

# A narrative review of tissue-resident memory T cells and their role in immune surveillance and COVID-19

L. CHEN<sup>1</sup>, B. WEI<sup>2</sup>, D.-L. DI<sup>2</sup>

<sup>1</sup>Department of Hematology, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, China

<sup>2</sup>Department of Immunology, Weifang Medical University, Weifang, Shandong, China

**Abstract.** – Most effector T cells will undergo programmed apoptosis after an immune response and some of them may become memory T cells. According to the distribution and functional status, the memory T cells can be divided into effector central memory T cells (TCM), effector memory T cells (TEM) and tissue-resident memory T cells (TRM) cells. TRM cells, including CD4+ TRM and CD8+ TRM cells, colonize various barrier surfaces and are no longer involved in lymphocyte recycling, closely monitored for local perturbations in homeostasis throughout the body as a critical component of the first defense line. When pathogenic microorganisms invade the body, TRM cells can quickly produce a defense response to initiate innate immunity and adaptive immunity by producing cytokines or killer molecules to resist viral and bacterial infections. In addition, TRM cells are also involved in cancer surveillance and play an essential role in maintaining cancer-immune equilibrium. The high frequency of TRM cells in tumor tissues often means favorable survival for patients. The latest research proves that TRM cells also play an important role in vaccine development and pathological features of COVID-19. This article will summarize the biological functions of TRM cells and aims at providing references for further research on their mechanism and for targeting the best treatment of clinical disease.

*Key Words:*

Tissue-resident memory T cells, Immune surveillance, Vaccine development, COVID-19.

## Introduction

The immune memory is an essential feature of the adaptive immune response, a successful immune response can induce the formation of memory T cells. Memory T cells are the most

abundant lymphocyte population in the body that can survive for a long time and provide protection against pathogens invasion. When the body encounters the same pathogen, these cells will rapidly activate and clear them. Memory T cells can generate a more effective immune response than naïve T cells<sup>1,2</sup>. According to the different phenotypes, circulation pathways and effector functions, the classical memory T cells can divide into two groups, namely central memory T cells ( $T_{CM}$ ) and effector memory T cells ( $T_{EM}$ ).  $T_{CM}$  cells and  $T_{EM}$  cells circulate between the blood and peripheral lymphoid organs continuously and survive for a long time<sup>3,4</sup>. In 2001, a unique type of T cell that resided in the lung tissue of a mouse influenza model was reported first. When mice are infected with respiratory viruses, this antigen-specific T cell, known as tissue-resident memory T cells ( $T_{RM}$ ), the third type of memory T cells<sup>5,6</sup>, rapidly activates and fights the infection. Most  $T_{RM}$  cells are abundant in the barrier sites such as the skin, lung, stomach, liver, bladder, and genitourinary tract, serving as the “first line” of defense<sup>7-11</sup>.  $T_{RM}$  cells are present in large numbers in the mouse epidermis, exhibit slow crawling behavior and extend long dendritic projections into the spaces between adjacent cells<sup>12</sup>.  $T_{RM}$  cell formation can also be observed in chronically bacterially infected intestinal mucosa or bronchi of the lungs<sup>13,14</sup>. Recent studies<sup>15</sup> have found that  $T_{RM}$  cells exist in immune-privileged areas such as brain tissue. Virus-specific  $T_{RM}$  cells can be dispersed in the brain parenchyma or sites that have previously encountered infectious viruses, these cells closely monitor the resuscitation of neurotropic viruses<sup>16</sup>.  $T_{RM}$  cells also play an important role in tumorigenesis, various inflammatory diseases and vaccine development.

### The Identification of T<sub>RM</sub> Cells

According to the expression of CD molecules, T<sub>RM</sub> cells can be divided into two types: CD4<sup>+</sup>T<sub>RM</sub> and CD8<sup>+</sup>T<sub>RM</sub>. CD4<sup>+</sup>T<sub>RM</sub> cells mainly settle in the epithelial layer of the mucosal barrier, such as the basement membrane and lymph nodes. After recognizing invading pathogens, CD4<sup>+</sup>T<sub>RM</sub> cells trigger an immune response by interacting with antigen-presenting cells, releasing inflammatory cytokines to activate natural killer cells, B cells, or other immune cells to clear pathogens. More research<sup>17</sup> suggests that CD4<sup>+</sup>T<sub>RM</sub> cells present in chronically inflamed lesions play an important role in inflammatory responses, thus targeting CD4<sup>+</sup>T<sub>RM</sub> may be a viable therapy for treating mucosal inflammatory diseases. CD4<sup>+</sup>T<sub>RM</sub> cells promote chronic inflammation and fibrosis in lung tissue exposed to aspergillus fumigatus by producing effector cytokines, play an important role in the pathological changes of chronic lung inflammation, inhibit the function of CD4<sup>+</sup>T<sub>RM</sub> cells, and alleviate pulmonary fibrotic lesions<sup>18</sup>. CD4<sup>+</sup>T<sub>RM</sub> cells in lung tissue help CD8<sup>+</sup>T cells through IL-21 to kill influenza virus-infected target cells<sup>19</sup>. CD4<sup>+</sup>T<sub>RM</sub> cells generated by vaginal mucosal cells infected with the herpes simplex virus (HSV) can cause antagonist viral infection by releasing IFN- $\gamma$ <sup>20</sup>. HSV-specific CD4<sup>+</sup>T<sub>RM</sub> cell accumulation has been found in dermal cells around the isthmus of skin follicles, this depends on persistent CCL5 chemokine signaling and mediates skin inflammatory responses<sup>21</sup>. The primary function of CD4<sup>+</sup>T<sub>RM</sub> cells is to provide local protection against infections, but they are detrimental in organ transplantation. For example, CD4<sup>+</sup>T<sub>RM</sub> cells detected in kidney transplantation mouse models proliferate locally and produce IFN- $\gamma$  to induce renal transplant rejection. Therefore, T<sub>RM</sub> cells can be used as targets for therapeutic inhibition of rejection, and targeted inhibition of T<sub>RM</sub> may improve the survival rate of transplantation<sup>22</sup>. Unlike CD4<sup>+</sup>T<sub>RM</sub> cells, which mainly mediate inflammatory responses, CD8<sup>+</sup>T<sub>RM</sub> cells are closely related to the anti-tumor process. CD8<sup>+</sup>T<sub>RM</sub> cells are usually located in various barrier tissues and monitor tumor development.

Compared with traditional  $\gamma\delta$  T cells, CD8<sup>+</sup>T<sub>RM</sub> cells in the skin and mucous membranes can survive stably long, act as “sentinels” to trigger antigen-specific immunity, release cytotoxic molecules (e.g., perforin and granzyme) and directly lyse infected cells<sup>23,24</sup>. Many pathogens can accumulate on the mucosal surface of the gastrointestinal tract and cause infection. Once *Yersinia*

infects the gastrointestinal tract, IFN- $\beta$  and IL-12 released by intestinal macrophages can recruit CD8<sup>+</sup>T<sub>RM</sub> cells in the inflammation areas, so the number of T<sub>RM</sub> cells can be significantly reduced in IL-12-deficient mice<sup>25</sup>.

