on IL-15. In this regard, it is interesting to note that the major cytokines controlling homeostatic proliferation in both lymphopenic and lymphoreplete hosts all utilize the common γ -chain receptor subunit that is used for signalling by IL-2, IL-4, IL-7 and IL-15. Thus, perhaps strain differences in the production of T_{VM} cells are based on which common γ -chain-containing receptors are most highly expressed or used in that background strain. This remains speculation until the relevant cytokines and their receptors can be more conditionally and temporally controlled in the BALB/c strain, and their peripheral compartments examined for the gain or loss of T_{VM} cells.

Human T_{VM} cells

Although memory T cell subsetting in mice has become something of a 'cottage industry' in and of itself, a reasonable test of physiological and clinical relevance is whether or not an identified subset has a corresponding correlate in humans. Definitive proof of identical human equivalents of T_{IM} and T_{VM} cells is yet to be produced, but reasonable correlates have been identified. Although almost all T cells in human cord blood have a naive phenotype, a substantial number of T_{IM}-like cells were identified in spleen samples obtained from pre-term infants^{30,66,67}. Furthermore, PLZF is highly expressed in both thymic and splenic human fetal CD4⁺ T cells^{67,68}, consistent with the possibility of T_{IM} cell development, IL-4-mediated expansion of T_{VM} cells or both scenarios. Features of these fetal cells, such as EOMES expression and rapid IFN γ production⁶⁷, are again consistent with either T_{IM} or T_{VM} cell classifications.

In cord and adult blood, a human cell population that has phenotypical and functional similarities (including in terms of trafficking behaviour) to mouse T_{VM} cells was recently

described^{28,69} (TABLE 1). Unlike in the mouse, in which CD5 levels are essentially fixed upon egress from the thymus^{70,71}, CD5 expression on human T cells decreases as the T cell undergoes differentiation⁷². Furthermore, T cells that express the lowest levels of CD5 are the most sensitive to IL-15 and have the highest expression of CD122 (REF. 73). A small but reproducible population of CD5^{low}CD122^{hi} T cells can readily be found within the human CD45RA⁺CD27⁻ compartment (with high CD45RA expression and a lack of CD27 expression being canonical markers of naive and memory T cells, respectively⁷⁴). In addition, these CD5^{low}CD122^{hi} cells display the following features: first, increased expression of NUR77 (also known as NR4A1), a molecule for which expression correlates with the strength of TCR signal the cell receives⁷⁵ (suggesting a higher basal TCR–MHC self-affinity); second, expression of NK cell-associated markers, such as NKG2A, killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and KIR3DL2 (REF. 69); and third, expression of both EOMES⁷⁶ and T-bet^{28,69}. Collectively, these markers indicate a human cell that possesses the mouse T_{VM} cell-like characteristics of a higher affinity for self, a responsiveness to IL-15, and a capacity for bystander cytokine and killing functions²⁸. This phenotype places these putative T_{VM} cells squarely within the human CD8⁺ T cell subset known as terminally differentiated effector memory cells re-expressing CD45RA (TEMRA; also known as terminal effectors). As can be surmised from the name, these cells are believed to be largely terminally differentiated. Indeed, their short telomeres — the shortest found among human CD8⁺ T cell subsets⁷⁷ — is consistent with this. However, we and others noted the presence of a substantial pool of CCR7⁺ T cells that are included in many analyses of TEMRA⁶⁹. Furthermore, this CCR7⁺ population is where the majority of EOMES⁺ cells are found, and these CCR7⁺ cells both increase in number with age and show increased trafficking to the liver with age (J.T.W., unpublished observations). Lastly, these cells can be found in both adult and cord blood²⁸. Although this is not formal proof, it is at least consistent with the idea that at least some of the cells in this population could be derived from antigen-inexperienced precursors.

