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REVIEW

Stem memory T cells (TSCM)—their role in cancer and HIV immunotherapies

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Stem memory T cells (TSCM) have been described in mice, non-human primates and in humans, constituting approximately 2–4% of the total CD4$^+$ and CD8$^+$ T-cell population in the periphery. TSCM represent the earliest and long-lasting developmental stage of memory T cells, displaying stem cell-like properties, and exhibiting a gene profile between naïve and central memory T cells. Their self-renewal capacity and long-term survival has sparked interest in the cancer and human immunodeficiency virus (HIV) fields. How and when the formation of TSCM occurs during the immune response to pathogens and the therapeutic potential of these cells are currently being investigated. This review will explore the potential role of TSCM to be used as, or targeted by, immunotherapies and vaccines for treatment of cancer and HIV.
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Memory T cells have an important role in the adaptive immune response to infectious diseases and cancer.[1–5] Following exposure to antigen, naïve T cells undergo proliferative expansion and differentiation into memory T-cell subsets, culminating into terminally differentiated effector T cells.[6,7] As T cells mature, they progressively acquire effector functions and lose the ability for self-renewal and survival.[4] A minority will survive the contraction phase and become long-lived memory T cells, which have the ability to acquire effector functions upon reinfection[5,6] (Figure 1).

Memory T cells have been characterized by their phenotypic and functional profiles into T-cell subsets, typically central memory (CM) and effector memory (EM) T cells (Table 1). Phenotypically, CM and EM cells are divided respectively by the presence or absence of lymph node homing receptors CD62L (L-selectin) and C–C chemokine receptor 7 (CCR7) on their surface.[8,9] Naïve and CM T cells express CD62L and CCR7 for migration to secondary lymphoid organs, and in the absence of these molecules, EM and effector cells can accumulate in peripheral tissues. CM and EM can also be divided by the level and type of cytokine secretion, with CM cells having a greater proliferative and interleukin-2 (IL-2)-producing ability, whereas EM have increased secretion of effector cytokines including interferon-γ (IFN-γ) and IL-4[8,9] (Figure 2).

CM T cells are relatively long-lived memory cells, which are able to differentiate into shorter-lived EM T cells upon antigen stimulation[4,7,9] and, to a lesser extent, in response homeostatic cytokines (IL-7 and IL-15).[10,11] Following the theory of a hierarchical system of memory, transition from naïve to CM to EM T cells has been described as progressively acquiring the capacity to respond to homeostatic cytokines, tissue homing receptors, antiapoptotic molecules and acquiring effector function, whilst losing the expression of lymph node homing receptors (CCR7 and CD62L) and the capacity for proliferation and IL-2 production.[12] Transitional memory T cells, which can be distinguished from other memory T-cell subsets through the additional use of CD27 surface receptor expression,[10] have been described as having functional and transcriptional characteristics in between CM and EM T cells.[13]

More recently, the notion that CM T cells demonstrate stem cell-like characteristics with their capacity to self-renew and also to generate more differentiated progeny from antigen stimulation[14] has been challenged by the discovery of an earlier stage memory T cell[15,16] (Figure 3). This novel T-cell subset, termed stem memory T cells (TSCM) has been detected in CD4$^+$ and CD8$^+$ T-cell populations of mice,[17] non-human primates (NHP)[16,18] and humans.[15,17] TSCM display stem cell-like properties and constitute a small proportion of the memory T-cell subset, approximately 2–4% of the total CD4$^+$ and CD8$^+$ T-cell population in the blood.[15,18] TSCM have been described as representing the earliest and longest lasting developmental stage of memory T cells[15] and exhibiting a gene profile which is between naïve and CM T cells.[15,18]

