Charles University

Faculty of Science

Study program: Special Chemical and Biological Programmes

Branch of study: Molecular Biology and Biochemistry of Organisms

Michaela Přibíková

Homeostatic memory and virtual memory CD8$^+$ T cells

Homeostatické paměťové a virtuální paměťové CD8$^+$ T lymfocyty

Bachelor‘s Thesis

Supervisor: Mgr. Ondřej Štěpánek, Ph.D.

Prague, 2017

Poděkování

Především bych ráda poděkovala mému školiteli Mgr. Ondřeji Štěpánkovi, Ph.D. za cenné rady a trpělivost při psaní této bakalářské práce. Ráda bych také poděkovala všem lidem z laboratoře adaptivní imunity a mým blízkým.

Prohlášení:

Prohlašuji, že jsem tuto práci vypracovala samostatně na základě konzultací se svým školitelem a použití uvedené literatury. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 15. 5. 2017

Abstract

This work sums up current knowledge of so-called homeostatic proliferation-induced memory $CD8^+$ T cells (HP) and virtual memory $CD8^+$ T cells. These populations do not represent true immunological memory because they are generated in the absence of a cognate foreign antigen. However, both HP-memory and virtual memory T cells share some phenotypical and functional features with true memory T cells, including the ability provide rapid immune response during infection. HP-memory T cells are generated via homeostatic proliferation during experimentally induced lymphopenia. Virtual memory T cells might arise via homeostatic proliferation during neonatal or age-related periods of lymphopenia, however, they can be generated also in healthy lymphoreplete hosts. Based on detail analysis of these two populations, I concluded that HP-memory $CD8^+$ T cells and virtual memory $CD8^+$ T cells most likely use identical differentiation program and represent the same T cell population.

Keywords: homeostatic proliferation, lymphopenia, $CD8^+$ T cells, immunological memory, virtual memory cells

Abstrakt

Tato práce shrnuje dosavadní poznatky zabývající se homeostatickou proliferací vzniklými paměťovými buňkami (HP) a virtuálními paměťovými $CD8^+$ T buňkami. Tyto dvě buněčné populace nepředstavují pravou imunologickou paměť, protože se tvoří v nepřítomnosti cizího antigenu. Nicméně, HP-paměťové i virtuální paměťové T buňky sdílejí některé fenotypické a funkční vlastnosti s pravými paměťovými buňkami, včetně schopnosti poskytnout rychlou imunitní odpověď během infekce. HP-paměťové buňky vznikají homeostatickou proliferací během lymfopenie. Virtuální paměťové buňky mohou vznikat také homeostatickou proliferací během neonatální nebo stařecké lymfopenie, ale objevují se i v jedincích, kteří mají normální počet lymfocytů v krvi. Na základě podrobné analýzy těchto dvou buněčných populací jsem došla k závěru, že HP-paměťové buňky a virtuální paměťové buňky pravděpodobně využívají stejný diferenciační program a představují stejnou populaci buněk.

Klíčová slova: homeostatická proliferace, lymfopenie, $CD8^+$ T buňky, imunologická paměť, virtuální paměťové buňky

Abbreviations

B6	C57BL6 mice
GF	germ free
HP	homeostatic proliferation
IFN-γ	interferon gamma
LMCV	*lymphocytic choriomeningitis virus*
Lm.OVA	Ovalbumin expressing *Listeria monocytogenes*
LN	Lymph nodes
MHC	Major histocompatibility complex
OVA	Ovalbumin
SPF	specific pathogen free
T_{CM}	central memory T cells
TCR	T-cell receptor
TM	true memory
VM	virtual memory
VSV	*vesicular stomatitis virus*

Outline

1. Introduction

T cells play a major role in adaptive immune responses. Conventional T cells use their T cell antigen receptor (TCR) to recognize antigens, i.e., peptides derived from proteins of invading pathogens, which are presented by antigen presenting cells. The hallmark of the immune protection by T cells is that each T-cell clone expresses a unique TCR and recognizes a different set of antigens. Conventional T cells express either CD4 or CD8 invariant coreceptor. $CD4^+$ T cells recognize antigens presented by MHCII (major histocompatibility complex), while $CD8^+$ T cells recognize antigens presented by MHCI.

After a T cell matures in the thymus, it migrates to peripheral lymphoid tissues as a naïve (i.e., antigen inexperienced) cell. Upon infection, a few T cell clones recognize antigens derived from the pathogen via their TCRs. Activation of the TCR signaling pathway induces rapid proliferation and differentiation into short-lived effector T cells and memory T cells which facilitate long-term protection against the same pathogen.

The memory T cells can be distinguished from naive T cells using specific surface markers such as CD44, which is expressed in memory, but not naïve mouse T cells. Moreover, memory T cells exhibit specific antigenic responses that differentiate them from naive T cells. For instance, memory T cells, but not naïve T cells, rapidly secrete interferon gamma (IFN-γ) after TCR stimulation (1). Interestingly, antigen inexperienced $CD8^+$ T cells with apparent memory phenotype have been described. Antigen-independent formation of memory-phenotype $CD8^+$ T cells can be induced by the lymphopenic environment. Because the differentiation into $CD8^+$ memory phenotype T cells in lymphopenia is coupled with homeostatic proliferation (1), such memory-phenotype T cells were named homeostatic proliferation-induced (HP) memory T cells. Recently, antigen inexperienced memory-phenotype T cells were identified in normal lymphoreplete mice (2). These T cells were called virtual memory (VM) T cells (3).

In this thesis, I will review current knowledge of HP memory and VM $CD8^+$ T cells. The main aim is to find out whether or not HP memory and VM T cells represent the same T cells population (i.e., they use identical differentiation program).

2. Memory $CD8^+$ T cells

Once naïve $CD8^+$ T cells recognize invading pathogen-derived peptide MHC I complexes, $CD8^+$ T cells undergo proliferation and differentiation to form a population of an effector cells. Effector cells rapidly eliminate pathogen-infected cells by using multiple mechanisms (i.e., IFN-γ and tumor necrosis factor (TNF-α) production, direct cell killing). After resolving the infection the majority of effector cells die by apoptosis. However, a small, heterogeneous population of long-lived memory $CD8^+$ cells remain (4–6).