Antigen-inexperienced CD4⁺ T_{VM}

We have focused on CD8⁺ T cells for the main reason that antigen-inexperienced memory CD4⁺ T cells have yet to be described in mice. Although memory CD4⁺ T cells are found in unmanipulated mice, all of these are CD49d^{hi} (REF. 27), which indicates that they have developed as a result of antigen encounter. Perhaps most tellingly, MHC class II-restricted TCR-transgenic mice have no CD44^{hi} T cells in the absence of secondary TCRs (that is, when maintained on a RAG-knockout background)⁴², whereas the majority of MHC class I-restricted TCR-transgenic mice do³. This is consistent with the interpretation that memory CD4⁺ T cells do not arise in an antigen-inexperienced fashion within a wild-type lymphoreplete mouse. Although the reasons for this are unknown, it may well be related to the substantially lower expression of CD122 on CD4⁺ T cells²⁷ relative to CD8⁺ T cells, which makes CD4⁺ T cells less susceptible to IL-15-mediated proliferation, much like the CD8⁺ T cells in the BALB/c host. While this would seem to suggest that all memory-phenotype CD4⁺ T cells are the result of antigen experience, the possibility remains that differentiating the memory CD4⁺ T cell populations in mice requires different markers. Although the search for CD4⁺ T_{VM} cells in mice has not been successful so far, there is

reason to believe that these cells may exist in humans. Recent studies from the laboratory of Mark Davis⁷⁸ suggest that some form of antigen-inexperienced CD4⁺ T cells most likely exist, and it is now a question of refining the search, as one group has done by analysing the complementarity-determining region sequences of naive and memory-phenotype CD4⁺ T cells⁷⁹.

Concluding remarks

A reasonable conclusion from the data on both T_{IM} cells and T_{VM} cells is that the host seems 'determined' to develop memory-phenotype CD8⁺ T cells in the presence or absence of antigen exposure. As with any biological phenomenon, this is either because evolution favoured the creation of these cells to benefit the organism or because they are the epiphenomenal result of some other evolutionarily conserved mechanism. Any answer to this question is clearly speculative, but the benefits of the spontaneous production of memory-phenotype T cells with non-antigen-dependent protective capacity seem obvious. Organisms go through periods of lymphopenia, both during the neonatal period and after thymic involution^{2,80,81}. The organism is at greater risk from infection during these time periods; the fewer T cells that an organism has at its disposal, the less likely the organism is to have T cell clones that are specific for any given pathogen, which increases the need for a broader T cell response^{57,82}. The production of T_{IM} cells and T_{VM} cells would be evolutionarily beneficial as a mechanism for generating CD8⁺ T cells that can respond robustly in conditions in which the T cell compartment is compromised. The high expression of chemokine receptors (namely, CXCR3) and adhesion receptors (namely, CD11a, CD44 and LY6C) on T_{IM} and T_{VM} cells suggests that they would be among some of the first cells to be recruited to sites of infection. The ability of T_{VM} cells to carry out bystander killing makes them even better suited to early pathogen control. As infections are increasingly encountered over time, it is in the best interests of the organism to favour the memory cells that can respond to that infection again. Antigen-inexperienced memory subsets, such as T_{IM} and T_{VM} cells, would be expected to eventually make way for the antigen-experienced memory cells, an inverse correlation that is consistent with published data⁴⁴. Furthermore, the fact that the CD8⁺ T cells with the highest self-affinity are converted into T_{VM} cells may even provide a way in which the organism can protect itself against autoimmunity, given that memory-phenotype T cells have decreased sensitivity to antigen and increased sensitivity to cytokines⁸³. Thus, although questions remain regarding the development and function of antigen-inexperienced memory CD8⁺ T cells, they seem to be unquestionably useful to the organism. The data summarized in this article argue that the T_{IM}–T_{VM} cell paradigm will be useful as antigen-inexperienced memory T cells are further studied in both mice and humans.

Acknowledgments

The authors wish to thank C. Suhr, S. Jameson and D. Hildeman for their helpful communications. This work was funded by US National Institutes of Health grants AI101205 and AI066121.