TSCM cells share common phenotypic characteristics with naïve T cells as they are CD45RA$^+$, CD45RO$^-$, CCR7$^+$ and CD27$^+$; however, they can be distinguished from naïve T cells by a high expression of CD95 and CD122 (IL-2Rβ)[15,19,20] (Table 1). CD95 and CD122 are cellular surface markers, which are also expressed by other memory T-cell subsets.[18] Human TSCM cells also express higher antiapoptotic molecule B-cell lymphoma 2, chemokine (C-X-C motif) receptor CXCR3, CXCR4, lymphocyte function-associated antigen-1 and a lower expression of CD38 and CD31 compared with naïve T cells.[15]

TSCM have been demonstrated to exhibit characteristics closer to those of conventional memory T cells compared with naïve T cells. The T-cell receptor (TCR) rearrangement excision circles, which are

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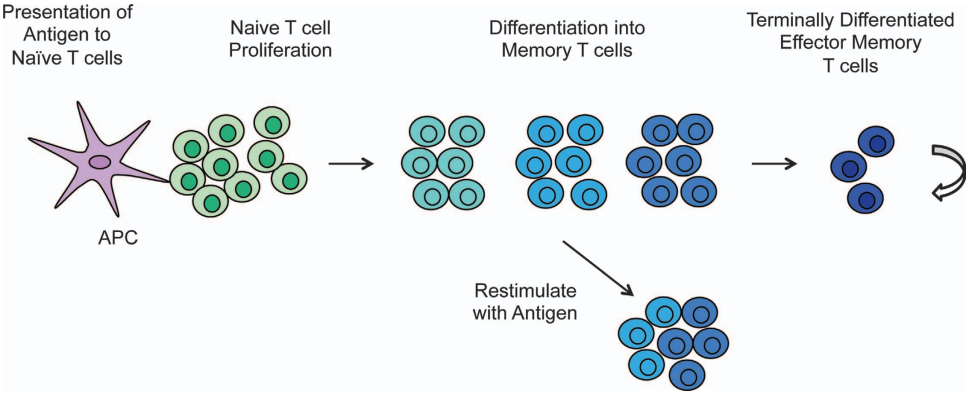

Figure 1 Following antigen exposure, naïve T cells undergo proliferative expansion and differentiate into memory T-cell subsets, which culminate into terminally differentiated effector T cells. A majority of memory T cells will survive the contraction phase and become long-lived memory T cells, which have the ability to acquire effector functions upon antigen re-exposure. APC, antigen-presenting cell.

Table 1 Phenotypic characteristics of T-cell subsets

Naive	*TSCM*	*CM*	*EM*
CD45RA+	CD45RA+	CD45RA−	CD45RA−
CD45RO−	CD45RO+	CD45RO+	CD45RO+
CCR7+	CCR7+	CCR7+	CCR7−
CD62L+	CD62L+	CD62L+	CD62L−
CD27+	CD27+	CD27+	CD27−
CD28+	CD28+	CD28+	CD28+/−
CD95−	CD95+	CD95+	CD95+
CD122−	CD122+	CD122+	CD122+

Abbreviations: CM, central memory; EM, effector memory; TSCM, stem memory T cell.
In $CD4^+$ T-cell subsets, transitional memory (TM) T cells can be distinguished from EM by the presence of CD27[10,20,35] and have functional properties and transcriptional characteristics in between CM and EM T cells.[13] Phenotypic characterization was assessed through personal observation and previous publications.[10,20,22,35]

diluted during clonal proliferation, were found to be of a lower copy number in TSCM compared with naïve T cells, similar to other memory T cells (CM and EM). This indicated that TSCM had undergone multiple rounds of division.[15] In addition, upon TCR stimulation, TSCM are antigen experienced and exhibit effector activity including tumor necrosis factor alpha, IFN-γ and IL-2 secretion, whereas naïve T cells were reported to remain relatively quiescent.[15] Gattinoni *et al.*[15] demonstrated TSCM to differentiate into CM and EM T-cell subsets, and compared with CM and EM T cells, showed TSCM to have a greater self-renewal capacity in the presence of IL-15 homeostatic signals and to be longer lived.[15]