T cells with memory phenotype are resting cells, which create at least two subpopulations, "central" (T_{cm}) and "effector" (T_{em}) cells (7). Central memory and effector memory cells are defined based on their differential expression of CCR7 and CD62L. T_{cm} express high levels of these receptors and home to secondary lymphoid tissues. In contrast, T_{em} do not express CCR7 and CD62L and localize mostly to nonlymphoid tissues and to the spleen (8). In this thesis, I will focus on T cells with an apparent central memory phenotype.

Memory $CD8^+$ T cells have protective advantage over naïve T cells. The secondary response of memory $CD8^+$ T cells is faster than primary response because they are present in larger numbers. However, the magnitude of primary response of memory T cells to antigen in comparison to naïve T cells is not clear. Some studies showed that memory $CD8^+$ T cells respond more rapidly than their naïve counterparts (9–11). By contrast, some recent studies found that memory $CD8^+$ T cells have lower proliferative responses in the same host after antigen stimulation than naïve T cells on a per-cell basis (12–14).

The characteristic hallmark of memory $CD8^+$ T cells is high expression of CD45RO in humans and CD44 in mice (7,15). Murine memory $CD8^+$ T cells can be identified as $CD44^{hi}$, $CD122^{hi}$, and $Ly6C^{hi}$ (16). Another important hallmark distinguishing memory $CD8^+$ T cells from naïve T cells is rapid expression of IFN-γ after antigenic stimulation (1), a key proinflammatory cytokine in most types of infection (17).

3. HP-memory CD8$^+$ T cells

Activation of naïve T cells by cognate antigens induces formation of effector and memory T cells that mediate immediate and long-term immune protection, respectively. However, it has been reported that memory-like T cells can arise in the absence of foreign antigens via homeostatic proliferation (HP) during lymphopenia (i.e., condition of low lymphocyte levels) in mice. These so called HP memory T cells share gene expression profile and some phenotypic features with antigen-induced memory (true memory; TM) T cells, although they do not constitute the bona fide immunological memory (1).

3.1 Generation of HP-memory T cells

3.1.1 TCR:self-pMHC interactions are essential for homeostatic proliferation of naïve T cells

Homeostatic proliferation (also called lymphopenia-induced proliferation) of naïve T cells during lymphopenia is relatively slow, depends on low-affinity MHC/self-peptide complexes and is driven by various cytokines. Homeostatic proliferation of naïve T cells is not associated with up-regulation of activation-induced antigens, such as CD25 or CD69 (18,19). It was observed that certain clones of naïve TCR transgenic cells are not able to undergo homeostatic proliferation (e.g. H-Y cells) (20) and some clones undergo homeostatic proliferation faster than others. T cells with low level of self-reactivity (e.g. 2C, P14 or F5 cells) of their TCRs to MHC/self-peptide molecules undergo slower homeostatic proliferation than T cells with high level of self-reactivity TCR (e.g. OT-I cells) (21).

Three different clones (OT-I, F5, and 2C) were used to analyze homeostatic responses and competition among naïve CD8$^+$ T cells. By transferring naïve T cells into RAG$^-$ mice, it was observed that only OT-I cells expressed high levels of CD44, CD122, and Ly6C (i.e., markers specific for memory phenotype T cells.). OT-I T cells in F5/RAG$^-$ or 2C/RAG$^-$ recipient mice undergo slow proliferation not associated with up-regulation of CD69, which are characteristic hallmarks of homeostatic proliferation and this is the evidence that OT-I cells had access to MHC/self-peptide in these mice (22,23).

These experiments showed that self-reactivity (measured as levels of a self-reactivity marker, CD5) of a particular T-cell clone correlates with its rate of homeostatic proliferation and ability to form HP memory. This indicates that HP memory formation is driven by TCR:self-pMHC interactions (22,24–26).

3.1.2 The role of IL-7 and other cytokines

It is known that particular cytokines are crucial for T cell development, survival, activation, and homeostasis. Homeostatic proliferation of naïve T cells is enhanced by several cytokines, including IL-7, IL-15, and IL-4. These cytokines are able to enhance homeostatic proliferation of $CD8^+$ T cells *in vitro*. However, it was found that naïve T cells die in few days after a transfer to IL-$7^{-/-}$ hosts, whereas they undergo homeostatic proliferation in IL-$15^{-/-}$ and IL-$4^{-/-}$ hosts. This is the evidence that only IL-7 is essential for survival and homeostatic proliferation of naïve T cells *in vivo* (27,28).

In contrast to naïve T cells, memory phenotype $CD8^+$ T cells ($CD44^{hi}$) express high levels of receptors for both IL-7 and IL-15. IL-7 or IL-15 alone are sufficient to stimulate homeostatic proliferation in $CD44^{hi}$ T cells, suggesting that once a naïve T cell becomes an HP memory T cells, it is not exclusively dependent on IL-7 (29).

3.2 Gene-expression profile of HP-memory T cells

Goldrath et al. (16) analyzed naïve OT-I TCR transgenic cells at different time-points of homeostatic proliferation. They observed that the majority of OT-I T cells expressed high levels of CD44, CD122, and Ly6C after 15 days. However, after 40 days, the majority of cells expressed same levels of these memory markers as naïve T cells. They suggested that conversion of naïve T cells to HP-memory phenotype T cells is reversible process (16).

In contrast, Goldrath et al. also analyzed naïve OT-I TCR transgenic $CD8^+$ T cells at different time-points of homeostatic proliferation to investigate whether T cells in lymphopenic hosts display a unique gene-expression signature. After 6 days in lymphopenic host, only 60% of the genes specific for memory cells were expressed at a higher level in the HP population than in naïve cells. In contrast, 95% of the genes correlated with memory expression 4 months later (1,18).

It was observed that naïve T cells acquire gene-expression profile identical to antigen-experienced memory cells, over the homeostatic proliferation time course in lymphopenic host. This analysis did not reveal a unique gene-expression profile for HP-memory cells (18).

The single important difference between HP-memory cells and TM T cells is distinct expression of α4-integrin (CD49d), a component of homing receptors VLA-4 and LPAM. HP-memory cells express low levels of CD49d ($CD49d^{lo}$) and their CD49d expression seems to be even lower than that of naive cells. In contrast, this integrin is normally expressed at high levels on TM cells ($CD49d^{hi}$) (3).