The primary surface markers of T<sub>RM</sub> cells are CD103 and CD69. CD103, also known as  $\alpha$ E-type integrin, is a marker molecule of T<sub>RM</sub> cells. CD103 and integrin  $\beta$ 7 form a heterodimeric receptor for E-cadherin through which T<sub>RM</sub> cells adhere to the peripheral tissue for a long time. Studies<sup>26</sup> have shown that T<sub>RM</sub> cells in mucosal parts such as skin, lung, salivary glands, and reproductive tract express a high level of CD103 molecules, which play an important role in the residency of T<sub>RM</sub> cells in local tissues. CD69 is a membrane-bound type II C-lectin receptor, which is abundantly expressed on the surface of T<sub>RM</sub> cells and  $\gamma\delta$  T cells. The expression of CD69 can be detected within 1 hour after the T cell surface antigen recognition receptor (TCR) recognizes the antigen. Therefore, CD69 is generally considered a marker of T<sub>RM</sub> cells activation. CD69 can bind to galectin-1 on the surface of adjacent cells for the adhesion of T<sub>RM</sub> cells in peripheral tissues. CD69 molecule also participates in many physiological and pathological processes such as cell adhesion, apoptosis, inflammatory response or tumor metastasis<sup>27</sup>.

The binding of Sphingosine-1-phosphate (S1P) with its receptor (S1P1) is an important pathway for lymphocyte outflow from the thymus and peripheral lymphoid tissues. Drugs such as FTY720 which are antagonists of S1P1 can reduce T cell outflow significantly<sup>28</sup>. CD69 is a natural antagonist of S1P1 that can bind to S1P1 on the cell surface, leading to its internalization and degradation and preventing T<sub>RM</sub> cells from flowing out of lymphoid tissues. Other studies<sup>29</sup> have shown that CD103 is also expressed on the surface of other T cells. The circulation of T cells can express CD69 under inflammation cytokines stimulation, so relying solely on CD103 or CD69 expression may not accurately identify T<sub>RM</sub> cells<sup>30</sup>. In recent years, it has been found that CD44 is abundantly expressed on the surface of T<sub>RM</sub> cells. CD44 is a transmembrane glycoprotein that can interact with extracellular matrix components, such as fibronectin and laminin and is widely involved in cell adhesion, migration or other activities. The continued expression of CD44 suggests that it may influence the function of T<sub>RM</sub> cells, although we cannot distinguish T<sub>RM</sub> cells from other T cells only by CD44. CD44 is maintained at high

levels on the surface of  $T_{RM}$  cells, suggesting that it may be necessary for  $T_{RM}$ , although its specific effects have not been elucidated<sup>31</sup>. The analysis of  $T_{RM}$  cells relied on the expression of single or few markers so far, which often makes it difficult to know which of the tumor-infiltrating lymphocytes (TIL) are actual  $T_{RM}$  cells, and the use of high throughput technologies (e.g., single-cell RNA sequencing and mass spectrometry flow cytometry) may help to identify  $T_{RM}$  cells<sup>32</sup>.

### Regulation and Differentiation of $T_{RM}$ Cells

The formation of  $T_{RM}$  cells involves several key steps, including the activation of naïve T cells by antigen-presenting cells in the lymph node, the migration and infiltration of T cells, the activation of  $T_{RM}$ -specific transcription factors, and various factors such as cytokines, chemokines, and adhesion molecules can affect the formation of  $T_{RM}$  cells.

When stimulated by antigens, naïve T cells are activated in lymph nodes by antigen presenting cells (e.g., dendritic cells or macrophages), then further differentiate and mature under the influence of pro-inflammatory cytokines and perform immune functions. Most of them will undergo programmed apoptosis after completing their mission and some will differentiate into memory T cells.  $T_{CM}$  and  $T_{EM}$  can circulate in various tissues and organs of the body through blood and lymph.  $T_{RM}$  cells settle down in peripheral organ tissue and no longer participate in lymphocyte recycling. A mouse skin infection model confirmed that dendritic cells in lymph nodes could activate naïve  $CD8^+$ T cells through alpha V-type integrin and MHC-I molecules and induce them to differentiation into  $T_{RM}$  cells through TGF- $\beta$ . Results of open chromatin sequencing confirmed that the TGF- $\beta$  signaling mediated by DCs expressing alpha v-type integrins is closely related to  $T_{RM}$  formation<sup>33</sup>. Driven by inflammatory signals in infected tissues, the endothelium abundantly expresses adhesion molecules, such as ICAM-1 and VCAM-1, which bind to LFA-1 and VLA-4 expressed by T cells, effectively increasing T cells infiltration and subsequent  $T_{RM}$  formation<sup>34</sup>. Epithelial infiltration is a key factor in  $T_{RM}$  cell formation, largely dependent on signaling from G protein-coupled receptors such as CXCR3. Chemokines CXCL9 and CXCL10 are highly expressed in inflamed tissues and can recruit T cells through CXCR3 to promote T cell infiltration in local epithelial tissues. However, CXCR3 genetically deficient  $CD8^+$ T cells are not entire-

ly excluded from epithelial cells, implying that other chemokines and adhesion factors may also act in a tissue-specific manner<sup>35,36</sup>. The efficient localization of  $T_{RM}$  cells in lung tissue relies on IFN- $\gamma$  and chemokines produced by  $CD4^+$ T cells. However, this requirement does not apply to all tissues, as virus-specific  $T_{RM}$  cells can develop and persist in the skin and brain without  $CD4^+$ T cell help<sup>37,38</sup>.  $T_{RM}$  cells in lung tissue are essential for the antagonism of respiratory viral infections. Monocytes, macrophages and dendritic cells in lung tissue are involved in the early differentiation of  $T_{RM}$  cells, which present influenza virus antigens to activate naïve T cells. With the help of CCR2, naïve T cells chemotaxis to the lesion area and differentiate into  $T_{RM}$  cells, and the content of  $T_{RM}$  cells in the lungs' tissues in CCR2<sup>-/-</sup> mice was significantly reduced<sup>39</sup>.

Currently, there is great interest in how different antigen-presenting cells promote the formation of  $T_{RM}$  cells. Although the traditional dendritic cells play a critical role in activating naïve T cells, increasing evidence suggests that monocyte-derived APCs (MoAPCs) play an important role in forming  $T_{RM}$  cells. MoAPCs provide the necessary chemokines and cytokines for the activation, differentiation, localization and survival of  $T_{RM}$  cells, inflammatory MoAPCs secrete TNF which can significantly increase the formation of  $T_{RM}$  cells<sup>40</sup>. A recent study by Farber et al<sup>41</sup> found that Stem cell memory T (Tscm) cells in humans and mice with high self-proliferating and multi-lineage differentiation potential may contribute to the formation of  $T_{RM}$  cells, the phenotype of Tscm cells is  $CD45RA^+/CD45RO^-$  and high levels of CD27, CD28, IL-7R and CCR7 on the cell surface. Tscm cells can be differentiated into  $T_{CM}$  cells,  $T_{EM}$  cells and  $T_{RM}$  cells at peripheral tissue sites under specific conditions.

$T_{RM}$  cells activate innate and adaptive immunity by producing effector molecules and amplify the immune response by recruiting other immune cells<sup>42</sup>. The generation of  $T_{RM}$  cells is affected by various cytokines, and the most studied are TGF- $\beta$  and IL-15. TGF- $\beta$  normally binds to latency-related proteins and exists in the body in a latent form, requiring specific proteases (e.g., matrix metalloproteinases) to hydrolyze and activate<sup>43</sup>. Mackay et al<sup>44</sup> have shown that cytokines, such as TGF- $\beta$  and IL-15, can activate Hobbit and Brip1 transcription factors of mouse T cells, promote T cells to express CD69 molecules, and mediate the development of  $T_{RM}$  cells in the skin and gut, liver and kidney, enabling the resident