Gattinoni *et al.*[15] investigated gene expression in $CD8^+$ memory T-cell subsets and found progressive changes moving from naïve to TSCM to CM and EM cells. These changes included a decrease in the expression of genes that encoded transcription factors for inhibiting T-cell activation and differentiation (lymphoid enhancer-binding factor1, forkhead box P1 and LAG1 homolog) from naive to EM cells, whereas transcripts encoding regulators of effector differentiation and senescence (including eomesodermin and T-box 21), cytotoxic molecules (granzyme A and perforin) and also T-cell senescence (killer cell lectin-like receptor subfamily G, member 1, KLRG1) were increased from naïve to EM cells.[15] These results were consistent with a notion of hierarchical system of memory differentiation where TSCM were the least differentiated of the memory T-cell subsets. It would also be valuable to examine the degree of overlap between the TCR repertoire of TSCM and the TCR repertoire of other memory T cells within an individual[21,22] as, discussed in this review, TSCM show promise for use in immunotherapies and vaccines.

The formation of TSCM during the immune response to pathogens and the therapeutic potential of these cells are currently being investigated. This review will discuss the role of TSCM in the development of immunotherapies and vaccines for cancer and human immunodeficiency virus (HIV).

THE POTENTIAL OF TSCM IN CANCER IMMUNOTHERAPY

TSCM have shown properties of self-renewal and superior antitumor responses compared with other memory T-cell subsets in adoptive T-cell therapy studies in mice.[15,17,23] It is qualities like these that make TSCM an attractive possibility for cancer therapies. These characteristics may be able to reduce the current therapeutic limitations of adoptive immunotherapies including poor T-cell engraftment, poor persistence and an inability to mount a long-lasting immune response.[4,24]

The use of TSCM in adoptive immunotherapy

Novel techniques for the generation of TSCM for use in adoptive immunotherapies have been demonstrated by Gattinoni *et al.*,[23] where $CD8^+$ TSCM were generated through the induction of Wnt-β-catenin signaling. Naïve $CD8^+$ T cells were primed in the presence of TWS119 an 'inhibitor of the serine-theronine kinase glycogen synthase kinase-3β (GSK-3β)',[23] which mimics Wnt signaling. Using the pmel mouse model,[25] the effect of TWS119 on $CD8^+$ T cells was assessed and found to inhibit T-cell differentiation. TWS119 also induced a dose-dependant reduction in T-cell killing activity and IFN-γ production, whilst maintaining the ability to produce IL-2, further implying TWS119 was a negative regulator of T-cell differentiation. Treatment with TWS119 increased the proportion of cells that were $CD44^{low}CD62L^{high}$ $CD8^+$ T cells, which were characterized as TSCM. Phenotypically, they expressed stem cell antigen-1 (Sca-1), CD122 and B-cell lymphoma 2, and they were able to secrete IFN-γ and IL-2 upon antigen encounter and underwent cell division after adoptive transfer.[23]

Importantly, these TSCM persisted and assisted in tumor destruction and demonstrated self-renewal capacity; following secondary transfer, $CD44^{low}CD62L^{high}Sca\text{-}1^{high}$ T cells were able to regenerate all of the T-cell subsets.[23] Compared with CM and EM T cells, TSCM cells displayed enhanced antitumor properties, triggering the destruction of tumors in mice, and improved survival. Results from

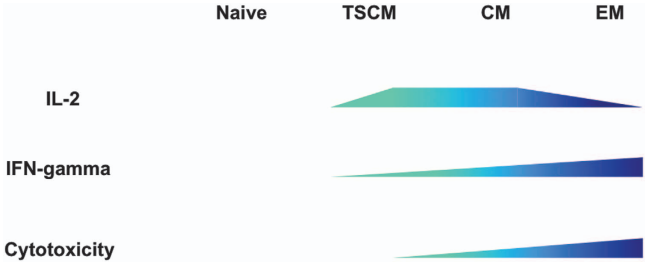

Figure 2 T-cell subsets are commonly characterized by their phenotypic and functional profiles. Memory T-cell subsets in particular have often been described by their effector functions, where CM and EM cells can be distinguished by CM cells having a greater proliferative and IL-2-producing capacity whilst EM have an increased secretion of effector cytokines, including IFN-gamma and enhanced cytotoxicity.

