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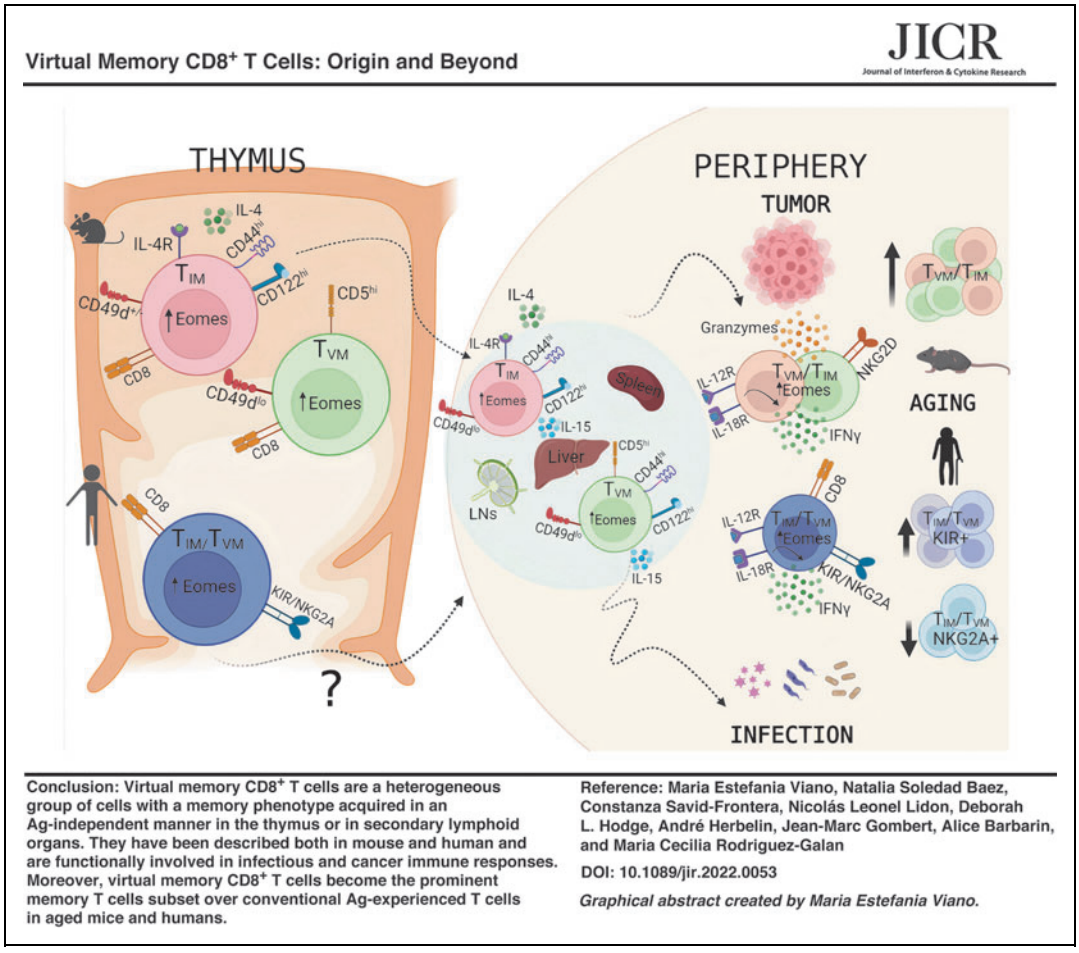
Virtual Memory CD8$^+$ T Cells: Origin and Beyond

Maria Estefania Viano,[1] Natalia Soledad Baez,[1] Constanza Savid-Frontera,[1] Nicolás Leonel Lidon,[1] Deborah L. Hodge,[2] André Herbelin,[3,4] Jean-Marc Gombert,[3–5] Alice Barbarin,[3,6] and Maria Cecilia Rodriguez-Galan[1]



Conclusion: Virtual memory CD8$^+$ T cells are a heterogeneous group of cells with a memory phenotype acquired in an Ag-independent manner in the thymus or in secondary lymphoid organs. They have been described both in mouse and human and are functionally involved in infectious and cancer immune responses. Moreover, virtual memory CD8$^+$ T cells become the prominent memory T cells subset over conventional Ag-experienced T cells in aged mice and humans.

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Graphical abstract created by Maria Estefania Viano.

[1]Inmunología, CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
[2]National Institutes of Health, Bethesda, Maryland, USA.
[3]Inserm U1313, Poitiers, France.
[4]Université de Poitiers, Poitiers, France.
[5]Service d'Immunologie et Inflammation, CHU de Poitiers, Poitiers, France.
[6]CHU de Poitiers, Poitiers, France.

The presence of $CD8^+$ T cells with a memory phenotype in nonimmunized mice has been noted for decades, but it was not until about 2 decades ago that they began to be studied in greater depth. Currently called virtual memory $CD8^+$ T cells, they consist of a heterogeneous group of cells with memory characteristics, without any previous contact with their specific antigens. These cells were identified in mice, but a few years ago, a cell type with characteristics equivalent to the murine ones was described in healthy humans. In this review, we address the different aspects of its biology mainly developed in murine models and what is currently known about its cellular equivalent in humans.

Keywords: T_{VM} cells, T_{IM} cells, Ag-specific T cells, infection, cancer, human T_{VM} cells

General Aspects of Memory-Like $CD8^+$ T Cells

Traditionally, both in mouse and human, it has been considered that $CD8^+$ T cells housed in secondary lymphoid organs (SLO) transit through a single differentiation pathway that begins as ''naive'' T cells (T_N). Then, after the recognition of their cognate antigen (Ag), they are activated and differentiate into effector T cells (T_{EFF}), whose number contracts after the antigen/pathogen is eliminated, leaving a ''pool'' of conventional Ag-experienced memory T cells (T_{MEM}) (Sallusto and others 1999; Boyman and others 2009; Mueller and others 2013). The T_{MEM} cell population consists mainly of effector memory T cells (T_{EM}), which are mainly found in peripheral tissues, especially in mucosa, and recirculate by blood and lymph. These cells respond quickly to newly encountered Ags, and central memory T cells (T_{CM}) that are found mainly in lymph nodes (LNs) and are responsible for self-renewal and supply of T_{EFF} cells (Boyman *et al.* 2009, Gerlach et al. 2015, Mueller *et al.* 2013, Sallusto *et al.* 1999).

More recently, another population of memory cells has been defined, which does not recirculate and resides mainly in the tissues where the primary infection has occurred and is called resident memory T cells (T_{RM}) (Mueller and others 2013; Gerlach and others 2015).

Two decades later, a new population of $CD8^+$ T cells was identified in unmanipulated mice and exhibited phenotypic characteristics similar to conventional memory T cells by rapidly responding to stimuli, either innate or through their TCRs (Thiele and others 2020). What is surprising about these cells is that they achieve this ''memory-like'' phenotype and respond as quickly as T_{MEM} without previously having contacted their specific Ags (Sallusto and others 1999). All these features defined these cells as ''memory-like'' $CD8^+$ T cells and currently are called virtual memory cells (T_{VM} cells). Similar to T_{MEM} cells, T_{VM} cells express high levels of CD44 and CD122 (β chain of IL-2/IL-15 receptor), as well as high levels of Eomesodermin (Eomes), a T-box family transcription factor known for regulating $CD8^+$ T effector and memory cell fate and function (Pearce and others 2003; Intlekofer and others 2005).

The high CD44 and CD62L expression in T_{VM} cells, combined with the absence of markers specifically distinguishing them from T_{MEM} cells, led to T_{VM} being mistakenly included in the T_{CM} cell group for many years (Lee and others 2013a). Fortunately today, T_{VM} can be differentiated since memory-like cells show low expression of CD49d, a component of the VLA-4 and LPAM homing receptors, which is only positively regulated in T_{MEM} cells after strong TCR antigen recognition (Haluszczak and others 2009; Lee and others 2013a; Quinn and others 2020). This point is quite critical, especially considering it has been reported that the vast majority (≥85%) of cells believed to be T_{CM} are actually $CD49d^{lo}$ and therefore T_{VM} cells (Quinn and others 2020).

Even though the existence of memory-like T cells in SLO of nonimmunized animals has long been described, the source and composition of these cells were undefined for many years. Originally, it was suggested that T_{VM} cells are specific for Ags derived from the microbiota. However, commensal microorganisms are not involved in the generation of T_{VM} cells since this population exists in SLO from both germ-free (GF) (Huang and others 2005; Haluszczak and others 2009) and feral mice (Moudra and others 2021) in similar frequency.

Today, an abundance of new evidence has emerged that clarifies some of these ''unknowns.'' Kedl and others proposed the term ''Virtual Memory'' (T_{VM}), based on the similarity to a computing term that means ''alternative use of disc space,'' to describe this novel repertoire of Ag-inexperienced memory T cells present in unprimed mice (Haluszczak and others 2009; White and others 2016, 2017).

When analyzing the frequency of memory-like T cells in nonimmunized mice, this number could reach up to 15%–20% of total $CD8^+$ T cells in SLO (Lee and others 2011, 2013a; White and others 2017). The evaluation of memory-like $CD8^+$ T cells in SLO is quite complex since it represents a heterogeneous group of cells from different origins. This diverse cellular pool comprised the following: (1) $CD8^+$ innate T cells (T_{IM}) that develop in the thymus and depend on IL-4 for their maturation. Contrary to conventional T_N cells, T_{IM} cells acquire a memory phenotype within the thymus without previous cognate Ag encounter. (2) Lymphopenia-induced memory cells (T_{LIM}) or homeostatic proliferation memory T cells (T_{HP}) arise from homeostatic mechanisms in situations of extreme lymphopenia, which are mediated by either irradiation or genetic T cell deficiency.

T_{LIM} can also result from physiological lymphopenia occurring in the neonatal period in mice (Min and others 2003; Schuler and others 2004). The generation of T_{LIM} cells can occur without foreign antigen activation and is thought to be driven by reduced competition for limiting resources, including IL-7 and low-affinity TCR ligands (Tan and others 2001). (3) T_{VM} cells develop in the periphery; however, these cells arise from specific precursors in the thymus and appear soon after birth in normal mice (Lee and others 2011, 2013a; White and others 2017; Quinn and others 2020). T_{VM} cells highly depend on IL-15 for their generation/maintenance mainly through IL-15 *trans* presentation by $CD8\alpha^+$ dendritic cells (Sosinowski and others 2013).

There have been a considerable number of publications over the years reporting Ag-independent memory cells with

an array of defining names such as innate T cells, memory-like T cells, bystander T cells, virtual memory T cells, and Ag-inexperienced $CD8^+$ T cells being the most commonly used. However, currently, there is consensus to call all 3 cell subsets (T_{IM}, $T_{HP/LIM}$, and T_{VM}) ''Virtual Memory'' when they reside in SLO since they cannot be distinguished at a phenotypic level (White and others 2017). In this review, we have compiled the current knowledge about T_{IM} and T_{VM} cell populations and address different aspects of their biology. Distinction between T_{HP}/T_{LIM} and T_{VM} have been mainly addressed by the reviews from *Pribikova and others (2018) and Hussain and Quinn (2019). In this work, we have also included a complete section about their more recently described human counterparts.