References

1. Dobber R, Hertogh-Huijbregts A, Rozing J, Bottomly K, Nagelkerken L. The involvement of the intestinal microflora in the expansion of CD4⁺ T cells with a naive phenotype in the periphery. *Dev Immunol.* 1992; 2:141–150. [PubMed: 1386544]
2. Le Campion A, et al. Naive T cells proliferate strongly in neonatal mice in response to self-peptide/self-MHC complexes. *Proc Natl Acad Sci USA.* 2002; 99:4538–4543. [PubMed: 11917110]
3. Haluszczak C, et al. The antigen-specific CD8⁺ T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *J Exp Med.* 2009; 206:435–448. [PubMed: 19188498]
4. Goldrath AW, Bevan MJ. Low-affinity ligands for the TCR drive proliferation of mature CD8⁺ T cells in lymphopenic hosts. *Immunity.* 1999; 11:183–190. [PubMed: 10485653]
5. Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J Exp Med.* 2000; 192:557–564. [PubMed: 10952725]
6. Kieper WC, Jameson SC. Homeostatic expansion and phenotypic conversion of naive T cells in response to self peptide/MHC ligands. *Proc Natl Acad Sci USA.* 1999; 96:13306–13311. [PubMed: 10557316]
7. Surh CD, Sprent J. Homeostatic T cell proliferation: how far can T cells be activated to self-ligands? *J Exp Med.* 2000; 192:9–14.
8. Cho JH, et al. An intense form of homeostatic proliferation of naive CD8⁺ cells driven by IL-2. *J Exp Med.* 2007; 204:1787–1801. [PubMed: 17664294]
9. Stoklasek TA, Colpitts SL, Smilowitz HM, Lefrançois L. MHC class I and TCR avidity control the CD8 T cell response to IL-15/IL-15R α complex. *J Immunol.* 2010; 185:6857–6865. [PubMed: 21041729]
10. Sandau MM, Winstead CJ, Jameson SC. IL-15 is required for sustained lymphopenia-driven proliferation and accumulation of CD8 T cells. *J Immunol.* 2007; 179:120–125. [PubMed: 17579029]
11. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. *Nat Immunol.* 2000; 1:426–432. [PubMed: 11062503]
12. Tan JT, et al. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci USA.* 2001; 98:8732–8737. [PubMed: 11447288]
13. Guimond M, et al. Interleukin 7 signaling in dendritic cells regulates the homeostatic proliferation and niche size of CD4⁺ T cells. *Nat Immunol.* 2009; 10:149–157. [PubMed: 19136960]
14. Napolitano LA, et al. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat Med.* 2001; 7:73–79. [PubMed: 11135619]
15. Fry TJ, et al. A potential role for interleukin-7 in T-cell homeostasis. *Blood.* 2001; 97:2983–2990. [PubMed: 11342421]
16. Ernst B, Lee DS, Chang JM, Sprent J, Surh CD. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity.* 1999; 11:173–181. [PubMed: 10485652]
17. Cho JH, Kim HO, Surh CD, Sprent J. T cell receptor-dependent regulation of lipid rafts controls naive CD8⁺ T cell homeostasis. *Immunity.* 2010; 32:214–226. [PubMed: 20137986]
18. Varshney P, Yadav V, Saini N. Lipid rafts in immune signalling: current progress and future perspective. *Immunology.* 2016; 149:13–24. [PubMed: 27153983]
19. Takada K, Jameson SC. Self-class I MHC molecules support survival of naive CD8 T cells, but depress their functional sensitivity through regulation of CD8 expression levels. *J Exp Med.* 2009; 206:2253–2269. [PubMed: 19752186]
20. Goldrath AW, Luckey CJ, Park R, Benoist C, Mathis D. The molecular program induced in T cells undergoing homeostatic proliferation. *Proc Natl Acad Sci USA.* 2004; 101:16885–16890. [PubMed: 15548615]
21. Wyss L, et al. Affinity for self antigen selects T_{reg} cells with distinct functional properties. *Nat Immunol.* 2016; 17:1093–1101. [PubMed: 27478940]