of T<sub>RM</sub> cells in the inflammation tissues. The residence of other lymphocytes such as NK cells and NKT cells in the liver also requires the help of the Hobbit and Brim1 transcription factors<sup>45</sup>. TGF- $\beta$  and IL-15 can promote the development and differentiation of KLRG1-negative epithelial cells into CD103<sup>+</sup>CD8<sup>+</sup> T<sub>RM</sub> cells, which play an essential role in skin and mucosal immune defense<sup>46</sup>. TGF- $\beta$  can induce the expression of CD103 molecule in memory CD8<sup>+</sup>T cells in mice, the binding between CD103 and E-cadherin promotes long-term retention of T<sub>RM</sub> cells in the gastrointestinal or reproductive tract and responds to pathogens or foreign proteins rapidly<sup>47,48</sup>. Stromal cells induce the expression of integrins by secreting TGF- $\beta$ , and integrins  $\alpha\beta6$  and  $\alpha\beta8$  promote the residency of Langerhans cells and CD8<sup>+</sup>T<sub>RM</sub> cells in epithelial tissues, constituting the body's first line of defense against pathogens<sup>49</sup>. The IFN- $\gamma$  produced by CD4<sup>+</sup>T cells can recruit circulating T cells to skin and mucous membranes through CXCR3 molecules, effectively resisting parasitic infection. The transcription factor T-bet can promote CD8<sup>+</sup>T<sub>RM</sub> cells accumulating in the lungs, effectively antagonizing influenza virus infection<sup>37,50</sup>. Woon et al<sup>51</sup> have shown that IL-15 can down-regulate the expression of SIP1 through Kruppel-like factor 2 transcription factor after tonsils are infected by Epstein Barr virus or cytomegalovirus, then recruiting and promoting CD8<sup>+</sup>T<sub>RM</sub> cells to kill the infected target cells, which help protecting the body from viral infections.

### T<sub>RM</sub> Cells in COVID-19

Responsiveness and clearance of respiratory pathogens ability in older adults have significantly reduced, resulting in higher rates of lung infection and higher mortality. Loss of homeostatic of CD8<sup>+</sup> T<sub>RM</sub> caused by aging may mediate pulmonary pathological changes, which may be related to influenza virus and 2019 coronavirus (COVID-19) patients<sup>52</sup>. Clinical data show that the elderly prone to develop into severe patients after being infected with COVID-19, and they are often accompanied by sequelae such as pulmonary function damage after discharge. The pathological lung changes caused by infection are heterogeneous. There is a high correlation between the severity of pulmonary sequelae and the immune system changes. Virus-specific tissue-resident CD8<sup>+</sup>T<sub>RM</sub> cells were enriched in the patient's respiratory system and positively correlated with the degree of lung damage, these

cells continued to cause lungs' damage after the patient's recovery. The mouse respiratory virus infection model confirmed that the removal of CD8<sup>+</sup>T<sub>RM</sub> cells could effectively improve mice lungs' function. This result indicates that the CD8<sup>+</sup>T<sub>RM</sub> cells resident in the lungs' tissue may play adverse effects, such as pathological damage and lung tissue fibrosis<sup>53</sup>. The results of single-cell immunoassay of bronchoalveolar lavage (BAL) samples from patients with COVID-19 confirmed that antigen-driven CD8<sup>+</sup>T<sub>RM</sub> and CD4<sup>+</sup>Th17 cells in patients with mild COVID-19 show actively immune functions. In contrast, T<sub>RM</sub> cells are often highly dysregulated in critical COVID-19 patients and fall into a naïve state stressed by inflammation-related changes<sup>54</sup>. In acute phase COVID-19 patients, immune cells such as granulocytes, monocytes, CD11c<sup>+</sup>NK cells, and CD4<sup>+</sup>T effector cells increased significantly, most immune cells returned to normal when the patient entered the recovery period except for CD127<sup>+</sup> granulocytes and CD8<sup>+</sup>T<sub>RM</sub> cells, and SARS-CoV-2-specific CD8<sup>+</sup>T cells persisted in the nasal mucosa for at least 2 months, suggesting that COVID-19 has long-term effects on the respiratory tract<sup>55</sup>. Several COVID-19 associated genes have been identified through genome-wide association studies (GWAS), single-cell transcriptome analysis of bronchoalveolar lavage fluid (BALF) from COVID-19 patients revealed that CXCR6 and CCR9 genes were favorable for patients, and CXCR6 tends to be low expressed in T<sub>RM</sub> cells in the lung<sup>56</sup>. T<sub>RM</sub> cells can induce chronic airway inflammation and fibrosis. Pulmonary fibrosis is a common sequela associated with inflammatory cytokines storm. JAK kinase is an important pathway for cytokine release, while Tofacitinib is an important JAK inhibitor developed by Pfizer to inhibit T<sub>RM</sub> cell function, and it might be a potential drug candidate for the treatment of COVID-19<sup>57</sup>.

However, other views show that T<sub>RM</sub> may play a protective role for COVID-19 patients. Unlike most immune cells in lungs that remain stable, CD8<sup>+</sup>T<sub>RM</sub> cells are susceptible to the effects of age, and decreased levels of IFN- $\gamma$  and GM-CSF in the elderly often result in lower levels of CD8<sup>+</sup>T<sub>RM</sub> cells. A study by Nguyen et al<sup>58</sup> comprehensively assessed the immune response following exposure to the influenza virus and SARS-CoV-2. The results indicate that the absence of pathogen-specific T<sub>RM</sub> cells in lungs' tissue with ageing resulted in a weakened early antiviral immune response, which created a significant



effect opportunity for respiratory pathogens invasion. Another study<sup>59</sup> used a 3D human lung tissue model to determine the protective effect of pre-existing T cells against SARS-CoV-2 in lungs' tissue. The results confirmed the protective function of IFN- $\gamma$  induced  $T_{RM}$  and IgA<sup>+</sup> plasma cells for SARS-CoV-2 infection.

### $T_{RM}$ Cells in Cancer-Immune Equilibrium

The immune system plays an important role in tumor immune surveillance. Various immune cells, such as T cells and NK cells, can check the presence of malignant cells in tissues. Once abnormal cells are detected, immune cells can eliminate them from the host. However, the immune system may reach a compromise with tumor cells under certain circumstances. Immune cells will inhibit the growth and spread of tumor cells without destroying them, keeping tumor cells and immune cells in a state of balance called "cancer-immune equilibrium", a state that may be maintained for long periods, possibly for decades. Under the constant immune stress, tumor cells will undergo a process of immunoediting in which tumors can evade immune surveillance,  $T_{RM}$  cells play an important role in maintaining cancer-immune equilibrium, although the specific immune mechanisms remain unclear<sup>60</sup>. In the melanoma transplant mouse model, researchers found that  $T_{RM}$  cells can promote cancer-immune equilibrium to confine the tumor in the epidermal layer of the skin. About 40% of mice transplanted with melanoma cells had no visible skin lesions long after tumor cell inoculation and tumor specific CD69<sup>+</sup>CD103<sup>+</sup> $T_{RM}$  cells may associate with this balance. In contrast, mice lacking  $T_{RM}$  cells were more likely to develop tumors, and these mice often contained melanoma cells in the epidermal layer of their skin. In vivo imaging of mice shows that tumor cells are monitored by  $T_{RM}$  cells and tumor specific  $T_{RM}$  cells can significantly inhibit tumor development. The depletion of  $T_{RM}$  cells led to tumor growth in about 20% of mice, indicating that  $T_{RM}$  cells can effectively inhibit the development of tumors. Hence,  $T_{RM}$  cells play an important role in tumor immune surveillance by maintaining "cancer-immune equilibrium", which is expected to provide a new target for future anti-cancer immunotherapies<sup>61</sup>.  $T_{RM}$  cells isolated from melanoma patients manifest an anti-tumor capacity through directly killing tumor cells or secreting IFN- $\gamma$  to amplify the immune response. The immune checkpoint molecules, such as PD-1 and LAG3, are highly expressed

on the surface of  $T_{RM}$  cells in melanoma tissue. These  $T_{RM}$  cells are likely to initiate a response to anti-PD-1 and anti-LAG-3 treatments<sup>62</sup>. The transcription factor Runx3 is a key regulator for  $T_{RM}$  cell differentiation, which promotes the generation of  $T_{RM}$  cells in different tissue environments and inhibits the expression of recycling-related genes. Runx3-deficient mice show higher tumor growth rates and mortality, overexpression of Runx3 can enhance  $T_{RM}$  cell aggregation, delayed tumor growth and prolonged mouse survival; this may help treat melanoma patients<sup>63</sup>.