T_{IM} Cells, Phenotype, Origin, and Differentiation

As is the case of conventional single positive CD8 thymocytes (SP8), T_{IM} cells also require particular developmental conditions when it comes to MHC class I (MHC I) and cytokine interactions. Several laboratories have offered valuable information about these points. For instance, it has been demonstrated that MHC Ib-restricted $CD8^+$ T cells are more prone to develop into an ''innate-like'' phenotype than MHC Ia-restricted T cells (Urdahl and others 2002; Cho and others 2011). Even more, it has been reported that innate $CD8^+$ T cells can be positively selected by MHC class Ib molecules expressed on hematopoietic cells (HCs), and not necessarily by thymic epithelial cells (TEC), as is the case for development of conventional single positive CD8 (SP8) thymocytes (Cho and others 2011; Huang and others 2013; Urdahl and others 2002).

Other investigators have arrived at similar conclusions using $Kb^{-/-}Db^{-/-}$ mice expressing MHC class Ib, but lacking MHC class Ia. Urdahl and others demonstrated that selection of SP8 thymocytes (specific for L. monocytogenes), that acquired a $CD44^{hi}$ activated phenotype was the result of intrathymic interactions of MHC-class Ib but not MHC-class Ia restricted $CD8^+$ T cells with MHC class I-expressing HCs (Urdahl and others 2002). Moreover, the adaptor molecule SAP (SH2D1A) is required for innate $CD8^+$ T cell selection on HCs in ITK KO mice (Horai and others 2007). Cho and others (2011) expanded this concept by using transgenic (Tg) mice expressing a TCR specific for the listerial peptide LemA (D7 Tg) presented by MHC-linked H2-M3 (M3), a MHC class Ib molecule.

The authors show that M3-restricted $CD8^+$ T cells can be successfully selected by either TECs or HCs. Interestingly, the same M3-restricted $CD8^+$ T precursors selected from 2 distinct cell types led to clones with different phenotype and functional characteristics. While M3-restricted $CD8^+$ T cells selected on TECs have a less activated phenotype with high expression of β7 integrin that efficiently migrates to the gut, the same precursors selected by HCs preferentially present features of innate cells (Cho and others 2011). Because both types of T cells generated expressed distinct patterns of integrin receptors, the authors speculate that they occupy different immunological niches and could ultimately play unique roles during an immune response (Cho and others 2011).

Due to the fact that innate $CD8^+$ T cells carry an effector/memory phenotype ($CD44^{hi}CD122^{hi}$), one concern that has arisen is the possibility that T_{IM} cells represent mature T cells from SLO, which have migrated to the thymus as previously described by our and other laboratories (Chau and others 2002; Hale and Fink 2009; Hodge and others 2012). To answer that question, Rafei and others (2011) have used the RAG2p-GFP mouse model (B6 background). This system allows one to discern between local (GFP^+) or recirculating T cells (GFP^-). Rafei and others (2011) report that up to 10% of total SP8 thymocytes (GFP^+) develop into the innate phenotype (GFP^+ $CD44^{hi}$) in steady-state conditions and are not recirculating mature T cells from SLO.

Interestingly and contrary to other types of innate T cells that develop in the thymus, T_{IM} cells present a nonrestricted TCR repertoire (Rafei and others 2011). In this work, by using OT-I RAG2p-GFP mice, the investigators demonstrate that expression of a given TCR could give rise to both conventional and innate SP8 thymocytes. This demonstrates that the generation of both lineages is not dictated merely by the nature of the TCR and it is possible that conventional and innate SP8 thymocytes may have overlapping TCR repertoires (Rafei and others 2011).

Signaling through MHC class I is important in the selection of conventional and T_{IM} cells, but a role has also been reported for TCR signaling-associated molecules (kinases). In this context, data presented by Atherly and others (2006) indicate that conventional $CD8^+$ T cell maturation is highly dependent on signaling through ITK and RLK, members of the Tec family tyrosine kinases. ITK and RLK, are expressed on thymocytes and regulate T cell receptor signaling thresholds during positive and negative selection.

The investigators showed that ITK KO and RLK/ITK double KO mice are almost devoid of conventional SP8 thymocytes and contain a large number of SP8 thymocytes that express consensus lineage markers indicative of T_{IM} cells ($CD44^{hi}$, $CD122^+$, and $EOMES^{hi}$). These cells also produce large amounts of IFNγ *ex vivo* and depend on IL-15 for maturation (Atherly and others 2006). Similar results were published by Broussard and others (2006), who additionally demonstrated that when ERK is reconstituted in ITK KO mice, they exhibited normalized thymic features and SP8 cells appeared phenotypically more similar to conventional SP8 cells from wild-type (WT) mice.

Most of the current knowledge about T_{IM} biology was gained through studies in mutant mice where this population is dramatically expanded. For instance, after the discovery that mice deficient in Tec family proteins have biased thymic development toward T_{IM} cells, new studies began to appear showing that deficiency in other T cell signaling molecules or transcription factors can also enrich the thymic innate CD8 T cell lineage. In the review by Lee and others (2011), the authors present a complete summary of specific molecules downstream of the TCR pathway involved in innate CD8 T cell development.

In this context, Nayar and others (2012) evaluated the role of IFN regulatory factor 4 (IRF4), a transcription factor that is upregulated following TCR stimulation in WT T cells, and observed that, in contrast to WT thymocytes, activation of SP8 IRF4 KO cells leads to a high and rapid expression of Eomes and the acquisition of a memory phenotype. These data may indicate that, contrary to innate SP8 cells, in conventional SP8

*__Correction added__ on October 17, 2022 after first online publication of September 9, 2022: The references in this sentence originally cited Drobek and others (2018) and Hussain and others (2019). These have been updated to Pribikova and others (2018) and Hussain and Quinn (2019). Pribikova and others (2018) is added to the reference list.

thymocytes, TCR activation induces a high ITK signaling, which in turn promotes IRF4 upregulation and suppression of Eomes expression (Nayar and others 2012).

The reason why different mutations bias SP8 development through the T_{IM} lineage was quite mysterious until it was shown that these particular mutations lead to an overproduction of IL-4 by thymic NKT or $CD4^+$ T cells. IL-4 produced by NKT or $CD4^+$ T cells acts in a cell-extrinsic way promoting Eomes expression on SP8 thymocytes, a hallmark of innate CD8 T cells (Lee and others 2011; Min and others 2011). In this context, Carty and others investigated the signaling pathways required for IL-4-dependent Eomes induction in T_{IM} cells.

They demonstrated that IL-4 is sufficient to drive Eomes expression through STAT6- and Akt-dependent pathways. Very interestingly, the authors suggest that IL-4 signaling pathways may direct cell fate when TCR signals are limiting as IL-4 has little effect on Eomes induction when T cells receive a strong TCR signal; however, IL-4 effectively promotes Eomes during attenuated TCR stimulation (Carty and others 2014).

A study performed by Lee and others demonstrated that in several mouse strains (especially BALB/c mice), a subpopulation of thymic iNKT cells, named NKT2 cells, was abundant and able to produce IL-4 in the absence of stimulation. The authors demonstrate that these physiological amounts of IL-4 produced by NKT2 cells are sufficient to induce a ''memory-like'' phenotype in SP8 thymocytes during steady-state conditions (Lee and others 2013b); moreover, the transcription factor promyelocytic leukemia zinc finger (PLZF) was ultimately responsible for this effect (D'Cruz and others 2010; Weinreich and others 2010). IL-4 production is not exclusive to NKT cells since it has been reported that other $PLZF^+$ T cells can also drive innate CD8 T cell development (Weinreich and others 2010; Min and others 2011). Particularly, levels of PLZF in the thymus seem to be crucial to generate T_{IM} cells.

Park and others reported that the amounts of PLZF are able to control not only the generation but also the subset composition of thymic iNKT cells. The authors demonstrate that compared to WT mice, $PLZF^{GFPcre+/wt}$ BALB/c mice (mice that produced lower quantity of PLZF) present a dramatic decrease in the frequency of $PLZF^{hi}$ NKT2 cells, leading to lower numbers of innate SP8 thymocytes (Park and others 2019). Data addressing the different components that participate in T_{IM} thymic development are schematically summarized in Fig. 1.

Exploring the role of Eomes in T_{IM} cell formation, Istaces and others (2019) performed an exhaustive analysis at the transcriptomic level, which highlighted a distinct epigenetic program during conventional and unconventional memory $CD8^+$ T cell formation. The investigators demonstrated that even though T_{IM} cells acquire classical features of memory cells, they are only partially programmed toward memory fate at the epigenetic level. They also provide evidence that Eomes contributes to this epigenetic programming in T_{IM} cells (Istaces and others 2019).

To further analyze these data, the investigator developed a transgenic mouse model that overexpressed Eomes in developing thymocytes. Their results demonstrated that SP8

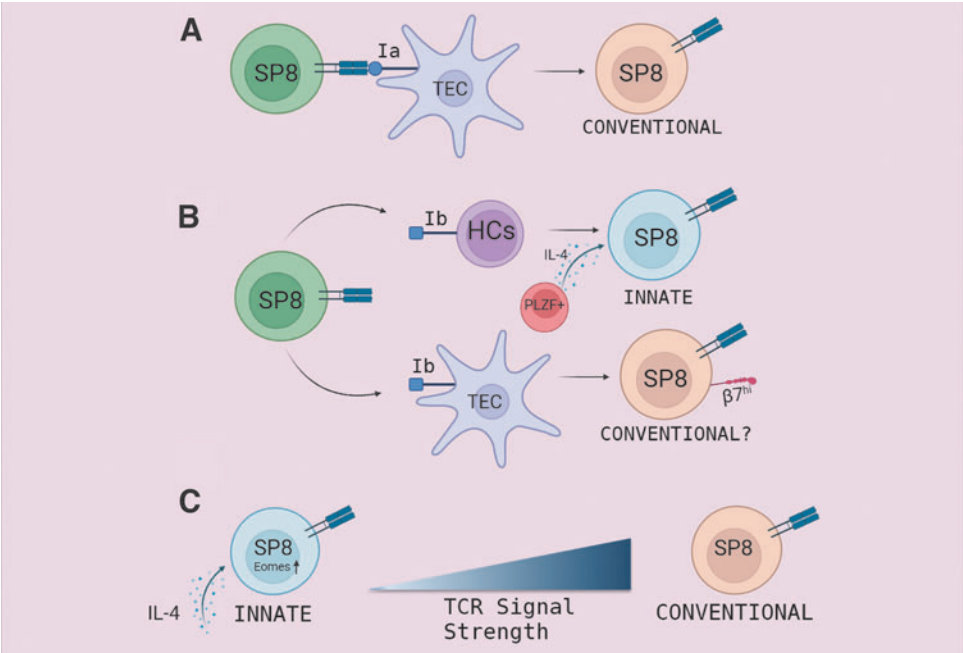

FIG. 1. Mechanisms involved in thymic differentiation of T_{IM} cells. T cell development in the thymus produces many lineages of mature T cells. **(A)** When the TCR of a single positive CD8 (SP8) cell recognizes an MHC-Ia molecule expressed on TEC, it results in the development of a conventional $CD8^+$ T cell. **(B)** In the context of MHC-Ib expression, SP8 thymocytes can be successfully selected either by TECs or HCs. While SP8 cells selected on TECs have ''a more naive'' phenotype, the same precursors selected by HCs and exposed to interleukin-4 (IL-4) derived from PLZF-expressing thymocytes convert into T_{IM} cells. **(C)** Reduced TCR signaling in SP8 cells in the presence of IL-4 induces Eomes expression and T_{IM} phenotype acquisition. IL-4 has little effect on Eomes induction when T cells receive a strong TCR signal giving rise mainly to conventional naive SP8 cells. TEC, thymic epithelial cell; HC, hematopoietic cell.

cells in this mouse model presented most of the phenotypic, functional, and transcriptional features of T_{IM} cells. They also identified the transcription factor RUNX3 and the epigenetic regulator BRG1 as partners that interact with EOMES to promote innate memory cell phenotype acquisition (Istaces and others 2019).

