Phenotypic and Immunometabolic Aspects on Stem Cell Memory and Resident Memory CD8+ T Cells

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The immune system, smartly and surprisingly, saves the exposure of a particular pathogen in its memory and reacts to the pathogen very rapidly, preventing serious diseases. Immunologists have long been fascinated by understanding the ability to recall and respond faster and more vigorously to a pathogen, known as “memory”. T-cell populations can be better described by using more sophisticated techniques to define phenotype, transcriptional and epigenetic signatures and metabolic pathways (single-cell resolution), which uncovered the heterogeneity of the memory T-compartment. Phenotype, effector functions, maintenance, and metabolic pathways help identify these different subsets. Here, we examine recent developments in the characterization of the heterogeneity of the memory T cell compartment. In particular, we focus on the emerging role of CD8+ T RM and T SCM Cells, providing evidence on how their immunometabolism or modulation can play a vital role in their generation and maintenance in chronic conditions such as infections or autoimmune diseases.

Keywords: CD8 T RM cells, CD8 T SCM cells, metabolism, differentiation, infectious diseases

INTRODUCTION

T lymphocytes are an essential component of adaptive immunity known for their role in protecting against infectious diseases caused by invasive pathogens and cancer cells but also for their crucial role as important target cells in immunotherapy (1–5).

Memory T cells differ in their naive counterparts for several functional characteristics identified in booster reactions due to a second encounter with the pathogen. These T memory populations are organized in a developmental hierarchy, where stem cell memory T-cells (T SCM) renew themselves and generate long-lived central memory T-cells (T CM) and short-lived memory T-cells (T EM) (6–10). However, the mechanisms underlying the increased multipotency of T SCM cells relative to T CM cells were not clearly defined in the molecular terms (11).

The principal characteristics of the memory T-cell responses include several aspects such as an increased pool of specific memory T cells thanks to the recognition of pathogen-derived-antigens by TCR, a faster and more potent response to the infection; a pre-programming to generate a “tailor-
made” set of effector cell types optimized to combat pathogens, which includes the role of T follicular helper cell (TFH) responses involved in helping humoral immunity; the presence of memory T cells in barrier tissues as sentinels in detecting and fighting infection. This last characteristic is dominated by resident memory T cells (TRM) that are maintained in non-lymphoid tissues and do not exchange with populations in the circulation. Since this population is not represented in the blood, it is difficult to evaluate its generation and persistence.

Once T cells are not properly primed or generated, they gradually lose their ability to resolve the chronic infection. Such T-cell memory populations are likely to disappear over time or become permanently dysfunctional, favoring the persistence of the pathogen. As a result, T lymphocyte responses can become dangerous, resulting in excessive tissue damage, collectively referred to as immunopathology, which may determine the immune system’s persistent systemic alteration, affecting the development of memory cells and their response to the re-encounter with the same pathogen.

The aim of this review is to focus on the role of T<sub>SCM</sub> and T<sub>RM</sub> cell subsets in the course of infectious and autoimmune diseases and how the metabolic program can impact the features of the immune response.

**THE BASIC CONCEPT OF CD8<sup>+</sup> T CELL MEMORY SUBSETS**

The major T cell subsets are classified into CD4<sup>+</sup> T and CD8<sup>+</sup> T cells based on cell functions and cell surface markers. CD4<sup>+</sup> T cells, also known as T helper (Th) cells, play an essential role in the adaptive immune response to pathogens due to their multiple roles in immune response orchestration (12), such as helping B cells to produce antibodies, recruiting granulocytes to infected sites, and producing cytokines and chemokines. Many distinct CD4<sup>+</sup> T cell subsets have been identified since the identification of Th1 by Mosmann et al. (12–15). CD8<sup>+</sup> T cells are essential for immune defense against intracellular pathogens (16, 17) and tumoral cells.

For several years, memory T cells were divided into two main principal subsets: central memory T cells (T<sub>CM</sub>) and effector memory T cells (T<sub>EM</sub>) (18). This initial classification was based on the expression of the lymphoid-homing molecules CCR7 and L-selectin (CD62L), while T<sub>EM</sub> were identified on the bases of the reduced expression of these receptors relative to T naive (T<sub>N</sub>) and T<sub>CM</sub> and for their presence as recirculating cells between the blood and peripheral tissues. After this first characterization of the different subsets, additional surface markers have been added, such as the mutually exclusive expression of the two isoforms of the CD45R molecule, CD45RA or CD45RO. In different species, such as mice, T<sub>EM</sub> has been shown to retain lower expression of molecules involved in long-term persistence (19), human primates (20, 21) and humans (22, 23), while upregulating transcription factors (TFs) mediating terminal differentiation with decreased self-renewal and multipotent capacity compared with T<sub>CM</sub> (7).

Furthermore, T<sub>EM</sub> cells have limited expansion potential and are unable to enter the lymph nodes from the blood, but are marked by the expression of genes associated with cytotoxicity and can perform rapid effector functions when renewing TCR signalling (24). For a long time, T<sub>EM</sub> cells were thought to be superior for penetrating and examining peripheral tissues; however, recent research indicates that T<sub>EM</sub> cells and T<sub>EM</sub>-like cells are largely excluded from human and mouse non-lymphoid tissues (NLTs) (23, 25–27).

T<sub>CM</sub> cells can be distinguished by the high-level expression of CD62L and CCR7 lymphoid localization markers and their capacity to recirculate between blood and lymphoid tissues. They are considered to be multipotent, and at least one subset of this cell pool shows increased expansion potential when the antigen is encountered (8). Two novel memory subsets have emerged during the past decade, further highlighting the inherent diversity among these subtypes. In particular, a noncirculating lineage of T cells, the T<sub>RM</sub>, express tissue-residency markers, CD69 and CD103, and reside in peripheral tissues (28–30). This subset is located at the entry point of invading pathogens to provide the first rapid defense line (28–30). While, T<sub>SCM</sub>, defined in humans by surface expression of CD122, CD95, and CXCR3 within the CD45RA<sup>+</sup>CD45RO<sup>+</sup>CCR7<sup>+</sup>CD62L<sup>+</sup> naive-like compartment, are characterized by increased homeostatic capacity for automatic renewal, increased proliferation capacity, and multipotent development potential compared to T<sub>CM</sub> and T<sub>EM</sub>; moreover, this subset is capable of generating differentiated in vitro in cells, including T<sub>CM</sub> and T<sub>EM</sub> or as adoptive transfer in xenogeneic mouse models, as well as in humans (8, 9, 20, 31–33). Although this subset was initially identified in mice as part of an allogeneic bone marrow transplantation (34) or induced by T naive (T<sub>N</sub>) activated by the inhibition of glycogen synthase kinase 3b (GSK-3b) (35).

Although T<sub>SCM</sub> and T<sub>CM</sub> have the ability to retain the expression of associated naive genes while simultaneously displaying the rapid production of cytokine upon stimulating T-cell receptor (TCR), the T memory subsets as a whole are often represented as having mutually exclusive properties, primarily a potential similar stem-like potential (T<sub>SCM</sub> and T<sub>CM</sub>) versus immediacy of effector functions/cytotoxicity (T<sub>EM</sub>) (7, 8, 22, 36, 37).

