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Roles of Virtual Memory T Cells in Diseases

Joon Seok [1,*,†], **Sung-Dong Cho** [2,†], **Seong Jun Seo** [1], **Su-Hyung Park** [2,3,*]

[1]Department of Dermatology, Chung-Ang University Hospital, Chung-Ang University College of Medicine, Seoul 06974, Korea
[2]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea
[3]The Center for Epidemic Preparedness, KAIST Institute, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea

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***Correspondence to**
Su-Hyung Park
Laboratory of Translational Immunology and Vaccinology, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Daejeon 34141, Korea.
Email: park3@kaist.ac.kr

Joon Seok
Department of Dermatology, Chung-Ang University Hospital, Chung-Ang University College of Medicine, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Korea.
Email: seokjoon923@gmail.com

[†]Joon Seok and Sung-Dong Cho contributed equally to this work.

ORCID iDs
Joon Seok
https://orcid.org/0000-0002-8016-8385
Sung-Dong Cho
https://orcid.org/0000-0002-7140-6863
Seong Jun Seo
https://orcid.org/0000-0003-2915-839X

ABSTRACT

Memory T cells that mediate fast and effective protection against reinfections are usually generated upon recognition on foreign Ags. However, a "memory-like" T-cell population, termed virtual memory T (T_{VM}) cells that acquire a memory phenotype in the absence of foreign Ag, has been reported. Although, like innate cells, T_{VM} cells reportedly play a role in first-line defense to bacterial or viral infections, their protective or pathological roles in immune-related diseases are largely unknown. In this review, we discuss the current understanding of T_{VM} cells, focusing on their distinct characteristics, immunological properties, and roles in various immune-related diseases, such as infections and cancers.

Keywords: Virtual memory T cells; Infectious disease; Cancer

INTRODUCTION

Classically, it was believed that $CD8^+$ T cells comprise populations of naive $CD8^+$ T (T_N) cells, effector T (T_{EFF}) cells, and memory T (T_{MEM}) cells, which remain quiescent while awaiting Ag-specific activation (1). A recent report described a $CD8^+$ T-cell population in unmanipulated mice, which could rapidly respond to innate or TCR-mediated stimuli, and showed similar immunological characteristics with conventional T_{MEM} cells (2). Remarkably, these cells achieved "memory-like" phenotype, and responded rapidly as T_{MEM} cells, without activation by previously encountered Ags (3). Based on these characteristics, this subset of $CD8^+$ T cells is considered "memory-like" and has been termed virtual memory (T_{VM}) cells (4). It was considered that microbiota-derived Ags might be responsible for T_{VM}-cell development, but this theory was disproved by the finding that T_{VM} cells exist at equal frequency in secondary lymphoid organs from both germ-free and feral mice (4,5).

T_{VM} cells express high levels of CD44, CD122, and Eomesodermin (Eomes), which are known to regulate the fate and function of $CD8^+$ T_{EFF} and T_{MEM} cells (6) (**Fig. 1**). For many years, T_{VM} cells were misclassified as central memory T (T_{CM}) cells due to their high CD44 and CD62L expression levels, and the absence of specific markers to distinguish T_{VM} cells from T_{MEM} cells (7). T_{VM} cells rarely express CD49d, which is only upregulated in T_{MEM} cells after TCR stimulation by its cognate Ag (4). T_{CM} and T_{VM} can be distinguished based on CD49 level.

Su-Hyung Park
https://orcid.org/0000-0001-6363-7736

Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

Eomes, Eomesodermin; KO, knockout; LCMV, lymphocytic choriomeningitis virus; LM-OVA, ovalbumin-expressing Listeria monocytogenes; NKR, NK cell receptor; TCM, central memory T; TEFF, effector T; TIM, innate memory T; TMEM, memory T; TMP, memory precursor T; TN, naive CD8+ T; TRM, resident memory T; TVM, virtual memory T; WT, wild-type.

Author Contributions

Conceptualization: Seok J, Cho SD, Park SH; Supervision: Park SH; Visualization: Cho SD; Writing - original draft: Seok J, Cho SD, Seo SJ, Park SH; Writing - review & editing: Seok J, Park SH.

Previous publications have described these memory-like cells using a variety of names—most commonly T_{VM} cells, memory precursor T (T_{MP}) cells, and innate memory T (T_{IM}) cells (2). Among them, T_{IM} cells develop from naïve $CD8^+$ T cells in the thymus, abundant with IL-4 secreted by $PLZF^+$ cells including αβ iNKT cells and γδ NKT cells (8-10). T_{VM} cells that originated from specific precursors in the thymus develop in the periphery and appear in mice shortly after birth (11). Since T_{IM}, T_{VM}, and T_{MP} cell populations cannot be separated phenotypically in the periphery, they are currently referred to as "virtual memory" cells from a functional point of view (3).

In this review, we present the recent knowledge regarding T_{VM}-cell populations, and discuss their distinct characteristics, immunological properties, and roles in various immune-related diseases.