Following these comprehensive reports in genetically modified animals, the most curious question was whether innate CD8 T cells existed in WT animals, and if so, if similar mechanisms operated in normal mice. In this regard, Weinreich and others (2010) investigated the phenotype of SP8 thymocytes in BALB/c and C57BL/6 (B6) mice. They found that BALB/c mice present a larger percentage of $PLZF^+$ thymocytes than B6 mice and this finding was in accordance with increased numbers of the memory-phenotype SP8 cells in BALB/c over B6 mice (Weinreich and others 2010). Further studies from this laboratory demonstrated that the large percentage of $CD44^{hi}$, $CD122^{hi}$ SP8 cells (T_{IM}) in BALB/c mice was IL-4 dependent since IL-4 KO BALB/c mice had lower numbers of thymic T_{IM} cells than WT BALB/c mice (Weinreich and others 2010).

The same laboratory further investigated this point. They speculated that because iNKT lineage diversification is different between mouse strains, the frequency of T_{IM} cells should also be dissimilar. They compared 6 commonly used different inbred strains of mice and showed variability in the frequency and number of total thymic iNKT cells. To this point, B6 presented more NKT1 cells and fewer NKT2, whereas BALB/c presented larger number of NKT2 cells (Lee and others 2013b). From these results, the authors concluded that higher numbers of T_{IM} cells correlated with a higher NKT2/NKT1 index in the different strains of mice (Lee and others 2013b).

Innate CD8 T cell development can be reproduced *in vitro*. Rafei and others (2013) have developed a novel *in vitro* model that is suitable for analysis of positive selection and T cell differentiation based on co-culture of $CD69^{neg}$ (preselected) DP thymocytes from OT-I–transgenic mice with peptide-pulsed OP9 stromal cells. By using synthetic peptides that are described to induce positive selection in OT-I cells (Santori and others 2002), they report that culture of DP OT-I cells in the presence of recombinant IL-4 (rIL-4) induced high levels of CD69, PD-L1, Eomes, and CD44, some of the consensus markers of innate polyclonal TCR$\alpha\beta$ $CD8^+$ T cells (Rafei and others 2013).

In alignment with this observation, our laboratory has reported that co-culture of either DP $CD69^{neg}$ or $CD69^{pos}$ OT-I cells with a bulk population of thymocytes obtained from *Trypanosoma cruzi*-infected mice was sufficient to generate SP8 cells with innate characteristics in 48 h. We demonstrated that this effect is IL-4 and IL-15 dependent. Moreover, in the context of *T. cruzi* infection, SP4 $CD44^{hi}$ cells and thymic myeloid cells are responsible for the local production of IL-4 and IL-15 at the thymus, respectively (Baez and others 2019). Data confirming that T_{IM} cells are dependent not only on IL-4 but also IL-15 for their development were shown by Atherly and others. Their investigative analysis demonstrated that the high accumulation of SP8 $CD44^{hi}$ cells observed in the thymus of ITK KO mice was significantly reduced in the absence of IL-15 in $ITK^{-}/^{-}$ $IL\text{-}15^{-}/^{-}$ mice (Atherly and others 2006).

It is not well known how T_{IM} cells are exported from the thymus; however, in the review by White and others (2017) a possible explanation is offered. They speculate that IL-15 can upregulate the expression of the KLF2 transcription factor in T_{IM} cells, which in turn induces the expression of sphingosine 1-phosphate receptor 1 (S1P1), a crucial molecule that allows mature thymocytes to egress the thymus and migrate to SLO. To expand this topic, our preliminary data tracking thymocytes after being intrathymically (i.t.) stained with the dye eFluor670 (eF) indicate that during *T. cruzi* infection, a reduced number of mature SP4 and SP8 thymocytes are exported to SLO compared to control-uninfected mice.

When we analyzed $CD8^+$ T eF^+ cells in LNs and spleen 5 days after i.t. injection, we found that about 15–25% of the cells adopt a memory-like phenotype ($CD44^{hi}$), as previously reported for WT mice (Haluszczak and others 2009). Interestingly, only 5 days after exportation, a higher proportion of $CD8^+$ T eF^+ cells became T_{MEM} or T_{EFF} ($CD44^{hi}CD49d^{hi}$) than T_{VM} ($CD44^{hi}CD49d^{lo}$) in *T. cruzi*-infected mice compared to control mice (unpublished data). We have not yet determined if these $CD44^{hi}CD49d^{lo}CD8^+eF^+$ cells are T_{IM} cells or T_{VM} cells that convert upon arriving to SLO since both populations are phenotypically indistinguishable once they arrive to the periphery.

Other than these few reports, there is a substantial lack of information on the mechanism that T_{IM} cells utilize to leave the thymus and reach SLO. This important issue certainly deserves a more in-depth analysis, especially in the context of pathological triggers.

The information that accumulated over the years in relation to the origin and development of T_{IM} cells led us to think that the fate of a developing T cell is not quite predictable and even random. The process is highly dependent on the type of presenting cell they contact during positive selection and the surrounding cytokine microenvironment. Still, what type of signals SP8 receive from TECs or HCs, which makes them divert to different lineages, requires deeper investigation.

T_{VM} Cells, Phenotype, Origin, and Differentiation

The T_{VM} population is composed of cells from different origins. This was demonstrated by Kurzweil and others (2014) in a study where they used mice deficient in Nedd4 family-interacting protein 1 (Ndfip1 KO mice) and showed that even though overproduction of IL-4 in the periphery leads to an expanded T_{VM} population, not all memory-like cells are IL-4 dependent as ablation of IL-4 only partially affects the number of T_{VM} cells. This topic was also evaluated by Akue and others (2012), By using 2 well-characterized peptide/MHC complexes (B8R/Kb and HSVgB/Kb), the authors evaluated the frequency of T_{VM} cells with these specificities in unmanipulated mice and demonstrated that their numbers are reduced (but not eliminated) in IL-4 KO mice.

These results support the concept that part of the T_{VM} cell population in SLO could be T_{IM}, which are actually IL-4 dependent and migrate from the thymus to SLO, and once there, they become undistinguished from other T_{VM} cell types (Akue and others 2012). However, this is not completely true since the composition of IL-4-dependent memory-like cells in SLO is not exclusively of T_{IM} cells

(Park and others 2016). In a work reported by Park and others (2016), an IL-4/anti-IL-4 antibody complex (IL-4C) was administered for over 1 week, resulting in an induction of innate CD8 T cell-like phenotype in peripheral $CD8^+$ T cells.

The investigators then asked if IL-4-dependent T_{VM} cells could arise in SLO; then, they adoptively transferred $CD44^{lo}CXCR3^-$ (naive) or $CD44^{hi}CXCR3^+$ (T_{VM}) $CD8^+$ T cells into B6 mice, followed by IL-4C treatment for 1 week. After this period of time, Park and others (2016) showed that IL-4C treatment induced proliferation of both types of transferred cells and simultaneously caused differentiation of naive CD8 T cells into $CD44^{hi}CXCR3^+$ cells during their proliferation. These results indicate that IL-4 is able to induce both memory-like $CD8^+$ T cells from naive $CD8^+$ T cells and expand pre-existing T_{VM} cells in the periphery (Park and others 2016).

Along the same line of investigation, Tripathi and others (2016) asked if the composition and origin of T_{VM} cells between mice from different genetic backgrounds are similar. This point of inquiry was based on the report that BALB/c mice carry a larger number of T_{IM} cells in the thymus and more IL-4-dependent T_{VM} in periphery than C57BL/6 mice (Lee and others 2011). This is mainly due to the fact that BALB/c mice have a larger number of IL-4 producer NKT2-type cells in the thymus than C57BL/6 mice (Lee and others 2013b). In their report, by using IL-4 KO or IL-15 KO mice from both mouse strains, Tripathi and others (2016) demonstrated that the T_{VM} compartment in BALB/c mice relies more on IL-4 than IL-15, whereas the T_{VM} compartment in C57BL/6 mice is more highly dependent on IL-15 and minimally on IL-4.

Moreover, IL-15 is known to be responsible for the long-term survival of memory CD8 T cells. In addition, the prominent CD122 expression of T_{VM} cells provides them with a competitive advantage to respond to these cytokines. In accordance with these data, T_{VM} cells are highly enriched in the liver, representing up to 60%–70% of total memory T cells present in the organ (Nakagawa and others 2004). This is physiologically important as the liver is a rich source of IL-15 and provides T_{VM} cells access to this cytokine (Correia and others 2009).

Although IL-15 is very important in the development and maintenance of T_{VM} cells, reduction in T_{VM} cells in the absence of IL-4 and/or IL-15 is not complete. As such, it is proposed that IL-4 and IL-15 are not the only regulators of T_{VM} cell homeostasis. Indeed, Martinet and others (2015) demonstrated that the phenotype, function, and age-dependent expansion of T_{VM} cells are greatly disturbed in the absence of type I IFN signaling. In this context, the authors showed that type I IFNs are able to directly activate Eomes gene expression, a key transcription factor expressed by T_{VM} cells as mentioned earlier. This study points out that type I IFNs represent an important cytokine during T_{VM} and T_{IM} maturation (Martinet and others 2015).

One question that has arisen over the years is whether T_N and T_{VM} cells share the same TCR profile or do they develop from different T cell clones. In this matter, Haluszczak and others (2009) investigated the TCR repertoire of T_{VM} cells residing in SLO. By using different class I MHC tetramers loaded with various peptide antigens (OVA-K^b/SIINFEKL, vaccinia virus B8R-K^b/TSYKFESV, and HSV1gB-K^b/SSIEFARL), they showed that $CD8^+$ T cells present in SLO of unimmunized animals display a mix of naive and memory phenotype for each clone, but at different ratios. They determined that the percentage of cells with a memory phenotype ($CD44^{hi}$ cells) varied from 10% of OVA-specific $CD8^+$ T cells to 30%–40% in HSV1-specific $CD8^+$ T cells (Haluszczak and others 2009).

These results agree with what was stated for T_{IM} cells in the previous section, where it is demonstrated that an SP8 cell with the same TCR can give rise to a naive or an innate T cell according to the maturation context in which it is surrounded (positive selection by TEC vs. HC, levels of IL-4, TCR signaling strength, etc.). Haluszczak and others (2009) also compared the frequency of $CD44^{hi}$ versus $CD44^{lo}$ cells in gnotobiotic (germ free) and specific pathogen-free (SPF) animals. Notably, they showed that the MHC-I tetramer-bound populations from GF animals contained $CD44^{hi}$ cells with a similar frequency to those found in SPF mice (Haluszczak and others 2009). These data demonstrate that microbial antigens do not contribute to the appearance of memory-phenotype $CD8^+$ T cells in SLO.

Similar peptide/MHC class I tetramers experiments were later performed by the same laboratory to evaluate T_{VM} cell frequencies to 5 different epitopes recognized in the B6 response to murine cytomegalovirus (MCMV). By using 3 highly immunodominant epitopes and 2 that are hardly detectable in the acute MCMV response, the authors concluded that there was a lack of correlation between the frequency of T_{VM} cells and the immunodominance characteristics of the tetramer specificities (Akue and others 2012).