An important aspect of CD8<sup>+</sup> T cells is their ability to adapt their functions to the basic type of pathogens encountered. Firstly, the recognition of the antigen, T cells, through the clonal expansion process, become biased to recognize pathogens to which they have already been exposed (38). Moreover, remodelling of the epigenetic landscape enables the memory cells that form in this process to perform effective functions more rapidly (39). The distribution of the memory compartment of the CD8<sup>+</sup> T cells at different body sites maximizes the chances of early recognition of pathogens during a renewed infection (40). In accordance with the concept that the CD8<sup>+</sup> storage T-cell pool can provide fast effector functions and has the ability to renew clonal expansion, this cell pool is very diverse at epigenetic, transcriptional and protein expression levels.
It is believed that these memory populations are organized in a developmental hierarchy, according to which TSCM renew themselves and generate a long-lasting TCM, TEM cells, and TRM cells (6–10) (Figure 1). However, the mechanisms underlying the improved multi-potency of TSCM cells compared to TCM cells have not been clearly defined in molecular terms (11).

In addition to the CD8+ systemic memory T-cell pool, one can distinguish a TRM cell pool from CD8+ in tissues that permanently reside in non-lymphoid tissues (NLTs).

By a continuous migration and monitoring process that is limited to distinct anatomical compartments, such as stroma or organ parenchyma, TRM cells patrol tissues to scan for foreign invaders (41, 42). After encountering the antigen, TRM cells quickly induce a local alarm state, resulting in the recruitment of other immune cells and local production of antibacterial and antiviral proteins by epithelial cells (43, 44).

In keeping with this “pathogen alert” function, TRM cells produce not only cytotoxic molecules, such as granzyme B and perforin but also cytokines such as IFN-γ and TNF, which may affect the behaviour of neighbouring cells (45–50). In addition, the existence of TRM cells that express minimal levels of cytolytic molecules and therefore rely primarily on this pathogen, warning function, has been reported in several human tissues (51–54).

It has been proven that the differentiation of T cells from memory can be corrupted under conditions of persistent antigenic stimulation, this finding was primarily observed in chronic viral infections and progressive malignant tumours, which promote a state of T-cell exhaustion which is characterized by an organized loss of effective function, altered proliferation and increased regulation of inhibitor receptors (55). This dynamic process takes place over a period of weeks after initiation (56, 57) and involves a genome-wide accumulation of epigenetic changes (58, 59).

Recent studies have shown that exhausted T-cell (TEX) populations are developmental and functionally heterogeneous, incorporating stem-like progenitors that express T-cell factor 1 (TCF1), which produce highly differentiated TEX cells that are basically dysfunctional and do not have TCF1 (60–63). It is important to note that the therapeutic benefits of blocking immune checkpoints in the context of chronic viral infections and various cancers are believed to function via these TCF1+ progenitors, which appear to be susceptible to interventions that specifically target the inhibitory receptor programmed death-1 (PD-1) (60, 62, 64–67).

Current data suggest that exhausted and functional memory T cells originate from distinct populations of stem-like progenitors engaged in distinct destinies. However, the precise nature of these stem-like progenitors, which shape the
adaptive immune response and influence the outcome of many globally relevant diseases, has remained unclear. Very recently, Galletti et al. (68), with the aim of a complete and impartial approach, have mapped the origins of dysfunctional and functional CD8+ human memory T-cells. Their data allow the identification of two distinct subsets of CCR7+ progenitors in healthy individuals that are distinguished by the expression of PD-1 and TIGIT. They identified a progenitor committed the cells to generate a dysfunctional subset with an exhausted progeny expressing these two inhibitor receptors. In contrast, they identified another progenitor cell committed to generating a more functional progeny where the absence of these inhibitory receptors was observed. Therefore, the transcriptional assessment of the distinct subset PD-1"TIGIT" also explained most of the differences between the TSCM and TCM cells, providing a clearer view of how human CD8+ memory T-cells differentiate.

Recent advances in single-cell technologies at the proteomic and transcriptomic levels, along with genome-scale epigenetic profiling studies, provided new information that is refining our current understanding of gene expression programs and the developmental origin of memory T cells. In particular, single-cell transcriptomic has identified subsets of human and murine T cells that simultaneously co-express stem and effector genes. In addition, the epigenetic profiling of stem T cells has made it possible to identify several locus effectors in these cells which remain epigenetic, while having limited transcriptional activity, explaining their ability to quickly recall an effective response (69, 70).

T cell memory has a unique set of functional properties and potentials that set it apart from many of its sisters. This is not to say that every T-cell in memory is its own subset; rather, it is to say that one phenotypic or functional feature does not imply another.

METABOLIC REGULATION OF MEMORY T-CELL DIFFERENTIATION AND FUNCTION

Very recently, the role of metabolism in the immune system has become more evident. Inactivated naive T cells migrate continuously under immune surveillance via secondary lymphoid organs at the expense of ATP and thus require basal replacement biosynthesis. Resting T cells are more dependent on energy-efficient processes such as the β-oxidation of fatty acids and the oxidation of pyruvate and glutamine via the tricarboxylic acid cycle (TCA cycle). To maintain this energy-providing basal metabolism, resting T cells need specific signals via TCR or cytokine receptors such as IL-7R (71). Furthermore, resting T cells do not have a static and well-determined metabolism but undergo dynamic regulation. For example, environmental signals can control the utilization of nutrients in resting T cells, causing them to become trophic and influencing the ability to initiate cell proliferation and resistance to apoptosis. IL-7R regulates glucose absorption mainly thanks to the PI3k-Akt-mTOR pathway, which promotes the trafficking of Glut1 on the cell surface (72, 73). As a result, IL-7R regulation of glycolysis is critical for T lymphocyte basal metabolism in vivo, as its deletion results in cell atrophy and the inability to maintain glycolysis in vivo (74).

Unlike naive T lymphocytes, T memory (TM) lymphocytes usually divide every 2-3 weeks undergoing intermittent cell division. These lymphocytes necessarily require the combination of IL-15 and IL-7 for their proliferation and survival to which a high level of CD127 and CD122 molecules is associated, respectively regulated by FOXP1 and by T-bet and comestodermin (17, 75).

Furthermore, TM cells and their responses are dependent on lipid oxidation, and although the homeostasis of the various subgroups is very similar, there are important differences. In fact, CD4+ TM cells appear to be less dependent on IL-15 signal than CD8+ TM cells, and this is probably partly determined by the low expression of CD122 on CD4+ TM cells (76).

Homeostasis of memory T cells is determined by IL-15 and/or IL-7, which keep them in a precarious state of quiescence (G1 phase of the cell cycle). Aerobic glycolysis and mitochondrial metabolism have important and well-defined roles in the differentiation of TM cells, but mitochondrial metabolism supports their quiescent, survival, and functions (77, 78). Increased mitochondrial function and acetate uptake mediate rapid recall responses by memory T cells, both in TCM and TRM cells. Thus, metabolism influences entry and exit from quiescence to regulate their generation, survival, and function (79, 80).

Although it is clear why the transition from quiescent and naive T cells to proliferating T cells results in a series of altered metabolic demands, it is less clear why differentiated active T cell pool memory cells necessitate a carefully orchestrated transition to invert the cell focus on vigorous expansion and equip emerging memory cells for sustained maintenance and the enhanced metabolic capacity required to mount a rapid recall response. Differentiating effectors involve a spectacular reprogramming of metabolic functions, where the requirements for increased biosynthesis and energy use are met in the most obvious way by induction aerobic glycolysis (oxidative phosphorylation, characteristic of the catabolic state of naive cells, is also maintained but plays a proportionately lesser role).

