



Legend of the Sentinels: Development of Lung Resident Memory T Cells and Their Roles in Diseases

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SARS-CoV-2 is wreaking havoc around the world. To get the world back on track, hundreds of vaccines are under development. A deeper understanding of how the immune system responds to SARS-CoV-2 re-infection will certainly help. Studies have highlighted various aspects of T cell response in resolving acute infection and preventing re-infections. Lung resident memory T (T_{RM}) cells are sentinels in the secondary immune response. They are mostly differentiated from effector T cells, construct specific niches and stay permanently in lung tissues. If the infection recurs, locally activated lung T_{RM} cells can elicit rapid immune response against invading pathogens. In addition, they can significantly limit tumor growth or lead to pathologic immune responses. Vaccines targeting T_{RM} cells are under development, with the hope to induce stable and highly reactive lung T_{RM} cells through mucosal administration or “prime-and-pull” strategy. In this review, we will summarize recent advances in lung T_{RM} cell generation and maintenance, explore their roles in different diseases and discuss how these cells may guide the development of future vaccines targeting infectious disease, cancer, and pathologic immune response.

Keywords: tissue-resident memory T cells, lung, infection, asthma, cancer, vaccine

INTRODUCTION

The COVID-19 pandemic is ravaging the world. By the end of November 2020, there are over 60 million cumulative cases globally, and the number of deaths has exceeded one million (1). This disease is caused by SARS-CoV-2, which is mainly transmitted through air-borne droplets, leading to severe pulmonary diseases and systemic damage (2). Up to now, the treatment for COVID-19 is very limited, and no specific antiviral drug has been developed. Multiple candidate COVID-19 vaccines are undergoing clinical trials (3).

In general, most COVID-19 vaccines in clinical trials focus on humoral immunity, which exerts antibodies to prevent the virus from invading cells. However, antibodies alone may not be sufficient to prevent SARS-CoV-2 infection. One reason is that extracellular antibodies cannot completely clear the cells infected by virus (4). The final elimination of the virus depends on the supplement of cellular immunity, that is, the role of T cells, which help B cells produce neutralizing antibodies and can directly kill virus-infected cells. The second is that the memory B cell response tends to be short-

lived (5), whereas the T cell response can last for many years. Recent researches have demonstrated that patients who recovered from the severe acute respiratory syndrome (SARS) still had long-lasting memory T-cells but reduced antibody responses (6, 7). Therefore, vaccines against SARS-CoV-2 should focus on activating the adaptive branch of the immune system and explicitly focus on inducing long-term memory T cells. Given that many respiratory viruses are controlled by tissue immune cells that may not be present in the blood, the tissue-resident memory T (T_{RM}) cells infiltrated in the lungs that can recognize foreign antigens locally and provide a rapid immune response will be an area of concern.

Actually, CD8+T cells retained for a long time after influenza virus infection were observed in mouse lungs as early as 2001 (8). Extensive studies in mouse models have determined that the lungs are enriched in T_{RM} cells against a variety of viral and bacterial antigens brought by respiratory infections or vaccination. Specific T_{RM} cells were also detected in the respiratory tract of patients with influenza or tuberculosis (TB) (9). These pathogen-specific T_{RM} cells produced by prior exposure can control acute re-infections and achieve long-term immunity. In mouse model, an intranasal recombinant vaccinia virus boosting regimen has generated SARS-CoV-specific lung resident memory CD8+T cells. When re-stimulated, these T_{RM} cells can effectively release a variety of effector cytokines and cytotoxic molecules that prevent extensive virus replication and limit the alveolar damage (10). Another study suggested that the administration of SARS vaccine intranasally induced CD4+ T_{RM} cells in the respiratory tract of mice, which offered the protective immunity against death (11). Regarding SARS-CoV-2, recent published single-cell profiles have indicated that the CD8+ T cells in bronchoalveolar lavage fluids (BALFs) of patients with severe infection exhibited a less proportion of tissue-resident phenotypes than those in moderately infected patients (12). Hence a vaccine that induces the production of lung T_{RM} cells is an ideal candidate for generating a strong and rapid immune response against SARS-CoV-2.