CHARACTERISTICS OF T_{VM} CELLS

In mice, depending on the strain, different cytokines play crucial roles in the generation of T_{VM} cells. In BALB/c background mice, T_{VM}-cell development is absolutely dependent on IL-4, as shown by the finding that helminth infection does not induce an increase of T_{VM} cells in IL-4 knockout (KO) or IL-4R KO mice (12,13). On the other hand, helminth infection could increase the frequency of T_{VM} cells in C57BL/6 mice lacking IL-4 or IL-4R (12,14). It was demonstrated that T_{VM}-cell expansion during helminth infection in C57BL/6 mice was dependent on IL-15, and concluded that IL-4 was not a direct driver of T_{VM} cell proliferation in C57BL/6 mice with helminth infection (15). Unexpectedly, IL-4 exposure during development

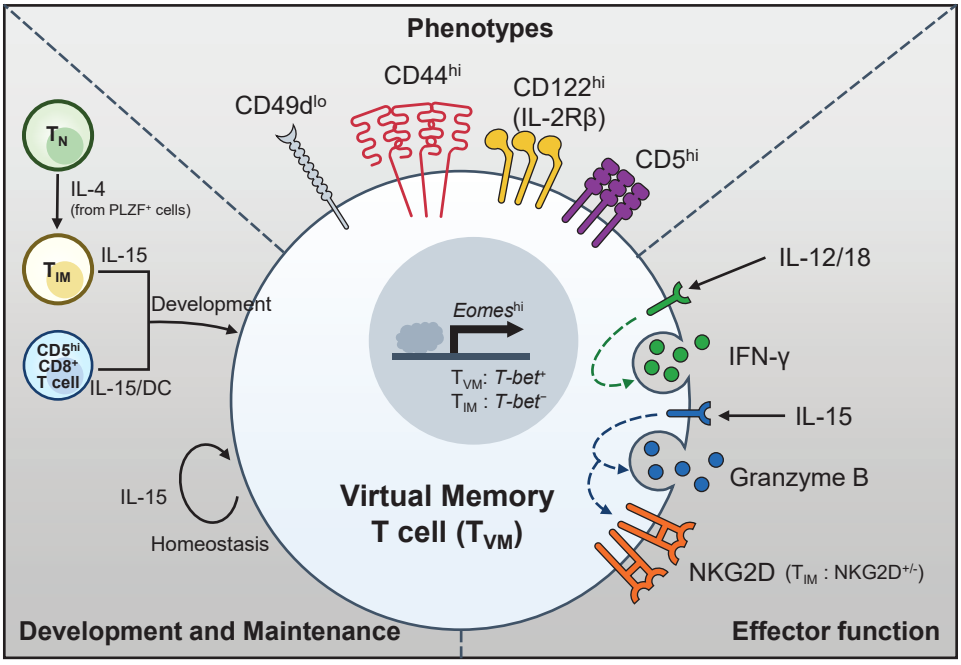

Figure 1. Characteristics of T_{VM} cells. T_{VM} cells originate from either IL-4 dependent T_{IM} cells or $CD5^{hi}CD8^+$ T cells. T_{VM} cells maintain their population dependent on IL-15. With the high expression level of Eomes at the transcriptome level, T_{VM} cells highly express CD44, CD122, and CD5. However, CD49d, a marker for cognate Ag experience, is downregulated in T_{VM} cells. T_{VM} cells have various cytokine responsiveness. For example, IL-12/18 stimulates T_{VM} cells and triggers the production of proinflammatory cytokines including IFN-γ production, and IL-15 induce NKG2D-dependent innate-like cytotoxicity. T-bet and NKG2D levels varies between T_{IM} and T_{VM}.
DC, dendritic cell.

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and homeostasis reduces $CD8^+$ T cells inherent ability of $CD8^+$ T cells to generate IFN-γ rapidly but increase a potent proliferative response to lymphocytic choriomeningitis virus (LCMV) infection in BALB/c mice (16). These findings imply that immune response to LCMV is induced by IL-4 conditioning, which also enhances the quantity but not the quality of memory-like $CD8^+$ T cells (16).

Furthermore, the TCR repertoire of T_{VM} cells reveals a TCR bias (11,17,18). Supporting the notion that T_{VM} differentiation is TCR-dependent, T_{VM} cells reportedly exhibit elevated amounts of Nur 77 in humans and CD5 in mice, which are surrogate markers of TCR signal intensity and indicators of increased TCR self-reactivity (19,20). It is believed that high self-peptide reactivity during T_{VM} cell growth is responsible for the elevated cytokine sensitivity of T_{VM} cells in the blood, which is at least partly due to the expression of Eomes (3). A previous study demonstrated that increased TCR responsiveness to self-ligands leads to Eomes upregulation in T_{VM} cells during thymic maturation (11). Additionally, it has been shown that Eomes binds to the il2rb promoter, thereby activating it and increasing CD122 expression (21). Therefore, it appears that the elevated reactivity of self-peptide MHC in T_{VM} cells leads to upregulation of Eomes, which causes increased CD122 expression, driving the IL-15 sensitivity and dependency of T_{VM} cells. Type I IFN signaling also reportedly increases Eomes expression in T_{VM} cells (22). In fact, IFN signaling led to Eomes-dependent increases of both peripheral T_{VM} cells and thymic T_{IM} cells, while $IFNAR^{-/-}$ mice exhibited drastically decreased T_{VM} cells (22).

T_{VM} cells are sensitive to cytokines including IL-12, IL15, and IL-18 (23,24) (**Fig. 1**). Previous reports showed that stimulation of T_{VM} cells with IL-12 and IL-18 can lead to IFN-γ production in an Ag-independent manner (23,25). As previously mentioned before, T_{VM} cells induce bystander activation in a manner dependent on IL-15 (23,26). When Ag-irrelevant T_{VM} cells having TCRs specific to irrelevant Ag were adoptively transferred into IL-15 KO mice and then challenged with ovalbumin-expressing *Listeria monocytogenes* (LM-OVA), the transferred T_{VM} cells displayed significantly lower expression levels of NKG2D, granzyme B, and IFN-γ, compared to the wild-type (WT) mice transferred with same cells (23).