To further understand the origin of these non-IL-4-dependent T_{VM} cells, the authors performed tetramer enrichment assays on thymic and peripheral lymphoid tissues from mice 1 to 4 weeks after birth and demonstrated that $tetramer^+$ T_{VM} cells appeared in the periphery in advance of the thymus, thereby supporting the idea that memory-like cells are generated in SLO after being exported from the thymus (Akue and others 2012). They also found that the frequency of pre-existing T_{VM} cells is stable in both steady-state conditions and after a greatly expanded Ag-driven memory $CD8^+$ T cell immune response. In addition, T_{VM} cells can be maintained long term by undergoing basal proliferation (Akue and others 2012).

Even though it is well accepted that T_{VM} cells develop in SLO, the concept that the thymus is not involved has been changing in recent years. White and others (2016) provide evidence that T cells emerging from the thymus with high affinity for self-antigens (these cells express high levels of CD5) are more likely to become T_{VM} cells when reaching SLO than their $CD5^{lo}$ counterpart. Their results revealed that the bulk population of T_{VM} cells shows a statistically significant increase in the expression of CD5 than in the bulk population of naive cells in unprimed B6 mice, suggesting that the affinity of a T cell to its selecting ligands during thymic positive selection could dictate the fate of a SP8 thymocyte toward the T_{VM} lineage (White and others 2016).

To demonstrate preferential conversion to the T_{VM} phenotype, White and others (2016) adoptively transferred $CD44^{lo}$ $CD5^{hi}CD8^+$ T cells into lymphoreplete WT mice, and after 3 weeks, they show that the donor cells acquired a T_{VM} phenotype ($CD44^{hi}$ $CD49d^{lo}$), and this conversion seems to occur irrespective of the TCR since transferred $CD44^{lo}$ $CD5^{hi}$ TCR transgenic gBT cells were significantly more

likely to became T_{VM} cells than transferred $CD44^{lo}CD5^{lo}$ gBT cells. These data reinforce the concept that the origin of T_{VM} initiates at the thymus since a cell with one particulate TCR could undergo thymic egression with a $CD5^{hi}$ or $CD5^{lo}$ phenotype depending on the signal strength received during positive selection. This could ultimately define its fate in SLO.

Aligned with these data, Drobek and others (2018) explored this concept at the TCR signaling level. By using T cells from CD8.4 knock-in mouse (T cells that carry a CD8 molecule formed by the extracellular portion of CD8α fused to the intracellular part of CD4) (Erman and others 2006), the authors generated T cells that strongly bind Lck, an initiating TCR signal transduction kinase. They demonstrated that when CD8.4 is associated with a low self-reactivity ($CD5^{lo}$) TCR, like F5 (specific for the influenza Ag NP68), T cells did not preferentially develop into the T_{VM} lineage.

Instead, when they associate CD8.4 co-receptor to $CD8^+$ T cells from OT-I cells, whose TCR has a strong self-reactive avidity ($CD5^{hi}$), most CD8.4 cells became T_{VM} (up to 80%), indicating that only the most highly self-reactive T cells have the potential to develop into T_{VM} cells (Drobek and others 2018). Based on their data, Drobek and others (2018) speculated that naive and T_{VM} cell compartments could contain T cell clones with different TCR repertoires. To probe their hypothesis, they utilized a Vβ5 transgenic mouse model with fixed TCRβ from the OT-I TCR (Fink and others 1992) that generates a polyclonal population of T cells with one type of TCRβ chain, but variable TCRα chains (Drobek and others 2018).

When they analyzed the frequency of T_{VM} versus T_N cells, they observed that, while the frequency of $TCRV\alpha3.2^+$ T cells is slightly enriched for the T_{VM} cell subset, the $TCRV\alpha8.3^+$ T cells have lower frequency of T_{VM} cells than the overall population. Accordingly, OVA-specific $TCRV\alpha2^+$ had higher levels of CD5 than $TCRV\alpha8.3^+$ cells (Drobek and others 2018). More recent work by Miller and others (2020) has extended those previous findings using a clonal approach. The authors sequenced the complete TCR repertoires of T_N cells and T_{VM} cells from SLO of B6 mice expressing a fixed transgenic TCRβ chain and variable TCRα chains. Their data revealed that the TCR repertoire of T_{VM} cells is largely dissimilar to that of T_N cells, and interestingly, was highly recurrent between individual mice (Miller and others 2020).

By using retrogenic mice expressing either TCR obtained from several recurrent T_{VM} or T_N $CD8^+$ T cell clones, they report that the memory phenotype in the periphery was only adopted by $CD8^+$ T cells that expressed T_{VM}, but not T_N TCRs. Interestingly, not every $CD8^+$ T cell that carries a high-frequency T_{VM} TCR clone adopts a memory-like phenotype, suggesting that T_{VM} cell development is also restricted to limited niches in SLO (Miller and others 2020). Collectively, the information from these works show that the generation of a T_{VM} cell has both fixed and stochastic aspects that depend upon first, the positive selection received in the thymus, and second, the possibility of accessing specific niches in SLO. The origin, phenotype, location, and cytokine requirement of different subsets of T_{VM} cells discussed in this section are schematically summarized in Fig. 2.

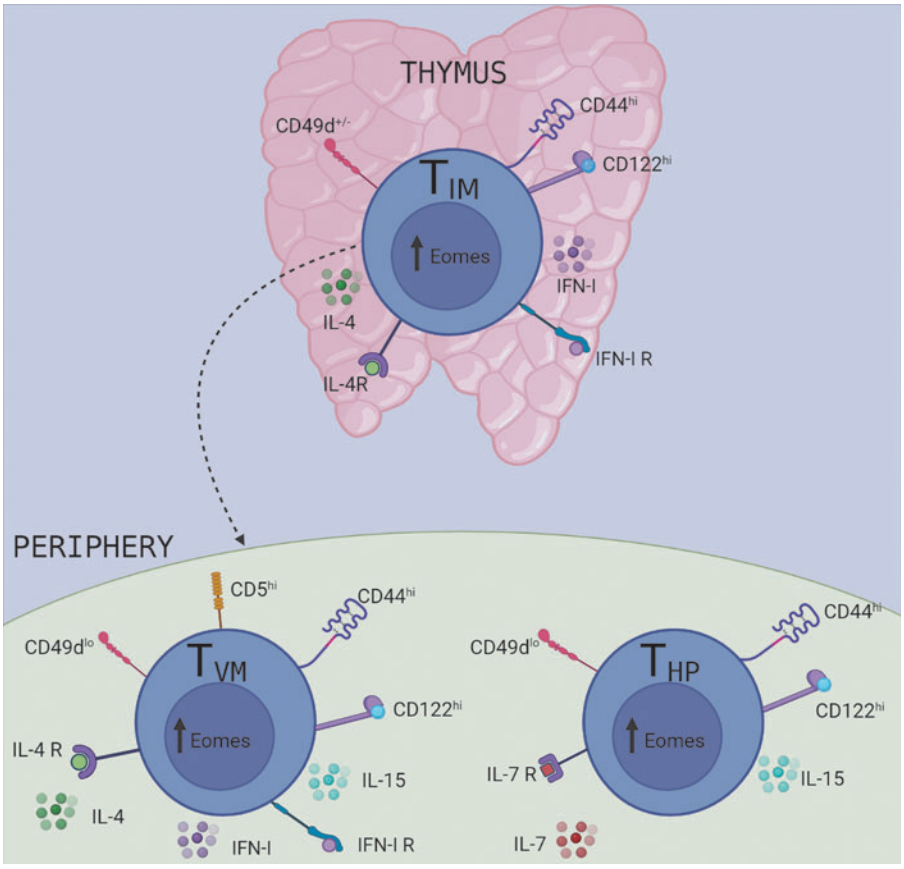

FIG. 2. Heterogeneous origin and types of cell subsets that compose the T_{VM} population. T_{IM} cell development from SP8 thymocytes when they are exposed to IL-4 and MHC-Ib in the thymus. T_{VM} cells are generated in the periphery from conventional naive cells that emerge from the thymus with a high self-recognition avidity ($CD5^{hi}$) and their generation is requisitely dependent on IL-15 signaling. While T_{VM} cells and T_{IM} cells were originally distinguished from one another by thymic differential expression of CD44 and CD49d and their dependence on IL-15 versus IL-4, once they emerge into the periphery, the 2 populations are phenotypically indistinguishable. HP memory $CD8^+$ T cells (T_{HP}) are produced by homeostatic mechanisms rather than conventional priming and express markers typical of T_{VM} cells (high expression of CD44 and CD122 and low expression of CD49d). T_{HP} can be considered part of the T_{VM} pool that resides in SLO and is dependent on IL-7 for survival.

By a different approach, Smith and others (2018) provided new evidence on the origin and fate of T_{VM} cells. They use a CD4 promoter-driven tamoxifen-inducible cre (CD4cre-ERT2) that is able to drive expression of the red fluorescent protein TdTomato (RFP) in $CD8^+$ T cells undergoing thymic selection at the DP stage of T cell development. By this strategy, they can permanently ''timestamp'' different waves of $CD8^+$ T cells made in the thymus every time mice are exposed to tamoxifen (Tx). Interestingly, the authors could evaluate peripheral waves of $CD8^+$ T cells at fetal, neonatal, and adulthood stages (Smith and others 2018). They showed that in 8-week-old mice, those that received Tx at 1 day of life had 5 times more T_{VM} cells that preferentially localized in the liver than those who received Tx at 28 days of life.

An important question they asked was whether $CD8^+$ T cells from 1-day-old Tx-exposed mice could more easily become T_{VM} cells due to the well-known neonatal lymphopenia that occurred when reaching SLO. To answer this question, the authors transplanted a newborn timestamp thymus into an adult lymphoreplete timestamp mouse and simultaneously evaluated waves of newborn (RFP) and adult (YFP) thymocytes at the same time and in the same peripheral environment. They reported that 4 weeks after Tx exposure, RFP^+ cells that originated from the newborn transplanted thymus and matured in adult hosts had a similar proportion of T_{VM} cells to that seen in the intact neonatal mice (Smith and others 2018).

This result correlates with their RNA-seq data that demonstrate $CD8^+$ T cells from 1-day-old Tx mice express a significantly higher proportion of genes typically found in effector and memory cells, while in 28-day-old Tx mice, $CD8^+$ T cells express more genes characteristic of naive and late memory cells (Smith and others 2018).

Another very interesting question that Smith and others (2018) posed is how these cells behave during an infectious state. They reported that cells produced early in life (mostly T_{VM} cells) proliferate and differentiate more quickly than those produced later in life (most T_N cells) at 5 days post-LM infection. Moreover, cells from 1-day-old Tx mice present a greater proportion of terminally differentiated short-lived effector cells and make more IFNγ in the early phases of the infection than cells from 28-day-old Tx mice (Smith and others 2018).

One concern that arises from T_{VM} cells is whether they could trigger autoimmunity since they recognized self-Ags with high avidity. In response to this point, Drobek and others (2018) demonstrate that T_{VM} cells are tolerant to self-Ags they encountered in the thymus during positive selection. They show that OT-I cells co-cultured with dendritic cells loaded with the endogenous Ags Catnb and Mapk8 [proposed as positive selecting Ags for OT-I T cells (Santori and others 2002)] do not show significant response in *in vitro* proliferative assays or *in vivo* during infection with LM-Catnb (Drobek and others 2018).