3.3 Immune response of HP-memory T cells

As mentioned, HP-memory $CD8^+$ T cells acquire some functional and phenotypic characteristics similar to antigen-specific memory $CD8^+$ T cells, such as up-regulation of memory cell markers or rapid production of IFN-γ. It is known, that antigen-specific memory $CD8^+$ T cells facilitate rapid response to pathogens during secondary infections. However, the important question is if HP-memory $CD8^+$ T cells can offer better immune protection against pathogenic infections than naïve T cells.

3.3.1 Comparable protective effects of HP-memory TM T cells

Protective capacity of HP-memory $CD8^+$ T cells was observed by using a double adoptive transfer system. Both HP-memory and TM populations were generated from naïve ovalbumin-reactive (OVA) OT-I transgenic $CD8^+$ T cells in sublethally irradiated mice or in mice infected with OVA expressing *Listeria monocytogenes* (Lm.OVA), respectively. Subsequently, the cells were transferred into healthy wild type (WT) recipients and infected with Lm.OVA. The bacterial clearance was observed 5 days after infection. HP-memory $CD8^+$ T cells provided as robust protection against Lm.OVA infection as TM T cells on a per-cell basis (30).

3.3.2 Functional differences between TM and HP T cells are revealed in a competitive setup

Although monoclonal OT-I HP memory T cells and TM T cells showed comparable expansion and protection against Lm.OVA when transferred into separate recipients (30), some differences were reported when these two memory cell types were cotransferred into a single host (31).

To analyzed numbers, phenotype, and localization of HP-memory and TM cells Cheung et al. generated these cells with distinct congenic markers CD45.1 and CD45.2, respectively, and transferred both populations in equal numbers into WT recipients and infected them with Lm.OVA 1 day later (31). They observed that despite the earlier expansion of HP-memory cells, the expansion of TM cell is more robust. Interestingly, it was shown that HP-memory cells rapidly up-regulated CD69L and localized to lymph nodes (LN), whereas TM T cells localized to the spleen during infection (8,31).

These data indicated that TM cells show superior antigenic responses to HP cells when they compete with each other for some limiting resources, potentially the antigen, costimulation signals, cytokines, or space.

3.3.3 Protective capacity of HP-memory cells depends on CD4⁺ T cells help

It was observed that $CD4^+$ T cells are required for the establishment and maintenance of memory $CD8^+$ T cells (32). Because it was shown that HP-memory T cells have similar properties with TM cells, the next step was to find out whether $CD4^+$ T cells are important for homeostatic proliferation of HP-memory $CD8^+$ T cells.

OT-I cells transferred into irradiated MHC class II-deficient (i.e., devoid of $CD4^+$ T cells) mouse underwent homeostatic proliferation and formed HP memory T cells. However, after adoptive transfer into WT recipients and Lm.OVA challenge, HP-memory T cells from MHC class II-deficient mouse did not provide better protection against the Lm.OVA than naïve T cells. In contrast, TM and HP-memory cells from WT mouse, generated in the presence of $CD4^+$ T cells, were capable of mediating protection against Lm.OVA challenge. Nevertheless, all HP-memory cells from MHC class II-deficient host, HP-memory cells, and TM cells from WT host expressed high levels of CD44, CD122, and Ly6C and showed rapid production of IFN-γ after activation. These results indicate that generation of defective HP-memory cells from MHC class II-deficient host could not be caused by the failure of induction of the HP memory differentiation program.

In summary, although $CD4^+$ T cells are essential for the function and protective capacity of HP-memory $CD8^+$ T cells, they are not necessary for their formation. However, how $CD4^+$ T cells affect functionality of HP-memory cells remains to be elucidated (30,33,34).

3.4 HP-memory T cells in humans

3.4.1 Presence of HP-memory T cells in humans is unclear

The demonstration that HP-memory $CD8^+$ T cells are present in mice leads to a question if populations of these cells exist in humans. Some studies observed presence of innate population of memory-phenotype T cells in the spleen and cord blood of fetus. However, it is difficult to find out the origin and function of these human memory-phenotype T cells and it is still unknown what happened with these cells after the end of pregnancy (35,36). In summary, presence of HP-memory $CD8^+$ T cells in humans and healthy organism remains unclear (3).

3.4.2 Potential role of HP-memory T cells in cancer immunotherapy

HP-memory $CD8^+$ T cells can induce protective immunity. Moreover, Dummer et al. observed that homeostatic proliferation of T cells during lymphopenia can also induce effective antitumor autoimmunity (37). Melanoma cells were injected into sublethally irradiated and nonirradiated WT mice followed by a transfer of 5×10^7 LN cells. After almost 2 months, they observed that sublethally irradiated mice had significantly smaller tumors than control mice (37).

In summary, this study (37) and some others (38,39) indicated that HP-memory T cells may be potentially useful in cancer immunotherapy and for immunotherapy of immunocompromised people.

4. Virtual memory CD8$^+$ T cells

Halusczak et al. have described a population of central memory phenotype (CD44$^+$ CD62L$^+$) CD8$^+$ T cells that has not previously encountered its cognate antigen in unmanipulated WT mice (3). These cells were termed virtual memory CD8$^+$ T cells

4.1 Generation of VM CD8$^+$ T cells

4.1.1 Origin of VM CD8$^+$ T cells

There are two possible explanations why some naïve T cells differentiate into VM CD8$^+$ T cells. First, they could differentiate from naïve T cells with relatively high affinity for self-antigens. Second, VM CD8$^+$ T cells may be generated by physiological homeostatic mechanism during neonatal or age-related lymphopenia (3,40,41).

4.1.2 Factors important for VM T cells formation

Sosinowski et al. (40) and others (42) observed that VM CD8$^+$ T cells develop in the periphery rather than in the thymus in WT C57/BL6 (B6) mice. VM CD8$^+$ T cells are minimally dependent on IL-4, whereas IL-15 has a key role in their formation because these VM T cells are substantially reduced in IL-15$^{-/-}$ mice (40). Likewise, formation and/or maintenance of VM T cells also requires expression of CD122, a subunit of IL-15 receptor, on CD8$^+$ T cells (2,40). Administration of IL-15/Rα complexes induced rapid expansion and/or de novo generation of VM T cells. Next, Sosinowski et al. observed that VM CD8$^+$ T cells expressed higher levels of a transcriptional factor Eomes than naïve T cells. They proposed that Eomes expression is another important factor in the development of VM T cells, because of the loss of VM T cells in T cell-specific Eomes deficient mice. Their hypothesis is that proliferation of T cells and conversion to VM CD8$^+$ T cells are caused by higher expression of Eomes which induces higher expression of CD122 and higher sensitivity to IL-15 presented by CD8α$^+$ dendritic cells (40).

