Going virtual as a memory-phenotype T lymphocyte

Bin Yang[1], Benjamin G. Dewals[2,3]

[1]Department of Laboratory Medicine, Department of Developmental Immunology, Division of Molecular Hematology, Faculty of Medicine, Lund University, Lund, Sweden
[2] Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine – FARAH, University of Liège, Liège, Belgium.
[3]Walloon Excellence in Life Sciences and Biotechnology (WELBIO) Department, WEL Research Institute, Wavre, Belgium.

*Correspondence: bgdewals@uliege.be (B.G. Dewals).

Abstract

Conventional or “true” memory CD8$^+$ T cells (T_{TM}) arise from immunologically naive T cells which circulate in the periphery after selection in the thymus. During infection or immunization by a foreign antigen, naive T cells can receive antigen-specific activation signals after recognition of MHC-antigenic peptide complexes. But the CD8$^+$ T cell population in immunologically naive hosts is not restricted to circulating naive T cells expecting cognate antigen encounter. Indeed, memory-phenotype T cells (T_{MP}) develop in absence of foreign antigen encounter and therefore exist in naive, pathogen-free as well as germ-free conditions. T_{MP} have been shown to mediate bystander cell killing through innate mechanisms, as well as rapidly respond to cognate antigen stimulation. While the existence of foreign antigen-inexperienced T_{MP} is now well acknowledged in laboratory mice, and also recognized in humans, the extensive nomenclature used for their description challenges the overall understanding of their multiple functions in health and disease. This article discusses the current understanding and controversies on the origin, maintenance and functions of the various populations recognized as T_{MP} and highlights some potential challenges for deciphering their fate.

Introduction

Foreign Ag exposure results in the clonal expansion of Ag-specific effector T cells after MHC-peptide recognition, which is followed by a contraction phase during which most T cells die and a small proportion of those Ag-specific T cells acquire a long-lived memory phenotype, namely conventional or "true" memory $CD8^+$ T cells (T_{TM}) [1]. Based on the expression of specific combinations of surface receptors and ligands, their longevity, as well as their effector, proliferative and migration characteristics, T_{TM} can be divided in three major subsets: central memory (T_{CM}), effector-memory (T_{EM}), and tissue resident-memory (T_{RM}) T cells [2]–[12]. There also exist more sub-populations characterized as long-lived effector cells, "peripheral" memory T cells, or stem cell-like memory T cells. We could extend the list with exhausted T cells (T_{EX}), which are not *per se* 'memory'.

When non-self Ag are absent and the host is *per definition* immunologically "naive", each circulating T lymphocyte has its own unique TCR, which is specific for an unencountered antigen. Although naive T cells (T_N) do not express memory markers, memory-like $CD8^+$ T cells can also be found in the periphery, as well as in the thymus of naive hosts [13]–[15]. Thus, these memory-phenotype T cells (T_{MP}) are immunologically "naive" but share several common features with T_{TM}: they are αβ T cells undergoing normal T cell receptor (TCR) rearrangement, they are present in the thymus and periphery, they upregulate memory markers such as CD44, CD122 (IL-2 receptor β), CXCR3, as well as transcription factors like EOMES and/or T-BET. However, T_{MP} downregulate the α4 integrin (CD49d), in contrast to T_{TM} [16]. Based on how these cells are induced and their localization, these Ag-inexperienced T_{MP} have been divided in three main subsets: homeostatic memory $CD8^+$ T cells (T_{HM}), found in lymphopenic conditions; innate memory $CD8^+$ T cells (T_{IM}) which were identified in the thymus; and virtual memory $CD8^+$ T cells (T_{VM}) which are found in the periphery. Of note, literature also reports several redundant denominations of cells having similar characteristics like "Ag-inexperienced memory T cells" or "regulatory $CD8^+$ T cells".

Unconventional T_{MP}: what do we understand?

T_{HM} cells – *a response to lymphopenia.* Resting T_N are selected in the thymus to become tolerant to self-antigens. However, the maintenance of T_N lymphocytes in the periphery also relies on a combination of low affinity TCR signals, along with homeostatic IL-7 stimulation [17]–[19]. Such "tonic" signals ensure that the T_N compartment is maintained with constant T cell number. Ionizing radiation or *Rag* deficiencies can cause lymphopenic conditions, which can also be experimentally induced by T cell antibody-based depletion. In lymphopenic hosts, both thymic *de novo* production of T_N and homeostatic stimulation of residual or adoptively transferred peripheral T cells can regenerate the T cell compartment. Adoptive transfer of T_N in a T lymphopenic environment not only results in the persistence of the donor cells, but also in their homeostatic proliferation and their conversion into memory-like T cells [17],[20]–[27], balancing the T cell pool [28],[29]. Although both $CD4^+$ and $CD8^+$ T cells can replenish the periphery by homeostatic proliferation, $CD8^+$ T cells are less dependent on thymus to recover normal cell numbers. Moreover, a recent study pointed that T_N recovery occurs after irradiation but only if the thymus was present, whereas surviving T cells after irradiation of athymic mice only gave rise to T_{MP}, which were also more responsive to homeostatic stimulation [30]. Thus, lymphopenia results in a replenishment of a T cell compartment enriched in T_{MP}, also affecting the TCR repertoire in the periphery. In term of phenotype, T_{HM} cells express CCR7 and CXCR5 and directly response to "tonic" self-antigen stimulation and homeostatic signals in specific lymphopenic conditions [16],[31]–[34] (**Table 1, Fig. 1**).

T_{IM} – *a thymic tale.* T_{MP} have been identified in thymic single-positive $CD8^+$ T cells (SP8) of some transgenic mouse strains, with increased EOMES expression but not T-BET [35],[36]. These cells were referred to as T_{IM} and were initially identified in the thymus of mice lacking the TEC kinase IL-2-inducible T cell kinase (ITK) or receptor like kinase (RLK) (**Table 1**) [37]. ITK-deficient and RLK/ITK double deficient mice are almost devoid of conventional SP8 thymocytes and contain a large number of SP8 cells that express consensus lineage markers indicative of T_{IM}. These cells develop in the thymus and acquire an effector phenotype in absence of foreign antigen encounter and share some characters with T_{TM} [38]–[44]. Importantly, the generation of T_{IM} is dependent on IL-4 produced in the thymus, via a PLZF-dependent mechanism and involving invariant NK-T lymphocytes (iNKT), present in BALB/c, DBA2 and CBA mice but not in 129 or C57BL/6 mice [45],[46], although iNKT seem to be dispensable in ITK-deficient mice in driving T_{IM} generation [47]. Phenotypically, T_{IM} display $CD44^+$ $CD122^+$ $EOMES^+$, and originate from SP8 naive T cells [48] (**Fig 1**). IL-4 is essential and sufficient to induce and expand T_{IM} with CXCR3 upregulation via EOMES in a cell-nonautonomous fashion [49],[50]. However, it remains unclear whether the IL-4-dependent conversion of developing $CD8^+$ T cells into T_{IM} within the thymus is stochastic or requires additional unidentified stimuli. Interestingly, T_{MP} in C57BL/6 mice develop from SP8 cells upregulating EOMES which is triggered by recognition of self-ligands during their maturation in the thymus [51]. However, potential combined effects of IL-4 and self-ligand recognition in the development of T_{IM} remains elusive.

T_{VM} – *a memory in naive cells*? Beyond lymphopenic-induced T_{HM} or thymic T_{IM}, T_{MP} are also circulating in the periphery of naive SPF or GF mice [16], meaning microbial antigens are not required for their differentiation. These cells were named virtual memory T cells (T_{VM}) and have a phenotype similar to T_{HM} and T_{IM}, with increased expression of CCR5, CXCR3, CXCR5 and variably express CCR2 [15],[52] (**Table**

1, Fig 1). T_{VM} form the majority of T_{MP} in unimmunized mice and around 10–20% of total $CD8^+$ T cells in the peripheral blood and secondary lymphoid organs in C57BL/6 mice, and even more abundant in BALB/c mice [53]–[57]. Although T_{IM} seem to express more CD49d (integrin α4 chain) when compared to T_{VM} in periphery [53], it remains imprecise to distinguish T_{IM} from T_{VM}.

Thus, T_{VM} were essentially defined as peripheral T_{MP} (meaning in circulating blood and SLOs) in a naive host (i.e. unexposed to its cognate antigen). Such definition infers that the history of antigenic exposure is known, which challenges the clear definition of such a population in humans. Nonetheless, the identification of human T_{VM} has been made as we will discuss in a following section. Another implication is that T_{IM} and T_{HM} cells potentially represent some particular T_{VM} present in the thymus or developing in response to homeostatic conditions, respectively. Finally, tissue-residence of T_{VM} is thus *per definition* a contradiction, but we will not discuss further T_{RM} cells and their potential development in absence of non-self stimulation.

Origin and maintenance of T_{VM}: a self-ligand tale?

How and when T_{VM} are being generated and are maintained over time are important questions in the understanding of how these cells function. Several key studies provided important insights in the dynamic of T_{VM} generation and maintenance throughout life, in particular how cytokine signals and TCR engagement are involved in these processes.

T_{VM} over time. Using inducible fate-mapping mice, newborn-derived $CD8^+$ T cells were shown to undergo more proliferation and preferentially develop into T_{VM} when compared to adult-derived $CD8^+$ T cells. Thus, $CD8^+$ T cells during the young age have a more inherent propensity to develop into T_{VM} [58], although T_{VM} are also produced in the adult age. In addition, although lymphopenia-induced proliferation might contribute to T_{VM} differentiation [59],[60], a lymphopenic environment in newborns does not seem to be required for the formation of T_{VM}. Importantly, neonatal $CD8^+$ T cells have also been shown to mediate enhanced innate-like bystander protection against unrelated pathogens, a mechanism controlled by a balance between BACH2 and AP-1 transcription factor activities [61]. How the neonatal period represents a T_{VM}-prone environment in the development of $CD8^+$ T cells remains to be explored. What seems to exist is a layered immune development of T cells during life where neonatal T cells are more prone to respond to inflammatory signals, whereas adult T cells have more avid TCR for antigenic responses [59].

Besides neonatal T_{VM} development, these cells also accumulate during aging at a period when thymic functions decline [17]. Whereas early studies reported T_{CM} cell accumulation with age as a potential result of lifelong foreign antigen exposure [54], such accumulation was rather explained by a lifelong accumulation of T_{VM} and not conventional memory T cells [62]. During aging, T_{VM} become senescent but not exhausted, similarly to aged T_N [63], and an elevated spare respiratory capacity (SRC, a higher mitochondrial energy reserve) was observed, together with anti-apoptotic Bcl-2 expression [64]. Thus, T_{VM} have a potential to be long-lived although their general lifespan is yet to be clarified. Asymmetric cell division is an important mechanism to generate T cell diversity and maintain a memory T cell pool. When compared with T_N, T_{VM} showed higher asymmetric cell division rate, likely contributing to the maintenance of the pool and to the response to primary influenza infection [65],[66]. Nevertheless, when investigating how the age, genetic background and microbiota composition could influence the gene expression program in both T_{VM} and T_{IM}, only few differences could be observed [67].