22. Atherly LO, et al. The Tec family tyrosine kinases Itk and Rlk regulate the development of conventional CD8⁺ T Cells. *Immunity*. 2006; 25:79–91. [PubMed: 16860759]
23. Berg LJ. Signalling through TEC kinases regulates conventional versus innate CD8⁺ T-cell development. *Nat Rev Immunol*. 2007; 7:479–485. [PubMed: 17479128]
24. Broussard C, et al. Altered development of CD8⁺ T cell lineages in mice deficient for the Tec kinases Itk and Rlk. *Immunity*. 2006; 25:93–104. [PubMed: 16860760]
25. Horai R, et al. Requirements for selection of conventional and innate T lymphocyte lineages. *Immunity*. 2007; 27:775–785. [PubMed: 18031697]
26. Weinreich MA, Odumade OA, Jameson SC, Hogquist KA. T cells expressing the transcription factor PLZF regulate the development of memory-like CD8⁺ T cells. *Nat Immunol*. 2010; 11:709–716. [PubMed: 20601952]
27. Sosinowski T, et al. CD8α⁺ dendritic cell *trans* presentation of IL-15 to naive CD8⁺ T cells produces antigen-inexperienced T cells in the periphery with memory phenotype and function. *J Immunol*. 2013; 190:1936–1947. [PubMed: 23355737]
28. White JT, et al. Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. *Nat Commun*. 2016; 7:11291. [PubMed: 27097762]
29. Lee YJ, Jameson SC, Hogquist KA. Alternative memory in the CD8 T cell lineage. *Trends Immunol*. 2011; 32:50–56. [PubMed: 21288770]
30. Jameson, SC., Lee, YJ., Hogquist, KA. *Advances in Immunology*. Frederick, WA., editor. Vol. 126. Academic Press; 2015. p. 3-213.Ch. 4
31. Van Kaer L. Innate and virtual memory T cells in man. *Eur J Immunol*. 2015; 45:1916–1920. [PubMed: 26013879]
32. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol*. 2011; 12:21–27. [PubMed: 21113163]
33. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol*. 2012; 30:647–675. [PubMed: 22224763]
34. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol*. 2011; 11:445–456. [PubMed: 21681197]
35. Qiu Y, Peng K, Liu M, Xiao W, Yang H. CD8αα TCRαβ intraepithelial lymphocytes in the mouse gut. *Dig Dis Sci*. 2016; 61:1451–1460. [PubMed: 26769056]
36. Fukuyama T, et al. Histone acetyltransferase CBP is vital to demarcate conventional and innate CD8⁺ T-cell development. *Mol Cell Biol*. 2009; 29:3894–3904. [PubMed: 19433445]
37. Verykokakis M, Boos MD, Bendelac A, Kee BL. SAP protein-dependent natural killer T-like cells regulate the development of CD8⁺ T cells with innate lymphocyte characteristics. *Immunity*. 2010; 33:203–215. [PubMed: 20674402]
38. Lee YJ, Holzapfel KL, Zhu J, Jameson SC, Hogquist KA. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nat Immunol*. 2013; 14:1146–1154. [PubMed: 24097110]
39. Weinreich MA, et al. KLF2 transcription-factor deficiency in T cells results in unrestrained cytokine production and upregulation of bystander chemokine receptors. *Immunity*. 2009; 31:122–130. [PubMed: 19592277]
40. Lee YJ, et al. Tissue-specific distribution of iNKT cells impacts their cytokine response. *Immunity*. 2015; 43:566–578. [PubMed: 26362265]
41. Koschella M, Voehringer D, Pircher H. CD40 ligation *in vivo* induces bystander proliferation of memory phenotype CD8 T cells. *J Immunol*. 2004; 172:4804–4811. [PubMed: 15067057]
42. Moon JJ, et al. Naive CD4⁺ T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity*. 2007; 27:203–213. [PubMed: 17707129]
43. Akue AD, Lee JY, Jameson SC. Derivation and maintenance of virtual memory CD8 T cells. *J Immunol*. 2012; 188:2516–2523. [PubMed: 22308307]
44. Lee JY, Hamilton SE, Akue AD, Hogquist KA, Jameson SC. Virtual memory CD8 T cells display unique functional properties. *Proc Natl Acad Sci USA*. 2013; 110:13498–13503. [PubMed: 23898211]