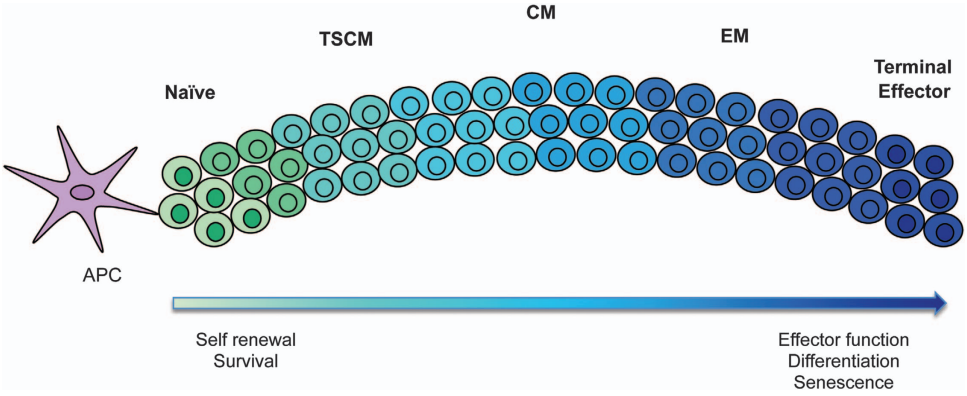

Figure 3 Following the theory of a linear hierarchical system, upon antigen exposure, naïve T cells proliferate and differentiate into memory T cells. Transition into more differentiated memory T-cell subsets has been described as progressively acquiring the capacity to respond to cytokines, tissue homing receptors, antiapoptotic molecules and acquiring effector functions, whilst also losing the expression of lymph node homing receptors, the capacity for proliferation, IL-2 production, self-renewal and survival. APC, antigen-presenting cell.

these adoptive transfer experiments were in combination with tumor antigen vaccination and exogenous IL-2, but provided principal for a superior antitumor response by TSCM compared with other memory T-cell subsets. Thus suggesting Wnt signaling is able to induce the generation of TSCM-like cells with rapid recall and proliferative ability, and that it could provide novel insights into the future design of vaccines and adoptive immunotherapies.

Further studies by Gattinoni *et al.*[15] indicated that following the adoptive transfer of TSCM into NSG mice (NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ) TSCM had greater replicative and survival ability compared with naive and memory T-cell subsets.[15] The investigators used Wnt signaling to generate mesothelin-specific TSCM, CM and EM T cells and adoptively transferred each T-cell subset with mesothelin-specific CD4$^+$ T cells into NSG mice, which had luciferase-expressing M108 mesotheloima (established for 3 months). Adoptively, transferring EM cells did not prolong survival of the mice and mediated poor antitumor responses. Transfer of CM cells was shown to be more effective than EM cells, although all mice died within 40 days post treatment. Transfer of TSCM, however, was associated with tumor regression and cure.[15] This study illustrated an enhanced antitumor activity for TSCM compared with CM and EM cells, with the potential for these cells to be used therapeutically.

Of further importance for adoptive immunotherapy research, Cieri *et al.*[17] demonstrated the induction of human TSCM from naïve precursors.[17] This study generated CD8$^+$ TSCM (defined as CD62L$^+$CCR7$^+$CD45RA$^+$CD45RO$^+$IL-7Rα$^+$CD95$^+$) through CD3 and CD28 engagement and culturing with IL-7 and IL-15 cytokines. These cells also express CD45RO, which indicates that IL-7 and IL-15 generated TSCM are more differentiated than the originally reported TSCM.[15] These TSCM did however closely cluster with naturally occurring TSCM, analyzed through gene expression and were characterized as between naïve T cells and CM. Cieri *et al.*[17] demonstrated that these cells could proliferate, differentiate and self-renew after antigen encounter.