T_{VM} cells express NK cell receptors (NKRs), and seem to constitute a cell subset that is functionally different from the $CD8^+$ T cells that display NK cell markers with aging and during infection (23,27,28). Compared to NKR^-CD8^+ T cells, human $CD8^+$ T cells that express NKRs (e.g., NKG2A, KIR2DL, and KIR3DL) produce lower effector cytokine levels in response to TCR-mediated stimulation (29). However, $KIR/NKG2A^+$ $CD8^+$ T cells can recognize MHC class I-deficient target cells, and trigger TCR-independent cytotoxicity via CD16 ligation and increased degranulation (30). Consequently, $KIR/NKG2A^+$ T cells seem to exhibit decreased TCR-mediated responses, together with increased innate responsiveness on a functional level. Although NKG2D activity on T_{MEM} and T_{VM} cells is a sign of senescence and TCR-mediated malfunction, it paradoxically promotes improved innate response. Increased granzyme and perforin production following NKG2D interaction on memory phenotypic $CD8^+$ T cells has been used to detect TCR-independent cytotoxicity (31,32). In cases where inhibitory Ly49 is expressed on memory phenotype T cells, this molecule reduces TCR-mediated T-cell activation without reducing responsiveness to IL-15 (33). Collectively, these results illustrate the reciprocal TCR-mediated and innate-like functionality of T cells.

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ROLE OF T_{VM} CELLS IN AGING

Unlike T_N cells, T_{VM} cells in young mice have the ability to produce cytokines and proliferate rapidly upon TCR stimulation or cytokine stimulations (27). However, the proportion of dysfunctional T_{VM} cells increases with age (27). A study in young and aged germ-free C57BL/6 and BALB/c mice revealed that the frequency of peripheral T_{VM} cells increases with age, regardless of genetic background (5). Moreover, it has been suggested that various hygienic conditions, such as cohousing laboratory mice and feral mice, showed minimal effects on the T_{VM} cell numbers in the blood (5). These results imply that there is a common homeostatic mechanism exists during aging, which is independent of genetic background and commensal microbiota.

The long-term maintenance of T_{VM} cell function in aged nonimmunized mice is of interest to understand the characteristics of T_{VM} cells. A previous study demonstrated that T_{VM} cells from aged OT-I or WT mice exhibited a number of characteristics not seen in younger mice (34). Aged T_{VM} cells showed a robust response to homeostatic cytokines, but also a selective reduction of their ability to replicate in response to TCR signals. Notably, this impairment was found to be associated with increased apoptosis in response to peptide stimulation, but decreased apoptosis in response to homeostatic cytokines in aged T_{VM} cells compared to aged T_N cells (34). Interestingly, in contrast to the response in young mice, among aged mice, the *de novo* response to influenza virus was dominated by T_{VM} cells, consistent with the age-related increase of T_{VM} cells (35). Aging-related changes in T_{VM}-cell function and frequency may also be explained by increases of cytokine levels (including IL-6, IL-15, and IL-18) with age (36,37).

According to a previous study, helminth infection does not stimulate the proliferation of aged T_{VM} cells, which is likely at least partly because aged mice exhibit an impaired Th2-related immune response to helminth infection (38). They also showed that T_{VM} cells from aged mice display an intrinsic defect in cytokine sensing. These findings indicate that the absence of T_{VM} cell expansion following helminth infection is explained by defective intrinsic T_{VM}-cell cytokine responsiveness, in conjunction with dysregulated helminth infection responses in aged mice. Furthermore, it was discovered that while young T_N and T_{VM} cells showed extensive proliferation, aged T_{VM} cells, not aged T_N cells, showed a severely diminished proliferative capacity primarily due to decreased cell division upon CD3 stimulation (27). Upon evaluating the proliferation of young and aged T_N and T_{VM} cells in response to IL-15 stimulation, they observed vigorous proliferation of only the young and aged T_{VM} cells (27). This indicated the independent regulation of TCR- and cytokine-dependent proliferation responses in T_{VM} cells. Further experiments involving adoptive transfer in mice revealed that young T_N and T_{VM} cells transferred to an aged environment acquired a proliferative defect and reduced functionality. Moreover, when aged T_N and T_{VM} cells were transferred to a young environment, more T_{VM} cells were recovered than T_N cells (27). This suggests that aged T_{VM} cells exhibit enhanced survival, possibly due to increased expression of receptors for homeostatic cytokines and integrins (23). Evaluation of exhaustion-related signatures revealed that age-related changes in T_{VM} cells were indicative of immune senescence, not indicative of T-cell exhaustion (27), which suggest that the inflammaging features of elderly individuals permits T_{VM} cells to survive relatively well, because T_{VM} cells respond better to cytokines than to TCR-mediated stimulation.

Collectively, the accumulated evidence regarding T_{VM} cells in the aging process suggests that these cells are highly dependent on innate-like immune responses, which continue to act efficiently via cytokines, rather than on Ag-specific responses, which become impaired.