T_{VM} and cytokines. Signalling through common γ (γc) chain cytokines, including the interleukins IL-2, IL-4, IL-7 and IL-15 represent an essential driver in the homeostatic maintenance of memory T cells, including T_{MP} [68]. Homeostatic-driven proliferation requires IL-7 signals to maintain T_{HM} cells, which seem to keep their memory-phenotype despite some report of transient acquisition of memory following homeostatic proliferation [24],[69]–[72]. Besides IL-7, it is now clear that homeostatic proliferation of T_N can be driven by elevated amounts of other γc cytokines like IL-2 and IL-15. IL-15 is required for sustained lymphopenia-induced proliferation of $CD8^+$ T cells [73], and irradiation-induced lymphopenia enhances T cell memory formation via IL-15 [74]. At least for memory $CD8^+$ T cells, the homeostatic expansion can be supported by either IL-7 or IL-15 [75]–[79]. Recipient mice with deficiency of *Il2ra*, *Il2rb*, or *Il2rg* makes adoptively-transferred T_N undergo massive proliferation and rapidly acquire the characteristics of T_{MP} in presence of high amounts of IL-2 or a mixture of both IL-2 and IL-15, together with recognition of self-pMHC ligands [27],[80],[81]. T_N seem to effectively respond to IL-2 receptor signalling to convert into T_{HM} or T_{VM}. In addition, deficiency of suppressor of cytokine signalling-1 (SOCS-1) in $CD8^+$ T cells resulted in increased responsiveness to IL-15 and self-ligands, resulting in expansion of T_{MP} [81],[82]. During homeostatic proliferation, the degree of proliferation is dictated by the number of T_N with sufficiently high affinity for self-ligands in presence of peripheral IL-15 [83], and IL-15 is trans-presented by $CD8\alpha^+$ dendritic cells (DCs) to T_N to induce T_{VM} differentiation [53].

Type I and II interferons (IFN), IL-12 and IL-18, also enhance memory $CD8^+$ T cell survival and/or proliferation mediated through the induction of IL-15 [84]. Interestingly, the lack of IFN-γ receptor signalling in $CD8^+$ T cells promoted the differentiation into T_{MP} without affecting cell numbers [85],[86]. While type I IFN enhance or reduce memory T cell proliferation, depending on the dose [87],[88], signalling via IRF9 regulates the homeostasis and function of T_{VM} by driving EOMES expression [89]. Hence, type I IFN signalling is required to maintain the T_{VM} pool. In addition, T_{VM} have been reported as the main IFN-γ producers at steady state, regulating $CD8^+$ T cell avidity during primary response to foreign antigens and establishment of T_{TM} [90].

IL-4 – a particular scenario. Among key cytokines, IL-4 has been extensively demonstrated to maintain and expand T_{VM} in SLOs, and such IL-4 intriguing effects on T_{VM} were confirmed in IL-$4^{-/-}$ and IL-4R$\alpha^{-/-}$ mice in both BALB/c and C57BL/6 backgrounds [60],[91]–[94], although T_{VM} can arise in absence of IL-4 and IL-4Rα in the C57BL/6 background at steady-state [95]. In immunocompetent mice, IL-4 signalling in T_{VM} results in their expansion in the SLOs mainly explained by the induction of cell proliferation with a small contribution of cell recruitment, rather than conversion from T_N [93],[94]. IL-4 can drive EOMES expression and innate-like memory phenotype through cooperation between STAT6- and AKT-dependent pathways, in settings of attenuated TCR stimulation in CD8$^+$ T cells cultured *in vitro* [96]. Importantly, EOMES can directly drive BCL-2 expression to mediate the survival of low-affinity memory precursor [97]. Hence, although the exact mechanisms explaining how IL-4 drives T_{VM} expansion are yet to be elucidated, IL-4 could induce via EOMES an anti-apoptotic mechanism to maintain T_{MP}. Nevertheless, the requirement of IL-4 signals is not absolute as IL-4 deficiency on T_{VM} could be compensated by type I interferons to maintain their homeostasis [98]. The contribution of IL-4 and IL-15 in promoting T_{VM} maintenance seems to depend on the mouse background, where T_{VM} rely more on IL-4 in BALB/c mice, while IL-15 was shown to be essential in maintaining T_{VM} in C57BL/6 mice [99]. In-depth investigation of T_{VM} expressing Ly-6C and/or Sca-1 markers in response to type I IFN and in presence of IL-4 and/or IL-15 were shown to contribute to predominant Ly-6C$^+$ subsets, while type I IFNs and IL-15 contributed to Sca-1$^+$ and Sca-1$^-$ subsets, respectively [86]. Of note, Ly-6C$^+$ T_{VM} were also enriched after whole body irradiation, suggesting type I IFN in response to ionization results in phenotypic changes in T_{VM} [30].

Transcription regulation. T_{VM} induction, maintenance and functions are likely associated to a specific transcription factor program, in which EOMES plays an important role. Istaces and collaborators showed that T_{IM} acquired only a fraction of the fully active enhancer repertoire of T_{TM} [100], in which EOMES is recruited to RUNX3-bound regions and induces epigenetic changes in enhancer regions. However, the global epigenetic profile was not unique for T_{IM}. Epigenetic regulator BRG1 was shown to be critically involved in the program of acquisition of memory features by CD8$^+$ T cells under steady state and upon *in vivo* injection of IL-4 – anti-IL-4 antibody complexes (IL-4c) [100]. In addition, deletion of the histone methyltransferase DOT1L in the T cell lineage strongly increased the number of T_{VM} by controlling TCR signalling and regulating a network of other transcriptional and epigenetic regulators, like EZH2 [101],[101]. Moreover, the ubiquitin-like modifier (UFM1) E3 ligase (UFL1) suppressed BATF activity and apoptosis to promote T_{VM} survival and function [102]. Completing the role of EOMES regulation, a recent study suggested AIOLOS (IKZF3) represses the expression of EOMES and CD122, resulting in a tight regulation of T_{VM} generation [103].

Self-ligand recognition: a main driver. When we envision the origin and maintenance of T_{VM}, a long-lasting key question has been whether TCR engagement contribute or not. One of the first observation supporting the role of TCR signalling was that T_{VM} convert more likely from T cells expressing high levels of CD5, a marker associated with self-reactivity [83],[104]. Expression levels of CD5 on T cells are directly proportional to the affinity of the TCR [105], and the strength of the TCR affinity has been shown to determine the relative "fitness" of naive T cells to compete for factors such as IL-7 to support cell survival and homeostatic proliferation [106]. Moreover, $CD5^{high}$ T cells were shown to be better poised for reactivity and differentiation than $CD5^{low}$ cells, and more reactive to antigenic stimulation [107],[108], and spontaneously differentiate into T_{MP} [109],[110]. Supporting the hypothesis that self-ligands contribute to T_{IM}/T_{VM} differentiation, TCR repertoire was shown to be distinct between T_{VM} and T_N [51],[111],[112]. Drobek and colleagues showed that a strong homeostatic TCR signal could significantly expand the T_{VM} compartment but was not sufficient to enable all cells to assume a memory phenotype, implying that TCR specificity for self is still an important parameter in T_{VM} generation. Indeed, only naive T cells expressing T_{VM}-enriched TCR clones led to their differentiation into T_{VM} [51], and T_{VM}, including IL-4-responding T_{VM}, expressed enriched CDR3 sequences with physico-chemical characteristics associated with self-reactivity [94]. Thus, self-ligand recognition might be essential for T_{VM} maintenance and clonal expansion induced by cytokines. Moreover, T_{VM} differentiation was associated with the upregulation of EOMES in the thymus in C57BL/6 mice, suggesting that T_{VM} differentiation is already triggered in the thymus before they receive further signals after thymic egress [51]. Deficiency of *Themis*, a protein regulating thymic selection, resulted in the expansion of a T_{MP} population expressing $V\alpha 3.2^+$ TCR, having increased responsiveness to IL-15 [113]. Moreover, deficiency in DOCK2, which contributes to cellular signalling events by activating small G-proteins, resulted in increased sensitivity to TCR stimulation by low-affinity peptides and T_{VM} expansion [114]. Together, these studies strongly support that self-ligand recognition governs the generation of T_{VM}, implying that some self-reactive $CD8^+$ T cells do evict thymic selection, as recently reported [115]. Thus, we could risk to make some parallels with $Foxp3^+$ regulatory T cells [116], and consider T_{VM} might have regulatory functions. The following section further discusses some important data supporting such consideration.

T_{VM} functions in inflammation, infection and cancer.

By definition, T_{VM} have not encountered foreign antigens but displayed an enhanced capability to respond to their cognate antigen when compared to their T_N counterparts. These cells are capable of mediating potent immunological protection against a bacterial challenge even in the absence of their cognate antigen [117], of which bystander protection is maintained by IL-15 in C57BL/6 mice [83]. In addition, IL-4 can also promote type 1 immune response and IFN-γ in T_{VM}, and regulate the $CD8^+$ T cell response to acute viral infection [91]. In the thymus, thymic IL-4 and IL-15 expression triggered by Th1-associated *Trypanosoma cruzi* infection induced an adequate niche for development of innate memory CD8$^+$ T cells as early as the DP stage in WT mice [118]. In the periphery, CXCR3 regulates the recruitment of memory CD8$^+$ T cells to sites of inflammation [119], and CCR2$^+$ T_{VM} were shown to rapidly infiltrate the lung in a CXCR3-dependent manner early after a primary infection with a high dose of influenza virus, promoting early viral control by producing IFN-γ and granzyme B [56]. During helminth exposure, IL-4 drives the expansion and activation of IFN-γ-producing T_{VM} directly associated with the enhanced control of a subsequent viral infection in the lung [93],[94]. Similarly, T_{VM} expanded by helminth infection were shown to confer protection against subsequent bacterial infection via an IFN-γ-dependent mechanism [120]. These studies provided a new prospect where type 1 and type 2 immune axes converge to drive the activation of immune protection through T_{MP} **(Fig 2**).

Besides their function in response to viral or bacterial infections, T_{VM} also display enhanced responses against tumours. Donor T_{VM} in a mouse model of prostate adenocarcinoma constituted a substantial fraction of the tumour-infiltrating CD8$^+$ T cells and the majority expressed high densities of the inhibitory receptor PD-1, which may functionally impact antitumor immunity or directly be impacted by anti-PD-1 or anti-PD-L1 checkpoint blockade antibodies [51]. Chemotherapy-treated cancer cells directly activated T_{VM} in MHC-I-independent manner requiring cell–cell contact and activation of the PI3K pathway, which preferentially promoted T_{VM} tumour-infiltrating and potent granzyme B-dependent cytotoxicity [121]. In addition, systemic expression of IL-12 and IL-18 and an enrichment of T_{VM} displaying antitumor characteristics were found in a melanoma and pancreatic ductal adenocarcinoma tumour models [122], which was abolished in the absence of IFN-γ.

The combination of a hyperresponsive differentiation state with the expression of self-reactive TCRs could imply that T_{VM} would be less self-tolerant than T_N and represent a risk for inducing autoimmunity. However, T_{VM} retain self-tolerance phenotypic traits and are less able to induce autoimmune diabetes compared to T_{TM}, while they have a similarly low ability to drive experimental diabetes as T_N [111]. In general, T_{VM} do not upregulate the high-affinity IL-2 receptor alpha chain CD25 and express low CD49d, suggesting T_{VM} activation can be tightly controlled by potential counter-inhibitory mechanisms. Importantly, our work demonstrated that IL-4 drives CD22 expression in expanding T_{VM} [94]. While CD22 is a canonical marker expressed on B cells, its expression in T_{VM} was shown to limit IL-4-driven T_{VM} expansion resulting in reduced responsiveness to stimulation of CD22$^+$ T_{VM}, such reduced production of IFN-γ and GZMB. But beyond intrinsic regulation of T_{VM} activation, CD22$^+$ T_{VM} could also regulate T cell-induced immunopathology, suggesting they could have regulatory functions. In addition, Foxp3$^+$ regulatory CD4$^+$ T cells limited expansion of T_{VM} *via* restraining IL-15 trans-presentation by DCs to maintain stable T_{VM}

population [123], suggesting multiple mechanisms of regulation of the T_{VM} response are involved. While mostly protective, T_{VM} have also been associated with disease. Hence, local activation of T_{VM} expressing high levels of CD44 caused alopecia areata (a chronic inflammatory skin disease) [124]. Whether such pathological T_{VM} response escaped tolerance against self-ligand recognition is unclear.