In summary, these data indicated that formation of VM CD8$^+$ T cells depends on IL-15, CD8α$^+$ dendritic cells, intrinsic expression of CD122, and Eomes (40,42).

Sosinowski et al. (40) characterized VM $CD8^+$ T cells in B6 mice which have very low levels of IL-4 (43). In contrast to this study, Tripathi et al. (44) characterized VM $CD8^+$ T and their dependence on IL-4 and IL-15 in both B6 mice and Balb/c mice. They observed that virus-specific VM cells in Balb/c mice were significantly more reduced in the absence of IL-4 than in the absence of IL-15. However, naïve T cells were not reduced in the absence of either IL-4 or IL-15 or both. In summary, the development and/or maintenance of VM $CD8^+$ T cells in Balb/c mice depends more on IL-4 than on IL-15, whereas development and/or maintenance of VM $CD8^+$ T cells in B6 mice largely depends on IL-15 and minimally on IL-4 (44).

A memory-phenotype IL-4 dependent population of so-called *innate memory* $CD8^+$ T cells develops in the thymus of Balb/c, but not WT B6, mice (43,45). It is not clear if the VM $CD8^+$ T cells observed by Tripathy et al. (44) in Balb/c mice and the innate memory T cells represent one or two independent lineages. Kurzweil et al. (43) observed, that deficiency in Ndfip1 adaptor protein leads to increased production of IL-4 in the periphery and subsequent generation/expansion of VM $CD8^+$ T cells in the periphery, but not in the thymus (43). This suggests that IL-4 can induce and/or expand memory phenotype T cells in the thymus as well as in the periphery (Fig. 1).

The relationships between the three experimentally observed populations of antigen-independent memory-like T cells, i.e., canonical IL-15-dependent VM T cells in B6 mice, thymic IL-4-dependent innate memory T cells in Balb/c mice, and peripherally IL-4 induced memory-phenotype in $Ndfip1^{-/-}$ B6 mice, remain to be addressed.

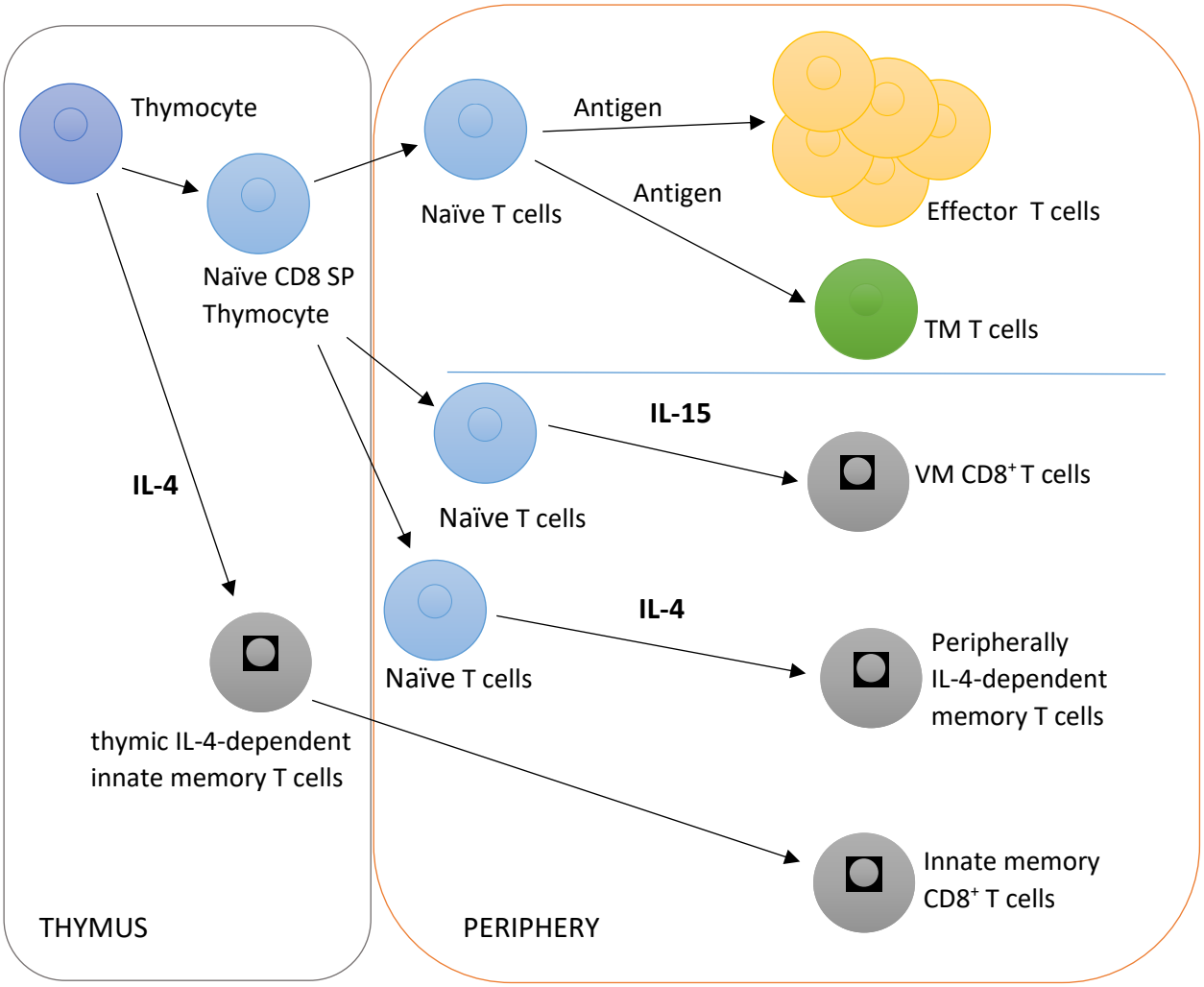

Fig. 1 Conversion of naïve T cells in the thymus and in the periphery under the control of IL-4 and IL-15 (modified from Lauvau a Goriely, 2016 (46))

4.1.3 The role of TCR in the generation of VM CD8⁺ T cells

White et al. showed (2) that expression of CD5 (i.e., a self-reactivity marker) determines propensity of naïve T cells to become VM $CD8^+$ T cells. They isolated naïve T cells expressing high or low levels of CD5 from unmanipulated WT mice and transferred them into lymphoreplete WT mice. After 3 weeks, they observed that 30% $CD5^{hi}$ T cells, but only 5% $CD5^{lo}$ T cells, acquired VM phenotype. These data indicated that VM $CD8^+$ T cells are preferentially derived from T cells receiving stronger tonic TCR signals from self-antigens. However, it remains unclear whether TCR specificity plays an important role in the generation of VM $CD8^+$ T cells (2). Importantly, this is the only experiment showing generation of VM population in lymphoreplete mice so far.