To confirm these data in a murine model of autoimmunity, Drobek and others (2018) sorted either T_N or T_{VM} cells from OVA-specific clones V14-C1 and V14-C2, so both types of cells express the same TCRs. They then adoptively transferred these cells into RIP.OVA mice (mice that exhibit detectable ovalbumin in pancreatic islets) followed by infection with LM-OVA. Outcomes, in the case of both clones, demonstrate that naive T cells were more efficient in inducing autoimmune diabetes than T_{VM} cells. To explain this effect, the authors speculate that T_{VM} cells could develop suppressive mechanisms that contribute to self-tolerance, such is the case of lower expression of both CD49d integrin and CD25 upon activation (Drobek and others 2018).

A recent publication by Hou and others (2021) demonstrates a very revealing phenomenon in the field of immunology, which is that T_{VM} cells could give rise to tissue-resident memory cells (T_{RM}) during the course of influenza infection. The author presents evidence that T_{VM} cells that recognize viral antigen can rapidly migrate to the lungs during the first 24 h postinfection period to provide early infection control, but are retained in the organ and give rise to T_{RM} independent of SLO (Hou and others 2021).

The knowledge that is emerging in the field of T_{IM} and T_{VM} cells in recent years is providing enlightening evidence about the role of these Ag-independent (or not) T cells in the immune system. These latest findings lead one to think about the flexibility of the immune system in adapting different lineages of T cells according to the system's need, especially in infectious and pathological contexts.

Effector Mechanisms of T_{IM}/T_{VM} Cells

One of the greatest enigmas about T_{VM} cells is which cytotoxic mechanisms operate in these cells. This topic has been approached with some laboratories, through ingenious methodologies and strategies, shedding light on this mystery. An interesting question is how T_{VM} cells distinguish their targets and whether the recognition and signaling through the TCR are involved. We will mention in more detail in the next section how early IFNγ production by T_{IM}/T_{VM} cells participates in the control of pathogen dissemination and burden load; however, other classical $CD8^+$ T cell mechanisms, such as perforin/granzyme release, are less investigated. Moreover, typical NK ''killing'' receptors, such as NKG2D, have been proposed to participate in T_{VM} cell effector mechanisms. In the following section, we comment on reports that address the role of these T_{VM} effector molecules and their actions.

Chu and others (2013), by using Nur77-GFP reporter mice (whose GFP level is proportional to the TCR stimulus strength and independent of inflammatory signals), demonstrated that after 48 h post-LM-OVA infection, T_{VM} cells (termed bystander-activated $CD8^+$ T cells by this group) slightly upregulated GFP expression compared to naive $CD8^+$ T cells from the same mice. However, GFP expression was much lower than in mice treated with αCD3 as a positive control and potent inducer of TCR signaling (Chu and others 2013). Their data support the idea that a weak TCR signal could activate T_{VM} cells. However, as demonstrated by other laboratories (see Role of T_{VM} cells in infectious processes section), T_{VM} cells could respond to Ag stimulation during primary and secondary immune responses giving rise to T_{EFF} and T_{CM} cells.

Another important question addressed by Chu and others (2013) is whether NKG2D is a bona fide effector receptor in T_{VM} cells. NKG2D is an activating receptor commonly expressed in NK cells and in activated memory $CD8^+$ T cells after TCR stimulation (Slifka and others 2000). RNA-seq data have shown that T_{VM} cells express IL-12R, IL-18R, IFNγ, granzyme B (GrzB), and NKG2D, molecules known to enhance innate-like effector functions (Hussain and

others 2019; White and others 2016). Exposure to IL-12 and IL-18 enables T_{IM}/T_{VM} cells to produce IFNγ early in Th1 inflammatory/infectious processes (Berg and others 2002, 2003; Haluszczak and others 2009), while GrzB and NKG2D expression can mediate Ag-independent cytotoxicity (Chu and others 2013).

Based on data demonstrating that T_{VM} cells are primed to rapidly respond to IL-12, IL-15, and IL-18 through constitutive receptor expression, Chu and others (2013) asked if exposure to these stimuli was able to induce both GrzB and NKG2D upregulation. Results demonstrated that IL-12, IL-15, and IL-18 can upregulate GrzB expression as early as 6 h after *in vitro* stimulation, but the cytokines are not primary regulators of NKG2D expression (Chu and others 2013). The situation might be different in an *in vivo* context as White and others (2016) reported that when gBT T_{VM} cells are adoptively transferred into IL-15 KO hosts and then challenged with LM-OVA, the transferred cells showed substantially reduced levels of GrzB, NKG2D, and IFNγ compared to the same cells transferred to WT mice.

In T_{MEM} cells, NKG2D is associated with a senescent phenotype (Prajapati and others 2018). However, despite being a marker of senescence and TCR-mediated dysfunction, NKG2D activity in T_{VM}-like cells ($CD8^+$ $CD25^-$) facilitates enhanced innate responsiveness (Tietze and others 2012). In this context, Chu and others (2013) presented evidence that T_{VM}-like cells can attack target cells in an NKG2D-dependent manner. In addition, a role for NKG2D in both infectious and tumor mouse models has been reported as follows: after primary influenza infection, a rapid arrival of non-Ag-specific OT-I cells is found in the lung, which are able to upregulate NKG2D, but not CD25, expression.

Accordingly, the *in vivo* blockage of NKG2D induces a lack of control in early viral replication in these mice (Sckisel and others 2014). In a tumor model, Tiezte and others (2012) co-treated Renca-bearing mice with anti-CD40/IL-2 and an NKG2D blocking antibody and demonstrated that blockade of NKG2D in mice receiving immunotherapy led to a significant decrease in the control of tumor growth. NKG2D could also be induced with other innate stimuli. To demonstrate this, Tiezte and others (2012) sorted $NKG2D^-$ $CD25^-CD8^+CD44^{high}$ T cells from congenic Ly5.1 mice and adoptively transferred them into WT C57BL/6 mice.

Two days after transfer, mice were treated with anti-CD40/IL-2, and NKG2D expression was analyzed 11 days later. The investigators found that $CD8^+$ T cells from immunotherapy-treated mice were able to expand and upregulate NKG2D expression (Tietze and others 2012). It is worth clarifying that, between the different subsets constituting the pool of T_{VM} cells in SLO as mentioned above, some laboratories have reported that IL-4-dependent T_{VM} cells exhibit reduced or absent NKG2D expression, although they do produce IFNγ after IL-12 + IL-18 stimulation (Ventre and others 2012; Jameson and others 2015).

Lee and others (2013a) have focused their attention on the functional characteristics of T_{VM} cells with reported differences from both naive and Ag-specific cells. For example, Lee and others (2013a) showed that *in vitro*, T_{VM} cells manifest certain T_{MEM} cell functions such as increased T-box transcription factor expression and advanced G1 cell cycle status and present naive-like properties, such as low IFNγ production after Ag stimulation. In all cases, Eomes expression seemed to be essential for the development of functional memory-like characteristics of the innate memory population. Eomes expression has been shown to bind to the *il2rb* promoter leading to increases in CD122 expression and then driving memory $CD8^+$ T cell sensitivity to IL-15 (Intlekofer and others 2005). Also, Eomes increases the ability of memory $CD8^+$ T cells to rapidly produce IFNγ (Intlekofer and others 2005).

NKG2D is not an exclusive functional mediator of T_{VM} cells. For instance Lanzer and others (2018) have demonstrated that T_{VM} cells obtained from the lung of aged mice infected with influenza virus developed a strong GrzB response and mediated viral clearance similar to that observed in young mice. In another study, Wang and others (2021) presented evidence that co-culturing T_{VM} cells with A20 lymphoma cells, pre-treated with the chemotherapeutic drug cytarabine (Ara-C) or doxorubicin (DOX), resulted in the ability of T_{VM} cells to substantially induce GrzB expression.

Moreover, adding a GrzB inhibitor (Z-AAD-CMK) to the co-cultures significantly reduced T_{VM}-medicated tumor cell death when compared to vehicle control-treated cells (Wang and others 2021). Collectively, data presented in this section demonstrate that T_{VM} cells are capable of exhibiting several mechanisms of cytotoxicity in different infectious or tumor settings. The fact that some types of $CD8^+$ T cells present as T_{VM} cells that develop into rapid effectors of the innate immune response, whereas other conventional $CD8^+$ T cells, with the same TCRs, can develop later during the adaptive immune response, is a fascinating and creative tool of the immune system.

Role of T_{IM}/T_{VM} Cells in Infectious Processes

T_{IM} and T_{VM} cells as an early source of IFNγ

During the early phase of certain infectious processes, innate cells are the main contributors of IFNγ, a critical cytokine for the control of multiple pathogens (Lukin and others 2000; Lertmemongkolchai and others 2001; Berg and others 2002, 2003). To date, T_{IM}/T_{VM}-like lymphocytes have been found to play a very important first-line defense role in viral (Sckisel and others 2014; Lee and others 2015), bacterial (Lertmemongkolchai and others 2001; Berg and others 2003; Chu and others 2013), and parasitic (Baez and others 2019) infections. For many years, it was believed that NK and NKT cells were the primarily sources of IFNγ in response to pathogen-derived inflammatory triggers occurring in the absence of antigenic immune responses.

However, more recently, Kambayashi and others (2003) demonstrated that other cell types were involved in early IFNγ production through identification of a population of spleen and lymph node IFNγ-secreting $CD8^+$ T cells in LPS-injected mice (Kambayashi and others 2003). Moreover, Kambayashi and others (2003) reported that production of IFNγ by this $CD8^+$ T cell population was MHC class I independent and restricted to $CD44^{hi}$ (memory phenotype) cells. IFNγ production by memory-like $CD8^+$ T cells was indirectly induced through macrophage/dendritic cell-derived IFNα/β, IL-15, IL-12, and IL-18 in an Ag-independent way (Yajima and others 2001; Kambayashi and others 2003; Haluszczak and others 2009; Bou Ghanem and others 2011; Martinet and others 2015).

Pathogen-associated molecular patterns and danger signals can promote the production of inflammatory cytokines by different innate immune cells, such as dendritic cells and macrophages that are capable of producing large amounts of IL-12 and IL-18 in the early phase of an infection. The

receptors for both IL-12 and IL-18 are constitutively expressed on T_{IM}/T_{VM} cells (White and others 2016, 2017), thus providing a mechanism whereby these innate cells can rapidly respond by producing large amounts of IFNγ early in infectious or Th1 inflammatory processes. Interestingly, a recent work demonstrates a higher frequency of IFNγ-producing T_{VM} cells in B6 than BALB/c mice after *in vitro* stimulation with IL-12/IL-18 due to a larger expression of IL-18R in T_{VM} cells from B6 mice (Moudra and others 2021).

The role of innate T_{IM}/T_{VM}-like cells during certain bacterial infections, such as Burkholderia pseudomallei (BP) and LM, where resistance is strictly dependent upon IFNγ production, has been appreciated for some time (Lertmemongkolchai and others 2001; Berg and others 2002). Approximately 20 years ago, 2 different laboratories demonstrated that *in vitro* exposure of splenocytes to BP or LM induced IFNγ production from pre-existing $CD8^+$ $TCR\alpha\beta^+$ $CD44^{hi}$ T cells and this effect was triggered by bacterially induced IL-12 and IL-18 (Lertmemongkolchai and others 2001).