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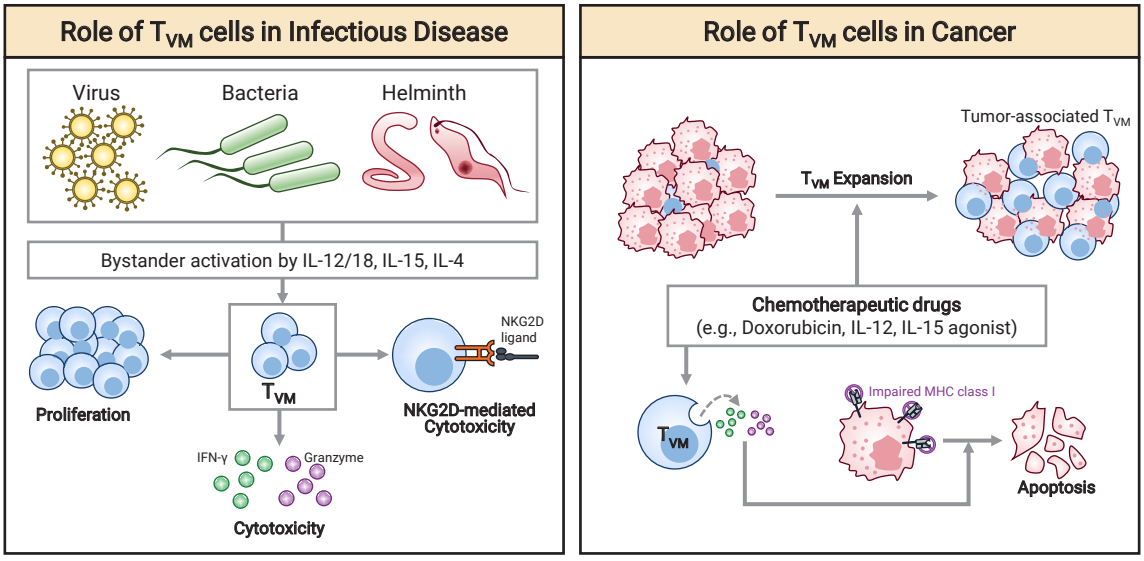

Figure 2. Roles of T_{VM} cells in infectious diseases and cancers. T_{VM} cells have diverse immunological roles. T_{VM} cells show protective roles in situations of infections with virus, bacteria, and helminth in a bystander manner. T_{VM} cells proliferate with cytokine stimulation and have cytotoxic effects. In particular, bystander activated T_{VM} cells mediate cytolytic activity in a NKG2D-dependent manner. In case of chemotherapy in cancer patients, T_{VM} cells expand and form a tumor-associated T_{VM} cell environment with higher clonality. Cancer cells with impaired MHC class I molecules undergo apoptosis with increased cytotoxicity of T_{VM} cells.

ROLE OF T_{VM} CELLS IN INFECTION

Like innate cells, T_{VM} cells reportedly play a protective role in first-line defense to viral, bacterial, and parasitic infections (12,13,35,39) (**Fig. 2**). In the past, NK and NKT cells had been thought to be the major IFN-γ producers by pathogen-derived inflammatory triggers (40). However, a previous study identified a population of IFN-γ–secreting $CD8^+$ T cells in the spleen and lymph nodes of LPS-injected mice, indicating that other immune cells are capable of early IFN-γ production (41). Moreover, it was shown that IFN-γ production by this $CD8^+$ T-cell population was restricted to $CD44^{hi}$ cells, which was independent of MHC class I (41). Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) can stimulate inflammatory cytokine production by various innate immune cells, including macrophages and dendritic cells that can produce IL-12 and IL-18 during the early phase of an infection. IFNα/β, IL-12, and IL-18 derived from macrophage and dendritic cells can indirectly stimulate Ag-independent IFN-γ production by memory-like $CD8^+$ T cells (4,41,42). T_{VM} cells constitutively express both IL-12 and IL-18 receptors (23), suggesting a mechanism through which T_{VM} cells can respond quickly to IFN-γ production in the early stages of infection. Moreover, a recent study revealed that *in vitro* stimulation with IL-12 and IL-18a yielded a higher frequency of IFN-γ–producing T_{VM} cells in C57BL/6 mice compared to BALB/c mice, due to the greater IL-18 receptor expression in T_{VM} cells of C57BL/6 mice (5).

Lee et al. (43) demonstrated that in the case of viral infection, IL-4 induced a large number of innate $CD8^+$ T cells that produced high levels of both IFN-γ and TNF. Upon LCMV Clone 13 infection, these innate $CD8^+$ T cells completely controlled the viremia and were dependent on IL-4, as IL-4 KO mice were incapable of clearing the virus (43). T_{VM} cells also play a role in bacterial infections, such as LM, and *Yersinia pseudotuberculosis* (12). Additionally, during the acute parasitic infection with *Trypanosoma cruzi*, thymic cells enriched with T_{IM} cells exhibit a substantial capacity to produce IFN-γ in response to stimulation with IL-12 and IL-18, and

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adoptive transfer of these cells can protect *T. cruzi*-infected mice (39). Hussain et al. (15) demonstrated that IL-15 is critical for the helminth-induced induction of T_{VM} cells in C57BL/6 mice, which was solely driven by proliferation of existing T_{VM} cells, with minimal contribution from naive cell differentiation. Moreover, compared with other $CD8^+$ T cells, the T_{VM}-cell population proliferated the most in response to helminth infection and IL-15 (15). However, it has also been reported that the high CD5 expression of T_N cells with IL-15 stimulation and helminth infections can convert T_N cells into T_{VM} cells in BALB/c mice (13). Therefore, the possibility of a species-specific effect must be considered.