45. Tripathi P, et al. IL-4 and IL-15 promotion of virtual memory CD8⁺ T cells is determined by genetic background. *Eur J Immunol.* 2016; 46:2333–2339. [PubMed: 27457412]
46. Renkema KR, et al. IL-4 sensitivity shapes the peripheral CD8⁺ T cell pool and response to infection. *J Exp Med.* 2016; 213:1319–1329. [PubMed: 27298446]
47. Fulton RB, et al. The TCR's sensitivity to self peptide- MHC dictates the ability of naive CD8⁺ T cells to respond to foreign antigens. *Nat Immunol.* 2015; 16:107–117. [PubMed: 25419629]
48. Kurzweil V, LaRoche A, Oliver PM. Increased peripheral IL-4 leads to an expanded virtual memory CD8⁺ population. *J Immunol.* 2014; 192:5643–5651. [PubMed: 24795452]
49. Martinet V, et al. Type I interferons regulate eomesodermin expression and the development of unconventional memory CD8⁺ T cells. *Nat Commun.* 2015; 6:7089. [PubMed: 25953241]
50. Ventre E, et al. Negative regulation of NKG2D expression by IL-4 in memory CD8 T cells. *J Immunol.* 2012; 189:3480–3489. [PubMed: 22942430]
51. Morris SC, et al. Endogenously produced IL-4 nonredundantly stimulates CD8⁺ T cell proliferation. *J Immunol.* 2009; 182:1429–1438. [PubMed: 19155490]
52. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody–cytokine immune complexes. *Science.* 2006; 311:1924–1927. [PubMed: 16484453]
53. Carlson CM, et al. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature.* 2006; 442:299–302. [PubMed: 16855590]
54. Takada K, et al. Kruppel-like factor 2 is required for trafficking but not quiescence in postactivated T cells. *J Immunol.* 2011; 186:775–783. [PubMed: 21160050]
55. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8⁺ memory T cells by opposing cytokines. *Science.* 2000; 288:675–678. [PubMed: 10784451]
56. Chu T, et al. Bystander-activated memory CD8 T cells control early pathogen load in an innate-like, NKG2D-dependent manner. *Cell Rep.* 2013; 3:701–708. [PubMed: 23523350]
57. Rudd BD, et al. Nonrandom attrition of the naive CD8⁺ T-cell pool with aging governed by T-cell receptor:pMHC interactions. *Proc Natl Acad Sci USA.* 2011; 108:13694–13699. [PubMed: 21813761]
58. Correia MP, et al. Hepatocytes and IL-15: a favorable microenvironment for T cell survival and CD8⁺ T cell differentiation. *J Immunol.* 2009; 182:6149–6159. [PubMed: 19414768]
59. Doherty DG. Immunity, tolerance and autoimmunity in the liver: a comprehensive review. *J Autoimmun.* 2016; 66:60–75. [PubMed: 26358406]
60. Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol.* 2013; 14:996–1006. [PubMed: 24048121]
61. Crispe IN. Liver antigen-presenting cells. *J Hepatol.* 2011; 54:357–365. [PubMed: 21084131]
62. Knolle P, et al. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. *J Hepatol.* 1995; 22:226–229. [PubMed: 7790711]
63. Zhang M, Xu S, Han Y, Cao X. Apoptotic cells attenuate fulminant hepatitis by priming Kupffer cells to produce interleukin-10 through membrane-bound TGF- β . *Hepatology.* 2011; 53:306–316. [PubMed: 21140375]
64. Lee WY, et al. An intravascular immune response to *Borrelia burgdorferi* involves Kupffer cells and iNKT cells. *Nat Immunol.* 2010; 11:295–302. [PubMed: 20228796]
65. Spellberg B, Edwards JE. Type 1/type 2 immunity in infectious diseases. *Clin Infect Dis.* 2001; 32:76–102. [PubMed: 11118387]
66. Byrne JA, Stankovic AK, Cooper MD. A novel subpopulation of primed T cells in the human fetus. *J Immunol.* 1994; 152:3098–3106. [PubMed: 8144905]
67. Min HS, et al. MHC class II-restricted interaction between thymocytes plays an essential role in the production of innate CD8⁺ T cells. *J Immunol.* 2011; 186:5749–5757. [PubMed: 21478404]
68. Lee YJ, et al. Generation of PLZF⁺ CD4⁺ T cells via MHC class II-dependent thymocyte–thymocyte interaction is a physiological process in humans. *J Exp Med.* 2010; 207:237–246. [PubMed: 20038602]
69. Jacomet F, et al. Evidence for eomesodermin-expressing innate-like CD8⁺ KIR/NKG2A⁺ T cells in human adults and cord blood samples. *Eur J Immunol.* 2015; 45:1926–1933. [PubMed: 25903796]