$T_{RM}$  cells play an essential role in preventing the development and spread of solid tumors. Unlike other T cells located in the tumor microenvironment, the binding of CD103 with E-cadherin contributes to a long-term residence and immune surveillance for  $T_{RM}$  cells.  $T_{RM}$  cells can rapidly recognize tumor-associated antigens (TAAs) on the surface of tumor cells, release cytotoxic particles to kill target tumor cells, and secrete pro-inflammatory cytokines to expand the immune response.  $T_{RM}$  cells play an important role in controlling tumor size, limiting tumor cell dissemination, and reducing the risk of recurrence in many solid tumors, and the high levels of  $T_{RM}$  cells are often associated with a favorable prognosis for tumor patients<sup>64</sup>. Studies<sup>65-67</sup> showed that  $T_{RM}$  cells are abundantly expressed in ovarian, breast, and bladder cancer tissues,  $T_{RM}$  cells in tumor tissue are closely related to the disease-free survival (DFS) and overall survival (Overall Survival, OS) of patients, so  $T_{RM}$  cells may be considered a marker of favorable prognosis in cancer patients. A subset of tumor-infiltrating lymphocytes (TILs) manifests phenotypic and functional characteristics of  $T_{RM}$  cells in non-small cell lung cancer (NSCLC). High infiltration of  $T_{RM}$ -like TILs is closely associated with a patient's survival, suggesting that  $T_{RM}$ -like TIL cells may contribute to anti-tumor immune responses<sup>68</sup>. CD8<sup>+</sup>CD103<sup>+</sup> $T_{RM}$  cells are closely related to the survival rate of patients with early-stage NSCLC. CD8<sup>+</sup>CD103<sup>+</sup>TILs isolated from NSCLC specimens show the phenotypic characteristics of  $T_{RM}$  cells with highly expressed immune checkpoint molecules such as PD-1 and TIM-3, these cells showed specific cytolytic activity and induced apoptosis function against tumor cells when blocking the interaction of PD-1 and PD-L1, the suppressive effect of  $T_{RM}$  cells on NSCLC supports the use of anti-PD-1 antibodies to reverse tumor cell-induced T cell depletion in NSCLC patients<sup>69</sup>. Breast tumor tis-

sue usually expresses high levels of TGF- $\beta$  and TNF, which may enhance the generation and maintenance of T<sub>RM</sub> cells. Byrne et al<sup>70</sup> have shown that triple-negative breast cancer patients with high T<sub>RM</sub> cells tend to be more sensitive to anti-PD-1 immunotherapy and have a favorable prognosis. Other studies<sup>71</sup> have shown that CD103<sup>+</sup>CD8<sup>+</sup>T<sub>RM</sub> cells accumulate in esophageal squamous cell carcinoma tissue, which can induce effective cell toxicity response and cytokine secretion. Immune checkpoint molecules, such as PD-1 and TIM-3, are highly expressed on the cell surface, eliciting anti-tumor immunity after receiving anti-PD-1 immunotherapy, suggesting that CD103<sup>+</sup>CD8<sup>+</sup>T<sub>RM</sub> cells may be a potential therapeutic target for ESCC patients.

The familiarity of T<sub>RM</sub> cells with the tumor microenvironment makes them one of choice in solid tumors treatment. However, in some cases, even if the tumor tissue contains a large number of T<sub>RM</sub> cells, the tumor will lose some of the original features and continue to grow. It remains unclear whether the efficacy of T<sub>RM</sub> cells is subsequently reduced. The researchers aim to understand how T<sub>RM</sub> cells survive in tumors by exploring cell metabolism. The tumor microenvironment (TME) is usually characterized by low oxygen and low glucose levels, and T<sub>RM</sub> cells can adapt to this microenvironment. Lin et al<sup>72</sup> have found that T<sub>RM</sub> cells in the tumor cell microenvironment can utilize exogenous free fatty acids instead of glucose to maintain their metabolic and immune functions. The energy obtained from fatty acids can support the long-term survival of T<sub>RM</sub> cells in TME, and the targeted inhibition of PD-L1 can increase the expression of the fatty acid-binding protein (Fabp) 4/5 in T<sub>RM</sub> cells, promote lipid uptake and prolong the survival of T<sub>RM</sub> cells. Therefore, it is necessary to study the metabolic properties of T<sub>RM</sub> cells in the tumors' microenvironment, which may reveal new anti-tumor therapeutics. Some T cells differentiate into effector T cells (Teff) after recognizing tumor cells in tumor cells, and some Teffs become exhausted by continuous stimulation by tumor antigens, which is called T cell exhaustion (Tex). Tex cells are characterized by loss of effector function and increased expression of inhibitory receptors (IRS), which is functionally distinct from Teff and Tmem. T cell exhaustion is one of the reasons for immune dysfunction in tumor patients: these dysfunctional Tex cells express immune checkpoint proteins, and by blocking the function of PD-1 they can reverse T cell

exhaustion, rejuvenate the dysfunctional or exhausted T cells, and re-release tumor-destroying molecular. Immune checkpoint receptor blockade therapy may be a promising therapy<sup>73</sup>. Similar to TEX cells, T<sub>RM</sub> cells also express high immune checkpoint molecules and may act as target cells for screening immune checkpoint antibody drugs to restore anti-tumor immunity. Hence, it may be too simple to describe T cells as "exhausted" based solely on the expression of specific immune checkpoints, which may not accurately reflect the cell's state<sup>74</sup>.

### T<sub>RM</sub> Cells in Vaccine Development

The role of T<sub>RM</sub> cells in the clearance of infectious agents is well established, and T<sub>RM</sub>-mediated immunity in many tissues does not depend on the persistence of antigens and appears to be long-lived, for example, T<sub>RM</sub> cells persist in the skin for up to 2 years. Thus, T<sub>RM</sub> cells appear to meet some key expectations for future vaccine targets to provide effective cellular immunity<sup>75</sup>.

Different reports suggest that T<sub>RM</sub> cells are closely related to the immune efficacy of tumor vaccines. In some tumor models, the route of vaccination is closely related to T<sub>RM</sub> production. Tumor vaccines administered directly on the mucosa can induce large numbers of T<sub>RM</sub> cells in the mucosa compared to intramuscular or subcutaneous injections route; therefore, T<sub>RM</sub> cells may represent a new marker for detecting tumor vaccine efficacy<sup>76</sup>. In head and neck cancer mouse models, intranasal vaccination with a mucosal vector (the B subunit of Shiga toxin) can produce local T<sub>RM</sub> cells and inhibit tumor cell growth. The number of T<sub>RM</sub> cells is correlated with the overall survival of mice and blocking TGF- $\beta$  can reduce the induction of T<sub>RM</sub> by the vaccine and reduce the efficacy of the tumor vaccine<sup>77</sup>. Uncovering the different molecular characteristics of T<sub>RM</sub> cells in various tissues may help developing T<sub>RM</sub>-cell-based vaccines. It is necessary to induce enough T<sub>RM</sub> cells in the lungs to generate adequate immunity to antagonism respiratory viruses, such as SARS-CoV-2; in fact, a key advantage of T<sub>RM</sub> cells is their ability to persist for a long time and act quickly when the body is re-infected by the virus<sup>78</sup>. Lung immunization with antigenic peptides and CpG adjuvant (amph-vaccine) significantly increased vaccine accumulation in the lung and mediastinal lymph nodes compared to conventional vaccines, while inducing T<sub>RM</sub> production and immune system resistance to viral or tumor attack<sup>79</sup>. Vaccinia virus (VACV) is