As previously stated, T_{VM} cells tend to respond to cytokines during infections. Nonetheless, these cells possess a fully functional TCR repertoire. It was demonstrated that OTI T_{VM} cells can protect against LM-OVA infection by decreasing bacterial colony-forming units in the spleen (23). Moreover, a comparable protection level was observed in a TCR transgenic mouse model that does not recognize bacteria in an Ag-specific manner, indicating that T_{VM} cells can mediate immune-protection against bacterial infection, regardless of the presence of their cognate Ag (23). In the Ag-independent context, the effector function of T_{VM} cells is largely dependent on IL-15. In IL-$15^{-/-}$ mice as recipients, only Ag-specific T_{VM} cells conferred a protective activity, whereas Ag-nonspecific T_{VM} cells exhibited compromised functional capacity (23). Taken together, these findings suggest that T_{VM} cells can rapidly respond in an Ag-specific or Ag-nonspecific manner in inducing immune response.

Quinn et al. (27) demonstrated that T_{VM} cells can give rise to T_{EFF} cells in response to TCR stimulation. However, T_{VM}-derived T_{EFF} cells produced predominantly IFN-γ, whereas T_N-derived T_{EFF} cells were more multifunctional by producing a broader spectrum of cytokines (27). Furthermore, T_{VM}-cell-derived T_{EFF} cells become short-lived effector cells, while T_{EFF} cells derived from T_N cells are more likely to be differentiated into stable T_{MEM} cells (25,44). When a previous report evaluated the secondary immune responses by Ag-specific T_{MEM} and T_{VM} cells, both subsets expanded equally, but T_{VM} cells produced significantly more T_{CM} cells than T_{MEM} cells (25). In addition, Hou et al. (45) demonstrated that $CCR2^+$ T_{VM} cells played a dominant role in providing early protection, while $CCR2^-$ T_{VM} cells had a greater capacity to produce resident memory T (T_{RM}) cells. These findings indicate that T_{VM} cells can respond in a TCR-specific or non-specific manner in inducing effector functions such as rapid IFN-γ production during the early phase of infections, and can also respond to a secondary challenge by predominant differentiation into T_{CM} or T_{RM} cell populations.

ROLE OF T_{VM} CELLS IN CANCER

In recent years, there has been growing interest in cellular immunotherapy as a means of harnessing the immune system to fight cancers, and in the production and anticancer effect of cancer Ag-specific T cells (46-48). However, cancers can evade these cells by downregulating or losing MHC I Ag presentation, making them less stimulating or even invisible to $CD8^+$ T cells, without impairing their growth or metastatic potential (49). Therefore, we need to focus on the anticancer activities of non-cancer-specific T cells. It have been shown that T_{VM}-like cells exhibit increased production of inflammatory cytokines in the absence of Ag immunization (31,50). However, these studies could not determine whether these cells were indeed T_{VM} cells, because of not using the phenotypic markers that currently define T_{VM} cells.

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Since defining T_{VM}-cell propulsion, researchers have recently examined its role in cancer immunology (**Fig. 2**). Miller et al. (11) examined the presence of T_{VM} clones in tumors and draining lymph nodes of prostate adenocarcinoma-bearing mice. After co-transfecting these mice with polyclonal T_{VM} and T_N cells for 4 months, the author discovered that T_{VM} cells constituted a substantial proportion of the $CD8^+$ T cells that infiltrated the tumors. The TCR repertoire of T_{VM} cells was significantly distinct from that of T_N cells, and was highly conserved among cancer-bearing mice. They further demonstrated that prostate cancers drove the recurrent enrichment of tumor-associated T_{VM} clones, which were uncommon in the periphery, but selectively enriched in prostate tumors. This means that T_{VM} cells quantifiably contribute to the immune infiltration of tumors.

Wang et al. (51) demonstrated that chemotherapy resulted in a large increase of T_{VM} cells in tumors. *In vitro* treatment of tumor cells with cytarabine or doxorubicin activated T_{VM} cells, causing them to produce large amounts of granzyme B, which killed target cells. It was also shown that treating tumors with chemotherapy activated T_{VM} cells in a humanized mouse model, in a manner that did not involve MHC class I in the tumors (**Fig. 2**).

T_{VM} CELLS IN HUMANS

As specific markers of Human T_{VM} cells, KIRs and/or NKG2A surface markers with effector memory T cells re-expressing CD45RA phenotypes and Eomes expression have been suggested, which were identified in cord blood and peripheral blood (30). T_{VM} cells have high cytotoxic potential due to their high perforin and granzyme B content, and the innate-like effector function of T_{VM} cells in cord blood has been demonstrated by their IFN-γ secretion in response to IL-12 and IL-18 (30). Similar to in the mouse model, that express Eomes and CD122 tend to be affected by cytokines such as IL-4, IL-15, and type I interferon in terms of homeostasis and development of human T_{VM} cells (52).