Cells need to reprogram their metabolism to leave rapidly expanding and reenter the memory pool (81). For the first time, it has been shown by pioneering studies that mTOR inhibition and enhanced fatty acid oxidation (FAO) induce an increase in CD8+ differentiated memory T cells (82–84).

Above all, memory cells’ metabolic status differs from that of naive T cells; changes in mitochondrial mass and FAO capacity (including alterations) result in memory cells that are much better able to undergo metabolic reprogramming, CD8+ memory T cells, for example, have increased mitochondrial mass and alternate breathing capacity (80, 85), which can be linked to the ability of cells to respond quickly to metabolic increases. Moreover, memory cells selectively express the carrier of Aquaporin-9 glycerol, which is involved in fatty acid storage (86).
The inability to achieve this metabolic change is related with decreased central memory retention or responsiveness (85, 86). Demonstrating that these “fit” memory cells appear to be favoured by mitochondrial remodelling (including networks of fused mitochondria) (78), and subsequent discoveries reveal that CD28 costimulation drives that process (87).

There is much more to be learned about how metabolic pathways regulate survival, differentiation and function in T lymphocytes; for example, the need for FAO in CD8⁺ T_CM is satisfied in a way that is difficult to understand: T_CM involves importing glucose and glycerol to synthesize and then metabolize triacylglycerides into what appears to be a futile metabolic cycle (i.e. those who consume as much energy as they produce) (88). Synthesis/storage (at times of abundance) and lipolysis (at times of need) ensure the continued survival of T_CM, but why this is preferred to direct uptake of free fatty acids (FFA) is unclear.

CD4⁺ T lymphocytes also require glucose absorption for sustained maintenance in a pathway controlled by signalling through Notch (89). However, it is unclear how similar metabolic needs are for maintaining CD4⁺ and CD8⁺ T_CM. The transcription activation of HIF-1α by hypoxia (or other indications) is associated with the promotion of the effector at the expense of the differentiation of memory (90, 91), but may also support epigenetic regulation (92). Similarly, the regulation of aerobic glycolysis can influence epigenetic transcription and control of gene expression (93, 94).

Interestingly, recent data suggest that there are alternative metabolic strategies for memory T lymphocyte populations. Studies in which CD8⁺ T cells were prevented from switching between aerobic glycolysis and oxidative phosphorylation during an immune response (through sustained HIF activation) showed a surprisingly robust generation of memory cells in lymphoid sites - particularly T_EM phenotype cells, suggesting that glycolysis dependency is sufficient to meet the metabolic requirements of at least some CD8⁺ recirculated memory T cells (95).

T_RM appears to use another mechanism to treat their metabolic process. As stated above, T_CM makes its own fatty acids for stimulating the FAO: the skin, whilst T_RM, on the other hand, clearly use the more direct route of absorption of free fatty acids (involving the fatty acid-binding proteins Fabp4 and Fabp5) to meet their FAO requirements, as noted by the finding that Fabp4/Fabp5 deficiency selectively affects cutaneous T_RM but not the generation of recirculated memory CD8⁺ T cells (45). It will be essential to determine whether this metabolic route is also used in other tissues. These findings illustrate the fact that there are obviously multiple ways to resolve metabolic needs for sustained maintenance of memory cells. It is unclear whether these alternatives will easily correspond to current subset designations.

GENERAL BACKGROUND OF T_SCM CELL

While T cell development on the individual cell level is well known, we still lack critical information about how the T cell compartment is formed. Broadly, the T cell compartment is highly heterogeneous and different subsets of T cells based on phenotypical and functional distinct can be identified in the peripheral blood, which is classified, as previously described, as either naïve or memory cells based on their expression (or lack thereof) of various cell-surface markers and receptors. The frequency of memory T cells is highly age-dependent, and it dynamically changes throughout an individual’s lifetime. During the first two decades of life, all T cells in the peripheral blood are naïve. Memory T cells develop over time in response to exposure to diverse antigens, and eventually, circulating memory T cell frequencies reach a plateau and remain stable throughout adulthood (96, 97). Ultimately the proportion and the functionality of memory T cells become altered during immune senescence, starting at 65–70 years of age (97–99).

Recently a subset of memory T cells with stem cell-like properties has been identified with a superior survival potential and stemness function (100). T_SCM in humans derived from naïve T cells with a multipotent function that can further differentiate into other T cell subsets with a high proliferative capacity and self-renewal flair. Additionally, T_SCM defined in humans by surface expression of CD122 (also known as IL−2RB), CD95 (also known as FAS), and CXCR3 within the CD45RA⁺CD45ROCCR7⁺CD62L⁺ naïve-like compartment, and they also express high levels of the co-stimulatory receptors CD27 and CD28, IL−7 receptor α–chain (IL−7Rα). These properties define their "stem cell-like" capacities (100, 101).

T_SCM cells have been described in peripheral blood and can be either CD4⁺ or CD8⁺. They are a small fraction of circulating T lymphocytes (≥2–3%). Notably, the frequency of circulating T_SCM cells can vary because of the different factors that will be explained in this review, but there is evidence to be heritable and associated with single-nucleotide polymorphisms (SNPs) at a genetic locus containing CD95 (102) that showed a potential role of FAS-signalling pathway in the regulation of T_SCM cell homeostasis. After many years of tracking the origin of different memory cells to address the stemness function, the studies recently showed that T_SCM cells differentiate directly from naïve precursors that can reconstitute the entire heterogeneity of memory T cell subsets (31, 32).

THE ROLE OF METABOLIC PATHWAY OF T_SCM CELLS IN INFECTIOUS DISEASES

T cells can be formidable soldiers in the fight against human diseases such as cancer and infection; there is a minority illustrious population due to the capacity to reconstitute a wide-ranging diversity of T cell compartment, robust proliferative potential, and extreme longevity such as T_SCM, which make them an ideal cell population to use in adoptive immunotherapy or the development of new vaccines against infectious diseases, this might ultimately allow for the widespread application of adoptive immunotherapy based on multipotent, highly proliferative, tumour-specific T_SCM. Finally, generating long-term T cell memory might be important for T cell-based vaccines designed to target intracellular pathogens, as well as immunotherapy. But we are still in the early stage of this
intention because there are many outstanding questions about this very small population, such as their metabolic requirements, epigenetic and transcriptional programs also anatomical niches. So far, the clinical exploitation of TSCM has been limited by their low numbers in the circulation (100, 103) and the lack of robust, clinical-grade manufacturing protocols capable of generating and maintaining this cell type \textit{in vitro}.

Here we will review all the available protocols in detail. In summary, all of these protocols are based on the fate decision that the naïve cell must make to go on a path for proliferation and effector production or memory development.

As explained before, the fate decision that naïve T cells have to make is highly dependent on cellular metabolism or transcriptional modification, but the key question in this critical decision time is how does an activated T cell retain the stem-like capacity to self-renew while simultaneously developing into more differentiated progeny?