$CD8^+CD122^+CD49d^{low}$ cells (i.e. T_{VM}) have also been reported as "regulatory $CD8^+$ T cells" [125], mediating killing of activated T cells via Fas/FasL-mediated cytotoxicity. Thus, these observations together with our work showing that $CD22^+$ T_{VM} protected *Rag*-deficient mice from inflammatory bowel disease induced by co-transfer naïve $CD4^+$ T cells furtherly explained how T_{VM} contribute to regulation of immunopathology [1]. Ly-49, a family of killer cell lectin-like receptor was also identified as a marker of $CD8^+$ "regulatory" T cells [126],[127]. Mechanistically, thymic eviction of immature autoreactive SP8 into the periphery was shown to drive self-tolerance of $mature^+$ CD8 T cells [115]. Such phenomenon could explain the observed association between self-reactivity and self-tolerance in T_{VM} biology, potentially driving dual functions of bystander effector and regulatory mechanisms.

Human T_{IM}/T_{VM}: how can we make sure?

Recent data reasonably suggested the existence of human T_{VM}, although the demonstration of Ag-inexperienced T cells in human will likely never be definitive. $CD8^+$ T cells expressing either KIR receptors or the inhibitory NKG2A receptor with an EMRA phenotype (effector memory cells re-expressing CD45RA, $CD45RA^+$ $CCR7^-$) in cord blood, exempt of viral infection or placental pathology [128], represent the most reliable demonstration of human T_{VM}. Supporting the idea of human T_{VM}, a substantial number of T_{VM}-like cells in the spleens of pre-term infants, with EOMES expression and rapid IFN-γ production upon restimulation [129]. Supporting the existence of T_{MP} during in early life, a recent study reported single cell transcriptome and TCR usage atlas from several foetal tissues during the second trimester of pregnancy. Interestingly, increasing proportions of $CD45RO^+$ memory T cells, T_{EM} and T_{RM} were observed during developmental progression across organs [130], in which substantially higher proportions of αβ TCR clones recognizing diverse antigens including self-antigens were identified, when compared to adult counterparts. PLZF is highly expressed in both thymic and splenic human foetal $CD4^+$ T cells, which potentially provide good conditions for T_{IM}/T_{VM} expansion [129],[131]. The notable difference is that CD5 expression on human T cells decreases as the T cell undergoes differentiation [132], and T cells expressing the lowest levels of CD5 are the most sensitive to IL-15 [132],[133]. These $CD5^{low}CD122^{hi}$ cells increased expression of NUR77, suggesting a higher basal TCR–MHC self-affinity, NKG2A, both EOMES and T-BET, and were $CD45RA^+KIR^+$ [83],[134]–[136] (**Table 1**). In an attempt to categorize human T_{VM}, two subsets were described as KIR^{+}/NKG2A^{+}CD45RA^{+}TIGIT$^{+/-}$CD226$^{+/-}$ [137], and RNA-seq revealed different gene signatures in these two subsets, notably for *Helios*, *Cd122* and *Tigit* expression [127],[137]. The subset TIGIT^{+}Helios^{+}NKG2A^{+}CCR7^{-}CD27^{-}CD28^{-} showed high cytotoxic ability (perforin and granzyme B), a less diverse TCR repertoire and regulatory functions in autoimmune diseases [48],[127]. Interestingly, NKG2A^{+} innate-like memory T cells have been shown to develop early in human life during gene therapy with *Il2rg* in X-linked severe combined immunodeficient individuals and were shown to be epigenetically poised to rapidly elicit effector cytokines in an antigen-independent manner [138]. However, these innate-like memory T cells upregulated both CD45RO and CD49d. In addition, expression of IL-15Rβ on T_{VM} is significantly higher than that on T_{N} in young adults, increased significantly with age [63]. In addition to CD8^{+} T_{MP}, CD4^{+} T_{MP} were found in large numbers in the foetal intestinal mucosa, showing effector memory while expressing significantly lower CD27 and CD28 than infant cells [139].

During infection, the challenge would be to distinguish functions mediated by human T_{VM} from bystander TCR-independent activities of T_{TM}. Whereas no study reported IL-4-induced expansion of human T_{VM} during helminth infection, increased levels of IL-15 during hepatitis A virus (HAV) infection resulted in a TCR-independent activation of memory CD8^{+} T cells, while HAV non-specific CD8^{+} T cells upregulated NKG2D and displayed innate-like cytotoxicity associated with liver injury [140],[141]. IL-15 was shown to upregulate CCR5 expression, which correlated with inflammation [142]. A population with T_{VM} characteristics negatively correlated with HIV DNA loads and positively correlated with circulating IFN-α2 and IL-15 in patients undergoing antiretroviral therapy, in which T_{VM} constitutively expressed high levels of cytotoxic granule components, including granzyme B, perforin and granulysin [132],[133]. The levels of HIV-1 DNA and cell-associated unspliced viral RNA correlated negatively with CCL4^{-} CCL5^{+} CD8^{+} terminally differentiated effector memory cells, in which a T_{VM} subset was enriched and showed superior cytotoxicity potentially driven by T-BET and RUNX3 [143].

Thus, whereas there is no real doubt about the existence of human T_{VM}, there remains a significant gap to fill in the understanding of their phenotype, function and regulation during homeostasis, and in disease.

Conclusion and Perspectives

The current literature converges in proposing that T_{VM} emerge from self-ligands recognition as the main driving force to achieve a memory phenotype. But there is no evidence that such thymic eviction of self-reactive T cells results in autoimmune reaction and immunopathology. Rather, T_{VM} maintenance and activation is tightly regulated through various intrinsic mechanisms such as the upregulation of the inhibitory receptor CD22 in response to IL-4, as well as extrinsic mechanisms, like their modulation via Foxp3$^+$ Tregs. Nonetheless, T_{VM} maintain their potential to mediate bystander functions, or even respond to their cognate Ag presentation more effectively than T_N. Nonetheless, while there have been extensive efforts to explore T_{VM} biology since their discovery, we have to recognize that while it is still too soon to provide a clear response to how much similar or different T_{VM} are from T_{TM}, it is important to recognize that a primary response to an antigen do not solely involve T_N, but also the participation of T_{MP} [4].

One aspect that we think would be worth clarifying, is the shift of paradigm in which a type 2 cytokine like IL-4 drives the activation of memory/effector properties in T_{VM}, such as increased IFN-γ or production of granzyme A. Although thymic IL-4 in some mouse strains has been demonstrated to drive T_{IM} expansion more than a decade ago, no clear mechanism in term of signalling and gene regulation has been identified. Do T_{MP} rely on IL-4 signals to drive EOMES directly? Or would an indirect phenomenon explain that IL-4 drives activation signals leading to more self-ligand recognition signals via their TCR? In addition, what happens to expanded T_{VM} after their activation, for instance during helminth infection? Could we expect an imprinting of long-lived T_{VM} which would contribute to surveillance of primary infections throughout the life, and would T_{VM} contribute to the regulation of inflammation induced by pathogens like parasitic worms, and contribute to disease tolerance? These are only a few examples of investigation that would need to be pursued, to which the hypothetical IL-4 responsiveness of human T_{VM} could also be added. There, the development by several groups of controlled human helminth infections could represent a unique opportunity to provide valuable information.

Conflict of interest disclosure

The authors have no conflict of interest to report.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study

References

1. **Kaech SM**, **Cui W**. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nature Reviews Immunology*. 2012; **12**:749–761.DOI: 10.1038/nri3307.

2. **Gebhardt T**, **Wakim LM**, **Eidsmo L**, **Reading PC**, **Heath WR**, **Carbone FR**. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nature Immunology*. 2009; **10**:524–530.DOI: 10.1038/ni.1718.

3. **Halle S**, **Halle O**, **Förster R**. Mechanisms and Dynamics of T Cell-Mediated Cytotoxicity In Vivo. *Trends in Immunology*. 2017; **38**:432–443.DOI: 10.1016/j.it.2017.04.002.

4. **Jameson SC**. The Naming of Memory T-Cell Subsets. *Cold Spring Harbor Perspectives in Biology*. 2021; **13**:a037788.DOI: 10.1101/cshperspect.a037788.

5. **Jameson SC**, **Masopust D**. Understanding Subset Diversity in T Cell Memory. *Immunity*. 2018; **48**:214–226.DOI: 10.1016/j.immuni.2018.02.010.

6. **Masopust D**, **Vezys V**, **Marzo AL**, **Lefrançois L**. Preferential Localization of Effector Memory Cells in Nonlymphoid Tissue. *Science*. 2001; **291**:2413–2417.DOI: 10.1126/science.1058867.

7. **Masopust D, Choo D, Vezys V, Wherry EJ, Duraiswamy J, Akondy R, Wang J, *et al.*** Dynamic T cell migration program provides resident memory within intestinal epithelium. *Journal of Experimental Medicine*. 2010; **207**:553–564.DOI: 10.1084/jem.20090858.

8. **Mueller SN**, **Gebhardt T**, **Carbone FR**, **Heath WR**. Memory T Cell Subsets, Migration Patterns, and Tissue Residence. *Annual Review of Immunology*. 2013; **31**:137–161.DOI: 10.1146/annurev-immunol-032712-095954.

9. **Muroyama Y**, **Wherry EJ**. Memory T-Cell Heterogeneity and Terminology. *Cold Spring Harbor Perspectives in Biology*. 2021; **13**:a037929.DOI: 10.1101/cshperspect.a037929.

10. **Reinhardt RL**, **Khoruts A**, **Merica R**, **Zell T**, **Jenkins MK**. Visualizing the generation of memory CD4 T cells in the whole body. *Nature*. 2001; **410**:101–105.DOI: 10.1038/35065111.

11. **Sallusto F**, **Lenig D**, **Förster R**, **Lipp M**, **Lanzavecchia A**. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999; **401**:708–712.DOI: 10.1038/44385.

12. **Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, Andrian UH von, *et al.*** Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nature Immunology*. 2003; **4**:225–234.DOI: 10.1038/ni889.

13. **Jameson SC**, **Lee YJ**, **Hogquist KA**. Innate memory T cells. *Adv Immunol*. 2015; **126**:173–213.DOI: 10.1016/bs.ai.2014.12.001.

14. **Thiele D**, **Gruta NL**, **Nguyen A**, **Hussain T**. Hiding in Plain Sight: Virtually Unrecognizable Memory Phenotype CD8+ T cells. *International Journal of Molecular Sciences*. 2020; **21**:8626.DOI: 10.3390/ijms21228626.

15. **White JT**, **Cross EW**, **Kedl RM**. Antigen-inexperienced memory CD8+ T cells: where they come from and why we need them. *Nature Reviews Immunology*. 2017; **17**:391–400.DOI: 10.1038/nri.2017.34.

16. **Haluszczak C, Akue AD, Hamilton SE, Johnson LDS, Pujanauski L, Teodorovic L, Jameson SC, *et al.*** The antigen-specific CD8+ T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *Journal of Experimental Medicine*. 2009; **206**:435–448.DOI: 10.1084/jem.20081829.