Recent studies have examined the phenotype of human T_{VM} cells in detail. A RNA-seq analysis of $CD8^+CD45RA^+NKG2A^+$ cells and $CD8^+CD45RA^+$pan-$KIR2D^+KIR3DL1/DL2^+$ cells revealed that these 2 cell populations have distinct characteristics (28). In particular, $CD8^+KIR^+$ cells have been shown to share characteristics with regulatory $CD8^+$ T cells, including higher CD122 expression (53). Similarly, another RNA-seq analysis of $CD8^+KIR^+$ T cells confirmed that KIR^+CD8^+ T cells are the functional equivalent of mouse $Ly49^+CD8^+$ T cells, and play a regulatory role in autoimmune disorders (54). Schattgen et al. (55) reported that, compared to $KIR2D^-NKG2C^-CD8^+$ T cells, a significant portion of $KIR2D^+NKG2C^{+/-}CD8^+$ cells are $Helios^+$ cells, supporting the demonstration that KIR^+ T_{VM} cells have a regulatory function.

Confirming the function of $KIR/NKG2A^+Eomes^+$ T_{VM} cells, when chemotherapy-treated human lymphoma cells were co-cultured with $CD8^+$ T cells from healthy donors, granzyme B increased along with the proportion of $KIR/NKG2A^+$ $Eomes^+$ T_{VM} cells (51). Additionally, $KIR/NKG2A^+Eomes^+$ T_{VM} cells have been discovered in solid tumors, such as ovarian and breast cancer (52). Jin et al. (56) found that $KIR/NKG2A^+Eomes^+$ T_{VM} cells were also increased in HIV patients receiving antiretroviral therapy, and exerted suppressive action in a KIR receptors dependent manner. These findings show that human T_{VM} cells play roles in various disease conditions.

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CONCLUDING REMARKS

T_{VM} cells have long been confounded with T_{CM} cells, such that their function within the immune system has not been fully understood. Over recent years, there has been growing interest in the discovery of human T_{VM} cells with features resembling mouse T_{VM} cells. Important new challenges are to learn to distinguish between the roles of T_{VM} cells and Ag-specific cells, and to understand the significance of the T_{VM}-cell population in various disease processes.

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REFERENCES

1. Harty JT, Badovinac VP. Shaping and reshaping $CD8^+$ T-cell memory. *Nat Rev Immunol* 2008;8:107-119.
PUBMED | CROSSREF
2. Thiele D, La Gruta NL, Nguyen A, Hussain T. Hiding in plain sight: virtually unrecognizable memory phenotype $CD8^+$ T cells. *Int J Mol Sci* 2020;21:8626.
PUBMED | CROSSREF
3. White JT, Cross EW, Kedl RM. Antigen-inexperienced memory $CD8^+$ T cells: where they come from and why we need them. *Nat Rev Immunol* 2017;17:391-400.
PUBMED | CROSSREF
4. Haluszczak C, Akue AD, Hamilton SE, Johnson LD, Pujanauski L, Teodorovic L, Jameson SC, Kedl RM. The antigen-specific $CD8^+$ T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *J Exp Med* 2009;206:435-448.
PUBMED | CROSSREF
5. Moudra A, Niederlova V, Novotny J, Schmiedova L, Kubovciak J, Matejkova T, Drobek A, Pribikova M, Stopkova R, Cizkova D, et al. Phenotypic and clonal stability of antigen-inexperienced memory-like T cells across the genetic background, hygienic status, and aging. *J Immunol* 2021;206:2109-2121.
PUBMED | CROSSREF
6. Hussain T, Quinn KM. Similar but different: virtual memory CD8 T cells as a memory-like cell population. *Immunol Cell Biol* 2019;97:675-684.
PUBMED | CROSSREF
7. Chiu BC, Martin BE, Stolberg VR, Chensue SW. Cutting edge: central memory CD8 T cells in aged mice are virtual memory cells. *J Immunol* 2013;191:5793-5796.
PUBMED | CROSSREF
8. Lee YJ, Jameson SC, Hogquist KA. Alternative memory in the CD8 T cell lineage. *Trends Immunol* 2011;32:50-56.
PUBMED | CROSSREF
9. Lee YJ, Holzapfel KL, Zhu J, Jameson SC, Hogquist KA. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nat Immunol* 2013;14:1146-1154.
PUBMED | CROSSREF
10. Jameson SC, Lee YJ, Hogquist KA. Innate memory T cells. *Adv Immunol* 2015;126:173-213.
PUBMED | CROSSREF
11. Miller CH, Klawon DE, Zeng S, Lee V, Socci ND, Savage PA. Eomes identifies thymic precursors of self-specific memory-phenotype $CD8^+$ T cells. *Nat Immunol* 2020;21:567-577.
PUBMED | CROSSREF
12. 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 Immunol* 2019;12:258-264.
PUBMED | CROSSREF