Another study showed that T-cell clones expressing endogenous TCRα chains in OT-I Rag^+ transgenic, but not monoclonal OT-I $Rag^{-/-}$, mice display conversion into VM $CD8^+$ T cells during aging (41). Based on their experiments, they concluded that TCR specificity plays an important role in age-related accumulation of VM $CD8^+$ T cells. However, the authors suggest that TCR specificity is not the main factor that determinates the generation of VM $CD8^+$ T cells in neonatal mice (41).

4.2 Gene-expression profile of VM $CD8^+$ T cells

Two phenotypic differences distinguish VM T cells from TM T cells, i.e., low expression of CD49d and slightly higher levels of CD122 (3,47). However, these markers were not validated in GF mice. White et al. (2) used deep RNA sequencing to compared gene expression profiles of VM $CD8^+$ T cells and naïve T cells. They found many genes that were differentially expressed between these two populations, including chemokine receptors (e.g. *Cxcr3*, *Ifngr1*), effector molecules (e.g. *Fasl, Grzb*), and adhesion proteins (e.g. *CD44, Ccr9*). However, they did not include TM T cells to their analysis and most of the genes differentially expressed between VM and naïve T cells are general markers of memory T cells. Moreover, they did not isolate the VM T cells from GF mice and thus it is not clear, whether they analyzed a pure population of VM T cells. Overall, the gene expression program of VM T cells is still poorly characterized (2).

4.3 Immune response of VM CD8$^+$ T cells

VM CD8$^+$ T cells display some, but not all, features of antigen-specific CD8 T cells which facilitate rapid response to pathogens during secondary infections (47). This finding leads to a question, whether VM CD8$^+$ T cells are capable of mediating protective responses to pathogens.

4.3.1 Production of IFN-γ by VM CD8$^+$ T cells

It was observed that TM and VM T cells expressed significantly higher levels of T-bet and Eomes (i.e., two key transcription factors of differentiation of memory CD8 T cells) than naïve T cells (47). These T-box transcription factors are positive regulators of IFN-γ production. Therefore, IFN-γ production was analyzed in OVA-specific TM, VM, and naïve T cells, following OVA peptide stimulation in vitro. It was observed that VM T cells display a capacity to produce IFN-γ in response to TCR stimulation that is intermediate between naïve (not producing IFN-γ) and TM (robust production of IFN-γ) CD8$^+$ T cells (47). Haluszczak et al. showed that production of IFN-γ by VM CD8$^+$ T cells after IL-12 and IL-18 stimulation is similar to TM CD8$^+$ T cells (3).

4.3.2 VM T cells can provide potent antigen-specific protective immunity against infection

Sosinowski et al. (40) transferred naïve and VM CD45.1 OT-I T cells into polyclonal CD45.2 hosts. CD45.2 mice were challenged with Lm.OVA and analyzed a few days later. They observed that VM population provides better protection against Lm.OVA than naïve population (40).

Lee at al. (47) used polyclonal Vβ5 CD8 T cells to examine VM T cells for protective ability against Lm.OVA infection. Cells were isolated and transferred into separate hosts which were challenged with virulent Lm.OVA. After a few days they observed that TM Vβ5, as well as VM T cells, provide more robust protection against Lm.OVA infection than naïve Vβ5 T cells (47).

Importantly, all published studies examined immune protection of VM CD8$^+$ T cells only against *Listeria monocytogenes* and thus it is not clear, whether VM CD8$^+$ T cells provide protection to a broad spectrum of pathogens. However, Decman et al. examined activation of CD44^{lo} naïve and CD44^{hi} VM T cells after *Lymphocytic choriomeningitis virus* (LCMV) and *Vesicular stomatitis virus* (VSV) challenge (48). CD44^{lo} or CD44^{hi} P14 (LCMV-specific T cell line)

$CD8^+$ T cells were transferred into WT recipient mice and then infected with LCMV. Similarly, $CD8^+$ OVA-specific T cells from OT-I $Rag2^{-/-}$ were sorted into $CD44^{hi}$ and $CD44^{lo}$ cells, transferred into congenic recipients and infected with VSV-OVA. After 8 days, they observed that the numbers of antigen-specific $CD44^{hi}$ T cell were significantly lower than numbers of $CD44^{lo}$ naïve T cells in both infection models. These data indicated that the response of VM T cells to viral infections is weaker than the response of naïve T cells (48). Unfortunately, this study did not analyze the effects of antigen-specific VM and naïve $CD8^+$ T cells on the clearance of the virus. It is not clear, why Decman et al. observed different results than the studies using *Listeria monocytogenes*. It can be caused by using monoclonal cells. Decman et al. used OT-I $CD8^+$ T cells from RagKO that developed in the absence of $CD4^+$ T cells which are essential for the function and protective capacity of HP-memory $CD8^+$ T cells and potentially also for VM T cells. The second possibility is that they used different pathogens (i.e., Decman et al. used viral pathogens (48), whereas other studies (40,47) used bacterial pathogens). It can be also caused by different timing of the analysis of the T-cell response. Decman et al. examined activation 8 days after the infection, whereas others (40,47) analyzed protection against Lm.OVA 3 – 5 days after the infection.