70. Azzam HS, et al. Fine tuning of TCR signaling by CD5. *J Immunol.* 2001; 166:5464–5472. [PubMed: 11313384]
71. Azzam HS, et al. CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity. *J Exp Med.* 1998; 188:2301–2311. [PubMed: 9858516]
72. Herndler-Brandstetter D, et al. Post-thymic regulation of CD5 levels in human memory T cells is inversely associated with the strength of responsiveness to interleukin-15. *Hum Immunol.* 2011; 72:627–631. [PubMed: 21539877]
73. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8⁺ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood.* 2003; 101:4260–4266. [PubMed: 12576317]
74. Larbi A, Fulop T. From “truly naive” to “exhausted senescent” T cells: when markers predict functionality. *Cytometry A.* 2014; 85:25–35. [PubMed: 24124072]
75. Moran AE, et al. T cell receptor signal strength in T_{reg} and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J Exp Med.* 2011; 208:1279–1289. [PubMed: 21606508]
76. Intlekofer AM, et al. Effector and memory CD8⁺ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol.* 2005; 6:1236–1244. [PubMed: 16273099]
77. Romero P, et al. Four functionally distinct populations of human effector-memory CD8⁺ T lymphocytes. *J Immunol.* 2007; 178:4112–4119. [PubMed: 17371966]
78. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4⁺ memory-phenotype T cells are abundant in unexposed adults. *Immunity.* 2013; 38:373–383. [PubMed: 23395677]
79. Marusina AI, et al. CD4⁺ virtual memory: antigen-inexperienced T cells reside in the naive, regulatory, and memory T cell compartments at similar frequencies, implications for autoimmunity. *J Autoimmun.* 2017; 77:76–88. [PubMed: 27894837]
80. Schüler T, Hämmerling GJ, Arnold B. Cutting edge: IL-7-dependent homeostatic proliferation of CD8⁺ T cells in neonatal mice allows the generation of long-lived natural memory T cells. *J Immunol.* 2004; 172:15–19. [PubMed: 14688303]
81. Lynch HE, et al. Thymic involution and immune reconstitution. *Trends Immunol.* 2009; 30:366–373. [PubMed: 19540807]
82. Schwab R, et al. Expanded CD4⁺ and CD8⁺ T cell clones in elderly humans. *J Immunol.* 1997; 158:4493–4499. [PubMed: 9127016]
83. Mehlhop-Williams ER, Bevan MJ. Memory CD8⁺ T cells exhibit increased antigen threshold requirements for recall proliferation. *J Exp Med.* 2014; 211:345–356. [PubMed: 24493801]
84. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med.* 1994; 179:347–351. [PubMed: 8270879]
85. Hamann D, et al. Phenotypic and functional separation of memory and effector human CD8⁺ T cells. *J Exp Med.* 1997; 186:1407–1418. [PubMed: 9348298]

Figure 1. A model of the development of innate memory and virtual memory T cells

Innate memory T (T_{IM}) cell development: in the thymus, naive $CD8^+$ T cells are exposed to interleukin-4 (IL-4) derived from promyelocytic leukaemia zinc finger (PLZF)-expressing thymocytes and convert into T_{IM} cells. In the periphery, the presence of T_{IM} cells is dependent on IL-15, either because it is necessary for T_{IM} cells to exit the thymus or because it is necessary for their survival once they are in the periphery. Although T_{IM} cells in the thymus do not express T-bet, all antigen-inexperienced memory-phenotype $CD8^+$ T cells in the periphery (defined by a $CD44^{hi}CD49d^{low}CD122^{hi}$ phenotype) express both eomesodermin (EOMES) and T-bet. This suggests that the majority of T_{IM} cells in the periphery have encountered IL-15, which results in their phenotype becoming indistinguishable from that of virtual memory T (T_{VM}) cells. T_{VM} cell development: naive $CD8^+$ T cells expressing T cell receptors (TCRs) with a strong affinity for self-peptide–MHC complexes express high levels of CD5, exit the thymus and convert into T_{VM} cells following their interaction with IL-15 that is trans-presented by lymphoid-resident $CD8\alpha^+$ dendritic cells (DCs). IL-4 may act on existing T_{VM} cells to further expand the T_{VM} cell population, which depends on IL-15 for survival. Dashed arrows indicate differentiation. IL-4R, IL-4 receptor.