13. Rolot M, Dougall AM, Chetty A, Javaux J, Chen T, Xiao X, Machiels B, Selkirk ME, Maizels RM, Hokke C, et al. Helminth-induced IL-4 expands bystander memory CD8$^+$ T cells for early control of viral infection. *Nat Commun* 2018;9:4516.
PUBMED | CROSSREF
14. 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.
PUBMED | CROSSREF
15. Hussain T, Nguyen A, Daunt C, Thiele D, Pang ES, Li J, Zaini A, O'Keeffe M, Zaph C, Harris NL Helminth infection-induced increase in virtual memory CD8 T cells is transient, driven by IL-15, and absent in aged mice. *J Immunol* 2023;210:297-309.
PUBMED | CROSSREF
16. Renkema KR, Lee JY, Lee YJ, Hamilton SE, Hogquist KA, Jameson SC. IL-4 sensitivity shapes the peripheral CD8$^+$ T cell pool and response to infection. *J Exp Med* 2016;213:1319-1329.
PUBMED | CROSSREF
17. Drobek A, Moudra A, Mueller D, Huranova M, Horkova V, Pribikova M, Ivanek R, Oberle S, Zehn D, McCoy KD, et al. Strong homeostatic TCR signals induce formation of self-tolerant virtual memory CD8 T cells. *EMBO J* 2018;37:e98518.
PUBMED | CROSSREF
18. Quinn KM, Zaloumis SG, Cukalac T, Kan WT, Sng XY, Mirams M, Watson KA, McCaw JM, Doherty PC, Thomas PG, et al. Heightened self-reactivity associated with selective survival, but not expansion, of naïve virus-specific CD8+ T cells in aged mice. *Proc Natl Acad Sci U S A* 2016;113:1333-1338.
PUBMED | CROSSREF
19. Ashouri JF, Weiss A. Endogenous Nur77 is a specific indicator of antigen receptor signaling in human T and B cells. *J Immunol* 2017;198:657-668.
PUBMED | CROSSREF
20. 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.
PUBMED | CROSSREF
21. Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, Mullen AC, Gasink CR, Kaech SM, Miller JD, et al. Effector and memory CD8$^+$ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol* 2005;6:1236-1244.
PUBMED | CROSSREF
22. Martinet V, Tonon S, Torres D, Azouz A, Nguyen M, Kohler A, Flamand V, Mao CA, Klein WH, Leo O, et al. Type I interferons regulate eomesodermin expression and the development of unconventional memory CD8$^+$ T cells. *Nat Commun* 2015;6:7089.
PUBMED | CROSSREF
23. White JT, Cross EW, Burchill MA, Danhorn T, McCarter MD, Rosen HR, O'Connor B, Kedl RM. Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. *Nat Commun* 2016;7:11291.
PUBMED | CROSSREF
24. Akue AD, Lee JY, Jameson SC. Derivation and maintenance of virtual memory CD8 T cells. *J Immunol* 2012;188:2516-2523.
PUBMED | CROSSREF
25. Lee JY, Hamilton SE, Akue AD, Hogquist KA, Jameson SC. Virtual memory CD8 T cells display unique functional properties. *Proc Natl Acad Sci U S A* 2013;110:13498-13503.
PUBMED | CROSSREF
26. 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. *J Immunol* 2013;190:1936-1947.
PUBMED | CROSSREF
27. Quinn KM, Fox A, Harland KL, Russ BE, Li J, Nguyen TH, Loh L, Olshanksy M, Naeem H, Tsyganov K, 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.
PUBMED | CROSSREF
28. Pieren DK, Smits NA, Hoeboer J, Kandiah V, Postel RJ, Mariman R, van Beek J, van Baarle D, de Wit J, Guichelaar T. Regulatory KIR$^+$ RA$^+$ T cells accumulate with age and are highly activated during viral respiratory disease. *Aging Cell* 2021;20:e13372.
PUBMED | CROSSREF

29. Björkström NK, Béziat V, Cichocki F, Liu LL, Levine J, Larsson S, Koup RA, Anderson SK, Ljunggren HG, Malmberg KJ. CD8 T cells express randomly selected KIRs with distinct specificities compared with NK cells. *Blood* 2012;120:3455-3465.
PUBMED | CROSSREF

30. Jacomet F, Cayssials E, Basbous S, Levescot A, Piccirilli N, Desmier D, Robin A, Barra A, Giraud C, Guilhot F, et al. Evidence for eomesodermin-expressing innate-like CD8$^+$ KIR/NKG2A$^+$ T cells in human adults and cord blood samples. *Eur J Immunol* 2015;45:1926-1933.
PUBMED | CROSSREF

31. Tietze JK, Wilkins DE, Sckisel GD, Bouchlaka MN, Alderson KL, Weiss JM, Ames E, Bruhn KW, Craft N, Wiltrout RH, et al. Delineation of antigen-specific and antigen-nonspecific CD8$^+$ memory T-cell responses after cytokine-based cancer immunotherapy. *Blood* 2012;119:3073-3083.
PUBMED | CROSSREF

32. Wong HC, Jeng EK, Rhode PR. The IL-15-based superagonist ALT-803 promotes the antigen-independent conversion of memory CD8$^+$ T cells into innate-like effector cells with antitumor activity. *OncoImmunology* 2013;2:e26442.
PUBMED | CROSSREF

33. Anfossi N, Robbins SH, Ugolini S, Georgel P, Hoebe K, Bouneaud C, Ronet C, Kaser A, DiCioccio CB, Tomasello E, et al. Expansion and function of CD8$^+$ T cells expressing Ly49 inhibitory receptors specific for MHC class I molecules. *J Immunol* 2004;173:3773-3782.
PUBMED | CROSSREF

34. Renkema KR, Li G, Wu A, Smithey MJ, Nikolich-Žugich 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;192:151-159.
PUBMED | CROSSREF

35. 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. *Immun Ageing* 2018;15:17.
PUBMED | CROSSREF

36. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and age-related diseases: role of inflammation triggers and cytokines. *Front Immunol* 2018;9:586.
PUBMED | CROSSREF