The self-renewal capacity of the CD8$^+$ TSCM cells was further tested in a graft-versus-host-disease mouse model where TSCM were demonstrated to engraft, expand and possess the ability to exhibit xenoreactivity over serial transplantations.[17] TSCM were the only T-cell subset examined that possessed this quality. The TSCM population before and after transplantation in this model was demonstrated to have a gene profile distinct and hierarchically superior to CM cells and the one that resembled the naturally occurring TSCM subset.

This study indicated that stimulation with IL-7 and IL-15 was required to support expansion of CD8$^+$ TSCM phenotype. Interference with the glycogen synthase kinase-3B/Wnt pathway was not found to be nearly as effective in expanding and maintaining TSCM generated from naïve precursors in this model, compared with the IL-7 and IL-15 method. Research by Gattinoni *et al.*[15,23] previously demonstrated targeting the Wnt/β-catenin pathway by GSK-3β inhibitors was able to assist the generation of TSCM; however, this system also inhibited T-cell proliferation.[4] Possible reasons for the differences in results of GSK-3β inhibitor (TWS119) seen by Cieri *et al.* in human TSCM compared to studies in mice by Gattinoni

et al.[15,23] may include a lack in the upregulation in Wnt dependent genes seen in the naïve precursors, which could possibly indicate posttranscriptional mechanisms of regulation or an alternate signaling pathway as discussed by Cieri *et al.*[17]

Cieri *et al.* also noted that the level of IL-7 was not limited in the generation of TSCM, which may have contributed to a higher level of TSCM being generated compared with other models. However, a system similar to the one described by Cieri *et al.*,[17] which is able to generate larger numbers of TSCM in a clinical setting, is desirable for the development of immunotherapies as TSCM have been demonstrated to have antitumor qualities superior to other T-cell subsets. This study by Cieri *et al.*[17] importantly illustrated the ability of TSCM cells to differentiate, expand, self-renew and be genetically modified providing a promising candidate for further studies on adoptive T-cell therapy.

The use of TSCM as a cellular therapy

Current cellular therapies for cancer include the transfer of antigen-specific T cells to the site of the tumor. This treatment has been performed by isolating tumor-infiltrating lymphocytes (TILs), which commonly demonstrate autologous tumor reactivity,[26] and expanding them before transfering back to the patient.[27] TILs have been commonly used to treat solid cancers such as melanomas. Clinical trials have shown attributes for the transfer of TILs, which correlated with improved responses including a short duration in culture (termed 'young' TILs), rapid expansion ability, longer telomere length[28] and a higher proportion of CM cells.[29,30] These studies suggested that a less differentiated T cell could improve therapeutic responses, whereas it was originally proposed that the most desirable cell for transfer would demonstrate cytolytic capabilities and be able to infiltrate peripheral tissues, suggesting $CD62L^-$ EM and effector T-cell populations. Whether the use of TSCM in human TIL therapies could provide a further improvement in treatment responses remains to be investigated.

Another cellular therapy for cancer involves the engineering of T cells to express chimeric antigen receptors (CARs). Peripheral blood lymphocytes can be engineered to express tumor-associated antigen-specific TCR described as a 'transmembrane receptor consisting in the tumor-associated antigen-binding domain of an immunoglobulin fused to an intracellular tail, which contains one or more immunostimulatory signaling molecules'.[31] The most investigated use of the CAR technology has been for CD19-specific CAR for B-cell leukemias and lymphomas, which has shown successful B-cell tumor eradication with different CD19 CARs.[32,33] Currently, clinical trials are testing the use of autologous T cells expressing CD19-specific CAR as a therapy for non-hodgkins lymphoma (reviewed in Aranda *et al.*[34] and listed www.clinicaltrials.gov). Some of these studies are using genetically modified CM T cells to express CD19-specific CAR. Clinical trials using genetically modified TSCM have not been reported to date; however, due to the low frequency of TSCM in the peripheral blood, expansion techniques may be required before transfer. Methods that are able to generate large quantities of TSCM, including pharmacological modulators of T-cell differentiation, have the ability to be coupled with genetically engineered T cells for use in cancer immunotherapies.