4.3.3 VM T cells can provide protection in the absence of cognate antigen

White et al. showed that VM $CD8^+$ T cells expressed molecules, IL-12R, IL-18R, IFN-γ, Granzyme B, and NKG2D (2). These proteins had been previously shown to enhance so-called bystander protection of memory CD8+ T cells (i.e., protection of memory T cells in the absence of their cognate antigen) (49). To examine if VM $CD8^+$ T cell can mediate bystander protection they sorted VM cells from OT-I (antigen-specific) and gBT-1 (antigen-irrelevant) transgenic mice, transferred them in 3KxRag$^{-/-}$ ($Rag^{-/-}$ mouse crossed to the 3K TCR transgenic mouse (50)) mice or IL-$15^{-/-}$ mice which were challenged with Lm.OVA one day later. Surprisingly, they observed that gBT-1 cells provided same protection against Lm.OVA challenge as OT-I cells in 3KxRag$^{-/-}$ mice. However, only OT-I cells provided protection against infection in IL-$15^{-/-}$ mice. In summary, these data indicated that VM $CD8^+$ T cells mediate bystander protective immunity and bystander protection of VM $CD8^+$ T cells is dependent on IL-15 (2).

However, Lee et al. observed protection of OVA-specific VM CD8$^+$ T cells against Lm.OVA, but not against Lm.WT, suggesting that the antigen-specific protection is stronger than the bystander protection (47).

4.4 Age-related accumulation of VM CD8$^+$ T cells

As mentioned above, VM CD8$^+$ T cells are cells with an apparent central memory phenotype. It was observed that VM CD8$^+$ T cells are present in highest frequency in 3-4 weeks old mice (42) and then accumulate during aging (41,51).

By analyzing CD44 and CD62L expression of CD8 T cells in 20 months old unprimed mice, it was observed that more than half of cells acquired central memory phenotype. The numbers of T_{cm} cells in aged mice were significantly higher compared to young (4 months) mice. However, it was observed that T_{cm} in blood, spleen, and peripheral lymph nodes of aged mice expressed lower levels of CD49d than naïve T cells. These data indicated that majority of abundant T_{cm} cells in aged mice are VM CD8$^+$ T cells (51).

4.5 Presence of VM CD8$^+$ T cells in humans

To date, VM CD8$^+$ T cells were characterized only in mice. Presence of these cells in humans is not well documented. However, some studies (2,52) addressed evidence whether humans produced an equivalent subset to murine VM CD8$^+$ T cells.

It was observed that KIR/NKG2A$^+$ (i.e., natural killer-like phenotype) CD8$^+$ T cells in healthy human adults share some similar characteristics with murine VM CD8$^+$ T cells, such as elevated expression of Eomes, T-bet, and CD122 or rapid production of IFN-γ after IL-12 and IL-18 stimulation (2,52). In addition, KIR/NKG2A$^+$ CD8$^+$ T cells represent 1% of human cord blood CD8$^+$ T cells, suggesting that they might arise in the absence of foreign antigen (2). It was observed that these cells expressed higher levels of Eomes than KIR/NKG2A$^-$ CD8$^+$ T cells. The frequency of KIR/NKG2A$^+$ Eomes$^+$ CD8$^+$ T cells was markedly elevated in the liver, which corresponds to the enrichment of murine VM CD8$^+$ T cells in the liver (2). Furthermore, it was examined if KIR/NKG2A$^+$ Eomes$^+$ CD8$^+$ T cells accumulate during aging. The spleen samples from healthy patients ranging between 30 and 70 years showed that the frequency of these cells correlates with age (2,52).

In summary, these data indicated that there might be population of antigen-inexperienced $CD8^+$ T cells in humans. This population may represent the human equivalent of murine VM $CD8^+$ T cells. KIR/$NKG2A^+$ $Eomes^+$ $CD8^+$ T express natural killer receptors. This suggested that they may have an important role in anti-tumoral and anti-infection protection in humans (2,52).

5. Comparison of HP-memory and VM CD8$^+$ T cells

HP-memory CD8$^+$ T cells and virtual memory CD8$^+$ T cells are two antigen-inexperienced memory-phenotype T cell populations, described from different mouse models (i.e., experimentally induced lymphopenia and steady-state unprimed mice, respectively). In this chapter, I will summarize facts presented in my thesis and compare reported features of these two populations to determine whether or not they actually represent a single CD8$^+$ T cell subpopulation.

Although the global gene-expression profile of HP-memory and VM T cells has not been directly compared, these two populations are characterized by the identical signature of surface markers (i.e., CD44^{hi}, CD122^{hi}, and CD49^{lo}). The low levels of CD49d distinguish HP and VM T cells from TM T cells. Moreover, both populations express higher levels of T-bet and Eomes than naïve T cells. These data are in line with the hypothesis that HP-memory and VM T cells use the same differentiation program.

Notable difference between HP-memory CD8$^+$ T cells and VM CD8$^+$ T cells is their dependence on different cytokines. It has been shown that particular cytokines enhanced homeostatic proliferation of naïve T cells *in vitro* (e.g. IL-7 and IL-15). However, only IL-7 was found as a crucial cytokine required for homeostatic proliferation of naïve T cells *in vivo* and for formation of HP-memory CD8$^+$ T cells. In contrast, generation and/or maintenance of VM T cells is dependent largely on IL-15. However, these studies examined rather factors leading to the generation of HP-memory and VM CD8$^+$ T cells than their cytokine requirements for survival or expansion. Because HP-memory and VM T cells are generated in different conditions (induced lymphopenia vs. physiological ontogenesis) by definition, the particular cytokines might have differential impact on formation of HP-memory and VM T cells. Nevertheless, once generated, HP-memory and VM T cells might still represent the identical cell type.

HP-memory T cells are formed from highly self-reactive clones. TCR:self-pMHC interactions are important for formation of these T cells. Formation of VM T cells correlates with self-reactivity which corresponds to HP-memory T cells. However, the role of TCR specificity in the generation of VM T cells is unclear and most likely is not the main factor that determinates the generation of VM CD8$^+$ T cells in neonatal mice. Formation of both HP-

memory and VM T cells from relatively highly self-reactive clones indicate that HP-memory and VM T cells may use the same differentiation program.

Importantly, both HP-memory and VM T cells are capable of producing IFN-γ after TCR and cytokine (i.e., IL-12 and IL-18) stimulation and they have the ability to provide better immune protection against *Listeria monocytogenes* than naïve T cells. These findings also correspond to the hypothesis that HP-memory and VM T cells use the same differentiation program.