Over the last decade, the existed protocol for isolation and \textit{in vitro} expansion of TSCM have been accomplished using modified human T cell expansion protocols by targeting different cellular pathways, such as replacing IL-2 with IL-7 and IL-15, or IL-21 (104, 105), promoting Notch signalling (106) or limiting reactive oxygen species (ROS) metabolism upon antioxidant treatment (106), pharmacological inhibition of GSK-3b (107), AKT (108) or BET proteins (109) also takes to the point as additional strategies, which targeting the suppression of genes associated with terminal effector differentiation, such as Tbet, BATF, and EOMES, and in the maintenance of stem-like genes, such as those encoding TCF1 and lymphoid enhancer binding factor 1 (LEF1) to address the naïve fate decision and force it to generate TSCM (Figure 2).

Physiologically, the generation of effector T cells and T cells with stem cell-like properties are linked. In fact, inhibition of lymphocyte differentiation to maintain long-lived, self-renewing antigen-experienced T cells with stem cell-like properties are strictly correlated. A Recent protocol to generate CD8$^+$ TSCM is by oxidative metabolism modulation using antioxidants such as N-acetylcysteine (NAC) that significantly increases the intracellular antioxidant molecule Glutathione and consequently decreases the ROS intracellular levels (106). NAC may regulate cellular behaviour in many ways, but the impressive observation is an increased level of Carnitine palmitoyl transferase 1 alfa (CPT1α) and TCF1 expression. From this study, it is now clear that the expression of TCF1 is marked as self-renewing human CD8$^+$ T cells (110); furthermore, CPT1 is a transmembrane enzyme of the mitochondrial outer membrane, which converts long-chain fatty acyl-CoA to long-chain acylcarnitine, following which carnitine acylcarnitine translocase, transports the fatty acid across the inner mitochondrial membrane and then enter fatty acid $\beta$-oxidation. Therefore, when naïve T cell use \textit{via} OxPhos and decreases dependence on glycolysis, it starts the long-live memory generation. A confirmation for this statement is the study of Seok et al. that demonstrated CPT1 can increase fatty acid oxidation and reduce mitochondrial membrane potential ($\Delta$Ym) (111), which is clearly one of the indispensable characteristics of TSCM. The induction of TCF1 and its downstream target ID3 and LEF1 genes are thought to be
responsible for the enhanced long-term persistence of stem-like memory T cells. Furthermore, NAC might scavenge ROS levels because it reacts slowly with free radicals such as $O_2^-$, $H_2O_2$ and $NO_3^-$ also, NAC decreases mTOR activity, which it explained as a master regulator of multiple metabolic processes, including glycolysis in T cells. As a result, and according to this explanation, the modulation of mTOR signalling by the simple addition of an inexpensive antioxidant such as NAC in the range of 10–20 mM can triggers the formation of T$_{SCM}$ (112).

An additional strategy in the protocol development to induce in vitro cells with T$_{SCM}$ properties is the activation of the Wnt/β-catenin pathway in naïve T cells stimulated through T cell receptor by inhibition of glycosynase kinase-3b (Gsk-3b) (107). While the Wnt/β-catenin pathway has been shown to play an essential role in various developmental processes such as asymmetric cell division, stem cell pluripotency, and cell fate specification (113–115), in addition, the Wnt/β-catenin pathway is clearly marked as the essential role of development of T lymphocytes in thymus. Indeed, the role of Wnt proteins in regulating mature T cell function has remained a relatively understudied area. As different Wnt signalling pathways have been described, but our focus is on the canonical Wnt pathway. Actually, Wnt signalling and β-catenin dependent gene expression play a critical role during the formation of different body regions in the early embryo. One of the most important results of Wnt signalling and the high level of β-catenin in certain cell types is the maintenance of stem cell pluripotency (116). Broadly, β-catenin is considered the centre of the Wnt signalling pathway. In addition, the transcriptional activity of the T cell factor (TCF) is induced by direct association with β-catenin (115, 117). However, the expression of β-catenin levels can be carefully modulated by Gsk-3b. The significantly effective way to rapidly accumulate β-catenin to almost 1.7 fold is inhibition of Gsk-3b by a molecule known as TWS119, which initiates the Wnt signalling pathway and controls TCF and LEF transcription factors (115) which acts as the main downstream effectors of the Wnt signalling pathway. The idea behind this protocol is that Wnt signalling functions as a mechanism in T cells to establish a pool of undifferentiated progenitor or stem cells that are more developed than naïve T cells for further differentiation or proliferation. Hence, such applications also require careful study of the regulation of WNT signalling in T cell development and fate. Indeed, small-molecule inhibitors and activators of WNT–β-catenin such as TWS119 are being developed for T$_{SCM}$ generation as therapeutic applications. Primary studies clearly showed that inhibition of GSK3β by 3 μM of TWS119 can arrest CD8$^+$ T cell’s development into effector cells, consequently maintaining T$_{SCM}$ phenotype, which can be used as a new vaccination design strategies and adoptive immunotheapies (107). As we explained before, maintaining the stemness function of T cells is dependent on cell metabolism and using more fatty acids as energy via OxPhos by decreasing mTOR. Taken together, an existence of cross-talk between mTOR and Wnt signalling comes to mind, which still remains unclear. Furthermore, Scholz et al. revealed that induction of T$_{SCM}$ is utterly independent from the Wnt signalling pathway (118), which has already been called into question as to the molecular mechanism of TWS119, but there is confirming evidence in mouse T cells that TWS119 inhibits mTORC1 (119). In any case, inhibition of mTORC1 can switch the metabolic program of activated naïve T cells toward an oxidative metabolism dependent on FAO, but in vitro experiment with TWS119 showed that all naïve T cells do not follow equally this issue (119). A point that needs to be addressed is how a cell’s intrinsic factors can stop differentiation upon mTORC1 inhibition. One of the important clues might be Krüppel-like-factor 2 (KLF2), which has been shown to maintain the expression of CCR7 and CD62L and has been suggested to be up-regulated upon mTORC1 inhibition (85, 120). Interestingly, KLF2 can positively regulate the expression of GSK3β, GATA6 and FOX3 based on STRING gene interaction data. In addition, the low expression of KLF2 in naïve T cells can make them a T$_{SCM}$ cell precursor potential (118).

These two investigations reveal that gene expression differs significantly between in vitro these two well-developed in vitro induced-T$_{SCM}$ protocols in vitro TWS119-induced T$_{SCM}$ demonstrated an upregulation of LAMP3 (CD63), providing them with a robust co-stimulatory signal, as well as a remarkable elevation of interferon responsive gene expression such as IFIT2, IFIT3, IFH6, and IFI27 Interestingly, mTOR signalling modulated induced-T$_{SCM}$, resulting in the upregulation of genes with cell metabolism control functions such as NQO1, TXNRD1, which can protect cells from oxidative stress and toxic quinones and may be linked to increased oxidative phosphorylation and fatty acid oxidation (118).