Studies have demonstrated TSCM to have the potential to improve several cancer therapies, with promising results of tumor regression shown in mice[15,23] and novel techniques for expansion of TSCM shown in a clinically relevant setting.[17] The use of TSCM as a therapeutic strategy for cancer will require investigation of the optimal timing and sequence of combination therapies, and theoretically the number and ratio of different T-cell subsets for different cancers to ensure the best use of the scarcer TSCM.

THE IMPORTANCE OF TSCM IN HIV-1 IMMUNOTHERAPY AND VACCINE RESEARCH

Several studies have demonstrated the important role of memory T cells in the adaptive immune response to viral infections.[1,35] $CD4^+$ T cells are a key target of infection by HIV-1, and the depletion of these cells causes the immune system to deteriorate and progress to AIDS.[36,37] HIV-1 uses cellular CD4 and a co-receptor, CCR5 or CXCR4 to enter cells (reviewed in Gorry and Ancuta[38]). These co-receptors are found on $CD4^+$ T-cell subsets with varying levels of expression, with a trend for an increase in CCR5 expression and a decrease in CXCR4 expression moving from naïve to TSCM to CM to EM cells.[20,39] The cellular tropism of HIV-1 can influence the size of the viral reservoir, with different $CD4^+$ T-cell subsets being described as cellular reservoirs for HIV-1.[10,40]

Important for HIV-1 research, CM and transitional memory $CD4^+$ T-cell subsets have been demonstrated as major HIV-1 cellular reservoirs, where maintenance of these cellular reservoirs was associated with T-cell survival and homeostatic proliferation (antigen driven and IL-7 mediated, respectively).[10] In addition, the more recently described TSCM $CD4^+$ T-cell subset,[15] has been demonstrated to support long-lived T-cell memory and potentially to be a long-lived cellular reservoir for HIV-1.[15,16,19]

Discoveries from NHP TSCM research

NHP models have been used widely in HIV-1 research as a tool to study viral pathogenesis, cellular immune responses, therapeutics and vaccine candidates using the related simian immunodeficiency virus (SIV) strain allowing the translation of knowledge to HIV-1/AIDS research.[41,42] NHP have also been used to examine the cellular distribution of TSCM in tissues and the role of TSCM in SIV infection models. NHP TSCM were found to constitute ~2–3% of the circulating $CD8^+$ and $CD4^+$ T cells, similar to percentages seen in humans, and were shown to have a distribution similar to naïve T cells with a tropism for secondary lymphoid tissues.[16] NHP TSCM were assessed for phenotypic and functional characteristics and resembled TSCM found in humans. Furthermore, following TCR stimulation, NHP TSCM were able to generate CM, EM and terminal effector cells, indicating NHP TSCM were a discrete memory subset and supported the notion that TSCM were precursors for CM, EM and terminal effector cells following a linear hierarchical system.

NHP SIV models were used to examine the presence of antigen-specific T-cell subsets during infection. SIV-specific $CD8^+$ T cells were examined using Mamu-A*01 pMHC1 multimers, which presented SIV-derived Gag CM9 or Tat-TL8 peptides.[16] Antigen-specific $CD8^+$ TSCM were found 21 days post infection indicating an early response to infection. These cells showed signs of activation ($HLA\text{-}DR^+$ and $CD38^{bright}$) and proliferation ($Ki\text{-}67^+$).

Examination of both CM9 and TL8 Mamu-A*01 epitopes allowed the examination of epitopes that are generally maintained during chronic infection (CM9 epitopes) and those that have been demonstrated to undergo escape mutation (TL8 epitopes) within 4–5 weeks post $SIV_{mac}239$ or $SIV_{mac}251$ infection.[16,43–45] These models were able to assess the relative antigen dependence of different T-cell subsets during infection. Once viral escape to TL8 epitopes occurred, there was a decrease in the frequency of TL8-specific $CD8^+$ T cells, whereas CM9-specific $CD8^+$ T cells were maintained throughout infection. Interestingly, after antigen loss, the number of TL8-specific CM and EM cells reduced quite considerably; however, the TSCM population

was maintained. Similar results were seen in the CM9 model, although the loss of CM9-specific CM and EM was at a slower rate, with maintenance of CM9-specific TSCM. This indicated that despite antigen loss, TSCM cells were maintained during SIV infection and suggested that they were an important precursor of T-cell memory even when a reduction in antigen occurred.