a double-stranded DNA virus used initially as a vaccine against smallpox. Although smallpox has become the only disease that has been successfully eradicated by vaccination in human history, research on VACV continues. Liu et al<sup>80</sup> demonstrate that non-replicating modified vaccine Ankara virus (MVA) can induce epidermal CD8<sup>+</sup> CD103<sup>+</sup> T<sub>RM</sub> cell production *via* the skin scarification (s.s.) pathway instead of traditional injectable routes, providing effective protective immunity for the organism. After immunization, CD8<sup>+</sup> T<sub>RM</sub> cells, produced in the liver by plasmodium attenuated sporozoites, can mediate adequate protection against malaria infection<sup>81</sup>. An important strategy to induce T<sub>RM</sub> cell generation is targeting delivery by the vector. Therefore, cervicovaginal immunization with papillomavirus vectors can induce the proliferation of CD8<sup>+</sup> T<sub>RM</sub> cells in cervicovaginal mucosa<sup>82</sup>. Recombinant influenza-HIV vectors combined with intravaginal mucosal immunization can induce the production of HIV-specific CD8<sup>+</sup> T<sub>RM</sub> in mice, these HIV-specific CD8<sup>+</sup> T<sub>RM</sub> cells persist in the vaginal epithelial compartment, recognize viral antigens, and rapidly initiate an immune response and clear virus-infected cells. These cells also upregulate endothelial vascular addressing expression and subsequent recruitment of immune cells, significantly enhancing the vaginal defense against HIV infection. Vaccines that induce CD8<sup>+</sup> T<sub>RM</sub> cells in cervicovaginal mucosa are expected to produce effective immunity against sexually transmitted HIV<sup>83</sup>. The chemokines CXCL9 and CXCL10 can promote the formation of HSV-specific T<sub>RM</sub> cells in vaginal mucosa and reduce disease severity<sup>84</sup>. It is necessary to induce the generation of T<sub>RM</sub> cells in pulmonary airways and peripheral lymphoid organs against multiple respiratory pathogens invasion, pulmonary immunization with polymeric polyethyleneimine may be an effective way to induce antigen specific CD8<sup>+</sup> T<sub>RM</sub> cells to the clearance of airway pathogens. These vaccine-induced cells will effectively mediate protective immunity against cowpox and influenza viruses<sup>85</sup>. A study<sup>86</sup> evaluates the effect of commercially influenza vaccines, including inactivated influenza virus (IIV, Fluzone) and live-attenuated influenza virus (LAIV, FluMist) vaccines, IIV vaccines can produce specific neutralizing antibodies. However, lung-localized virus-specific T<sub>RM</sub> cells stimulated by LAIV vaccines can provide long-term protection for the body, suggesting that lung T<sub>RM</sub> cells are important for protecting the population from sudden influenza pandemics. LAIV-induced

CD4<sup>+</sup> T<sub>RM</sub> and virus-specific CD8<sup>+</sup> T<sub>RM</sub> can mediate an effective immune response, which is important for protecting populations from sudden influenza pandemics by strengthening lung-specific immunity<sup>86</sup>. Modified Ankara Varicella (MVA) is a recently approved smallpox vaccine. Experiments in mice confirmed that immunization by skin scratch (s.s.) is more effective than other delivery routes to antagonism cowpox virus (VACV) invasion, and that CD8<sup>+</sup> T<sub>RM</sub> induced by MVA (s.s.) performs well in protecting mice from the challenge of lethal VACV infection<sup>87</sup>.

Some SARS-CoV-2 vaccines may not be satisfactory due to viral variants, so effective boosters are required to increase immune efficacy. Experiments in mice have demonstrated that intranasal immunization with adenoviral vectors can induce mucosal IgA and lung-resident memory T<sub>RM</sub> production compared to intramuscular administration of mRNA vaccines. The enhanced mucosal immunity can protect mice from SARS-CoV-2 infection, which may be a promising approach against respiratory viral infections<sup>88</sup>. These encouraging results demonstrate that T<sub>RM</sub> cells induced in a vaccine environment may provide deep protection against infectious pathogen challenges, and this seems to be an attractive strategy.

## Conclusions

T<sub>RM</sub> cells are a unique type of memory T cells in the body's immune system that play a rapid defense response in anti-infection, an important immune system component. The high expression of immune checkpoint molecules makes T<sub>RM</sub> cells ideal candidates for immunotherapy. T<sub>RM</sub> cells play an irreplaceable role in antitumor immune responses by immunosurveillance and COVID-19. T<sub>RM</sub> cells are also an indicator for detecting vaccine efficacy that contributes to vaccines' development. The formation, maintenance, and regulation of T<sub>RM</sub> cells deserve further study.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

## Funding

This product is financially supported by the Shandong Province Health Department (grant No. 2019WS589, 2017WS407)..