In the case of viral infections, Lee and others (2015) have shown that the large number of IL-4-induced innate $CD8^+$ T cells (currently called T_{IM} cells) present in $CIITA^{tg}$ mice produce high levels of both IFNγ and TNFα. These cells fully control the viremia upon infection with clone 13 of lymphocytic choriomeningitis virus (LCMV) and are dependent upon IL-4 since $CIITA^{tg}$IL-4KO mice are not capable of clearing the virus (Lee and others 2015).

Our laboratory reported similar results using a parasitic infection model. We demonstrated that during the acute phase of *T. cruzi* infection, thymic cells enriched with T_{IM} cells have substantial capacity to produce IFNγ after IL-12 + IL-18 stimulation and induce protection when adoptively transferred to *T. cruzi*-infected mice (Baez and others 2019).

IL-12+IL-18-induced IFNγ production in memory-like $CD8^+$ T cells seems to be regulated differently than in NK cells. Martinet and others (2015) demonstrated that after *in vitro* rIL-12 + rIL-18 stimulation, or following LM infection *in vivo*, memory-like $CD8^+$ T cells, and not NK cells, from IRF9 knockout (KO) mice produced significantly lower amounts of IFNγ than their WT counterparts.

Berg and others (2003) further explored this topic and found that besides their rapid capacity to produce IFNγ, innate $CD8^+$ T cells can also protect mice from *L. monocytogenes* by mechanisms that are TCR and IFNγ independent. Moreover, Hu and others (2007) demonstrated that adoptively transferred $CD8^+$ $CD44^{hi}$ cells from ITK KO mice exhibited enhanced response to *L. monocytogene* infection by reducing bacterial burden in IFNγ KO mice. Overall, these data support the concept that memory-like $CD8^+$ T cells act in a rapid and efficient manner early during infection phase to provide an additional source of IFNγ, which alongside innate cytotoxic mechanisms collaborate to resolve or resist infections until an adaptive immune response can initiate. Even more interesting is the notion that contrary to other innate cell types, T_{IM}/T_{VM} cells may also produce IFNγ through TCR signaling mechanisms. This is examined in the next section.

The TCR is involved in T_{IM}/T_{VM} cell immune response

As mentioned above, T_{IM}/T_{VM} cells are more prone to respond in a bystander manner to cytokine stimuli during the course of an infection rather than by TCR activation and signaling. However, these cells carry a wide TCR repertoire that is completely functional. Data that support this point were provided by White and others (2016), who demonstrated that OTI T_{VM} cells ($CD44^{hi}$ $CD49d^{lo}$ OTI cells) are able to protect against LM-OVA by inducing a substantial reduction in splenic bacterial CFUs. Interestingly, when they evaluated protection by a different TCR transgenic mouse model that does not recognize bacteria in an Ag-specific way (gBT cells), White and others (2016) observed a surprisingly high protection after LM-OVA infection, similar to the one mediated by OTI cells.

Thus, the investigators concluded that T_{VM} cells are capable of mediating potent immunological protection against bacterial challenge in the presence or absence of their cognate antigen (White and others 2016). Interestingly, this effect is highly dependent on IL-15 in an Ag-independent context; since using IL-$15^{-/-}$ mice as recipients, only the antigen-specific T_{VM} OTI-transferred cells demonstrate a protective effect in this system, while nonspecific gBT T_{VM} cells show a substantial reduction in granzyme B, NKG2D, and IFNγ expression, which compromises their functional capacity (White and others 2016). These latest data suggest that memory-like cells could rapidly respond early in infections, in an Ag-specific or nonspecific manner, to support the innate immune response until Ag-experienced adaptive immunity develops.

If recognition by the TCR in T_{VM} cells is possible, then it would be reasonable to ask whether T_{VM} cells can become effector cells during an infectious process and generate memory-phenotype progeny. In this matter, several laboratories have demonstrated that both T_{IM} and T_{VM} cell subsets are poised to produce IFNγ when they encounter cognate antigen. As a result, both cell types trigger an antigen-specific protective immune response against infection that is far better than $CD8^+$ T_N cells (Lee and others 2013a; White and others 2016).

Moreover, when comparing *in vitro* IFNγ production by OVA-specific T_N, T_{VM}, and T_{MEM} cells after a 5-h Ag stimulation, T_{VM} cells produced higher levels of IFNγ compared to T_N cells, but significantly lower than T_{MEM} cells (Lee and others 2013a). Lee and others (2013a) also tested the same populations after LM-OVA *in vivo* infection and observed that in the early infection phase, T_{VM} cells expand more rapidly than T_N cells, but this difference was lost at later times. Interestingly, when evaluating a recall response to LM-OVA, no advantage in the number of memory OVA-specific T_{VM} cells was observed compared to the memory T_N cell counterpart (Lee and others 2013a).

When studying cytokine production, Quinn and others (2018) demonstrated that following TCR stimulation, T_{VM} cells could give rise to T_{EFF} cells; however, T_{VM}-derived T_{EFF} cells produced predominantly more IFNγ alone compared to T_N-derived T_{EFF} that were more multifunctional through production of a broad spectrum of cytokines. Moreover, T_{EFF} cells that arise from T_{VM} cells adopt a short-lived effector cell phenotype, while T_N-derived T_{EFF} cells are more likely to develop into stable T_{MEM} populations (Lee and others 2013a; Smith and others 2018). When evaluating Ag-specific secondary immune responses by T_{MEM} and T_{VM} cells, Lee and others (2013a) showed that both subsets expand equally; however, T_{VM} cells produced significantly larger numbers of T_{CM} cells than T_{MEM} cells.

Collectively, these data demonstrate that T_{VM} cells are not only capable of responding in a TCR-specific manner to generate effector cells with rapid IFNγ production capacity early in infection, but are also able to respond to secondary challenge by differentiating mainly into T_{CM} cells.

Role of T_{IM}/T_{VM} in Cancer

Numerous studies on cancer immunotherapy show that the antitumor effects depend on the generation of antigen-specific T cells. However, there is evidence that antitumor properties can also be mediated by alternatively activated T cells that are not tumor specific. Tietze and others (2012) treated mice with anti-CD40 Ab + IL-2 and observed significant antitumor effects in 3 different murine tumor models. Their data correlated with a massive expansion of splenic $CD8^+CD44^{hi}CD122^{hi}CD25^-$ at 11 days after this regimen administration, where, as referred by the authors, no expression of CD25 on T cells mainly indicates activation independent of TCR engagement as demonstrated by the same authors in *in vitro* assays (Tietze and others 2012).

They reported that the $CD8^+$ memory-like T cells that responded to the treatment also exert high cytolytic activity toward tumor targets partially recognized through NKG2D (Tietze and others 2012). The authors also evaluated the phenotype of $CD8^+$ T cells present in melanoma biopsies from patients receiving local treatment with the TLR7 agonist imiquimod, a nonantigenic immunotherapy. Interestingly, immunohistologic analyses of the biopsies demonstrated a marked infiltration of $CD8^+CD25^-$ T cells within the tumors compared with tumors treated with the vehicle alone (Tietze and others 2012).

Similarly, Hu and others (2014) also reported, years ago, a substantial benefit in the antitumor activity of $NKG2D^+CD8^+$ T cells in 4T1 tumor-bearing mice after a nonantigenic treatment with doxorubicin (Dox) + IL-12. Their analysis shows a large number of $NKG2D^+CD8^+$ T cells colonizing the tumors of mice that received the treatment compared to control mice (Tietze and others 2012; Hu and others 2014). In addition, they demonstrate that blocking NKG2D completely reversed Dox plus IL-12-mediated inhibition of tumor growth (Tietze and others 2012; Hu and others 2014).

By their site, Xu and others (2013) show that a single dose of ALT-803, a complex of an interleukin (IL)-15 superagonist mutant and a dimeric IL-15 receptor, is able to eliminate 5T33P and MOPC-315P myeloma cells present in the bone marrow of tumor-bearing mice. Also, ALT-803 treatment promoted *in vivo* expansion of $CD8^+CD44^{high}$ that upregulates NKG2D, but not CD25 expression, and secretes large amounts of IFNγ (Xu and others 2013). Furthermore, ALT-803-activated $CD8^+$ memory-like T cells exhibited *in vitro* nonspecific cytotoxic activity against myeloma and other tumor cell lines (Xu and others 2013).

Even though the number of these ''memory-like'' cells described in these reports increased in response to alternative activation with inflammatory cytokines in the absence of immunization with specific Ags, whether these cells belong to the virtual $CD8^+$ T cell lineage is not determined in these studies, as they were not characterized with the markers that now define this population.

The role of T_{IM}/T_{VM} cells is particularly important in cancer immune response since once tumors miss their MHC class I expression, they cannot be recognized by $CD8^+$ T cells in a TCR-specific manner. This point is quite vital because the loss of MHC-I occurs frequently in many different types of human cancers (Garrido and others 2010; Challa-Malladi and others 2011). Wang and others (2021), by using several tumor models, demonstrated that chemotherapeutic treatment significantly increases T_{VM} TILs in tumors ($CD44^+CD122^+NKG2D^+Eomes^+CD49d^-$).

Interestingly, in their work, T_{VM} cells were activated in the presence of tumors cells treated *in vitro* with the chemotherapeutic drug Ara-C or Dox and produced large amounts of granzyme B, which can mediate the apoptosis of target cells (Wang and others 2021). The authors also validated their results in a humanized murine model and demonstrated that chemotherapy-treated human tumors also activated human T_{VM} cells, independent of tumor-derived MHC-I (Wang and others 2021).

In our group, we have demonstrated that IL-12 and IL-18 systemic expression in tumor-bearing OT-I mice are able to induce high infiltration of $CD8^+$ T cells with a T_{VM} phenotype into non-OVA B16 or pancreatic ductal adenocarcinoma tumor cells (KPC). Moreover, cells obtained from LNs of IL-12+IL-18-treated OT-I mice showed activation features after *in vitro* exposure and contact with KPC cells (unpublished data).

In the work previously mentioned by Miller and others (2020), the investigators examined the presence of T_{VM} recurrent clones in tumors and draining LNs from TRAMP-bearing mice. The author co-transfected polyclonal T_{VM} and T_N cells into the prostatic adenocarcinoma-bearing mice, and 4 months later analyzed the transferred cells. They found that T_{VM} cells represent a substantial fraction of the tumor-infiltrating $CD8^+$ T cells. They isolated $CD8^+$ T cells from the prostate tumors and using a TCR sequencing approach, they identified numerous T_{VM} cell clones that were enriched in TRAMP prostate tumors (Miller and others 2020).

Comparing the frequency of those intratumor T_{VM} clones with the ones present in SLO of tumor-free mice, they found that the prevalence of those clones is quite different between SLO and tumors. They conclude that TRAMP prostate tumors favor the recurrent enrichment of ''tumor-associated'' T_{VM} cells that are uncommon in the periphery (Miller and others 2020).

The control of tumor growth through Ag-independent pathways is a topic of growing interest, considering that several tumors lose the expression of MHC type I as an evasion mechanism, which makes the tumor less susceptible to Ag-specific lysis, but more susceptible to innate control mechanisms such as NK cells and now to T_{VM} cells.