17. **Sprent J**, **Surh CD**. Normal T cell homeostasis: the conversion of naive cells into memory-phenotype cells. *Nature Immunology*. 2011; **12**:478–484.DOI: 10.1038/ni.2018.

18. **Sun H**, **Sun C**, **Xiao W**, **Sun R**. Tissue-resident lymphocytes: from adaptive to innate immunity. *Cellular & Molecular Immunology*. 2019; **16**:205–215.DOI: 10.1038/s41423-018-0192-y.

19. **Tanchot C**, **Lemonnier FA**, **Pérarnau B**, **Freitas AA**, **Rocha B**. Differential Requirements for Survival and Proliferation of CD8 Naïve or Memory T Cells. *Science*. 1997; **276**:2057–2062.DOI: 10.1126/science.276.5321.2057.

20. **Clarke SRM**, **Rudensky AY**. Survival and Homeostatic Proliferation of Naive Peripheral CD4+ T Cells in the Absence of Self Peptide:MHC Complexes. *The Journal of Immunology*. 2000; **165**:2458–2464.DOI: 10.4049/jimmunol.165.5.2458.

21. **Ernst B**, **Lee D-S**, **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.DOI: 10.1016/S1074-7613(00)80092-8.

22. **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.DOI: 10.1016/S1074-7613(00)80093-X.

23. **Kieper WC**, **Jameson SC**. Homeostatic expansion and phenotypic conversion of naïve T cells in response to self peptide/MHC ligands. *Proceedings of the National Academy of Sciences*. 1999; **96**:13306–13311.DOI: 10.1073/pnas.96.23.13306.

24. **Murali-Krishna K**, **Ahmed R**. Cutting Edge: Naive T Cells Masquerading as Memory Cells. *The Journal of Immunology*. 2000; **165**:1733–1737.DOI: 10.4049/jimmunol.165.4.1733.

25. **Jameson SC**. Maintaining the norm: T-cell homeostasis. *Nature Reviews Immunology*. 2002; **2**:547–556.DOI: 10.1038/nri853.

26. **Hamilton SE**, **Wolkers MC**, **Schoenberger SP**, **Jameson SC**. The generation of protective memory-like CD8+ T cells during homeostatic proliferation requires CD4+ T cells. *Nature Immunology*. 2006; **7**:475–481.DOI: 10.1038/ni1326.

27. **Surh CD**, **Sprent J**. Homeostasis of Naive and Memory T Cells. *Immunity*. 2008; **29**:848–862.DOI: 10.1016/j.immuni.2008.11.002.

28. **Bell EB**, **Sparshott SM**, **Drayson MT**, **Ford WL**. The stable and permanent expansion of functional T lymphocytes in athymic nude rats after a single injection of mature T cells. *Journal of immunology (Baltimore, Md. : 1950)*. 1987; **139**:1379–84.

29. **Rocha B**, **Dautigny N**, **Pereira P**. Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios *in vivo*. *European Journal of Immunology*. 1989; **19**:905–911.DOI: 10.1002/eji.1830190518.

30. **Heidarian M**, **Kannan SK**, **Swanson W**, **Griffith TS**, **Harty JT**, **Badovinac VP**. Delineating the short- and long-term impact of ionizing radiation on antigen-inexperienced CD8+ T cell subsets. *JCI Insight*. 2025; **10**. Available at: https://insight.jci.org/articles/view/194201.DOI: 10.1172/jci.insight.194201.

31. **Surh CD**, **Boyman O**, **Purton JF**, **Sprent J**. Homeostasis of memory T cells. *Immunological Reviews*. 2006; **211**:154–163.DOI: 10.1111/j.0105-2896.2006.00401.x.

32. **Gruta NLL, Rothwell WT, Cukalac T, Swan NG, Valkenburg SA, Kedzierska K, Thomas PG, *et al.*** Primary CTL response magnitude in mice is determined by the extent of naive T cell recruitment and subsequent clonal expansion. *Journal of Clinical Investigation*. 2010; **120**:1885–1894.DOI: 10.1172/JCI41538.

33. **Takada K**, **Jameson SC**. Naive T cell homeostasis: from awareness of space to a sense of place. *Nature Reviews Immunology*. 2009; **9**:823–832.DOI: 10.1038/nri2657.

34. **Sprent J**, **Cho J-H**, **Boyman O**, **Surh CD**. T cell homeostasis. *Immunology & Cell Biology*. 2008; **86**:312–319.DOI: 10.1038/icb.2008.12.

35. **Weinreich MA**, **Takada K**, **Skon C**, **Reiner SL**, **Jameson SC**, **Hogquist KA**. KLF2 Transcription-Factor Deficiency in T Cells Results in Unrestrained Cytokine Production and Upregulation of Bystander Chemokine Receptors. *Immunity*. 2009; **31**:122–130.DOI: 10.1016/j.immuni.2009.05.011.

36. **Weinreich MA**, **Odumade OA**, **Jameson SC**, **Hogquist KA**. T cells expressing the transcription factor PLZF regulate the development of memory-like CD8+ T cells. *Nature Immunology*. 2010; **11**:709–716.DOI: 10.1038/ni.1898.

37. **Berg LJ**. Signalling through TEC kinases regulates conventional versus innate CD8+ T-cell development. *Nature Reviews Immunology*. 2007; **7**:479–485.DOI: 10.1038/nri2091.

38. **Urdahl KB**, **Sun JC**, **Bevan MJ**. Positive selection of MHC class Ib–restricted CD8+ T cells on hematopoietic cells. *Nature Immunology*. 2002; **3**:772–779.DOI: 10.1038/ni814.

39. **Salmond RJ**, **Brownlie RJ**, **Morrison VL**, **Zamoyska R**. The tyrosine phosphatase PTPN22 discriminates weak self peptides from strong agonist TCR signals. *Nature Immunology*. 2014; **15**:875–883.DOI: 10.1038/ni.2958.

40. **Dutton RW**, **Bradley LM**, **Swain SL**. T cell memory. *Annual Review of Immunology*. 1998; **16**:201–223.DOI: 10.1146/annurev.immunol.16.1.201.

41. **Bendelac A**, **Killeen N**, **Littman DR**, **Schwartz RH**. A Subset of CD4 $^+$ Thymocytes Selected by MHC Class I Molecules. *Science*. 1994; **263**:1774–1778.DOI: 10.1126/science.7907820.

42. **Kurepa Z**, **Su J**, **Forman J**. Memory Phenotype of CD8+ T Cells in MHC Class Ia-Deficient Mice. *The Journal of Immunology*. 2003; **170**:5414–5420.DOI: 10.4049/jimmunol.170.11.5414.

43. **Kawachi I**, **Maldonado J**, **Strader C**, **Gilfillan S**. MR1-Restricted Vα19 *i* Mucosal-Associated Invariant T Cells Are Innate T Cells in the Gut Lamina Propria That Provide a Rapid and Diverse Cytokine Response. *The Journal of Immunology*. 2006; **176**:1618–1627.DOI: 10.4049/jimmunol.176.3.1618.

44. **Andreotti AH**, **Schwartzberg PL**, **Joseph RE**, **Berg LJ**. T-Cell Signaling Regulated by the Tec Family Kinase, Itk. *Cold Spring Harbor Perspectives in Biology*. 2010; **2**:a002287–a002287.DOI: 10.1101/cshperspect.a002287.

45. **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.DOI: 10.1038/ni.2731.

46. **Lee YJ**, **Jameson SC**, **Hogquist KA**. Alternative memory in the CD8 T cell lineage. *Trends in Immunology*. 2011; **32**:50–56.DOI: 10.1016/j.it.2010.12.004.

47. **Huang W**, **Huang F**, **Kannan AK**, **Hu J**, **August A**. ITK tunes IL-4-induced development of innate memory CD8+ T cells in a γδ T and invariant NKT cell-independent manner. *Journal of Leukocyte Biology*. 2014; **96**:55–63.DOI: 10.1189/jlb.1AB0913-484RR.

48. **Viano ME, Baez NS, Savid-Frontera C, Lidon NL, Hodge DL, Herbelin A, Gombert J-M, *et al.*** Virtual Memory CD8 $^{+}$ T Cells: Origin and Beyond. *Journal of Interferon & Cytokine Research*. 2022; **42**:624–642.DOI: 10.1089/jir.2022.0053.

49. **Weinreich MA**, **Takada K**, **Skon C**, **Reiner SL**, **Jameson SC**, **Hogquist KA**. KLF2 transcription-factor deficiency in T cells results in unrestrained cytokine production and upregulation of bystander chemokine receptors. *Immunity*. 2009; **31**:122–130.DOI: 10.1016/j.immuni.2009.05.011.

50. **Lai D, Zhu J, Wang T, Hu-Li J, Terabe M, Berzofsky JA, Clayberger C, *et al.*** KLF13 sustains thymic memory-like CD8+ T cells in BALB/c mice by regulating IL-4–generating invariant natural killer T cells. *Journal of Experimental Medicine*. 2011; **208**:1093–1103.DOI: 10.1084/jem.20101527.

51. **Miller CH**, **Klawon DEJ**, **Zeng S**, **Lee V**, **Socci ND**, **Savage PA**. Eomes identifies thymic precursors of self-specific memory-phenotype CD8+ T cells. *Nature Immunology*. 2020; **21**:567–577.DOI: 10.1038/s41590-020-0653-1.

52. **Hou S, Shao T, Mao T, Shi J, Sun J, Mei M, Tan X, *et al.*** Virtual memory T cells orchestrate extralymphoid responses conducive to resident memory. *Science Immunology*. 2021; **6**.DOI: 10.1126/sciimmunol.abg9433.

53. **Sosinowski T**, **White JT**, **Cross EW**, **Haluszczak C**, **Marrack P**, **Gapin L**, **Kedl RM**. 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. *The Journal of Immunology*. 2013; **190**:1936–1947.DOI: 10.4049/jimmunol.1203149.

54. **Nikolich-Žugich J**. Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nature Reviews Immunology*. 2008; **8**:512–522.DOI: 10.1038/nri2318.

55. **Rudd BD, Venturi V, Li G, Samadder P, Ertelt JM, Way SS, Davenport MP, *et al.*** Nonrandom attrition of the naive CD8 $^+$ T-cell pool with aging governed by T-cell receptor:pMHC interactions. *Proceedings of the National Academy of Sciences*. 2011; **108**:13694–13699.DOI: 10.1073/pnas.1107594108.

56. **Hou S, Shao T, Mao T, Shi J, Sun J, Mei M, Tan X, *et al.*** Virtual memory T cells orchestrate extralymphoid responses conducive to resident memory. *Science Immunology*. 2021; **6**.DOI: 10.1126/sciimmunol.abg9433.

57. **Hussain T**, **Quinn KM**. Similar but different: virtual memory CD8 T cells as a memory-like cell population. *Immunology & Cell Biology*. 2019; **97**:675–684.DOI: 10.1111/imcb.12277.

58. **Smith NL, Patel RK, Reynaldi A, Grenier JK, Wang J, Watson NB, Nzingha K, *et al.*** Developmental Origin Governs CD8+ T Cell Fate Decisions during Infection. *Cell*. 2018; **174**:117-130.e14.DOI: 10.1016/j.cell.2018.05.029.

59. **Davenport MP**, **Smith NL**, **Rudd BD**. Building a T cell compartment: how immune cell development shapes function. *Nature Reviews Immunology*. 2020; **20**:499–506.DOI: 10.1038/s41577-020-0332-3.

60. **Akue AD**, **Lee J-Y**, **Jameson SC**. Derivation and Maintenance of Virtual Memory CD8 T Cells. *The Journal of Immunology*. 2012; **188**:2516–2523.DOI: 10.4049/jimmunol.1102213.