## References

- 1) Netea MG, Schlitzer A, Placek K, Joosten L, Schultze JL. Innate and Adaptive Immune Memory: an Evolutionary Continuum in the Host's Response to Pathogens. *Cell Host Microbe* 2019; 25: 13-26.
- 2) Enamorado M, Khouili SC, Iborra S, Sancho D. Genealogy, Dendritic Cell Priming, and Differentiation of Tissue-Resident Memory CD8(+) T Cells. *Front Immunol* 2018; 9: 1751.
- 3) Nguyen QP, Deng TZ, Witherden DA, Goldrath A W. Origins of CD4(+) circulating and tissue-resident memory T-cells. *Immunology* 2019; 157: 3-12.
- 4) Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999; 401: 708-712.
- 5) Hogan RJ, Usherwood EJ, Zhong W, Roberts AA, Dutton RW, Harmsen AG, Woodland DL. Activated antigen-specific CD8+ T cells persist in the lungs following recovery from respiratory virus infections. *J Immunol* 2001; 166: 1813-1822.
- 6) Masopust D, Vezys V, Marzo AL, Lefrançois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001; 291: 2413-2417.
- 7) Hartana CA, Ahlen BE, Broome A, Berglund S, Johansson M. Tissue-resident memory T cells are epigenetically cytotoxic with signs of exhaustion in human urinary bladder cancer. *Clin Exp Immunol* 2018; 194: 39-53.
- 8) Teijaro JR, Turner D, Pham Q, Wherry EJ, Lefrançois L, Farber DL. Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. *J Immunol* 2011; 187: 5510-5514.
- 9) Booth JS, Toapanta FR, Salerno GR, Patil S, Kader HA, Safta AM, Czinn SJ, Greenwald BD, Szein MB. Characterization and functional properties of gastric tissue-resident memory T cells from children, adults, and the elderly. *Front Immunol* 2014; 5: 294.
- 10) Pallett LJ, Davies J, Colbeck EJ, Robertson F, Hansi N, Easom N, Burton AR, Stegmann KA, Schurich A, Swadling L, Gill US, Male V, Luong T, Gander A, Davidson BR, Kennedy P, Maini MK. IL-2(high) tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. *J Exp Med* 2017; 214: 1567-1580.
- 11) Swaims-Kohlmeier A, Haaland RE, Haddad LB, Sheth AN, Evans-Strickfaden T, Lupo LD, Cordes S, Aguirre AJ, Lupoli KA, Chen CY, Ofotukun I, Hart CE, Kohlmeier JE. Progesterone Levels Associate with a Novel Population of CCR5+CD38+CD4 T Cells Resident in the Genital Mucosa with Lymphoid Trafficking Potential. *J Immunol* 2016; 197: 368-376.
- 12) Ariotti S, Beltman JB, Chodaczek G, Hoekstra ME, van Beek AE, Gomez-Eerland R, Ritsma L, van Rheenen J, Marée AF, Zal T, de Boer RJ, Haanen JB, Schumacher TN. Tissue-resident memory CD8+ T cells continuously patrol skin epithelia to quickly recognize local antigen. *Proc Natl Acad Sci USA* 2012; 109: 19739-19744.
- 13) Bergsbaken T, Bevan MJ. Proinflammatory microenvironments within the intestine regulate the differentiation of tissue-resident CD8+ T cells responding to infection. *Nat Immunol* 2015; 16: 406-414.
- 14) Takamura S, Yagi H, Hakata Y, Motozono C, McMaster SR, Masumoto T, Fujisawa M, Chikaishi T, Komeda J, Itoh J, Umemura M, Kyusai A, Tomura M, Nakayama T, Woodland DL, Kohlmeier JE, Miyazawa M. Specific niches for lung-resident memory CD8+ T cells at the site of tissue regeneration enable CD69-independent maintenance. *J Exp Med* 2016; 213: 3057-3073.
- 15) Smolders J, Heutinck KM, Franssen NL, Remmerswaal E, Hombrink P, Ten BI, van Lier R, Huitinga I, Hamann J. Tissue-resident memory T cells populate the human brain. *Nat Commun* 2018; 9: 4593.
- 16) Wakim LM, Woodward-Davis A, Bevan MJ. Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. *Proc Natl Acad Sci* 2010; 107: 17872-17879.
- 17) Turner DL, Farber DL. Mucosal resident memory CD4 T cells in protection and immunopathology. *Front Immunol* 2014; 5: 331.
- 18) Rakhra K, Abraham W, Wang C, Moynihan KD, Li N, Donahue N, Baldeon AD, Irvine DJ. Exploiting albumin as a mucosal vaccine chaperone for robust generation of lung-resident memory T cells. *Sci Immunol* 2021; 6: eabd8003.
- 19) Son YM, Cheon IS, Wu Y, Li C, Wang Z, Gao X, Chen Y, Takahashi Y, Fu YX, Dent AL, Kaplan MH, Taylor J J, Cui W, Sun J. Tissue-resident CD4(+) T helper cells assist the development of protective respiratory B and CD8(+) T cell memory responses. *Sci Immunol* 2021; 6: eabb6852.
- 20) Iijima N, Iwasaki A. T cell memory. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. *Science* 2014; 346: 93-98.
- 21) Collins N, Jiang X, Zaid A, Macleod BL, Li J, Park CO, Haque A, Bedoui S, Heath WR, Mueller SN, Kupper TS, Gebhardt T, Carbone FR. Skin CD4(+) memory T cells exhibit combined cluster-mediated retention and equilibration with the circulation. *Nat Commun* 2016; 7: 11514.
- 22) Abou-Daya KI, Tieu R, Zhao D, Rammal R, Sarcirbegovic F, Williams AL, Shlomchik WD, Oberbarnscheidt MH, Lakkis FG. Resident memory T cells form during persistent antigen exposure leading to allograft rejection. *Sci Immunol* 2021; 6: eabc8122.
- 23) Takamura S. Niches for the Long-Term Maintenance of Tissue-Resident Memory T Cells. *Front Immunol* 2018; 9: 1214.



- 24) Behr FM, Chuwonpad A, Stark R, van Gisbergen K. Armed and Ready: Transcriptional Regulation of Tissue-Resident Memory CD8 T Cells. *Front Immunol* 2018; 9: 1770.
- 25) Bergsbaken T, Bevan MJ, Fink PJ. Local Inflammatory Cues Regulate Differentiation and Persistence of CD8(+) Tissue-Resident Memory T Cells. *Cell Rep* 2017; 19: 114-124.
- 26) Reilly EC, Lambert EK, Buckley PM, Reilly NS, Smith I, Chaves FA, Yang H, Oakes PW, Topham DJ. T(RM) integrins CD103 and CD49a differentially support adherence and motility after resolution of influenza virus infection. *Proc Natl Acad Sci USA* 2020; 117: 12306-12314.
- 27) Cibrián D, Sánchez-Madrid F. CD69: from activation marker to metabolic gatekeeper. *Eur J Immunol* 2017; 47: 946-953.
- 28) Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 2004; 427: 355-360.
- 29) Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol* 2012; 30: 69-94.
- 30) Mackay LK, Braun A, Macleod BL, Collins N, Tebartz C, Bedoui S, Carbone FR, Gebhardt T. Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. *J Immunol* 2015; 194: 2059-2063.
- 31) Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol* 2018; 11: 64.
- 32) Amsen D, van Gisbergen K, Hombrink P, van Lier R. Tissue-resident memory T cells at the center of immunity to solid tumors. *Nat Immunol* 2018; 19: 538-546.
- 33) Mani V, Bromley SK, Äijö T, Mora-Buch R, Carizosa E, Warner RD, Hamze M, Sen DR, Chasse AY, Lorant A, Griffith JW, Rahimi RA, McEntee CP, Jeffrey KL, Marangoni F, Travis MA, Lacy-Hulbert A, Luster AD, Mempel TR. Migratory DCs activate TGF- $\beta$  to precondition naïve CD8+ T cells for tissue-resident memory fate. *Science* 2019; 366: eaav5728.
- 34) Agace WW. Tissue-tropic effector T cells: generation and targeting opportunities. *Nat Rev Immunol* 2006; 6: 682-692.
- 35) Slütter B, Pewe LL, Kaech SM, Harty JT. Lung airway-surveilling CXCR3(hi) memory CD8(+) T cells are critical for protection against influenza A virus. *Immunity* 2013; 39: 939-948.
- 36) Hickman HD, Reynoso GV, Ngudiankama BF, Cush SS, Gibbs J, Bennink JR, Yewdell JW. CXCR3 chemokine receptor enables local CD8(+) T cell migration for the destruction of virus-infected cells. *Immunity* 2015; 42: 524-537.
- 37) Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, Cauley LS, Craft J, Kaech SM. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. *Immunity* 2014; 41: 633-645.
- 38) Wakim LM, Woodward DA, Liu R, Hu Y, Villadangos J, Smyth G, Bevan MJ. The molecular signature of tissue resident memory CD8 T cells isolated from the brain. *J Immunol* 2012; 189: 3462-3471.
- 39) Dunbar PR, Cartwright EK, Wein AN, Tsukamoto T, Tiger LZ, Kumar N, Uddäck IE, Hayward SL, Ueha S, Takamura S, Kohlmeier JE. Pulmonary monocytes interact with effector T cells in the lung tissue to drive T(RM) differentiation following viral infection. *Mucosal Immunol* 2020; 13: 161-171.
- 40) Chu KL, Batista NV, Girard M, Watts TH. Monocyte-Derived Cells in Tissue-Resident Memory T Cell Formation. *J Immunol* 2020; 204: 477-485.
- 41) Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* 2014; 14: 24-35.
- 42) Retamal DA, Covián C, Pacheco GA, Castiglione MA, Bueno SM, González PA, Kalergis AM. Contribution of Resident Memory CD8(+) T Cells to Protective Immunity Against Respiratory Syncytial Virus and Their Impact on Vaccine Design. *Pathogens* 2019; 8: 147.
- 43) Travis MA, Sheppard D. TGF- $\beta$  activation and function in immunity. *Annu Rev Immunol* 2014; 32: 51-82.
- 44) Mackay LK, Minnich M, Kragten NA, Liao Y, Nota B, Seillet C, Zaid A, Man K, Preston S, Freestone D, Braun A, Wynne JE, Behr FM, Stark R, Pellicci DG, Godfrey DI, Belz GT, Pellegrini M, Gebhardt T, Busslinger M, Shi W, Carbone FR, Lier RA, Kallies A, Gisbergen KP. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. *Science* 2016; 352: 459-463.
- 45) Kragten N, Behr FM, Vieira BF, Remmerswaal E, Wesselink TH, Oja AE, Hombrink P, Kallies A, van Lier R, Stark R, van Gisbergen K. Blimp-1 induces and Hobit maintains the cytotoxic mediator granzyme B in CD8 T cells. *Eur J Immunol* 2018; 48: 1644-1662.
- 46) Mackay LK, Rahimpour A, Ma JZ, Collins N, Stock AT, Hafon ML, Vega-Ramos J, Lauzurica P, Mueller SN, Stefanovic T, Tschärke DC, Heath WR, Inouye M, Carbone FR, Gebhardt T. The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin. *Nat Immunol* 2013; 14: 1294-1301.
- 47) Schwartzkopff S, Woyciechowski S, Aichele U, Flecken T, Zhang N, Thimme R, Pircher H. TGF- $\beta$  downregulates KLRG1 expression in mouse and human CD8(+) T cells. *Eur J Immunol* 2015; 45: 2212-2217.
- 48) Casey KA, Fraser KA, Schenkel JM, Moran A, Abt MC, Beura LK, Lucas PJ, Artis D, Wherry EJ, Hogquist K, Vezys V, Masopust D. Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues. *J Immunol* 2012; 188: 4866-4875.