It is clear, that HP-memory $CD8^+$ T cells arise via homeostatic proliferation during experimentally induced lymphopenia. VM T cells occur in lymphoreplete hosts and they may be generated by physiological homeostatic mechanism during neonatal or age-related lymphopenia. However, VM T cells may arise upon an adoptive transfer of naïve T cells into young adult mice which implies that they can be generated in the absence of lymphopenia.

In summary, detail analysis (Table 1) and comparison of HP-memory and VM T cells and their common features (i.e., gene expression profile, immune response, role of TCR) suggested that these two populations most likely represent a single $CD8^+$ T cell subpopulation. In this scenario, VM T cells are naturally occurring HP-memory T cells.

	HP-memory T cells	**VM T cells**
Gene expression profile	$CD44^{hi}$, $CD122^{hi}$, $CD49d^{lo}$, $T\text{-}bet^{hi}$, $Eomes^{hi}$	$CD44^{hi}$, $CD122^{hi}$, $CD49d^{lo}$, $T\text{-}bet^{hi}$, $Eomes^{hi}$
Cytokines important for generation	IL-7	IL-15
Role of TCR	formed from highly self-reactive clones, TCR self-MHC interaction is required for formation	unclear, formation correlates with self-reactivity
Immune response	rapid IFN-γ production, robust protection against Lm.OVA infection	rapid IFN-γ production, robust protection against Lm.OVA infection

Table 1. Summary of characteristics of HP-memory and VM $CD8^+$ T cells

6. Conclusion

The relationship of HP-memory T cells, that are induced by experimental lymphopenia and VM $CD8^+$ T cells, that are found in lymphoreplete hosts, was unclear. The main aim of my thesis was to compare HP-memory and VM $CD8^+$ T cells to find out whether or not HP memory and VM T cells represent the same T cells population. After a thorough review of published studies, I concluded that HP-memory $CD8^+$ T cells and VM $CD8^+$ T cells have identical phenotypical and functional hallmarks and most likely use identical differentiation program. However, a thorough side-by-side analysis of gene expression profiles and TCR repertoires of HP-memory and VM memory T cells is required to definitely resolve this issue.

Functional characteristics of HP/VM $CD8^+$ T cells indicate that they may play an important role in anti-tumor and anti-infection immunity. Moreover, they can be potentially used in tumor immunotherapy. However, the biological role of HP/VM T cells is still poor understood. The putative presence of HP/VM memory T cells in humans is unclear and deserves to be experimentally addressed in great detail.

7. Bibliography

1. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. J Exp Med. 2000 Aug 21;192(4):549–56.

2. White JT, Cross EW, Burchill MA, Danhorn T, McCarter MD, Rosen HR, et al. Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. Nat Commun. 2016 Apr 21;7:11291.

3. Haluszczak C, Akue AD, Hamilton SE, Johnson LDS, Pujanauski L, Teodorovic L, 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 Feb 16;206(2):435–48.

4. Harty JT, Badovinac VP. Shaping and reshaping CD8+ T-cell memory. Nat Rev Immunol. 2008 Feb;8(2):107–19.

5. Jameson SC, Masopust D. Diversity in T Cell Memory: An Embarrassment of Riches. Immunity. 2009 Dec;31(6):859–71.

6. Slifka MK, Whitton JL. Activated and Memory CD8+ T Cells Can Be Distinguished by Their Cytokine Profiles and Phenotypic Markers. J Immunol. 2000 Jan 1;164(1):208–16.

7. Sallusto F, Geginat J, Lanzavecchia A. C ENTRAL M EMORY AND E FFECTOR M EMORY T C ELL S UBSETS : Function, Generation, and Maintenance. Annu Rev Immunol. 2004 Apr;22(1):745–63.

8. Jung YW, Rutishauser RL, Joshi NS, Haberman AM, Kaech SM. Differential Localization of Effector and Memory CD8 T Cell Subsets in Lymphoid Organs during Acute Viral Infection. J Immunol. 2010 Nov 1;185(9):5315–25.

9. Kaech SM, Ahmed R. Memory CD8(+) T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells. Nat Immunol. 2001 May;2(5):415–22.

10. Ahmed R, Gray D. Immunological Memory and Protective Immunity: Understanding Their Relation. Science. 1996 Apr 5;272(5258):54–60.

11. Cho BK, Wang C, Sugawa S, Eisen HN, Chen J. Functional differences between memory and naive CD8 T cells. Proc Natl Acad Sci U S A. 1999 Mar 16;96(6):2976–81.

12. Cho J-H, Kim H-O, Ju Y-J, Kye Y-C, Lee G-W, Lee S-W, et al. CD45-mediated control of TCR tuning in naïve and memory CD8+ T cells. Nat Commun. 2016 Nov 14;7:13373.

13. Mehlhop-Williams ER, Bevan MJ. Memory CD8 $^{+}$ T cells exhibit increased antigen threshold requirements for recall proliferation. J Exp Med. 2014 Feb 10;211(2):345–56.

14. Martin MD, Condotta SA, Harty JT, Badovinac VP. Population Dynamics of Naive and Memory CD8 T Cell Responses after Antigen Stimulations In Vivo. J Immunol. 2012 Feb 1;188(3):1255–65.

15. Sprent J, Surh CD. T C ELL M EMORY. Annu Rev Immunol. 2002 Apr;20(1):551–79.

16. Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. J Exp Med. 2000 Aug 21;192(4):557–64.

17. Paludan SR. Synergistic action of pro-inflammatory agents: cellular and molecular aspects. J Leukoc Biol. 2000 Jan;67(1):18–25.

18. Goldrath AW, Luckey CJ, Park R, Benoist C, Mathis D. The molecular program induced in T cells undergoing homeostatic proliferation. Proc Natl Acad Sci. 2004 Nov 30;101(48):16885–90.

19. Surh CD, Sprent J. Homeostatic T Cell Proliferation: Figure 1: How Far Can T Cells Be Activated to Self-Ligands? J Exp Med. 2000 Aug 21;192(4):F9–14.

20. 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 Aug;11(2):173–81.

21. Kieper WC, Burghardt JT, Surh CD. A Role for TCR Affinity in Regulating Naive T Cell Homeostasis. J Immunol. 2004 Jan 1;172(1):40–4.