61. **Watson NB, Patel RK, Kean C, Veazey J, Oyesola OO, Laniewski N, Grenier JK, *et al.*** The gene regulatory basis of bystander activation in CD8 $^+$ T cells. *Science Immunology*. 2024; **9**. Available at: https://www.science.org/doi/10.1126/sciimmunol.adf8776.DOI: 10.1126/sciimmunol.adf8776.

62. **Chiu B-C**, **Martin BE**, **Stolberg VR**, **Chensue SW**. Cutting Edge: Central Memory CD8 T Cells in Aged Mice Are Virtual Memory Cells. *The Journal of Immunology*. 2013; **191**:5793–5796.DOI: 10.4049/jimmunol.1302509.

63. **Quinn KM, Fox A, Harland KL, Russ BE, Li J, Nguyen THO, Loh L, *et al.*** Age-Related Decline in Primary CD8+ T Cell Responses Is Associated with the Development of Senescence in Virtual Memory CD8+ T Cells. *Cell Reports*. 2018; **23**:3512–3524.DOI: 10.1016/j.celrep.2018.05.057.

64. **Quinn KM, Hussain T, Kraus F, Formosa LE, Lam WK, Dagley MJ, Saunders EC, *et al.*** Metabolic characteristics of CD8+ T cell subsets in young and aged individuals are not predictive of functionality. *Nature Communications*. 2020; **11**:2857.DOI: 10.1038/s41467-020-16633-7.

65. **Borsa M, Barandun N, Gräbnitz F, Barnstorf I, Baumann NS, Pallmer K, Baumann S, *et al.*** Asymmetric cell division shapes naive and virtual memory T-cell immunity during ageing. *Nature Communications*. 2021; **12**:2715.DOI: 10.1038/s41467-021-22954-y.

66. **Lanzer KG**, **Cookenham T**, **Reiley WW**, **Blackman MA**. Virtual memory cells make a major contribution to the response of aged influenza-naïve mice to influenza virus infection. *Immunity & Ageing*. 2018; **15**:17.DOI: 10.1186/s12979-018-0122-y.

67. **Moudra A, Niederlova V, Novotny J, Schmiedova L, Kubovciak J, Matejkova T, Drobek A, *et al.*** Phenotypic and Clonal Stability of Antigen-Inexperienced Memory-like T Cells across the Genetic

Background, Hygienic Status, and Aging. *The Journal of Immunology*. 2021; **206**:2109–2121.DOI: 10.4049/jimmunol.2001028.

68. **Prlic M**, **Lefrancois L**, **Jameson SC**. Multiple Choices. *The Journal of Experimental Medicine*. 2002; **195**:F49–F52.DOI: 10.1084/jem.20020767.

69. **Goldrath AW**, **Bogatzki LY**, **Bevan MJ**. Naive T Cells Transiently Acquire a Memory-like Phenotype during Homeostasis-Driven Proliferation. *The Journal of Experimental Medicine*. 2000; **192**:557–564.DOI: 10.1084/jem.192.4.557.

70. **Ge Q**, **Hu H**, **Eisen HN**, **Chen J**. Different contributions of thymopoiesis and homeostasis-driven proliferation to the reconstitution of naive and memory T cell compartments. *Proceedings of the National Academy of Sciences*. 2002; **99**:2989–2994.DOI: 10.1073/pnas.052714099.

71. **Tanchot C**, **Campion AL**, **Martin B**, **Léaument S**, **Dautigny N**, **Lucas B**. Conversion of Naive T Cells to a Memory-Like Phenotype in Lymphopenic Hosts Is Not Related to a Homeostatic Mechanism That Fills the Peripheral Naive T Cell Pool. *The Journal of Immunology*. 2002; **168**:5042–5046.DOI: 10.4049/jimmunol.168.10.5042.

72. **Schluns KS**, **Kieper WC**, **Jameson SC**, **Lefrançois L**. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. *Nat Immunol*. 2000; **1**:426–432.DOI: 10.1038/80868.

73. **Sandau MM**, **Winstead CJ**, **Jameson SC**. IL-15 Is Required for Sustained Lymphopenia-Driven Proliferation and Accumulation of CD8 T Cells. *The Journal of Immunology*. 2007; **179**:120–125.DOI: 10.4049/jimmunol.179.1.120.

74. **Xu A, Bhanumathy KK, Wu J, Ye Z, Freywald A, Leary SC, Li R, *et al.*** IL-15 signaling promotes adoptive effector T-cell survival and memory formation in irradiation-induced lymphopenia. *Cell & Bioscience*. 2016; **6**:30.DOI: 10.1186/s13578-016-0098-2.

75. **Goldrath AW, Sivakumar PV, Glaccum M, Kennedy MK, Bevan MJ, Benoist C, Mathis D, *et al.*** Cytokine Requirements for Acute and Basal Homeostatic Proliferation of Naive and Memory CD8+ T Cells. *The Journal of Experimental Medicine*. 2002; **195**:1515–1522.DOI: 10.1084/jem.20020033.

76. **Tan JT**, **Ernst B**, **Kieper WC**, **LeRoy E**, **Sprent J**, **Surh CD**. Interleukin (IL)-15 and IL-7 Jointly Regulate Homeostatic Proliferation of Memory Phenotype CD8+ Cells but Are Not Required for Memory Phenotype CD4+ Cells. *The Journal of Experimental Medicine*. 2002; **195**:1523–1532.DOI: 10.1084/jem.20020066.

77. **Schluns KS**, **Williams K**, **Ma A**, **Zheng XX**, **Lefrançois L**. Cutting Edge: Requirement for IL-15 in the Generation of Primary and Memory Antigen-Specific CD8 T Cells. *The Journal of Immunology*. 2002; **168**:4827–4831.DOI: 10.4049/jimmunol.168.10.4827.

78. **Becker TC**, **Wherry EJ**, **Boone D**, **Murali-Krishna K**, **Antia R**, **Ma A**, **Ahmed R**. Interleukin 15 Is Required for Proliferative Renewal of Virus-specific Memory CD8 T Cells. *The Journal of Experimental Medicine*. 2002; **195**:1541–1548.DOI: 10.1084/jem.20020369.

79. **Cho J-H, Boyman O, Kim H-O, Hahm B, Rubinstein MP, Ramsey C, Kim DM, *et al.*** An intense form of homeostatic proliferation of naive CD8+ cells driven by IL-2. *The Journal of Experimental Medicine*. 2007; **204**:1787–1801.DOI: 10.1084/jem.20070740.

80. **Ramsey C**, **Rubinstein MP**, **Kim DM**, **Cho J-H**, **Sprent J**, **Surh CD**. The Lymphopenic Environment of CD132 (Common γ-Chain)-Deficient Hosts Elicits Rapid Homeostatic Proliferation of Naive T Cells via IL-15. *The Journal of Immunology*. 2008; **180**:5320–5326.DOI: 10.4049/jimmunol.180.8.5320.

81. **Davey GM, Starr R, Cornish AL, Burghardt JT, Alexander WS, Carbone FR, Surh CD, *et al.*** SOCS-1 regulates IL-15–driven homeostatic proliferation of antigen-naive CD8 T cells, limiting their autoimmune potential. *The Journal of Experimental Medicine*. 2005; **202**:1099–1108.DOI: 10.1084/jem.20050003.

82. **Ramanathan S**, **Gagnon J**, **Leblanc C**, **Rottapel R**, **Ilangumaran S**. Suppressor of Cytokine Signaling 1 Stringently Regulates Distinct Functions of IL-7 and IL-15 In Vivo during T Lymphocyte Development and Homeostasis. *The Journal of Immunology*. 2006; **176**:4029–4041.DOI: 10.4049/jimmunol.176.7.4029.

83. **White JT, Cross EW, Burchill MA, Danhorn T, McCarter MD, Rosen HR, O'Connor B, *et al.*** Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. *Nature Communications*. 2016; **7**:11291.DOI: 10.1038/ncomms11291.

84. **Tough DF**, **Zhang X**, **Sprent J**. An IFN-γ-Dependent Pathway Controls Stimulation of Memory Phenotype CD8+ T Cell Turnover In Vivo by IL-12, IL-18, and IFN-γ. *The Journal of Immunology*. 2001; **166**:6007–6011.DOI: 10.4049/jimmunol.166.10.6007.

85. **Sercan Ö**, **Stoycheva D**, **Hämmerling GJ**, **Arnold B**, **Schüler T**. IFN-γ Receptor Signaling Regulates Memory CD8+ T Cell Differentiation. *The Journal of Immunology*. 2010; **184**:2855–2862.DOI: 10.4049/jimmunol.0902708.

86. **Park HJ**, **Choi SM**, **Choi EA**, **Boo HJ**, **Lee JI**, **Jung KC**. IL-4, IL-15, and Type I Interferon Orchestrate the Shaping of the Heterogeneity of Virtual Memory CD8 T Cells. *European Journal of Immunology*. 2025; **55**. Available at: https://onlinelibrary.wiley.com/doi/10.1002/eji.202451765.DOI: 10.1002/eji.202451765.

87. **Zhang X**, **Sun S**, **Hwang I**, **Tough DF**, **Sprent J**. Potent and Selective Stimulation of Memory-Phenotype CD8+ T Cells In Vivo by IL-15. *Immunity*. 1998; **8**:591–599.DOI: 10.1016/S1074-7613(00)80564-6.

88. **Zhang X**, **Fujii H**, **Kishimoto H**, **LeRoy E**, **Surh CD**, **Sprent J**. Aging Leads to Disturbed Homeostasis of Memory Phenotype CD8+ Cells. *The Journal of Experimental Medicine*. 2002; **195**:283–293.DOI: 10.1084/jem.20011267.

89. **Martinet V, Tonon S, Torres D, Azouz A, Nguyen M, Kohler A, Flamand V, *et al.*** Type I interferons regulate eomesodermin expression and the development of unconventional memory CD8+ T cells. *Nature Communications*. 2015; **6**:7089.DOI: 10.1038/ncomms8089.

90. **Uhl LFK, Cai H, Oram SL, Mahale JN, MacLean AJ, Mazet JM, Piccirilli T, *et al.*** Interferon-γ couples CD8+ T cell avidity and differentiation during infection. *Nature Communications*. 2023; **14**:6727.DOI: 10.1038/s41467-023-42455-4.

91. **Renkema KR**, **Lee J-Y**, **Lee YJ**, **Hamilton SE**, **Hogquist KA**, **Jameson SC**. IL-4 sensitivity shapes the peripheral CD8+ T cell pool and response to infection. *Journal of Experimental Medicine*. 2016; **213**:1319–1329.DOI: 10.1084/jem.20151359.

92. **Park H-J**, **Lee A**, **Lee J-I**, **Park SH**, **Ha S-J**, **Jung KC**. Effect of IL-4 on the Development and Function of Memory-like CD8 T Cells in the Peripheral Lymphoid Tissues. *Immune Network*. 2016; **16**:126.DOI: 10.4110/in.2016.16.2.126.

93. **Rolot M, Dougall AM, Chetty A, Javaux J, Chen T, Xiao X, Machiels B, *et al.*** Helminth-induced IL-4 expands bystander memory CD8+ T cells for early control of viral infection. *Nature Communications*. 2018; **9**:4516.DOI: 10.1038/s41467-018-06978-5.

94. **Yang B, Piedfort O, Sanchez-Sanchez G, Lavergne A, Gong M, Peng G, Madrigal A, *et al.*** IL-4 induces CD22 expression to restrain the effector program of virtual memory T cells. *Science Immunology*. 2025; **10**. Available at: https://www.science.org/doi/10.1126/sciimmunol.adk4841.DOI: 10.1126/sciimmunol.adk4841.