- 49) Mohammed J, Beura L K, Bobr A, Astry B, Chicoine B, Kashem SW, Welty N E, Igyártó BZ, Wijeyesinghe S, Thompson EA, Matte C, Bartholin L, Kaplan A, Sheppard D, Bridges A G, Shlomchik WD, Masopust D, Kaplan DH. Stromal cells control the epithelial residence of DCs and memory T cells by regulated activation of TGF- $\beta$ . *Nat Immunol* 2016; 17: 414-421.
- 50) Glennie ND, Yeramilli VA, Beiting DP, Volk SW, Weaver CT, Scott P. Skin-resident memory CD4+ T cells enhance protection against *Leishmania* major infection. *J Exp Med* 2015; 212: 1405-1414.
- 51) Woon HG, Braun A, Li J, Smith C, Edwards J, Sierro F, Feng CG, Khanna R, Elliot M, Bell A, Hislop AD, Tangye SG, Rickinson AB, Gebhardt T, Britton WJ, Palendira U. Compartmentalization of Total and Virus-Specific Tissue-Resident Memory CD8+ T Cells in Human Lymphoid Organs. *PLoS Pathog* 2016; 12: e1005799.
- 52) Goplen NP, Cheon IS, Sun J. Age-Related Dynamics of Lung-Resident Memory CD8(+) T Cells in the Age of COVID-19. *Front Immunol* 2021; 12: 636118.
- 53) Cheon IS, Li C, Son YM, Goplen NP, Wu Y, Cassmann T, Wang Z, Wei X, Tang J, Li Y, Marlow H, Hughes S, Hammel L, Cox TM, Goddery E, Ayasoufi K, Weiskopf D, Boonyaratanakornkit J, Dong H, Li H, Chakraborty R, Johnson AJ, Edell E, Taylor JJ, Kaplan MH, Sette A, Bartholmai BJ, Kern R, Vassallo R, Sun J. Immune signatures underlying post-acute COVID-19 lung sequelae. *Sci Immunol* 2021; 6: k1741.
- 54) Wauters E, Van Mol P, Garg AD, Jansen S, Van Herck Y. Discriminating mild from critical COVID-19 by innate and adaptive immune single-cell profiling of bronchoalveolar lavages. *Cell Res* 2021; 31: 272-290.
- 55) Roukens A, Pothast CR, König M, Huisman W, Dalebout T, Tak T, Azimi S, Kruize Y, Hagedoorn RS, Zlei M, Staal F, de Bie FJ, van Dongen J, Arbous SM, Zhang J, Verheij M, Prins C, Does AM, Hiemstra PS, Vries J, Janse JJ, Roestenberg M, Myeni SK, Kikkert M, Yazdanbakhsh M, Heemskerk M, Smits HH, Jochems SP. Prolonged activation of nasal immune cell populations and development of tissue-resident SARS-CoV-2-specific CD8(+) T cell responses following COVID-19. *Nat Immunol* 2022; 23: 23-32.
- 56) Dai Y, Wang J, Jeong HH, Chen W, Jia P, Zhao Z. Association of CXCR6 with COVID-19 severity: delineating the host genetic factors in transcriptomic regulation. *Hum Genet* 2021; 140: 1313-1328.
- 57) Zununi VS, Hosseiniyan KS, Ahmadian E, Ardalan M. Targeting chronic COVID-19 lung injury; Tofacitinib can be used against tissue-resident memory T cells. *Biomed Pharmacother* 2022; 147: 112614.
- 58) Nguyen TH, Mcauley JL, Kim Y, Zheng MZ, Gherardin NA, Godfrey DI, Purcell DF, Sullivan LC, Westall GP, Reading PC, Kedzierska K, Wakim LM. Influenza, but not SARS-CoV-2, infection induces a rapid interferon response that wanes with age and diminished tissue-resident memory CD8(+) T cells. *Clin Transl Immunology* 2021; 10: e1242.
- 59) Goliwas KF, Wood AM, Simmons CS, Khan R, Khan SA, Wang Y, Berry JL, Athar M, Mobley JA, Kim YI, Thannickal VJ, Harrod KS, Donahue JM, Deshane JS. Local SARS-CoV-2 Peptide-Specific Immune Responses in Lungs of Convalescent and Uninfected Human Subjects. *medRxiv [Preprint]* 2022: 2021.09.02.21263042.
- 60) Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004; 22: 329-360.
- 61) Park SL, Buzzai A, Rautela J, Hor JL, Hochheiser K, Effern M, Mcbain N, Wagner T, Edwards J, Mcconville R, Wilmott JS, Scolyer RA, Tüting T, Palendira U, Gyorki D, Mueller SN, Huntington ND, Bedoui S, Hölzel M, Mackay LK, Waithman J, Gebhardt T. Tissue-resident memory CD8(+) T cells promote melanoma-immune equilibrium in skin. *Nature* 2019; 565: 366-371.
- 62) Edwards J, Wilmott JS, Madore J, Gide TN, Quek C, Tasker A, Ferguson A, Chen J, Hewaventti R, Hersey P, Gebhardt T, Weninger W, Britton WJ, Saw R, Thompson JF, Menzies AM, Long GV, Scolyer RA, Palendira U. CD103(+) Tumor-Resident CD8(+) T Cells Are Associated with Improved Survival in Immunotherapy-Naïve Melanoma Patients and Expand Significantly During Anti-PD-1 Treatment. *Clin Cancer Res* 2018; 24: 3036-3045.
- 63) Milner JJ, Toma C, Yu B, Zhang K, Omilusik K, Phan A T, Wang D, Getzler AJ, Nguyen T, Crotty S, Wang W, Pipkin ME, Goldrath AW. Runx3 programs CD8(+) T cell residency in non-lymphoid tissues and tumours. *Nature* 2017; 552: 253-257.
- 64) Park SL, Gebhardt T, Mackay LK. Tissue-Resident Memory T Cells in Cancer Immunosurveillance. *Trends Immunol* 2019; 40: 735-747.
- 65) Webb JR, Milne K, Watson P, Deleeuw RJ, Nelson BH. Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated with increased survival in high-grade serous ovarian cancer. *Clin Cancer Res* 2014; 20: 434-444.
- 66) Wang ZQ, Milne K, Derocher H, Webb JR, Nelson BH, Watson PH. CD103 and Intratumoral Immune Response in Breast Cancer. *Clin Cancer Res* 2016; 22: 6290-6297.
- 67) Wang B, Wu S, Zeng H, Liu Z, Dong W, He W, Chen X, Dong X, Zheng L, Lin T, Huang J. CD103+ Tumor Infiltrating Lymphocytes Predict a Favorable Prognosis in Urothelial Cell Carcinoma of the Bladder. *J Urol* 2015; 194: 556-562.
- 68) Marceaux C, Weeden CE, Gordon CL, Asselin-Labat ML. Holding our breath: the promise of tissue-resident memory T cells in lung cancer. *Transl Lung Cancer Res* 2021; 10: 2819-2829.
- 69) Djenidi F, Adam J, Goubar A, Durgeau A, Meurice G, De MV, Validire P, Besse B, Mami CF. CD8+CD103+ tumor-infiltrating lymphocytes are tumor-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. *J Immunol* 2015; 194: 3475-3486.