22. Ge Q, Bai A, Jones B, Eisen HN, Chen J. Competition for self-peptide-MHC complexes and cytokines between naive and memory CD8+ T cells expressing the same or different T cell receptors. Proc Natl Acad Sci. 2004 Mar 2;101(9):3041–6.

23. Murali-Krishna K. Persistence of Memory CD8 T Cells in MHC Class I-Deficient Mice. Science. 1999 Nov 12;286(5443):1377–81.

24. Ferreira C, Barthlott T, Garcia S, Zamoyska R, Stockinger B. Differential Survival of Naive CD4 and CD8 T Cells. J Immunol. 2000 Oct 1;165(7):3689–94.

25. Stritesky GL, Jameson SC, Hogquist KA. Selection of Self-Reactive T Cells in the Thymus. Annu Rev Immunol. 2012 Apr 23;30(1):95–114.

26. Azzam HS, Grinberg A, Lui K, Shen H, Shores EW, Love PE. CD5 Expression Is Developmentally Regulated By T Cell Receptor (TCR) Signals and TCR Avidity. J Exp Med. 1998 Dec 21;188(12):2301–11.

27. Surh CD, Sprent J. Homeostasis of Naive and Memory T Cells. Immunity. 2008 Dec;29(6):848–62.

28. Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, et al. IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc Natl Acad Sci. 2001 Jul 17;98(15):8732–7.

29. 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. J Exp Med. 2002 Jun 17;195(12):1523–32.

30. Hamilton SE, Wolkers MC, Schoenberger SP, Jameson SC. The generation of protective memory-like CD8+ T cells during homeostatic proliferation requires CD4+ T cells. Nat Immunol. 2006 May;7(5):475–81.

31. Cheung KP, Yang E, Goldrath AW. Memory-Like CD8+ T Cells Generated during Homeostatic Proliferation Defer to Antigen-Experienced Memory Cells. J Immunol. 2009 Sep 1;183(5):3364–72.

32. Shedlock DJ. Requirement for CD4 T Cell Help in Generating Functional CD8 T Cell Memory. Science. 2003 Apr 11;300(5617):337–9.

33. Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. Nat Immunol. 2003 Mar;4(3):225–34.

34. Hamilton SE, Jameson SC. The nature of the lymphopenic environment dictates protective function of homeostatic-memory CD8+ T cells. Proc Natl Acad Sci. 2008 Nov 25;105(47):18484–9.

35. Min HS, Lee YJ, Jeon YK, Kim EJ, Kang BH, Jung KC, et al. MHC Class II-Restricted Interaction between Thymocytes Plays an Essential Role in the Production of Innate CD8+ T Cells. J Immunol. 2011 May 15;186(10):5749–57.

36. Byrne JA, Stankovic AK, Cooper MD. A novel subpopulation of primed T cells in the human fetus. J Immunol. 1994 Mar 15;152(6):3098.

37. Dummer W, Niethammer AG, Baccala R, Lawson BR, Wagner N, Reisfeld RA, et al. T cell homeostatic proliferation elicits effective antitumor autoimmunity. J Clin Invest. 2002 Jul 15;110(2):185–92.

38. Hu H-M, Poehlein CH, Urba WJ, Fox BA. Development of antitumor immune responses in reconstituted lymphopenic hosts. Cancer Res. 2002 Jul 15;62(14):3914–9.

39. Dudley ME. Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes. Science. 2002 Oct 25;298(5594):850–4.

40. Sosinowski T, White JT, Cross EW, Haluszczak C, Marrack P, Gapin L, et al. CD8 + 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 Mar 1;190(5):1936–47.

41. Renkema KR, Li G, Wu A, Smithey MJ, Nikolich-Zugich J. Two Separate Defects Affecting True Naive or Virtual Memory T Cell Precursors Combine To Reduce Naive T Cell Responses with Aging. J Immunol. 2014 Jan 1;192(1):151–9.

42. Akue AD, Lee J-Y, Jameson SC. Derivation and Maintenance of Virtual Memory CD8 T Cells. J Immunol. 2012 Mar 15;188(6):2516–23.

43. Kurzweil V, LaRoche A, Oliver PM. Increased Peripheral IL-4 Leads to an Expanded Virtual Memory CD8+ Population. J Immunol. 2014 Jun 15;192(12):5643–51.

44. 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 Oct;46(10):2333–9.

45. Lee YJ, Jameson SC, Hogquist KA. Alternative memory in the CD8 T cell lineage. Trends Immunol. 2011 Feb;32(2):50–6.

46. Lauvau G, Goriely S. Memory CD8+ T Cells: Orchestrators and Key Players of Innate Immunity? Round JL, editor. PLOS Pathog. 2016 Sep 1;12(9):e1005722.

47. Lee J-Y, Hamilton SE, Akue AD, Hogquist KA, Jameson SC. Virtual memory CD8 T cells display unique functional properties. Proc Natl Acad Sci. 2013 Aug 13;110(33):13498–503.

48. Decman V, Laidlaw BJ, Doering TA, Leng J, Ertl HCJ, Goldstein DR, et al. Defective CD8 T Cell Responses in Aged Mice Are Due to Quantitative and Qualitative Changes in Virus-Specific Precursors. J Immunol. 2012 Feb 15;188(4):1933–41.

49. Chu T, Tyznik AJ, Roepke S, Berkley AM, Woodward-Davis A, Pattacini L, et al. Bystander-Activated Memory CD8 T Cells Control Early Pathogen Load in an Innate-like, NKG2D-Dependent Manner. Cell Rep. 2013 Mar;3(3):701–8.

50. Huseby ES, White J, Crawford F, Vass T, Becker D, Pinilla C, et al. How the T Cell Repertoire Becomes Peptide and MHC Specific. Cell. 2005 Jul;122(2):247–60.

51. Chiu B-C, Martin BE, Stolberg VR, Chensue SW. Cutting Edge: Central Memory CD8 T Cells in Aged Mice Are Virtual Memory Cells. J Immunol. 2013 Dec 15;191(12):5793–6.

52. Jacomet F, Cayssials E, Basbous S, Levescot A, Piccirilli N, Desmier D, et al. Evidence for eomesodermin-expressing innate-like CD8 $^{+}$ KIR/NKG2A $^{+}$ T cells in human adults and cord blood samples: Innate immunity. Eur J Immunol. 2015 Jul;45(7):1926–33.