95. **Tripathi P**, **Morris SC**, **Perkins C**, **Sholl A**, **Finkelman FD**, **Hildeman DA**. IL-4 and IL-15 promotion of virtual memory CD8+ T cells is determined by genetic background. *Eur J Immunol*. 2016; **46**:2333–2339.DOI: 10.1002/eji.201646404.

96. **Carty SA**, **Koretzky GA**, **Jordan MS**. Interleukin-4 Regulates Eomesodermin in CD8+ T Cell Development and Differentiation Freitas AA, ed. *PLoS ONE*. 2014; **9**:e106659.DOI: 10.1371/journal.pone.0106659.

97. **Kavazović I, Han H, Balzaretti G, Slinger E, Lemmermann NAW, Brinke A ten, Merkler D, *et al.*** Eomes broadens the scope of CD8 T-cell memory by inhibiting apoptosis in cells of low affinity Marrack P, ed. *PLOS Biology*. 2020; **18**:e3000648.DOI: 10.1371/journal.pbio.3000648.

98. **Choi SM**, **Park HJ**, **Boo HJ**, **Jung KC**, **Lee JI**. Characterization of CD8+ virtual memory T cells in IL-4 knockout mice using single-cell RNA sequencing. *Biochemical and Biophysical Research Communications*. 2024; **738**:150950.DOI: 10.1016/j.bbrc.2024.150950.

99. **Tripathi P**, **Morris SC**, **Perkins C**, **Sholl A**, **Finkelman FD**, **Hildeman DA**. IL-4 and IL-15 promotion of virtual memory CD8 $^+$ T cells is determined by genetic background. *European Journal of Immunology*. 2016; **46**:2333–2339.DOI: 10.1002/eji.201646404.

100. **Istaces N, Splittgerber M, Silva VL, Nguyen M, Thomas S, Le A, Achouri Y, *et al.*** EOMES interacts with RUNX3 and BRG1 to promote innate memory cell formation through epigenetic reprogramming. *Nature Communications*. 2019; **10**:3306.DOI: 10.1038/s41467-019-11233-6.

101. **Kwesi-Maliepaard EM, Aslam MA, Alemdehy MF, Brand T van den, McLean C, Vlaming H, Welsem T van, *et al.*** The histone methyltransferase DOT1L prevents antigen-independent differentiation and safeguards epigenetic identity of CD8 $^+$ T cells. *Proceedings of the National Academy of Sciences*. 2020; **117**:20706–20716.DOI: 10.1073/pnas.1920372117.

102. **Bhatt B, Kumar K, Shi H, Ganesan D, Anazodo F, Rathakrishnan A, Zhu H, *et al.*** UFL1 promotes survival and function of virtual memory CD8 T cells. *The Journal of Immunology*. 2025; **214**:446–459.DOI: 10.1093/jimmun/vkae042.

103. **Pokhrel S, Jones DM, Leonard MR, Tuazon JA, Read KA, Dileepan G, Warren RT, *et al.*** Aiolos restricts the generation of antigen-inexperienced, virtual memory CD8 $^{+}$ T cells. 2025. Available at: http://biorxiv.org/lookup/doi/10.1101/2025.06.11.659122.DOI: 10.1101/2025.06.11.659122.

104. **Mandl JN**, **Monteiro JP**, **Vrisekoop N**, **Germain RN**. T Cell-Positive Selection Uses Self-Ligand Binding Strength to Optimize Repertoire Recognition of Foreign Antigens. *Immunity*. 2013; **38**:263–274.DOI: 10.1016/j.immuni.2012.09.011.

105. **Tarakhovsky A**, **Kanner SB**, **Hombach J**, **Ledbetter JA**, **Müller W**, **Killeen N**, **Rajewsky K**. A Role for CD5 in TCR-Mediated Signal Transduction and Thymocyte Selection. *Science*. 1995; **269**:535–537.DOI: 10.1126/science.7542801.

106. **Kieper WC**, **Burghardt JT**, **Surh CD**. A role for TCR affinity in regulating naive T cell homeostasis. *J Immunol*. 2004; **172**:40–44.DOI: 10.4049/jimmunol.172.1.40.

107. **Fulton RB**, **Hamilton SE**, **Xing Y**, **Best JA**, **Goldrath AW**, **Hogquist KA**, **Jameson SC**. 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.DOI: 10.1038/ni.3043.

108. **Swee LK, Tan ZW, Sanecka A, Yoshida N, Patel H, Grotenbreg G, Frickel E-M, *et al.*** Peripheral self-reactivity regulates antigen-specific CD8 T-cell responses and cell division under physiological conditions. *Open Biol*. 2016; **6**:160293.DOI: 10.1098/rsob.160293.

109. **Drobek A, Moudra A, Mueller D, Huranova M, Horkova V, Pribikova M, Ivanek R, *et al.*** Strong homeostatic TCR signals induce formation of self-tolerant virtual memory CD8 T cells. *EMBO J*. 2018; **37**. Available at: https://www.ncbi.nlm.nih.gov/pubmed/29752423.DOI: 10.15252/embj.201798518.

110. **White JT, Cross EW, Burchill MA, Danhorn T, McCarter MD, Rosen HR, O'Connor B, *et al.*** Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. *Nat Commun*. 2016; **7**:11291.DOI: 10.1038/ncomms11291.

111. **Drobek A, Moudra A, Mueller D, Huranova M, Horkova V, Pribikova M, Ivanek R, *et al.*** Strong homeostatic TCR signals induce formation of self-tolerant virtual memory CD8 T cells. *The EMBO Journal*. 2018; **37**. Available at: https://www.embopress.org/doi/10.15252/embj.201798518.DOI: 10.15252/embj.201798518.

112. **Paprckova D, Niederlova V, Moudra A, Drobek A, Pribikova M, Janusova S, Schober K, *et al.*** Self-reactivity of CD8 T-cell clones determines their differentiation status rather than their responsiveness in infections. *Frontiers in Immunology*. 2022; **13**. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1009198/full.DOI: 10.3389/fimmu.2022.1009198.

113. **Prasad M, Wojciech L, Brzostek J, Hu J, Chua YL, Tung DWH, Yap J, *et al.*** Expansion of an Unusual Virtual Memory CD8+ Subpopulation Bearing Vα3.2 TCR in Themis-Deficient Mice. *Frontiers*

in Immunology. 2021; **12**. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.644483/full.DOI: 10.3389/fimmu.2021.644483.

114. **Mahajan VS, Demissie E, Alsufyani F, Kumari S, Yuen GJ, Viswanadham V, Huang A, *et al.*** DOCK2 Sets the Threshold for Entry into the Virtual Memory CD8+ T Cell Compartment by Negatively Regulating Tonic TCR Triggering. *The Journal of Immunology*. 2020; **204**:49–57.DOI: 10.4049/jimmunol.1900440.

115. **Badr ME**, **Zhang Z**, **Tai X**, **Singer A**. CD8 T cell tolerance results from eviction of immature autoreactive cells from the thymus. *Science*. 2023; **382**:534–541.DOI: 10.1126/science.adh4124.

116. **Richards DM**, **Kyewski B**, **Feuerer M**. Re-examining the Nature and Function of Self-Reactive T cells. *Trends in Immunology*. 2016; **37**:114–125.DOI: 10.1016/j.it.2015.12.005.

117. **Lee J-Y**, **Hamilton SE**, **Akue AD**, **Hogquist KA**, **Jameson SC**. Virtual memory CD8 T cells display unique functional properties. *Proceedings of the National Academy of Sciences*. 2013; **110**:13498–13503.DOI: 10.1073/pnas.1307572110.

118. **Baez NS, Cerbán F, Savid-Frontera C, Hodge DL, Tosello J, Acosta-Rodriguez E, Almada L, *et al.*** Thymic expression of IL-4 and IL-15 after systemic inflammatory or infectious Th1 disease processes induce the acquisition of ‘innate’ characteristics during CD8+ T cell development Sacks D, ed. *PLOS Pathogens*. 2019; **15**:e1007456.DOI: 10.1371/journal.ppat.1007456.

119. **Maurice NJ**, **McElrath MJ**, **Andersen-Nissen E**, **Frahm N**, **Prlic M**. CXCR3 enables recruitment and site-specific bystander activation of memory CD8+ T cells. *Nat Commun*. 2019; **10**:4987.DOI: 10.1038/s41467-019-12980-2.

120. **Lin JS**, **Mohrs K**, **Szaba FM**, **Kummer LW**, **Leadbetter EA**, **Mohrs M**. Virtual memory CD8 T cells expanded by helminth infection confer broad protection against bacterial infection. *Mucosal Immunology*. 2019; **12**:258–264.DOI: 10.1038/s41385-018-0100-x.

121. **Wang X**, **Waschke BC**, **Woolaver RA**, **Chen SMY**, **Chen Z**, **Wang JH**. MHC class I-independent activation of virtual memory CD8 T cells induced by chemotherapeutic agent-treated cancer cells. *Cellular & Molecular Immunology*. 2021; **18**:723–734.DOI: 10.1038/s41423-020-0463-2.

122. **Savid-Frontera C, Viano ME, Baez NS, Lidon NL, Fontaine Q, Young HA, Vimeux L, *et al.*** Exploring the immunomodulatory role of virtual memory CD8+ T cells: Role of IFN gamma in tumor growth control. *Frontiers in Immunology*. 2022; **13**. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.971001/full.DOI: 10.3389/fimmu.2022.971001.

123. **Costa ASD**, **Graham JB**, **Swarts JL**, **Lund JM**. Regulatory T cells limit unconventional memory to preserve the capacity to mount protective CD8 memory responses to pathogens. *Proceedings of the National Academy of Sciences*. 2019; **116**:9969–9978.DOI: 10.1073/pnas.1818327116.

124. **Seok J, Cho S-D, Lee J, Choi Y, Kim S-Y, Lee S-M, Kim S-H, *et al.*** A virtual memory CD8+ T cell-originated subset causes alopecia areata through innate-like cytotoxicity. *Nature Immunology*. 2023; **24**:1308–1317.DOI: 10.1038/s41590-023-01547-5.

125. **Akane K**, **Kojima S**, **Mak TW**, **Shiku H**, **Suzuki H**. CD8 $^{+}$ CD122 $^{+}$ CD49d $^{\text{low}}$ regulatory T cells maintain T-cell homeostasis by killing activated T cells via Fas/FasL-mediated cytotoxicity. *Proceedings of the National Academy of Sciences*. 2016; **113**:2460–2465.DOI: 10.1073/pnas.1525098113.

126. **Kim H-J**, **Wang X**, **Radfar S**, **Sproule TJ**, **Roopenian DC**, **Cantor H**. CD8 $^{+}$ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. *Proceedings of the National Academy of Sciences*. 2011; **108**:2010–2015.DOI: 10.1073/pnas.1018974108.

127. **Li J, Zaslavsky M, Su Y, Guo J, Sikora MJ, Unen V van, Christophersen A, *et al.*** KIR $^{+}$ CD8 $^{+}$ T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science*. 2022; **376**. Available at: https://www.science.org/doi/10.1126/science.abi9591.DOI: 10.1126/science.abi9591.

128. **Warren HS**, **Rana PM**, **Rieger DT**, **Hewitt KA**, **Dahlstrom JE**, **Kent AL**. CD8 T cells expressing killer Ig-like receptors and NKG2A are present in cord blood and express a more naïve phenotype than their counterparts in adult blood. *Journal of Leukocyte Biology*. 2006; **79**:1252–1259.DOI: 10.1189/jlb.0905536.