THE ROLE OF LONG-TERM MEMORY CELLS DURING CHRONIC INFECTIONS

During past years, the role of pathogen-specific T$_{SCM}$ cells in human acute and chronic infections caused by viruses, bacteria, and parasites has been increasingly considered. However, the T$_{SCM}$ development and formation mechanisms during natural immune responses against foreign pathogens remain poorly understood. The critical point of the robust immune response against pathogens is to have effectors not exhausted T cells; this requires continuously replenishment by less differentiated T cell subsets such as T$_{SCM}$ (60, 62, 121, 122). T$_{SCM}$ has recently been linked to a variety of disorders, including autoimmune diseases such as systemic lupus erythematosus (SLE) (123) and infectious diseases (124) and human T cell leukaemia (125). Therefore those subtype of T cells is also taken into account for tumour immunotherapy (126, 127) and allogeneic hematopoietic stem cell transplantation (31, 32).

Longevity and stemness function of T$_{SCM}$ can act as a double-edged sword as protective or pathogenic, as the evidence shows disrupting T$_{SCM}$ cell reservoirs in retroviral infections such as CD4$^+$ T$_{SCM}$ or in autoimmune diseases such as aplastic anaemia, rheumatoid arthritis, type I diabetes and SLE, might be a target in diseases controlling strategy. On the other hand, the protective role of T$_{SCM}$ in acute and chronic infections makes them optimal candidates for therapeutic exploitation in vaccination and adoptive T cell therapy against infectious diseases and cancer.
One of the best examples of the double-edged role of TSCM in human infectious diseases is controlling HIV or developing AIDS.

However, there is increasing evidence demonstrating that TSCM cells play a crucial role in HIV as a reservoir regardless of successful antiretroviral therapy (ART) (128–131), but immunometabolism data and stem cell-specific pathways can play a very important role in virus resistance since hematopoietic stem cells seem to be largely resistant to HIV-1. Hence, this theory cannot be far from the idea that at least a part of TSCM could have defence mechanisms protecting this cell subset from retroviral infection. A small amount of TSCM showed low expression of CCR5, which can be protected from HIV infection, but physiologically CD4+ TSCM expresses CCR5 (R5)- and CXCR4 (X4), which make them usual reservoirs for HIV, because silent or latent infection of these long-lived, self-renewing cells could provide an extremely stable niche for the virus which is modulated by SAMHD1 expression (132). Accordingly, it would need to be targeted TSCM in efforts to eradicate the virus that is appealing for many years after the discovery of TSCM as the stem cells of cellular immune memory from controlling this subset by Wnt-β-catenin to CRISPR-edited stem cells technology (133, 134).

Up to now, the role of HIV-1-specific CD8+ TSCM in immune defence is uncertain even if there is evidence that HIV-1-specific TSCM may contribute to HIV-1 restriction and naturally control the infection. Therefore, the proportions of total CD8+ TSCM in untreated HIV patients are inversely correlated with plasma viremia levels (135). As mentioned earlier, cell immunometabolism is fundamental for CD8+ memory formation. Surprisingly, a recent study found that HIV patients who could naturally control HIV infection had HIV-specific CD8+ T imprinted in their memory program, which is associated with their ability to use diverse metabolic resources; in contrast, reliance on glycolysis as a primary energy source (136). In addition, this dependency is in contrast with memory formation with stem cell function and development. Mueller, et al. could convincingly establish that IL15 can enhance the survival and function of HIV specific CD8+ T cells by increasing mitochondrial biogenesis (137), which we considered as a fait accompli to CD8+ TSCM formation. Taken together, those evidence unveil the importance of metabolic activity regulation and HIV-specific CD8+ TSCM formation and consequently induce HIV remission and provide a target for therapeutic vaccine development.

Inducing a long-lasting stem cell-like memory CD8+ T cell is not only for protecting human from an infection but also could be a novel target to inducing long-term memory, as yellow fever vaccination evidence shows rapid detection of YF-specific CD8+ T cells, but frequency of effector cells decreasing with time while YF-specific CD8+ TSCM maintained for more than 25 years and was capable of self-renewal ex vivo. Additionally, 17 years after vaccination, TSCM can be prominently distinguished based on their marker maintenance, such as CD58, CD95, CXCR3, KLRG1, CD11a+ high, IL-18Rα, granzyme A and IL-2Rβ (138). Accordingly, TSCM thought to be a pivotal T cell subset that have different qualities in terms of effector function, proliferative capacity as well as immune reconstitution and memory formation and it is believed that a TSCM net production can enhance adoptive immunotherapy which even very low numbers of T cells from the memory stem cell pool such as TCMs or TSCMs can reconstitute robust and long term maintained immune responses (11, 139). Given the TSCM role, a major challenge in vaccine development is determining the relative importance of TSCM cells and defining if effective vaccines should induce antigen-specific TSCM for maximal protection.

### TSCM AND AUTOIMMUNE DISEASES

TSCM cells provide long-term protective immunity for antitumour immunity, which is likely based on responsiveness to self-antigens; therefore, their involvement in autoimmunity is unavoidable (102, 140). The study of Hosokawa et al. showed that CD8+ TSCM cells played an essential role in the pathogenetic mechanism of aplastic anaemia (AA) and were expected to become a new therapeutic direction for this disease (141). An increased CD8+ TSCMs frequency at diagnosis is associated with responsiveness to immunosuppressive therapy, and an elevated CD8+ TSCMs population after immunosuppressive therapy correlates with treatment failure or relapse in AA patients.

A positive correlation between the frequencies of TSCM CD4+ and CD8+ cells is seen in AA, autoimmune uveitis and systemic lupus erythematosus (SLE) (141). SLE, a chronic, connective tissue multi-organ disorder that affects young women. Compared to healthy controls, there was a significant increase in the percentage of CD4+ and CD8+ TSCM cells in SLE patients. T Follicular helper cells (Tfh) increase antibodies produced by B cells, and TSCM cells play a role in SLE pathogenesis by maintaining Tfh cells (142).

Immune thrombocytopenia (ITP), an acquired autoimmune haemorrhagic disease, represents about 30% of all haemorrhagic diseases (143). Many researchers have conducted extensive studies on the pathogenic mechanisms of ITP. At present, there is growing evidence that abnormal cellular immunity plays a significant role in the development of ITP. Very recently, Cao et al., showed that the percentage of CD8+ TSCMs in peripheral blood before treatment in ITP patients was significantly higher than that in healthy controls, although some data overlapped in the two groups, whereas the percentages of the other T cell subsets did not exhibit significant differences (143).

Considering that TSCMs can generate all memory and effector T cells, the authors speculated that they could cause the progression of autoimmune diseases. Moreover, the variation in the percentage of TSCMs CD8+ is correlated with the effectiveness of the treatment. The percentage of CD8+ TSCM in peripheral blood of ITP patients was significantly reduced after glucocorticoid treatment, indicating that the imbalance of the ratio of CD8+ TSCM may be involved in the occurrence and development of ITP (143).
Type I diabetes (T1D) results from chronic autoimmune destruction of insulin-producing β-cells mediated by self-reactive T-cells. Many β-cell autoantigens are targets for autoimmunity. Among these, GAD65, (pro)insulin and the block-specific glucose-6-phosphatase subunit catalytic protein (IGRP) appear to be highly antigenic in man (144). The presence of a memory response to islet autoantigens may be related to the limited success of immunotherapies tested to date in T1D patients. Memory autoimmunity is highly resistant to modulation with immunosuppressant drugs and immunomodulatory molecules (145, 146), primarily designed to target activated and fast-proliferating T-effector cells. These raised the question of whether self-reactive and long-lasting TSCM precursors can serve as a reservoir of self-reactive clones to target activated and fast-proliferating T-effector cells. These population to design novel immunotherapy approaches. Selective targeting of self-reactive TSCM clones may be used to suppress more differentiated T-cells permanently.