Discoveries from human TSCM research

To investigate the infectivity of human CD4$^+$ T-cell subsets researchers have developed an *in-vitro* assay systems using Envelope (Env)-pseudotype GFP reporter viruses, which allows the investigation of the cellular tropism and infectivity of CD4$^+$ T-cell subsets by laboratory-derived and clinical isolates.[19,20,39,46,47] This assay system has the ability to detect alterations in cellular tropism between CCR5- and CXCR4-using viruses, using different clades of HIV-1, and examine any changes through disease pathogenesis and drug resistance which are important aspects for the design of therapeutics and characterizing the cellular reservoir of HIV-1.

Studies using GFP reporter viruses have shown CD4$^+$ TSCM, as well as other CD4$^+$ T-cell subsets, can be infected by CCR5- and CXCR4-using laboratory-adapted strains and clinical isolates.[19,20,39] CD4$^+$ TSCM have been shown to be latently infected in this assay system and also that infection of TSCM can be partially affected by the cellular restriction factor SAMHD1.[39] SAMHD1 is a cellular restriction factor that has been shown to block HIV-1 replication in myeloid, dendritic and resting CD4$^+$ T cells[48,49] as has been demonstrated to be active in CD4$^+$ T-cell subsets including CD4$^+$ TSCM.[39] SAMHD1 is able to block reverse transcription of HIV-1 by depleting deoxynucleoside triphosphates within cells, which reduces the amount of nucleotides available for reverse transcription.[50] Knockdown of SAMHD1 was able to increase infection of CD4$^+$ TSCM; however, there was still evidence of viral fusion rather than productive infection, which indicated that SAMHD1 was not the only factor contributing to abortive infection in CD4$^+$ TSCM (as discussed in Tabler *et al.*[39]).

Several studies have demonstrated that TSCM are able to differentiate into other memory T cell subsets whilst maintaining their own population through homeostatic self-renewal.[15,17] TSCM are also indicated to be the earliest and longest lasting subset of memory T cells, thus it has been hypothesized that these cells could contribute to long-term viral persistence of HIV-1.[19] Buzon *et al.*[19] examined this theory and demonstrated the susceptibility of CD4$^+$ TSCM cells to HIV-1 infection.[19] Using a CCR5 tropic HIV-1 isolate, CD4$^+$ TSCM cells showed a similar level of infection as CM cells[19] despite CD4$^+$ TSCM having a lower expression of CCR5 on their surface compared to CM.[19,20,39] Buzon *et al.*[19] also demonstrated HIV-1 RNA was detectable in CD4$^+$ TSCM from untreated HIV-1 participants.[19] Further examination revealed CD4$^+$ TSCM had low levels of HIV-1 restriction factors including TRIM5α, APOBEC3G and SAMHD1 and a low sensitivity for the cytotoxic effects of HIV-1 infection, thus indicating CD4$^+$ TSCM are susceptible to HIV-1 infection.

CD4$^+$ TSCM from highly active antiretroviral therapy (HAART) participants were also examined. The level of CD4$^+$ TSCM in HAART-treated participants was the same as in healthy donors; however, the per-cell level of HIV-1 DNA in CD4$^+$ T-cell subsets from infected participants was found to be highest in CD4$^+$ TSCM compared with other memory and naïve subsets. Although at a significantly lower level than that found in HAART participants, HIV-1 DNA was also detectable in CD4$^+$ TSCM from elite controllers (infected individuals whom are able to maintain undetectable HIV-1 replication in the absence of antiretroviral therapy[51]).