T_{VM} Cells During Aging

In young mice, T_{VM} cell functional capacity is optimum with the ability to rapidly proliferate and produce cytokines after TCR or innate stimulation compared to T_N, as previously mentioned. However, over time, the proportion of T_{VM} cells accumulates and becomes dysfunctional. Recent work examined peripheral T_{VM} cells in both young and aged germ-free B6 and BALB/c mice that found that the frequency of T_{VM} cells increases with age and is independent of genetic background. The investigators also examined different hygienic conditions that included cohousing laboratory and feral mice with results demonstrating only minimal effects on peripheral T_{VM} cells. This suggests that a common homeostatic mechanism exists during aging that is independent of mouse strain or commensal microbiota (Moudra and others 2021).

Several investigators have addressed the cause of these changes throughout the T_{VM} cell lifespan. Renkema and others (2014) evaluated the long-term maintenance of T_{VM} cells in unimmunized old mice. They found that T_{VM} cells from old OT-I or WT mice (≥14-month old) displayed several different characteristics not observed in younger mice (2–4-month old) (Renkema and others 2014). T_{VM} cell increased in frequency from 20% in young mice to up to 70% in old mice (Rudd and others 2011). Moreover, by using CD44, CD62L, and CD49d markers, Chiu and others (2013) determined that 90% of the T_{CM} $CD8^+$ T cells were indeed T_{VM} cells in aged mice.

This age-related accumulation in T_{VM} cells correlates with an increased proliferation capacity, exhibiting significantly higher propensity to divide 4 or more times in response to IL-7 and IL-15. In contrast, T_{VM} cells in old mice are less capable of proliferating in response to cognate peptide (TCR). Moreover, the cells undergo increased apoptosis specifically in response to peptide stimulation (Renkema and others 2014). However, preferential enrichment of T_{VM} cells with high avidity in older animals that exhibited strong antimicrobial function could compensate for functional defects during aging (Rudd and others 2011). Consistent with the age-associated increase in T_{VM} cells, it was reported that the *de novo* response to influenza virus in aged mice was dominated by T_{VM} cells, in contrast to the response in young mice (Lanzer and others 2018).

In a comparative study to evaluate functional defects characteristic of T_N versus T_{VM} cells with age, Quinn and others (2018) sorted T_N and T_{VM} cells from unimmunized WT mice, polyclonally stimulated *in vitro* with coated aCD3ε, and evaluated cell numbers up to 8 days post-stimulus. They found that, while young T_N and T_{VM} cells could extensively proliferate, only aged T_N cells exhibited a slight age-related defect; however, aged T_{VM} cells displayed a severe reduced proliferative capacity mainly caused by decreases in cell cycle division (Quinn and others 2018).

When they evaluated young and aged T_N and T_{VM} cell proliferation following *in vitro* IL-15 stimulation, Quinn and others (2018) found that only young and aged T_{VM} cells proliferated robustly. This indicated that in T_{VM} cells, TCR and cytokine-specific proliferative responses are regulated independently (Quinn and others 2018). To determine if environment is responsible for the defect observed in aged T_{VM} cells, the author performed adoptive transfer experiments by administering young T_N and T_{VM} cells into aged C57BL/6 recipient mice. Results indicated that, while T_N versus T_{VM} cell proportions were stable for over 2 months after transfer, both subsets exhibited a severe reduction in proliferative capacity.

In addition, adoptive transfer of aged T_{VM} cells to young WT recipient mice did not recover the T_{VM} cell defect (Quinn and others 2018). Evaluation of exhaustion-associated markers demonstrated that age-related changes in T_{VM} cells were not consistent with exhaustion, but rather senescence and associated with upregulation of NKRs, Bcl-2 expression, and increased phosphorylation of MAPK signaling pathway proteins (Quinn and others 2018). Based on these data, the author surmises that the ''inflammaging'' state commonly observed in elderly people allows T_{VM} cells to survive relatively well in the aged environment as they respond better to cytokines than to TCR engagement.

The collective data on the preferential accumulation of aged T_{VM} cells and their functional role in this stage suggest that, while aging, organisms become more dependent on innate immune responses that efficiently and rapidly act through cytokines rather than by an impaired Ag-specific response.

T_{IM}/T_{VM} Cell in Humans

Discovery and phenotype

In humans, demonstrating the existence of CD8 T^+ cells having a memory phenotype without encountering any antigen is quite challenging, and to date, only 4 studies have addressed this question using human cord blood samples. In 2006, a first study identified $CD8^+$ T cells expressing either KIR receptors or the inhibitory NKG2A receptor with an EMRA phenotype ($CD45RA^+$ $CCR7^-$) in cord blood, intact from viral infection or placental pathology (Warren and others 2006). Later, a second study identified $CD8^+$ T cells in fetal human thymus and spleen expressing CD45RA, CD161, and CD122 markers at their surface and the transcription factor Eomes intracellularly (Min and others 2011). In these first 2 studies, the identified T_{IM}/T_{VM} cells were functional, with the capacity to secrete IFNγ in response to a nonspecific stimulation by phorbol myristate acetate and ionomycin.

Later, Jacomet and others (2015) identified T_{IM}/T_{VM} cells expressing KIRs and/or NKG2A surface markers with an enriched EMRA phenotype and a marked Eomes expression. This result was recently confirmed by an independent study using the same markers to identify T_{IM}/T_{VM} cells in both cord blood and peripheral adult blood (Kasakovski and others 2021). T_{IM}/T_{VM} cells have a highly cytotoxic potential, as they have a high content of perforin and granzyme B (Jacomet and others 2015). Importantly, a new function enlightened by Jacomet and others (2015) was the secretion of IFNγ by T_{IM}/T_{VM} cells in response to a proinflammatory stimulation by IL-12 and IL-18, demonstrating the NK-like function of these cells in cord blood.

Currently, there is no consensus to specifically identify T_{IM}/T_{VM} cells in human peripheral blood; since early 2000, several studies have identified $CD8^+$ T cells with innate-like features and 2 methods seem to emerge concomitantly. The first method uses only surface cell markers: CD8, KIR, NKG2A, and CD45RA, and can be completed by the expression of CD62L and CD122 and the lack of CD27 and CCR7 markers (White and others 2016; Quinn and others 2020). The second method uses surface cell markers (CD8, KIR, and NKG2A) plus the expression of the transcription factor Eomes (Jacomet and others 2015, 2016; Barbarin and others 2017; Kasakovski and others 2021). This second method has the advantage of taking into account that Eomes expression is preferentially linked to the IFNγ secretion function in response to an innate stimulation by IL-12 and IL-18, as $CD8^+$ $Eomes^-$ $KIR/NKG2A^+$ T cells poorly secrete IFNγ (Daniel and others 2021).

In these 2 methods, the antibodies used are mainly a mix of anti-NKG2A, anti-panKIR2D (clone NKVSF1), and anti-KIR3DL1/DL2 (clones DX9 and 5.133), resulting in the identification of a heterogeneous population. In an attempt to better define T_{IM}/T_{VM} cells on a functional basis, Barbarin and others (2017) found an association between

CD49d and the IFNγ secretion, but not specific for the identification of T_{IM}/T_{VM} cells in humans. Another interesting marker could be the ecto-5′-nucleotidase CD73, which has been demonstrated to delineate a subset of polyfunctional memory T cells expressing Eomes, with increased survival and with the ability to develop into cells resembling tissue-resident memory T cells (Fang and others 2021).

Finally, 3 recent studies looked deeper into the phenotype of T_{IM}/T_{VM} cells.

The first study sorted separately $CD8^+$ $CD45RA^+$ pan-$KIR2D^+$ $KIR3DL1/DL2^+$ cells (T_{IM}/T_{VM} KIR^+ cells) and $CD8^+$ $CD45RA^+$ $NKG2A^+$ cells (T_{IM}/T_{VM} $NKG2A^+$ cells) and performed an RNA-seq analysis of these 2 subsets of T_{IM}/T_{VM} cells (Pieren and others 2021). The authors confirmed that these 2 subsets have different characteristics and suggested different functions. In particular, T_{IM}/T_{VM} KIR^+ cells were found to share common features with previously described regulatory $CD8^+$ T cells (Nakagawa and others 2018; Holderried and others 2021; Mishra and others 2021), with the expression of the transcription factor Helios and higher expression of CD122 and TIGIT.

This study identified TIGIT and CD226 as potential markers to better characterize T_{IM}/T_{VM} KIR^+ cells and T_{IM}/T_{VM} $NKG2A^+$ cells. Indeed, T_{IM}/T_{VM} KIR^+ cells are mainly $TIGIT^+$ $CD266^-$ and T_{IM}/T_{VM} $NKG2A^+$ cells are $TIGIT^-$ $CD226^+$. Furthermore, they also demonstrated a suppressive activity of T_{IM}/T_{VM} KIR^+ cells in an *in vitro* assay, but did not address the functionality of these 2 subsets in an innate-like stimulation assay (Pieren and others 2021).

The second study, combining gene expression profiles and TCR repertoires, identified a $CD8^+$ T cell subset ($CD45RA^+$ $CD45RO^-$ $CCR7^-$), expressing panKIR2D receptors with or without NKG2C (KLRC2) (Schattgen and others 2022). These T_{IM}/T_{VM} $KIR2D^+$ $NKG2C^{+/-}$ cells had a higher frequency of Helios-positive cells than $KIR2D^-$ $NKG2C^-$ $CD8^+$ T cells (Schattgen and others 2022), hence strengthening the hypothesis of a regulatory function for T_{IM}/T_{VM} KIR^+ cells. This last study also confirms the early study by Björkström and others (2012), showing that T_{IM}/T_{VM} KIR^+ cells displayed a restricted or biased TCR repertoire.

The third study also performed RNA-seq analysis of $CD8^+$ KIR^+ T cells and confirmed (1) their $TIGIT^+$ $Helios^+$ $NKG2A^+$ $CCR7^-$ $CD27^-$ $CD28^-$ phenotype with high cytotoxic content (perforin and granzyme B), (2) their less diverse TCR repertoire, and (3) their regulatory function in autoimmune diseases (Li and others 2022). Taken together, the phenotype $TIGIT^+$ $CD266^-$ of T_{IM}/T_{VM} KIR^+ cells and their biased TCR repertoire raised the question of their exhaustion status, as it was shown that $CD226^-$ $CD8^+$ T cells were dysfunctional (Weulersse and others 2020).

Finally, further characterization and properties of KIR^+ $CD8^+$ T cells, in particular $KIR2D^+$ $CD8^+$ T cells, were provided by Gimeno and others (2020). After culture in the presence of their HLA-C ligands, $KIR2DL2/L3/S2^+$ $CD8^+$ T cells showed notably higher expression of IFNγ, TGFβ, and perforin than $KIR2DL1/S1^+$ $CD8^+$ T cells. However, $KIR2DL1/S1^+$ $CD8^+$ T cells had a gene expression profile compatible with an active cancer immunosurveillance, whereas $KIR2DL2/L3/S2^+$ $CD8^+$ T cells had a gene expression profile suggesting a function of suppressive anti-tumor responses (Gimeno and others 2020).

Table 1 outlines the current knowledge on mouse T_{VM} and its human counterpart. Further studies are needed to determine where each T_{IM}/T_{VM} subset fits in the effector-memory and exhaustion differentiation continuum. This knowledge could aid in determining if these cells could be a target for immunotherapy.