37. Pangrazzi L, Naismith E, Meryk A, Keller M, Jenewein B, Trieb K, Grubeck-Loebenstein B. Increased IL-15 production and accumulation of highly differentiated CD8+ effector/memory T cells in the bone marrow of persons with cytomegalovirus. *Front Immunol* 2017;8:715.
PUBMED | CROSSREF

38. Humphreys NE, Grencis RK. Effects of ageing on the immunoregulation of parasitic infection. *Infect Immun* 2002;70:5148-5157.
PUBMED | CROSSREF

39. Baez NS, Cerbán F, Savid-Frontera C, Hodge DL, Tosello J, Acosta-Rodriguez E, Almada L, Gruppi A, Viano ME, Young HA, 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. *PLoS Pathog* 2019;15:e1007456.
PUBMED | CROSSREF

40. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. *In vivo* natural killer cell activities revealed by natural killer cell-deficient mice. *Proc Natl Acad Sci U S A* 2000;97:2731-2736.
PUBMED | CROSSREF

41. Kambayashi T, Assarsson E, Lukacher AE, Ljunggren HG, Jensen PE. Memory CD8$^+$ T cells provide an early source of IFN-γ. *J Immunol* 2003;170:2399-2408.
PUBMED | CROSSREF

42. Bou Ghanem EN, Nelson CC, D'Orazio SE. T cell-intrinsic factors contribute to the differential ability of CD8$^+$ T cells to rapidly secrete IFN-γ in the absence of antigen. *J Immunol* 2011;186:1703-1712.
PUBMED | CROSSREF

43. Lee A, Park SP, Park CH, Kang BH, Park SH, Ha SJ, Jung KC. IL-4 induced innate CD8$^+$ T cells control persistent viral infection. *PLoS Pathog* 2015;11:e1005193.
PUBMED | CROSSREF

44. Smith NL, Patel RK, Reynaldi A, Grenier JK, Wang J, Watson NB, Nzingha K, Yee Mon KJ, Peng SA, Grimson A, et al. Developmental origin governs CD8$^+$ T cell fate decisions during infection. *Cell* 2018;174:117-130.e14.
PUBMED | CROSSREF

45. Hou S, Shao T, Mao T, Shi J, Sun J, Mei M, Tan X, Qi H. Virtual memory T cells orchestrate extralymphoid responses conducive to resident memory. *Sci Immunol* 2021;6:eabg9433.
PUBMED | CROSSREF

46. Moon D, Tae N, Park Y, Lee SW, Kim DH. Development of bispecific antibody for cancer immunotherapy: focus on T cell engaging antibody. *Immune Netw* 2022;22:e4.
PUBMED | CROSSREF

47. Lee JB, Kim HR, Ha SJ. Immune checkpoint inhibitors in 10 years: contribution of basic research and clinical application in cancer immunotherapy. *Immune Netw* 2022;22:e2.
PUBMED | CROSSREF

48. Hupperetz C, Lah S, Kim H, Kim CH. CAR T cell immunotherapy beyond haematological malignancy. *Immune Netw* 2022;22:e6.
PUBMED | CROSSREF

49. Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer immune evasion through loss of MHC class I antigen presentation. *Front Immunol* 2021;12:636568.
PUBMED | CROSSREF

50. Xu W, Jones M, Liu B, Zhu X, Johnson CB, Edwards AC, Kong L, Jeng EK, Han K, Marcus WD, et al. Efficacy and mechanism-of-action of a novel superagonist interleukin-15: interleukin-15 receptor αSu/Fc fusion complex in syngeneic murine models of multiple myeloma. *Cancer Res* 2013;73:3075-3086.
PUBMED | CROSSREF

51. Wang X, Waschke BC, Woolaver RA, Chen SM, Chen Z, Wang JH. MHC class I-independent activation of virtual memory CD8 T cells induced by chemotherapeutic agent-treated cancer cells. *Cell Mol Immunol* 2021;18:723-734.
PUBMED | CROSSREF

52. Barbarin A, Cayssials E, Jacomet F, Nunez NG, Basbous S, Lefèvre L, Abdallah M, Piccirilli N, Morin B, Lavoue V, et al. Phenotype of NK-like CD8(+) T cells with innate features in humans and their relevance in cancer diseases. *Front Immunol* 2017;8:316.
PUBMED | CROSSREF

53. Mishra S, Srinivasan S, Ma C, Zhang N. CD8+ regulatory T cell – a mystery to be revealed. *Front Immunol* 2021;12:708874.
PUBMED | CROSSREF

54. Li J, Zaslavsky M, Su Y, Guo J, Sikora MJ, van Unen V, Christophersen A, Chiou SH, Chen L, Li J, et al. KIR^{+}CD8^{+} T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science* 2022;376:eabi9591.
PUBMED | CROSSREF

55. Schattgen SA, Guion K, Crawford JC, Souquette A, Barrio AM, Stubbington MJ, Thomas PG, Bradley P. Integrating T cell receptor sequences and transcriptional profiles by clonotype neighbor graph analysis (CoNGA). *Nat Biotechnol* 2022;40:54-63.
PUBMED | CROSSREF

56. Jin JH, Huang HH, Zhou MJ, Li J, Hu W, Huang L, Xu Z, Tu B, Yang G, Shi M, et al. Virtual memory CD8+ T cells restrain the viral reservoir in HIV-1-infected patients with antiretroviral therapy through derepressing KIR-mediated inhibition. *Cell Mol Immunol* 2020;17:1257-1265.
PUBMED | CROSSREF