The contribution of infected CD4$^+$ TSCM to the HIV-1 viral reservoir was found to be approximately 8% in HAART participants, which varied quite considerably between participants. The contribution to the viral reservoir was found to be 'inversely associated with HIV-1 DNA levels in the entire CD4$^+$ T-cell compartment' and could be affected by the size of the reservoir of CM and EM cells.[19] Interestingly, CD4$^+$ TSCM were not large contributors to the size of viral reservoir in HAART participants during the first year of therapy; however, there was an increase over time even though the contribution of CD4$^+$ TSCM to the CD4$^+$ T-cell pool did not change.[19] This study suggests that the infection of CD4$^+$ TSCM are maintained during HAART and thus are supporting viral persistence.

Furthermore, Buzon *et al.*[19] performed viral outgrowth assays from three participants who were on continuous HAART for a median of 28 months.[19] Replication component virus was obtained from CD4$^+$ TSCM in all three participants, demonstrating that HIV-1 DNA from CD4$^+$ TSCM is functionally capable of active viral gene expression. In addition, HIV-1 DNA isolated from CD4$^+$ TSCM and CM after to 4–8 years of HAART was found to be phylogenetically related to circulating plasma sequences from earlier disease stages (at the beginning of antiretroviral therapy).[19] The genetic distance was found to be lowest for HIV-1 DNA sequences from CD4$^+$ TSCM and CM comparing early and late stages of disease progression. This indicated that early viral strains are able to persist for years through the infection of CD4$^+$ TSCM and CM subsets. Interestingly, viral sequences from CD4$^+$ TSCM in early infection stages were identical to sequences isolated later in infection from CM, EM and terminally differentiated T cells.[19] This supported the notion that TSCM are earlier precursor cells for other more differentiated memory T-cell subsets and that long-lived CD4$^+$ TSCM have the potential to promote HIV-1 persistence.[19,52]

Early treatment prevention has been shown to enhance CD4$^+$ T-cell recovery[53] and to decrease the size of latent reservoir.[54,55] As TSCM have been shown to contribute to the reservoir under HAART and are permissive to HIV,[19] it will be important to determine whether early treatment interventions will prevent or decrease the infection of TSCM cells.

Novel research is combining the fields of cancer and HIV-1 TSCM cells and investigating the role of β-catenin inhibitors in HIV-1 research. β-catenin is able to stop the stem cells from differentiating into memory cells, and pharmaceutical drugs, which are able to inhibit this process, are currently being used to target cancer stem cells, as cancer stems have been shown to persist causing tumor recurrence after conventional treatments have killed proliferating tumor cells.[56,57] Treatments currently used for cancer may be effective against HIV-1-infected TSCM, allowing cell differentiation and potential reactivation of a long-term latent reservoir of HIV-1.[19] Current research demonstrates that pharmaceutical β-catenin inhibitors are able to promote differentiation of CD4$^+$ TSCM into a more differentiated, shorter-lived effector CD4$^+$ T cells.[58] As TSCM have been demonstrated to be a long-term reservoir for HIV-1, targeting cellular pathways affecting the stem cell-like properties of TSCM may be able to reduce long-term viral persistence in TSCM and have the potential to be used in combination with current HIV-1 therapy.

CONCLUSION

TSCM have been demonstrated to have stem cell-like properties of self-renewal and survival. They represent the least differentiated memory T-cell subset with properties in between that of naïve and CM cells. They have been shown to have superior antitumor

properties in adoptive immunotherapy cancer research with promise of enhancing the efficacy of current therapies. Whereas, in HIV-1, TSCM have been demonstrated to be a long-lived reservoir for HIV-1, potentially promoting viral persistence, and are thus proving to be an important target cell for the development of novel therapeutics. Combining the knowledge from both cancer and HIV-1 research, new strategies are being tested that target cellular pathways affecting some of the stem cell-like properties of TSCM in the hope of being able to either use or target TSCM in future immunotherapies and vaccines.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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