Further efforts are needed to clarify changes in health and disease stages among the different TSCM cell states.

### GENERAL BACKGROUND OF T RM CELLS

T RM cells are an essential part of the immune survey system, sensing alteration in homeostasis throughout the body and leading immune surveillance of most non-lymphoid tissues (148–151). T RM cells are involved in protection from infection and cancer, but also likely promoting autoimmunity, allergy, and inflammatory diseases and impede successful transplantation. The residence signature that marks T RM cells in several tissues is characterized by both increased expression of proteins that promote tissue retention and reduced expression of proteins involved in the recirculation in the blood and lymphatic vessels.

For instance, T RM cells exhibit reduced expression of the surface molecules such as S1PR1 and CCR7, which promote T cells to leave the NLT, an observation that is explained by a decreased expression of the transcription factor KLF2, which leads to transcription of S1PR1 and CCR7 (152). On the other hand, T RM cells express CD69 and, in the case of T RM cells located in epithelial tissues, the E-cadherin αE integrin binding (CD103, encoded by ITGAE gene), which both promote tissue retention (29).

T RM cells as all the memory T cells express CD44 and lack CD62L, while T RM cells are exclusively CD44 hi and CD62L low. They can display several tissue-specific markers, so that no single marker can uniquely identify a T RM population in all tissues.

Thus, the expression of CD69 and CD103 should be considered an imperfect marker for inferring tissue residence because the absence of their expression does not exclude the potential to leave NLTs (151, 153–156).

CD103, CD49a (VLA-1) and CD69 are the most commonly utilized markers to distinguish T RM cells from circulating memory cells (157). CD103 is a ligand for E-cadherin, widely expressed in the junctions between epithelial cells at barrier sites (158). CD49a is an integrin ligand for collagen IV which is located in the lamina dense of barriers tissues (159). Moreover, CD49a has an anti-apoptotic function for the collagen bounding T lymphocytes (160). CD69 is an S1P1 antagonist, countering signals for lymphocyte egression and circulation (161, 162). CD69 is expressed by the majority of T RM. CD103 is mainly expressed primarily by CD8+ T RM cells (163). Many other cell surface markers are associated with T RM cells, including CXCR6, CD101, PD-1, and CX3CR1.

Nevertheless, a large part of our current understanding of T RM cells relies on analyses of CD69+CD103+ T RM cells in epithelial tissues.

### ROLE OF T RM IN CHRONIC INFECTIOUS DISEASES

Consistent with their role as local sentinels, it has been demonstrated that T RM cells both prevent and exacerbate pathologies. For example, T RM cells protect local recurrent pathogens (148, 164) but these cells can also provide protection against the development of skin malignancies (165–167).

T RM cells can lead to immunotherapy-induced colitis (168), autoimmune disorders of the skin vitiligo (169) and psoriasis (170, 171) as well as other autoimmune and allergic diseases, and can play a central role in allograft rejection (172).

T RM constantly perform surveillance of the cells surrounding them and intimately interact with the components of their environment including epithelial cells and the extracellular matrix. For this reason, T RM are among the first line of defence and are early activated by the encountering of antigens. The involvement of T RM cells in a series of human diseases makes the design of therapeutic strategies that can modulate both their production and their activity an attractive objective, and to realize this goal, understanding how this cell pool is formed is essential (173).

While studies on the role of T RM in cancer are very extensive, the role of T RM in the context of chronic infections is less explored. In general, there is strong evidence that chronic tissue-specific infection is associated with a non-functional alteration of the T RM population evidenced by an exhausted phenotype. However, even in this context some studies have highlighted the possibility of using this subset for vaccination or therapeutic purposes against infectious diseases such as HIV and tuberculosis.

For instance, a recent study showed that in HIV patients, CD8+ T cells HIV specific with a T RM phenotype are abundant in HIV infected lymph nodes compared to peripheral blood. These CD8+ T RM cells show different specificity toward different HIV antigens, and this finding results in a different clonotype observed in different HIV infected lymph nodes (155).

The presence of HIV specific T RM cells in lymph nodes of HIV infected subjects makes these cells suitable targets of a...
vaccine against HIV. A recent study on murine model based on subcutaneous microneedles delivery array (MA) vaccination with recombinant E1, E3 deleted human adenovirus type 5 (AdHu5) vector encoding HIV-1 CN54 gag (AdHu5-CN54gag), induced in the skin the a subset of specific memory CD8⁺ cells with a T cells phenotype (CXCR3⁺, CD103⁺, CD49a⁺, CD69⁺) and a polyfunctional profile (174).

In a recent study on the tuberculosis pleural effusion (TPE), the authors found a heterogeneous population of T cells in which CD103⁺CD8⁺ T cells were the predominant subset of CD103⁺ T lymphocytes. Moreover, the frequency of CD103⁺ CD69⁺ memory CD8⁺ T cells in TPE was significantly higher than the frequency of these cells in peripheral blood. Altogether, CD103 and CD69 expressions define in TPE distinct CD8⁺ T cells -like subsets with different phenotypes and functions (175).

The consistent presence of T cells in lungs focused the attention of many scientists on these cells as a target for vaccine or host direct therapy against Mycobacterium tuberculosis infection (Mt). Moreover, different studies on possible mucosal TB vaccines against TB have been carried out on mouse models, revealing the marked presence of both CD8⁺ and CD4⁺ T cells in the lungs. In a study on murine models, the intranasal boosting with a Sendai virus vectored tuberculosis vaccine, SeV85AB, induced antigen-specific CD103⁺CD8⁺ T-cell protective response in the lung, associated with a reduction of mycobacterial burden both in lung and spleen compared to subcutaneous BCG alone vaccination (176). Nanoparticles (NPs) are an emerging vaccine technology, with recent oncology and infectious diseases successes. Hart et al., have associated Mt control by NPs due to the enhanced cellular immunity compared to BCG, including superior CD4⁺ and CD8⁺ T cell proliferation, T cells seeding in the lungs, and cytokine polyfunctionality (177). In another study, Bacillus subtilis spores coated with a fusion protein 1 ("FP1") consisting of Mt antigens Ag85B, ACR, and HBHA have been tested in a murine low-dose Mt aerosol challenge model (178). This vaccination model demonstrated an enhanced pulmonary control of Mt, as evidenced by reduced bacterial burdens in the lungs. Spore-FP1 immunization generated superior antigen-specific memory T-cell proliferation in both CD4⁺ and CD8⁺ compartments. Moreover, CD69⁺CD103⁺ T cells were found within the lung parenchyma after mucosal immunization, confirming the advantages of mucosal delivery (178). Finally, liposome-based subunit vaccine formulation of phosphatidyserine encapsulating, Mt antigens Ag85B, and ESAT-6, given mucosally, could generate T cells, that have been found in the lung parenchyma that are likely to be antigen specific, even if no formal evidence of their antigen specificity was demonstrated (179).

The administration of BCG by intranasal rote elicited the generation of both CD8⁺ CD69⁺CD103⁺ CXCR3⁺ T RM cells, able to improve the protection against Mt challenge when compared to the simple administration of subcutaneous BCG (180).