Figure 2. The influence of cytokine complexes on naive and memory $CD8^{+}$ T cell subsets
a | Interleukin-15 (IL-15)–IL-15 receptor subunit- α (IL-15R α) complexes induce a substantial expansion of cells with a virtual memory T (T_{VM}) phenotype. This most likely occurs through the combined effects of (i) stimulation and expansion of thymic-emigrant innate memory T (T_{IM}) cells, (ii) the conversion of naive $CD8^{+}$ T cells that have more moderate levels of CD5 expression (with lower self-affinity) and (iii) the expansion of the pre-existing T_{VM} cell pool. By contrast, IL-4 cytokine–antibody complexes promote the conversion of both naive and T_{VM} cells into distinct populations of ‘IL-4-induced’ memory-phenotype cells, expanding them in the process. **b** | The main phenotypical characteristics of these three memory-phenotype populations (namely, T_{VM} cells, T_{IM} cells and ‘IL-4-induced’ memory-phenotype cells) are shown. CXCR3, CXC-chemokine receptor 3; EOMES, eomesodermin.

Table 1

A comparison of the distinguishing characteristics of antigen-inexperienced memory-phenotype cells

Memory CD8 ⁺ T cell subset	Phenotype	Location	Precursor	Cell dependence	Cytokine dependence: development	Cytokine dependence: maintenance	Functions	Refs
Mouse virtual memory T cell	• CD44 ^{hi}	•	Periphery	CD8α ⁺ dendritic cells	IL-15	IL-15	• Increased IFN γ production in response to cognate antigen and stimulation with IL-12 and IL-18	3,27,28, 43,44
	• CD122 ^{hi}	•	Increase in percentage in the liver with age					
	• CD49d ^{low}							
	• CXCR3 ^{hi}							
	• NKG2D ⁺						• Increased protection from type 1 infections (for example, <i>Listeria monocytogenes</i> infection)	
	• T-bet ⁺						• Bystander killing	
Mouse innate memory T cell	• CD44 ^{hi}	Thymus and periphery	Unknown	Thymic PLZF ⁺ cells	IL-4	IL-15	• Increased IFN γ production in response to cognate antigen	26,29, 30,84
	• CD122 ^{hi}							
	• CD49d ^{low}							
	• CXCR3 ^{hi}							
	• NKG2D ⁻						• Increased protection in type 2 infections (for example, helminth infections)	
	• T-bet ⁻							
	• EOMES ⁺							

Memory CD8 ⁺ T cell subset	Phenotype	Location	Precursor	Cell dependence	Cytokine dependence: development	Cytokine dependence: maintenance	Functions	Refs
Human innate-memory CD8 ⁺ T cell	•	CD45RA ⁺	•	Periphery	IL-15-experienced phenotype (CD5 ^{low} EOMES ⁺)	IL-15-responsive phenotype (CD122 ^{hi} EOMES ⁺ CD27 ⁻)	•	28,69,72, 73,85
	•	CD45RO ⁻	•	Increase in percentage in the liver with age				Production of IFN γ in response to stimulation with IL-12 and IL-18
	•	CD27 ⁻						
	•	CD5 ^{low}						
	•	CD122 ^{hi}					•	Bystander killing phenotype (express NKG2A, KIRs, granzyme B and perforin ⁺)
	•	NUR77 ^{hi}						
	•	NKG2A ⁺						
	•	KIR ⁺						
	•	T-bet ⁺					•	Rapid killing <i>in vitro</i>
	•	EOMES ⁺						

CXCR3, CXC-chemokine receptor 3; EOMES, comesodermin; IFN γ , interferon- γ ; IL-, interleukin; KIR, killer immunoglobulin receptor; PLZF, promyelocytic leukaemia zinc finger.