- 70) Byrne A, Savas P, Sant S, Li R, Virassamy B, Luen S J, Beavis PA, Mackay LK, Neeson PJ, Loi S. Tissue-resident memory T cells in breast cancer control and immunotherapy responses. *Nat Rev Clin Oncol* 2020; 17: 341-348.
- 71) Han L, Gao QL, Zhou XM, Shi C, Chen GY, Song YP, Yao YJ, Zhao YM, Wen XY, Liu SL, Qi YM, Gao YF. Characterization of CD103(+) CD8(+) tissue-resident T cells in esophageal squamous cell carcinoma: may be tumor reactive and resurrected by anti-PD-1 blockade. *Cancer Immunol Immunother* 2020; 69: 1493-1504.
- 72) Lin R, Zhang H, Yuan Y, He Q, Zhou J, Li S, Sun Y, Li DY, Qiu HB, Wang W, Zhuang Z, Chen B, Huang Y, Liu C, Wang Y, Cai S, Ke Z, He W. Fatty Acid Oxidation Controls CD8(+) Tissue-Resident Memory T-cell Survival in Gastric Adenocarcinoma. *Cancer Immunol Res* 2020; 8: 479-492.
- 73) Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, Drake AM, Chen Z, Sen DR, Kurachi M, Barnitz RA, Bartman C, Bengsch B, Huang AC, Schenkel JM, Vahedi G, Haining WN, Berger SL, Wherry E J. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 2016; 354: 1160-1165.
- 74) Duhon T, Duhon R, Montler R, Moses J, Moudgil T, Miranda NF, Goodall CP, Blair TC, Fox BA, McDermott J E, Chang SC, Grunkemeier G, Leidner R, Bell RB, Weinberg AD. Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors. *Nat Commun* 2018; 9: 2724.
- 75) Lian CG, Bueno EM, Granter SR, Laga AC, Saavedra AP, Lin WM, Susa JS, Zhan Q, Chandraker AK, Tullius SG, Pomahac B, Murphy GF. Biomarker evaluation of face transplant rejection: association of donor T cells with target cell injury. *Mod Pathol* 2014; 27: 788-799.
- 76) Dumauthioz N, Labiano S, Romero P. Tumor Resident Memory T Cells: New Players in Immune Surveillance and Therapy. *Front Immunol* 2018; 9: 2076.
- 77) Nizard M, Roussel H, Diniz MO, Karaki S, Tran T, Voron T, Dransart E, Sandoval F, Riquet M, Rance B, Marcheteau E, Fabre E, Mandavit M, Terme M, Blanc C, Escudie J B, Gibault L, Barthes F, Granier C, Ferreira L, Badoual C, Johannes L, Tartour E. Induction of resident memory T cells enhances the efficacy of cancer vaccine. *Nat Commun* 2017; 8: 15221.
- 78) Christo SN, Evrard M, Park SL, Gandolfo LC, Burn TN, Fonseca R, Newman DM, Alexandre YO, Collins N, Zamudio NM, Souza GF, Pellicci DG, Chisanga D, Shi W, Bartholin L, Belz GT, Huntington ND, Lucas A, Lucas M, Mueller SN, Heath WR, Ginhoux F, Speed TP, Carbone FR, Kallies A, Mackay LK. Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity. *Nat Immunol* 2021; 22: 1140-1151.
- 79) Ichikawa T, Hirahara K, Kokubo K, Kiuchi M, Aoki A, Morimoto Y, Kumagai J, Onodera A, Mato N, Tumes D J, Goto Y, Hagiwara K, Inagaki Y, Sparwasser T, Tobe K, Nakayama T. CD103(hi) T(reg) cells constrain lung fibrosis induced by CD103(lo) tissue-resident pathogenic CD4 T cells. *Nat Immunol* 2019; 20: 1469-1480.
- 80) Liu L, Zhong Q, Tian T, Dubin K, Athale SK, Kupper TS. Epidermal injury and infection during poxvirus immunization is crucial for the generation of highly protective T cell-mediated immunity. *Nat Med* 2010; 16: 224-227.
- 81) Fernandez RD, Ng WY, Holz LE, Ma JZ, Zaid A, Wong YC, Lau LS, Mollard V, Cozijnsen A, Collins N, Li J, Davey GM, Kato Y, Devi S, Skandari R, Pauley M, Manton JH, Godfrey DI, Braun A, Tay SS, Tan PS, Bowen DG, Koch NF, Rissiek B, Carbone FR, Crabb BS, Lahoud M, Cockburn IA, Mueller SN, Bertolino P, Mcfadden GI, Caminschi I, Heath WR. Liver-Resident Memory CD8(+) T Cells Form a Front-Line Defense against Malaria Liver-Stage Infection. *Immunity* 2019; 51: 780.
- 82) çuburu N, Graham BS, Buck CB, Kines RC, Pang YY, Day PM, Lowy DR, Schiller JT. Intravaginal immunization with HPV vectors induces tissue-resident CD8+ T cell responses. *J Clin Invest* 2012; 122: 4606-4620.
- 83) Tan HX, Wheatley AK, Esterbauer R, Jegaskanda S, Glass JJ, Masopust D, De RR, Kent SJ. Induction of vaginal-resident HIV-specific CD8 T cells with mucosal prime-boost immunization. *Mucosal Immunol* 2018; 11: 994-1007.
- 84) Shin H, Iwasaki A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature* 2012; 491: 463-467.
- 85) Bivas BM, Gillard GO, Bar L, White KA, Webby RJ, Hovav AH, Letvin NL. Airway CD8(+) T cells induced by pulmonary DNA immunization mediate protective anti-viral immunity. *Mucosal Immunol* 2013; 6: 156-166.
- 86) Zens KD, Chen JK, Farber DL. Vaccine-generated lung tissue-resident memory T cells provide heterosubtypic protection to influenza infection. *JCI Insight* 2016; 1: e85832.
- 87) Pan Y, Liu L, Tian T, Zhao J, Park CO, Lofftus SY, Stingley CA, Yan Y, Mei S, Liu X, Kupper TS. Epicutaneous immunization with modified vaccinia Ankara viral vectors generates superior T cell immunity against a respiratory viral challenge. *NPJ Vaccines* 2021; 6: 1.
- 88) Lapuente D, Fuchs J, Willar J, Vieira AA, Eberlein V, Uhlig N, Issmail L, Schmidt A, Oltmanns F, Peter AS, Mueller SS, Irrgang P, Fraedrich K, Cara A, Hoffmann M, Pöhlmann S, Ensser A, Pertl C, Willert T, Thirion C, Grunwald T, Überla K, Tenbusch M. Protective mucosal immunity against SARS-CoV-2 after heterologous systemic prime-mucosal boost immunization. *Nat Commun* 2021; 12: 6871.