Adoptive transfer of these cells to naive, TCRα⁻β⁻ transgenic mice, prior to Mt infection, demonstrated parenchymal T cells, which expressed the T RM CXCR3⁺KLRG1⁻ phenotype, had far greater activity than circulating T-cells (18-fold vs 4-fold reduction in lung bacterial burden) (181).

An interesting finding regarding T RM cells in the tuberculosis murine model is the expression of PD-1, which is a key molecule to modulate the IFN-γ production and thus, prevent lethal lung disease. This finding was assessed by a recent study showing the generation of protective KLRG1⁻PD1⁺ T RM cells in lung after mucosal vaccination with BCG, but not after intradermal BCG administration (182).

T RM GENERATION AND HOMEOSTASIS IN THE BARRIER AND MUCOSAL TISSUES

T RM cells are a dedicated population of T cells that continuously recirculate through tissues or whether they can permanently reside and show decisive functions, organizing local protective immune responses. This migration, a feature of classical memory T cell subsets, is the basis of the differential expression of CCR7 (22). Once the pathogen is encountered, it expands in situ, acquiring marked cytotoxic activities, promoting the formation of a tissue antiviral environment and releasing numerous cytokines (183, 184). Indeed, T RM cells’ maturation foresees the encounter with the antigen at the tissue level resulting in the downregulation of CCR7 in the activated cells, and this process is a prerequisite for the formation of T RM (185-187). Furthermore, a study on a mouse model, based on the intranasal or intramuscular administration of CpG and nucleoprotein peptides (NP) from influenza A/HKx31 (H3N2) virus, showed the formation of NP-specific T RM in the respiratory tract tissues only in mice that had had intranasal administration of the NP (188).

Those functions generally require fundamental immunometabolism changes, metabolic homeostasis, and remodelling in T cells during life and ageing. All of this remains unexplored, but it is now clear that during humans ageing, progressive deterioration leads to impaired function and continual decline that results in an increased risk of disease and death (189); as an example, a recent study clearly shows how over the years there is a variation in the passage of immune cells in the lungs and how these cells respond specifically against classic influenza and SARS-CoV-2 virus (190). In fact, most immune cells in human lungs remain stable with age, unlike memory CD8⁺ T cells that decrease, particularly the T RM cells (191, 192). As seen in Nguyen et al. studies, a subgroup of CD103⁺ and CD69⁺ T RM cells in the lungs of donors under the age of 50 accounts for about 48% of the total memory CD8⁺ T cells, but in subjects over 50 years of age, it represents only 25% of CD8⁺ memory T cells (190).

These observations can be realized through ageing and T cell immunometabolism; for example, ageing is associated with reduced TCR diversity (193) and shifting from naive to memory subset, and these phenotypic changes result in an overall compromised response to new antigens. An example to prove this fact is that the overall frequency of influenza-specific CD8⁺ pulmonary memory T lymphocytes does not show variations with the donor’s age, while the subgroup of influenza-specific T RM lymphocytes with phenotype CD103⁺, CD69⁺ decreases with age. This decrease coincides with the
programs of tissue residency by increasing AMPK activation decisive in TRM cells and TCM CD8+ T cells is purinergic receptor P2RX7 (201, 200). For example, an essential requirement for the differentiation of barrier tissues and their microenvironments have different trophic and pH conditions that T RM cells should face. Moreover, the cell populations, the extracellular matrix, and the collagen type are different. For this reason, T RM cells can exploit diverse sources of energy by different metabolic pathways.

Metabolic reprogramming and metabolism changes are associated with adapting specific T cell subunits for survival and cell function, supporting cell function through energy supply and biosynthetic precursors. For example, cellular and transcriptional changes during an infection are responsible for developing different memory CD8+ T cells and antigen-specific CD8+ T cells. In addition, many changes can control CD8+ T cell metabolism in response to infection, driven by extracellular metabolites, such as transcriptional, translational, and epigenetic changes.

During acute infection, one of the most abundant CD8+ T RM cells become embedded in lymphoid and non-lymphoid peripheral tissues (151, 196). However, after pathogen clearance, only a few T cells remain as long-lived memory CD8+ T cells such as T RM or T SCRM, which are critically dependent on cellular metabolism to regulate CD8+ T cell differentiation and memory formation (197–200). For example, an essential requirement for the differentiation of T RM cells and T CM CD8+ T cells is purinergic receptor P2RX7 (201), which polymorphism on this gene plays a role in susceptibility to TB in the Asian population (202). In fact, non-lethal P2XR7 maintains mitochondrial homeostasis and TGF-β dependent programs of tissue residency by increasing AMPK activation compared with other tissue CD8+ T cells. CD8+ T RM appears to possess a distinct functional and metabolic profile that can depend on their environment and adapt to it. CD8+ T RM maintains long-term survival by using FAs which closely depend on extracellular factors such as extracellular ATP, which could influence the T RM metabolism and expression of CD69 and CD103 (201).

As an example, P2XR7 can affect the mTOR pathway to switching from glycolysis to OXPHOS (203). Unlike circulating memory CD8+ T that mainly depends on glycolysis to respond to pathogenic antigen (80), T RM depends on the uptake of exogenous FAs for subsequent FAO engagement and subsequently mitochondrial membrane potential and fatty acid metabolism for their homeostasis. Many microenvironment factors such as FA-rich sites, extracellular ATP (eATP) and many receptors can affect gene upregulation which eventually changes effector function and lipid metabolism on T RM. For example, the low-affinity P2RX7 receptor to eATP promotes the NLRP3 inflammasome activation (204). In contrast, P2RX7 can promote CD8+ T RM cell subsets through mitochondrial health (205) (Figure 2). Moreover, due to increased infected cell death or gene expression upregulation to change effector function in infection sites, the extracellular NAD+ concentration increases, promoting CD8+ T RM cell death by P2RX7 (206).

Another pathway used by T RM exploits free fatty acid (FFA). T RM cells showed a high expression of the fatty acid-binding receptors FABP4, FABP5, low-density lipid receptor, ApoE, and the CD36 scavenger receptor. These molecules are useful to enhance lipolysis for rapid exploiting the direct pathway of FFA uptake (45). The FFA can be used in oxidative phosphorylation and in FAO (45, 207). Thus, it is likely that T RM can switch their metabolic pathway from oxidative phosphorylation and glycolysis to FAO after antigen encounter and subsequent activation.

The master transcriptional regulators of T RM cells differentiation in different tissues are Hobit, Blimp1 (163), and Runx3 (208) at the transcriptomic level. The first two transcriptional factors are negative regulators of tissue egresses (209) and are involved in the T RM cells cytotoxic capability (210). Runx3 enhances the expression of CD103 and CD69 tissue retention molecules (208).

In conclusion, the fate decision by metabolism change in T RM cells remains largely unknown. Those cells could be a fascinating target not only for vaccination efficiency or controlling infection but also in tumour and autoimmune tissue disorders due to the double-edged sword character. Given this content, understanding in detail metabolic reprogramming to possible manipulation to enhance or decrease T RM longevity and function is crucial.