129. **Min HS, Lee YJ, Jeon YK, Kim EJ, Kang BH, Jung KC, Chang C-H, *et al.*** MHC Class II-Restricted Interaction between Thymocytes Plays an Essential Role in the Production of Innate CD8+ T Cells. *The Journal of Immunology*. 2011; **186**:5749–5757.DOI: 10.4049/jimmunol.1002825.

130. **He S, Luo C-L, Luo T, Chen H-T, Zhang S-F, Jiang J-X, Wang X-Y, *et al.*** Systemic immune activity occurs during human immune system maturation. *Cell*. 2025; **188**:7291-7308.e23.DOI: 10.1016/j.cell.2025.10.003.

131. **Lee YJ, Jeon YK, Kang BH, Chung DH, Park C-G, Shin HY, Jung KC, *et al.*** Generation of PLZF+ CD4+ T cells via MHC class II–dependent thymocyte–thymocyte interaction is a physiological process in humans. *Journal of Experimental Medicine*. 2010; **207**:237–246.DOI: 10.1084/jem.20091519.

132. **Herndler-Brandstetter D, Brunner S, Weiskopf D, Rijn R van, Landgraf K, Dejaco C, Duftner C, *et al.*** Post-thymic regulation of CD5 levels in human memory T cells is inversely associated with the strength of responsiveness to interleukin-15. *Human Immunology*. 2011; **72**:627–631.DOI: 10.1016/j.humimm.2011.03.028.

133. **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.DOI: 10.1182/blood-2002-11-3577.

134. **Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, Mullen AC, *et al.*** Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nature Immunology*. 2005; **6**:1236–1244.DOI: 10.1038/ni1268.

135. **Kasakovski D, Zeng X, Lai J, Yu Z, Yao D, Chen S, Zha X, *et al.*** Characterization of KIR + NKG2A + Eomes− NK-like CD8+ T cells and their decline with age in healthy individuals. *Cytometry Part B: Clinical Cytometry*. 2021; **100**:467–475.DOI: 10.1002/cyto.b.21945.

136. **Jacomet F, Cayssials E, Basbous S, Levescot A, Piccirilli N, Desmier D, Robin A, *et al.*** Evidence for eomesodermin-expressing innate-like CD8 $^{+}$ KIR/NKG2A $^{+}$ T cells in human adults and cord blood samples. *European Journal of Immunology*. 2015; **45**:1926–1933.DOI: 10.1002/eji.201545539.

137. **Pieren DKJ, Smits NAM, Hoeboer J, Kandiah V, Postel RJ, Mariman R, Beek J van, *et al.*** Regulatory KIR $^+$ RA $^+$ T cells accumulate with age and are highly activated during viral respiratory disease. *Aging Cell*. 2021; **20**. Available at: https://onlinelibrary.wiley.com/doi/10.1111/acel.13372.DOI: 10.1111/acel.13372.

138. **Vasandan AB, Abdelsamed HA, Boi SK, Ward G, Alli S, Mi T, Lan X, *et al.*** Innate-like memory T cells rapidly emerge in humans after gene therapy for SCID-X1. *Immunity*. 2025; **58**:1922-1930.e3.DOI: 10.1016/j.immuni.2025.07.002.

139. **Schreurs RRCE, Baumdick ME, Sagebiel AF, Kaufmann M, Mokry M, Klarenbeek PL, Schaltenberg N, *et al.*** Human Fetal TNF-α-Cytokine-Producing CD4+ Effector Memory T Cells Promote Intestinal Development and Mediate Inflammation Early in Life. *Immunity*. 2019; **50**:462-476.e8.DOI: 10.1016/j.immuni.2018.12.010.

140. **Kim J, Chang D-Y, Lee HW, Lee H, Kim JH, Sung PS, Kim KH, *et al.*** Innate-like Cytotoxic Function of Bystander-Activated CD8+ T Cells Is Associated with Liver Injury in Acute Hepatitis A. *Immunity*. 2018; **48**:161-173.e5.DOI: 10.1016/j.immuni.2017.11.025.

141. **Kim T-S**, **Shin E-C**. The activation of bystander CD8+ T cells and their roles in viral infection. *Experimental & Molecular Medicine*. 2019; **51**:1–9.DOI: 10.1038/s12276-019-0316-1.

142. **Seo I-H, Eun HS, Kim JK, Lee H, Jeong S, Choi SJ, Lee J, *et al.*** IL-15 enhances CCR5-mediated migration of memory CD8+ T cells by upregulating CCR5 expression in the absence of TCR stimulation. *Cell Reports*. 2021; **36**:109438.DOI: 10.1016/j.celrep.2021.109438.

143. **Hu W, Li Y-J, Zhen C, Wang Y-Y, Huang H-H, Zou J, Zheng Y-Q, *et al.*** CCL5-Secreting Virtual Memory CD8+ T Cells Inversely Associate With Viral Reservoir Size in HIV-1−Infected Individuals on Antiretroviral Therapy. *Frontiers in Immunology*. 2022; **13**. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.897569/full.DOI: 10.3389/fimmu.2022.897569.

144. **Martin MD**, **Badovinac VP**. Defining Memory CD8 T Cell. *Frontiers in Immunology*. 2018; **9**. Available at: https://www.frontiersin.org/article/10.3389/fimmu.2018.02692/full.DOI: 10.3389/fimmu.2018.02692.

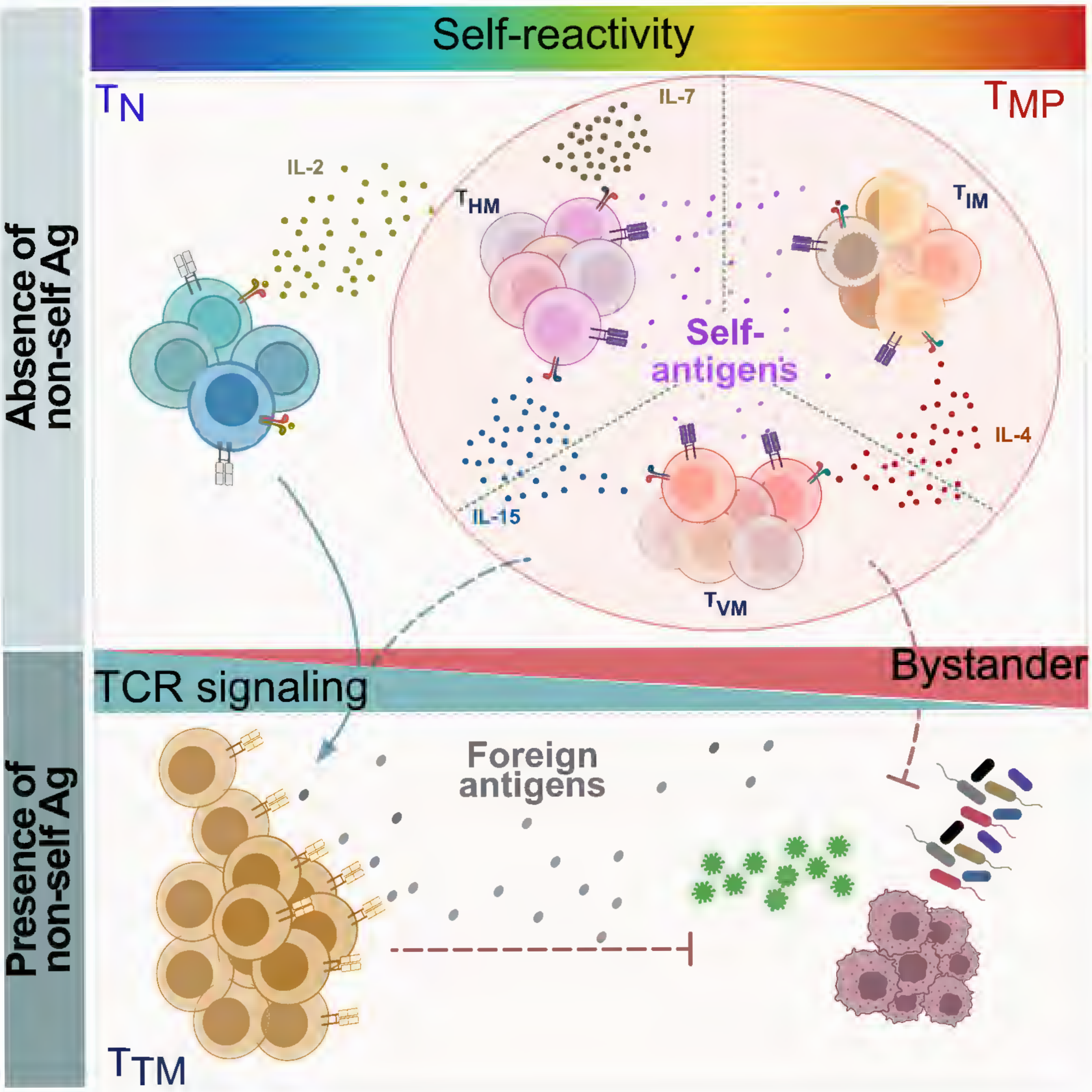
Figure legends

Figure 1. Origin and maintenance of T_{MP}. Summary of the origin, maintenance and the main phenotypes of T_{HM}, T_{IM} and T_{VM}. Contribution of thymic T_{IM} to peripheral T_{VM}, remains unclear. DN, double negative; DP, double positive; SP, single positive; APC, antigen-presenting cells; γc, common γ chain.

Figure 2. The contribution of T_{VM} in disease. T_{VM} acquire their memory phenotype in the thymus through recognition of self-ligands, associated with EOMES expression. In the periphery, T_{VM} are self-reactive but self-tolerant and are able to respond to bystander stimulation and infiltrate solid tumors to express high PD-1 [51]. Upon helminth infection, IL-4 can lead to T_{VM} activation, expansion and upregulation of CD22 [94]. IL-4-expanded T_{VM} contribute to clearance of bacterial coinfection and enhanced control of respiratory viral infection by differentiating into effector cells producing IFN-γ and GZMB, recruited to the lung and exhibiting cytotoxicity [93],[120].

Table 1. Phenotypic identification of T cells populations, based on foreign Ag experience [15],[52],[57],[62],[144].