Cytokines and $T_{IM/VM}$ cells

Despite few studies on the subject, by analogy with the mice model (Barbarin and others 2017), it is obvious that human T_{IM}/T_{VM} cells must be dependent on IL-15, IL-4, and type I interferon for their homeostasis/development. Indeed, T_{IM}/T_{VM} cells are enriched in CD122, the common β chain receptor for IL-2 and IL-15, and they express the transcription factor Eomes, both known to confer high sensitivity to IL-15. A recent *in vitro* study observed that after 10 days of culture, only IL-15 in combination with IL-2 increased the KIR^+ $CD56^-$ T cell frequency compared to IL-2 + IL-12 stimulation (David and others 2021).

For $KIR2DL3^+$ T_{IM}/T_{VM} cell subset, the authors demonstrated that the implied mechanism was the inhibition of their apoptosis (David and others 2021). In contrast, in an expansion *in vitro* model using HLA-C ligands, $panKIR2D^+$ $CD8^+$ T cells expanded preferentially in the presence of their ligand HLA-C1 and the proinflammatory cytokine IL-12, but surprisingly not in the presence of IL-15 (Gimeno and others 2020). This observation suggests that depending on the context and the subset of KIR cells, different cytokines are needed for T_{IM}/T_{VM} cell homeostasis/expansion.

IL-4 was also shown by Jacomet and others (2016) to participate in the homeostasis/development of T_{IM}/T_{VM} cells. This point will be further discussed below in the context of $iNKT/T_{IM}/T_{VM}$ cell axis described in chronic myeloid leukemia (CML).

Regarding type I IFN, in the mouse model and as mentioned earlier, it was shown that type I IFNs could induce T_{VM} cell differentiation (Martinet and others 2015). In humans, there is no direct evidence of a role of type I IFNs demonstrated, but type I IFNs were shown to promote IL-15Rα expression in human T cells, resulting in enhanced IL-15 signaling and increased cytotoxicity *in vitro* in a mixed lymphocyte reaction (Hansen and others 2011). Another study showed that adding IFNα to the proinflammatory IL-12 + IL-18 cytokine cocktail resulted in an IFNγ secretion similar to that found in response to the classical IL-12 + IL-18 stimulation in other innate-like T cell subsets (MAIT, iNKT, and Tγδ cells) (Gutierrez-Arcelus and others 2019).

Another indirect evidence comes from CML patients treated with IFNα before the rise of targeted therapy using tyrosine kinase inhibitors (TKI). A few numbers of patients were able to respond to the IFNα treatment and even maintained a treatment-free remission (TFR) for years (Bonifazi and others 2001). It is easy to hypothesize that the TFR obtained in these rare patients could be partially due to IFNα action on the immune system (de Castro and others 2003). Moreover, a recent study linked durable TFR of CML patients to a higher percentage of $T_{IM/VM}$ cells (Cayssials and others 2019). Despite the small number of subjects, two-thirds of them were patients with an IFNα treatment history, thus raising the question of IFNα in $T_{IM}/$

Table 1. Summary of Mouse and Human Virtual Memory CD8 T Cell Subsets Main Characteristics

Name	*Species*	*Membrane markers*	*Transcription factor signature*	*TCR repertoire*	*Response to TCR stimulation*	*Innate-like functions*	*Central/ peripheral differentiation*	*Homeostasis throughout life*	*Pathological context*
T_{VM}	Mouse	CD8, $CD44^{hi}$, $CD122^{hi}$, $CD49d^{lo}$, IL-4R, IFN-I R	$Eomes^{hi}$	Diverse, but nonsimilar to T_N repertoire	Yes, with low proliferation	Highly responsive to IL-15 IFNγ secretion in response to IL-12/ IL-18	IL-15 and Eomes dependent, mainly in periphery Precursors in the thymus	Increase with age	Bacterial and viral infections Tumor infiltration in several cancer models
T_{IM}/T_{VM}	Human	CD8, KIR^+ and/or $NKG2A^+$, CD62L, CD122, $CD45RA^+$, $CCR7^-$ $CD27^-$ as defined in Jacomet *et al.*, 2015	$Eomes^+$, $T\text{-}bet^+$	Unknown	Yes	IFNγ secretion in response to IL-12/ IL-18 CD107a degranulation induced by anti-CD16 or MHC class I- target cells Highly responsive to IL-15	Unknown, but present in cord blood, fetal thymus, and spleen	Not consensual	High antitumoral potential (CML, ovarian. and breast cancers) Expanded in HIV patients
T_{IM}/T_{VM} NKG2A+	Human	CD8, $NKG2A^+$, $CD45RA^+$, $TIGIT^-$, $CD226^+$ as defined in Pieren *et al.*, 2021	$Eomes^{int}$	Unknown	Yes, proliferation similar to other CD8 T cells	High IFNγ secretion in response to IL-12/IL-18	Unknown	Decline with age	High antitumoral potential, synergy with NK cells (NKG2A as a checkpoint inhibitor)
T_{IM}/T_{VM} KIR+	Human	CD8, KIR^+, $CD45RA^+$, $TIGIT^+$, $CD226^-$ as defined in Pieren *et al.*, 2021	$Eomes^{hi}$, Helios	Less diverse than other CD8 T cells	Yes, proliferation lower than other CD8 T cells	Low IFNγ secretion in response to IL-12/IL-18	Unknown	Increase with age	Putative CD8 Treg suppressive activity in autoimmune and infectious diseases

T_{VM} cells arising and long-term presence in the peripheral blood of these patients. Thus, IFNα could have an effect on T_{IM}/T_{VM} cells that remains to be further confirmed.

NK-like functions of innate CD8 T cells in humans: an antitumoral response?

Although it is well known that the diversity in the repertoire of KIR and the KIR/HLA-ligand interactions determine the susceptibility to autoimmunity, infections, or cancer (Takeshita and others 2013), only a few recent studies have identified $CD8^+$ T cells expressing KIR receptors with innate-like or Treg features and linked it with immunosurveillance of cancer or as possible therapeutic targets. For example, in the peripheral circulation of breast cancer patients, a $CD8^+$ $CD25^+$ $CD127^-$ T cell population was identified; this subset decreased with the advancement of cancer (Chakraborty and others 2018).

An elegant *in vitro* model showed that these cells were present mainly in the early stages of tumor development, expressed high level of KIR and low-level of NKG2A, and produced IFNγ (Chakraborty and others 2018), thus resembling the CD8 Treg KIR^+ T_{IM}/T_{VM} cells previously described. The authors hypothesized that $CD25^+$ KIR^+ $CD127^-$ $FOXP3^-$ $CD8^+$ T cells could impede tumor growth at an early stage before being deleted by $CD4^+$ Treg at a later stage (Chakraborty and others 2018).

In another study, the interaction of KIR3DL3 with its ligand HHLA2 was identified as a human immunosuppressive pathway (Wei and others 2021). In this work, $KIR3DL3^+$ $CD8^+$ T_{EMRA} cells could lyse NK-sensitive K562 targets and less-sensitive solid tumor cells by both TCR-dependent and TCR-independent mechanisms. Moreover, tumor mice models demonstrated that $KIR3DL3^-$HHLA2 interaction blockage restored the effector function of the $KIR3DL3^+$ cells and promoted antitumor immunity (Wei and others 2021). It remains to determine the respective contribution of $KIR3DL3^+$ NK cells and $KIR3DL3^+$ CD8 T cells to this antitumoral effect.

Regarding $NKG2A^+$ $CD8^+$ T cells, several studies demonstrated their importance in cancer immunosurveillance, but did not investigate the relevance of their innate-like functions in this context. As mentioned above, the antitumoral function of $NKG2D^+$ $CD25^-$ $CD8^+$ T_{IM} cells described in mice has been confirmed in human melanoma biopsies. In humans, memory $CD25^-$ $CD8^+$ T cells could be induced *in vitro* either by an IL-2 stimulation without TCR engagement or in melanoma by antigen-nonspecific immunotherapy treatment (Tietze and others 2012).

The strongest evidence linking T_{IM}/T_{VM} cells ($KIR/NKG2A^+$ $Eomes^+$) to an antitumoral function was established by the group of Gombert and Herbelin (Jacomet and others 2016). They demonstrated the implication of T_{IM}/T_{VM} cells in CML. First, they showed that $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cell subset was defective at disease diagnostic with the loss of antigen-independent cytotoxic activity and IFNγ production in response to innate-like stimulation with IL-12 + IL-18. The percentage and the IFNγ function of $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells were partially restored after tyrosine kinase (TKI) therapy in patients achieving disease control (Jacomet and others 2016). Indeed, T_{IM}/T_{VM} cells are targeted by the TKI dasatinib in humans and mice (Barbarin and others 2020).

Moreover, by analogy with the mice model, they hypothesized that $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cell deficit at CML diagnosis could be linked to iNKT cell deficit already observed, with an impaired IL-4 production by iNKT cells (Rossignol and others 2012). The positive correlation observed between Eomes expression in $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells and PLZF expression in iNKT cells, along with the direct effect of IL-4 *in vitro* on $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cell proliferation, strengthens this hypothesis (Jacomet and others 2016). Later, they demonstrated in a monocentric retrospective study that long-term TKI treatment discontinuation without relapse could be associated with an elevated presence of $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells in the blood, correlated with high NK cell percentage (Cayssials and others 2019). Supranormalized $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells were normally functional like in healthy donors, with an intact IFNγ secretion function (Cayssials and others 2019).

Taken together, $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells are a critical element for CML immunosurveillance. The role of $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells was also demonstrated in chemo-treated human lymphoma cells deprived of MHC-I molecules co-cultured *in vitro* with purified human $CD8^+$ T cells from healthy donors. An increase in the percentage of $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells, associated with an increase of granzyme B content, was observed (Wang and others 2021). $KIR/NKG2A^+$ $Eomes^+$ T_{IM}/T_{VM} cells were also observed in solid cancers: in the draining LNs from breast cancer patients and in primary tumor, carcinosis, and peritoneal ascites from ovarian cancer patients (Barbarin and others 2017).

Even though human T_{IM}/T_{VM} was mainly studied in the context of cancer, a few studies have reported their presence in other pathologies. Indeed, Jin and others (2020) observed an increase of these cells in HIV patients undergoing successful antiretroviral therapy, and demonstrated a suppressive activity dependent on KIR receptors *ex vivo*. Furthermore, Daniel and others (2021) showed that chronic allo-antigenic stimulation promotes the generation of T_{IM}/T_{VM} cells with a senescent/inflammaging signature (EMRA phenotype, $CD27^-$ $CD28^-$, and high perforin content).

Conclusions

The pool of memory $CD8^+$ T cells is composed of a variety of cell subtypes with different phenotypic and functional characteristics. Within this group, we can find the so-called Ag-independent memory cells or virtual memory cells. T_{VM} have long been confused with Ag-specific central memory T cells, which has caused for many years their true role not to be clarified within the immune system.

The discovery of human $CD8^+$ T cells with identical characteristics to murine T_{VM} cells has aroused enormous interest in recent years and has posed a new challenge in trying to understand not only their role in different pathological processes but also to discriminate what roles have been erroneously assigned to specific memory cells instead of virtual memory cells.

Numerous publications are currently emerging, which are clarifying more and more about the origin, functional characteristics and their role in different pathological processes, especially in infections and cancer, which will provide a new edge to understand their key role within the immune system.

Authors' Note

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Address correspondence to:
Dr. Maria Cecilia Rodriguez-Galan
Inmunología
CIBICI-CONICET
Facultad de Ciencias Químicas
Universidad Nacional de Córdoba
Córdoba 5000
Argentina

E-mail: maria.rodriguez.galan@unc.edu.ar

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