**T RM CELLS AND AUTOIMMUNE DISEASE**

T RM cells have been shown to play a role in autoimmune diseases, especially autoimmune skin diseases. In vitiligo, it showed the presence of T RM cells in the epidermis and dermis of lesional areas. These cells expressed CXCR3, whose binding with its ligands CXCL9 and CXCL10 drive the production of INF-γ, which represents an effector cytokine of this pathology. Moreover, these T RM cells produced high quantities of IFN-γ and GRZB after in vitro stimulation (211). The phenotype of vitiligo associated with T RM cells also displays the α chain of IL-15 receptor and CD122, which play an essential role in the pathological activity of T RM; in fact, the blocking of CD122 by monoclonal antibody leads to the repigmentation of vitiligo areas in an experimental mouse model (169).

Like vitiligo, psoriasis is an autoimmune skin disease, where IL-17 sustains its pathogenesis. CD8+ T RM cells from psoriatic lesions showed IL-17 production after in vitro restimulation. These cells display a specific phenotype (CD103+ CD49a+ CCR6+) characterized by the expression of IL23R and the absence of CXCR3 (211, 212). Several studies reported the involvement of T RM cells in other autoimmune diseases. CD8+ T RM cells specific for astrocytes-expressed protein play a crucial role in multiple sclerosis (MS) pathogenesis in a mouse model.
acids binding proteins (FABPs) in TRM cells (45, 226) determine CD8+ TRM cells or their survival (222). The signals from these two pathways is critical either in activated cells is linked to the ability of Treg to make TGF-β (219). Moreover, CD103+ cells were detected in the high metabolic disorders determine a more likely uncontrolled activation of CD8+ TRM cells can determine the production of IL-7, IL-15, and IL-17 that can trigger CD8+ TRM cells’ activation and enhance their proliferation and the maintenance of a stable pool of activated cells (183). CD8+ TRM cells in response to IL-7 and IL-15 can rapidly be activated by the JAK/STAT5 pathway, which profoundly influences the formation of long-lived memory T cells to enhance the anti-apoptotic BCL-2 molecule (217–219). Also, the PI3K/AKT pathway activation is associated with long-lived memory T cells (217, 220, 221). An appropriate balance of the signals from these two pathways is critical either in activated CD8+ TRM cells or their survival (222).

It was demonstrated that cross-talk between Treg and TRM cells is linked to the ability of Treg to make TGF-β available for the TRM formation and corroborated by similar expression of CXCR3, which allows both cell types to migrate toward the site of infection and inflammation (223, 224). The role of Treg can prevent pathological activation of TRM cells. Nevertheless, it happens that impaired function of Treg in the skin leads to the formation of TRM cells with autoimmune activity. To confirm this hypothesis, it was observed that the checkpoint inhibitor therapy used in patients with melanoma is associated with an increase in vitiligo disease (169, 225).

About metabolic pathways used by TRM cells during their activation in autoimmune diseases, skin TRM cells can exploit FFA and mitochondrial oxidative metabolism. Together with the fact that skin autoimmune disorders are often concomitant with metabolic diseases, these findings suggest that the metabolic disorders determine a more likely uncontrolled activation of CD8+ TRM cells due to an enhanced FFA metabolism. Evident lipid accumulation in droplets (45) and high expression of fatty-acid binding proteins (FABPs) in TRM cells (45, 226) determine the higher expression of transcripts of enzymes involved in lipid metabolism, which supports this hypothesis. In conclusion, the presence of dysfunctional CD8+ TRM cells in lesions caused by autoimmune diseases makes the TRM cells subset an important player in the onset and chronic assessment of these diseases and, at the same time, a promising target for new therapeutic strategies in autoimmune diseases.

**CONCLUDED REMARKS**

The recent application of high-level single-cell technologies and epigenetic profiling approaches has revealed that the stem and the effector properties of CD8+ T cells are no longer reciprocal. Transcriptional programming that equips a T cell with a multipotent development capability can coexist with effector programs and establishes a population of long-lived memory T cells that can respond against a previously exposed pathogen. Recent studies investigating these properties in T cell responses to chronic antigen sources illustrate an essential role of these

**T CELL MEMORY SUBSETS IN SARS-COV-2 INFECTION OR VACCINATION**

Within the T cell memory compartment, TRM and TSCM CD8+ T cells play a different role in SARS-COV-2 infection or vaccination. The first subset has been detected in the respiratory tract following coronavirus infection, where they employ a protective function (227). Moreover, activated profiles of lung CD8+ and CD4+ TRM cells were reported in COVID-19 patients, and these cells were frequently detected even ten months after infection (228, 229). Recovered patients acquire TRM cells with Th1 phenotype against COVID-19 (229). It was shown that, although resident T cells are elicited by COVID-19 infection, they do not present sufficient protection against secondary viral infection (230). On the other side, CD8+ TRM cells can significantly contribute to lung damage/pathology development, determined by the prolonged activities after respiratory viral infection (231). In fact, lung CD103+ CD69+ T cells of older patients with COVID-19 produce elevated inflammatory molecules associated with worse lung pathology and reduced lung function (232). It has been found that respiratory CD103+ CD69+ T cells exacerbate lung pathology and reduce lung function (232). Therefore, several therapeutic strategies have been developed to target the TRM cells. A recent study has evaluated the role of Tofacitinib (the FDA approved oral JAK2/1/3 inhibitor) targeting the tissue pathology caused by acute SARS-CoV-2 infection (233). SARS-CoV-2 specific memory TSCM cell responses are induced after recovery from SARS-CoV-2 infection or vaccination, according to increasing data. After ten months of infection, Jung et al. discovered a successful generation of TSCM cells in convalescent COVID-19 patients (234). Another study clearly showed that an antigen-specific population of TSCM is observed even after six months of vaccination with BNT162b2 vaccine, which provides a multipotent memory reservoir (235). It was recently revealed that stimulation of T cells shortly after BNT162b2 vaccination with spike epitopes leads to rapid production of TNF-α, which was independent of the neutralizing antibodies’ titer (236). The production of TNF-α, even within a few hours after TCR-stimulation, is most characteristic of naïve-like CD8+ T cells, similar to TSCM (237). The high production of TNF-α is not always related to a good immunological response, due to its involvement in several inflammatory diseases or the severity of long COVID patients (238–240). In conclusion, TRM and TSCM cells represent a double-edge sword in SARS-Cov-2 infection, and further studies are needed in order to delineate their role better.
coexisting characteristics in establishing therapeutic modalities based on selected subsets of T lymphocytes. The great interest in studying the impact of the metabolic activity of pathways in immune cells has opened a new window in understanding the requirements of metabolic pathways for differentiation/maintenance of CD8+ T cells, which can lead to the discovery of molecular targets capable of developing new anti-infective and/or anti-inflammatory drugs applicable to different contexts (241–244).

The outstanding issues surrounding these fundamental properties of T cells are of great translational importance and represent a question of intense investigation. These processes will likely provide more information on the cellular and molecular mechanisms of memory T-cell differentiation and exhaustion in acute and chronic pathogenic challenges, allowing the development of new therapeutic approaches that use the exquisite specificity and longevity of T cells.

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AUTHOR CONTRIBUTIONS

Conceptualization and Supervision, FD, NC; Writing-Review & Editing, BT MPLM, MSA, LM, GDB; Writing-Original Draft Preparation, NC, FD, MPLM; BT, MSA, LM; Figure preparation: MPLM, BT, GDB, MSA, LM. All authors have read and agreed to the submitted version of the manuscript.

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