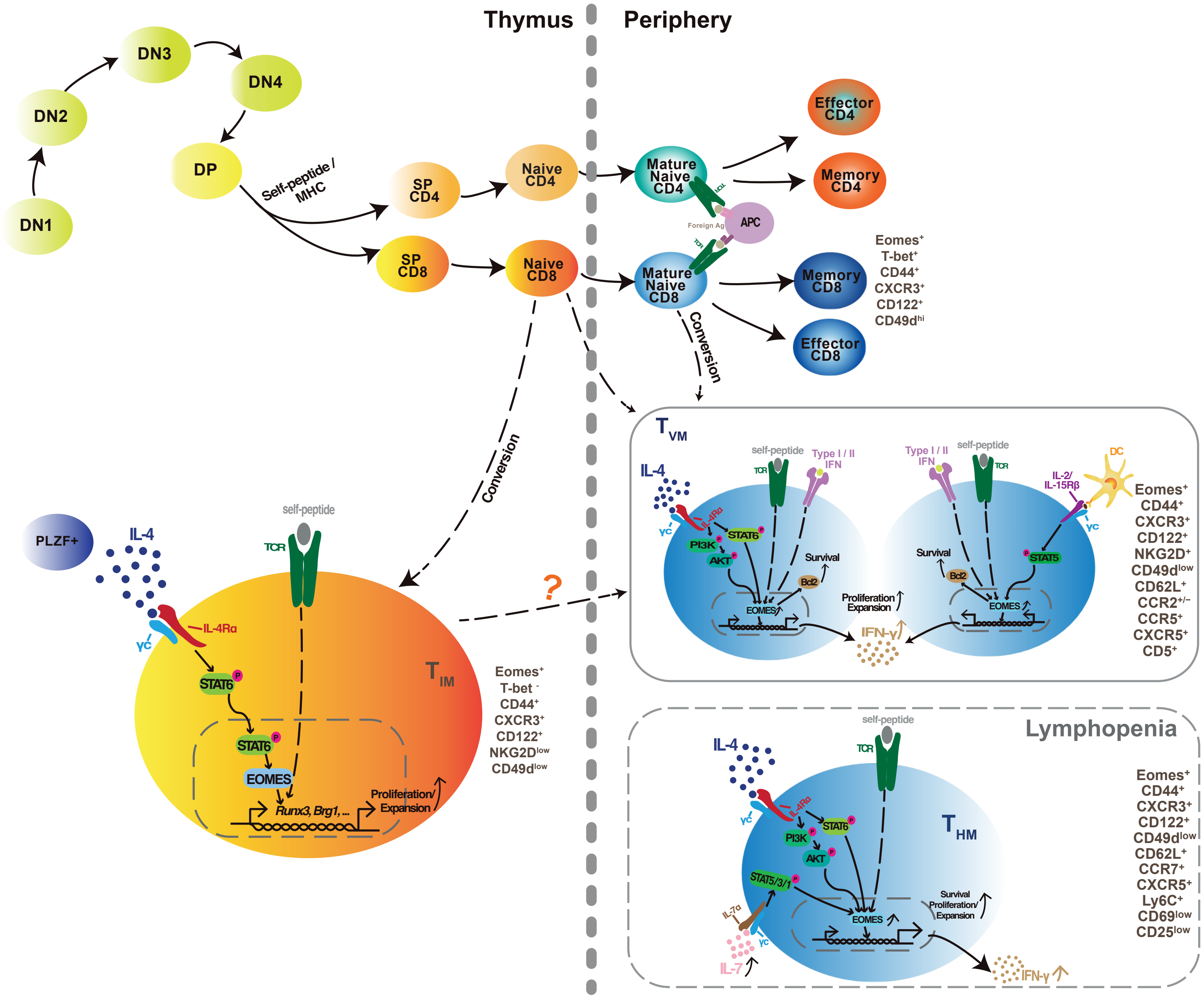
	Immunologically "naïve" host					**Antigen experienced host**			
	T_N	**T_{MP}**							
Cell types	Mouse T_N	Mouse T_{HM}	Mouse T_{IM}	Mouse T_{VM}	Human T_{VM}	Mouse T_{CM}	Mouse T_{EM}	Mouse T_{RM}	Human Effector memory Re-expressing CD45RA
Key markers	CD44^{low} CD49d^{low}	CD44^{hi} CD49d^{low}	CD44^{hi} CD49d^{low}	CD44^{hi} CD49d^{low}	CD45RA^{+} KIR^{hi} and/or NKG2A^{hi}	CD44^{hi} CD49d^{hi}	CD44^{hi} CD49d^{hi}	CD44^{hi}	CD45RA^{+}
Additional markers	CD62L^{hi} CCR7^{hi} Eomes^{low} CD122^{low} CD127^{hi} CXCR3^{low} CX3CR1^{low}	CD62L^{hi} CD122^{hi} CXCR3^{+} CCR7^{+} CXCR5^{+} Ly6C^{+} CD122^{+} CD69^{low} CD25^{low}	CD62L^{hi} CD122^{hi} Eomes^{hi} CXCR3^{hi} T-bet^{-} CD5^{+}	CD62L^{hi} CCR7^{low} Eomes^{hi} CD122^{hi} CD127^{hi} CXCR3^{hi} CX3CR1^{low} NKG2D^{+} T-bet^{+} CCR2$^{+/-}$ CCR5^{+} CXCR5^{+} CD5^{+}	CD45RO^{-} CCR7^{low} Eomes^{hi} CD27^{low} CD5^{low} CD122^{hi} NUR77^{hi} T-bet^{+}	CD62L^{hi} CCR7^{hi} Eomes^{hi} CD122^{int} CD127^{hi} CXCR3^{hi} CX3CR1^{low} T-bet^{low}	CD62L^{low} CCR7^{low} Eomes^{low} CD122^{low} CD127^{low} CXCR3^{hi} CX3CR1^{hi}	CD62L^{-} CCR7^{-} CD103^{hi} CD69^{hi} CD27^{low} CD49a^{+} CD127^{hi} CXCR3^{hi} CX3CR1$^{low/int}$ T-bet^{low} EOMES$^{-/low}$ (depending on tissue)	CD27^{-} CCR7^{-} CD127^{low}


Self-reactivity
T_N
T_{MP}
IL-7
IL-2
T_{HM}
T_{IM}
Self-antigens
IL-15
IL-4
T_{VM}
Absence of non-self Ag
TCR signaling
Bystander
Presence of non-self Ag
Foreign antigens
T_{TM}

Graphical abstract text :

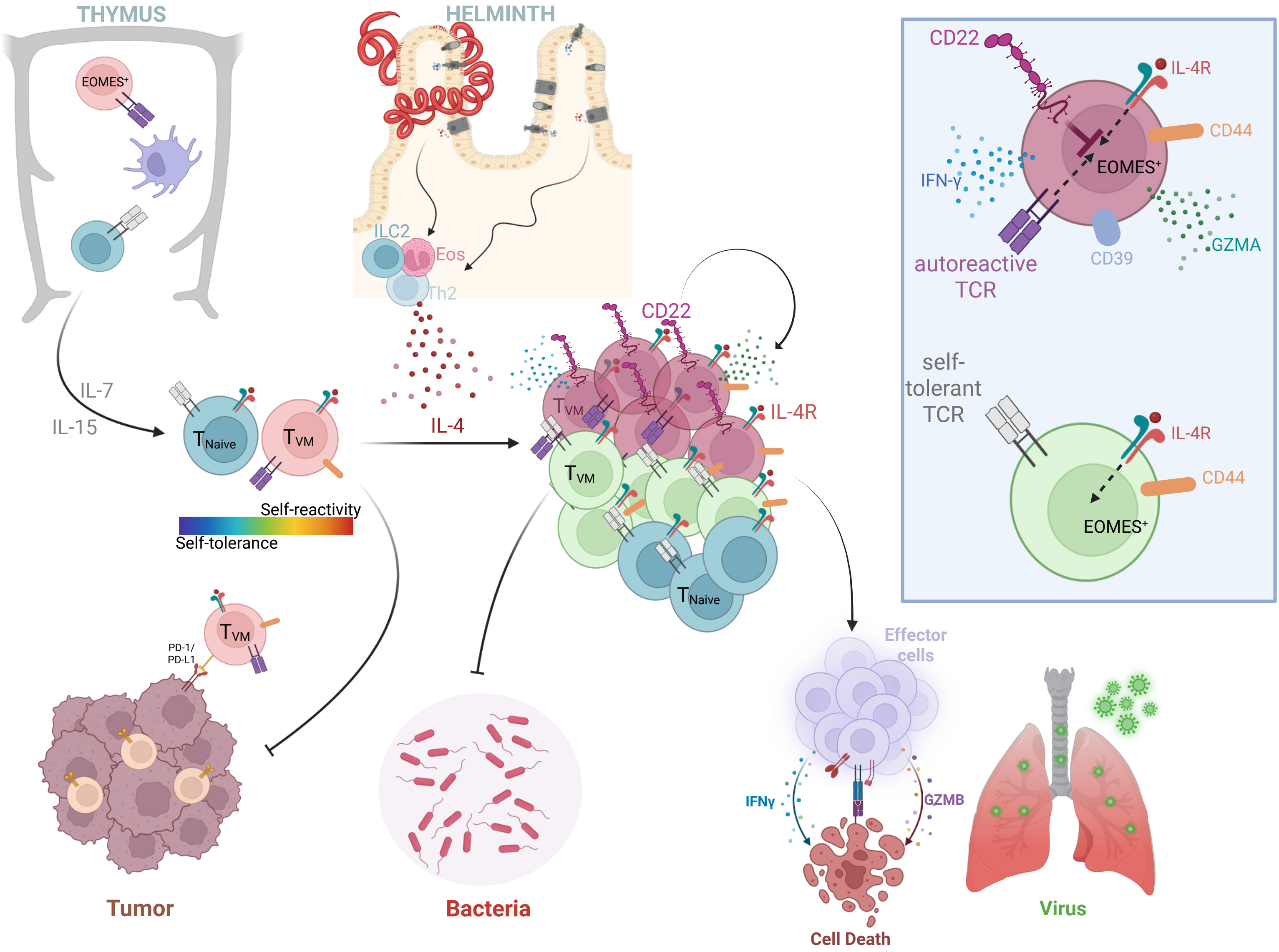
This review highlights how memory-phenotype CD8$^+$ T lymphocytes (T_{MP}) develop in immunologically naive hosts in absence of non-self antigens (Ag). We discuss the current understanding and controversies on the origin, maintenance and functions of the main populations recognized as T_{MP}, in comparison to naive (T_N) and Ag-specific true memory T cells (T_{TM}).

Fig01



Thymus
Periphery
DN1
DN2
DN3
DN4
DP
Self-peptide / MHC
SP CD4
SP CD8
Naive CD4
Naive CD8
Mature Naive CD4
Mature Naive CD8
TCR
Foreign Ag
APC
Effector CD4
Memory CD4
Memory CD8
Effector CD8
Eomes+
T-bet+
CD44+
CXCR3+
CD122+
CD49dhi
Conversion
Conversion
?
PLZF+
IL-4
self-peptide
TCR
γc
IL-4Rα
STAT6
P
STAT6
EOMES
Runx3, Brg1, ...
Proliferation/ Expansion
TIM
Eomes+
T-bet -
CD44+
CXCR3+
CD122+
NKG2Dlow
CD49dlow
TVM
IL-4
γc
IL-4Rα
PI3K
AKT
STAT6
self-peptide
TCR
Type I / II IFN
Survival
Bcl2
EOMES
Proliferation Expansion
IFN-γ
Type I / II IFN
self-peptide
TCR
DC
IL-2/ IL-15Rβ
γc
STAT5
Survival
Bcl2
EOMES
Eomes+
CD44+
CXCR3+
CD122+
NKG2D+
CD49dlow
CD62L+
CCR2+/−
CCR5+
CXCR5+
CD5+
Lymphopenia
self-peptide
TCR
IL-4
γc
IL-4Rα
STAT6
PI3K
AKT
STAT5/3/1
IL-7α
γc
IL-7
THM
EOMES
Survival Proliferation/ Expansion
IFN-γ
Eomes+
CD44+
CXCR3+
CD122+
CD49dlow
CD62L+
CCR7+
CXCR5+
Ly6C+
CD69low
CD25low

Fig02


THYMUS
EOMES+
IL-7
IL-15
T Naive
T VM
Self-reactivity
Self-tolerance
HELMINTH
ILC2
Eos
Th2
IL-4
CD22
T VM
IL-4R
T VM
T Naive
PD-1/
PD-L1
T VM
Tumor
Bacteria
Effector
cells
IFNγ
GZMB
Cell Death
Virus
CD22
IL-4R
CD44
IFN-γ
EOMES+
autoreactive
TCR
CD39
GZMA
self-
tolerant
TCR
IL-4R
CD44
EOMES+