There are other T_{RM} cells in the lungs with different roles, including T_{RM} cells that may cause pathological immune responses and tumor-infiltrating T_{RM} cells that can enhance anti-tumor immunity in the lungs (13). These T_{RM} cells under different immune microenvironment in the lungs act in various roles in immune defense, immune homeostasis, and immune surveillance. An in-depth understanding of the generation and maintenance of lung T_{RM} cells will provide new insights for the development of novel vaccine formation and delivery strategies and lung-specific immunoregulatory therapy.

This review will focus on the definition, generation, and different roles of lung T_{RM} cells in infection, pathological immune responses, and cancers, and discuss T_{RM} cell-related vaccination strategies combined with emerging cutting-edge discoveries.

HALLMARKS OF T_{RM} CELLS

T_{RM} cells, also known as non-circulating memory T cells, include both CD8+ and CD4+ subgroups. It refers to those memory T

cells that occupy long-term residency in local tissues such as lung, intestine, and skin. Through cell labeling, parabiosis, tissue transplantation, and other methods, the circulation trajectory of cells can be observed to determine T_{RM} cells (14–16). However, it is still a challenge to clearly distinguish T_{RM} cells from other cells *in vitro* by surface markers.

In recent years, with the development of transcriptomics, T_{RM} cells have been found to have unique transcriptional profiles and functional characteristics. The main hallmarks of T_{RM} cells that distinguish it from other circulating memory T cells are the ability to adhere to peripheral tissues and the lack of homing signals. Based on the research on both mouse and humans, the most used phenotypic marker defining T_{RM} cell subsets is CD69. Due to the competitive protein-protein interaction between CD69 and sphingosine-1-P receptors (S1PR), it inhibits the expression of S1PR and prevents S1P-mediated egress (17, 18). These cells also lack CD62L and CC-chemokine receptor 7 (CCR7), both of which direct cells into lymphoid tissue (19). On the flip side, CD44 up-regulated by T_{RM} cells is the receptor for hyaluronic acid and other ligands expressed in peripheral tissues, which can induce the retention of memory T cells in peripheral tissues (20). As another key T_{RM} cell marker, the integrin $\alpha E:\beta 7$ (CD103) is mainly expressed on CD8+ T_{RM} cells and some on CD4+ T_{RM} cells, which binds E-cadherin and anchors cells around epithelial cells (21). It is worth noting that T_{RM} cells in lungs can be defined by several major surface markers, but this subset itself is still heterogeneous in some way. The transcriptome analysis reveals the inconsistent changes in gene expression among different cells (19, 22, 23). Further elucidation of detailed mechanism of T_{RM} cell formation and maintenance will add to understanding of the phenotype of lung T_{RM} cells under different pathophysiological conditions.

DEVELOPMENT OF LUNG T_{RM} CELLS

The development of lung T_{RM} cells can be divided into several steps: 1) activation in lymphoid tissues and migration into inflammatory lung tissue guided by local cytokines, 2) expression of homing molecules and specific transcription factors and differentiation into lung resident memory T cells, 3) local maintenance in specific niches and replenishment from T_{CM} cells (**Figure 1**). So far, the focus on specific transcription factors and cell surface receptors has gradually revealed details in the fate determination mechanism of lung T_{RM} cells.

Activation and Migration

The inability to recirculate between lung and lymph nodes or bloodstream is a key determinant of lung T_{RM} cells (24, 25). However, these cells did not start in the lung tissue but migrated into it later. Under normal conditions, naïve T cells consecutively circulate throughout the body. When infection occurs, dendritic cells (DCs) migrate from infected respiratory sites into mediastinal lymph nodes (MdLN) and activate naïve T cells. Among these migrant DCs there are two subsets, and only airway localized CD103+ DCs can fully induce the differentiation of

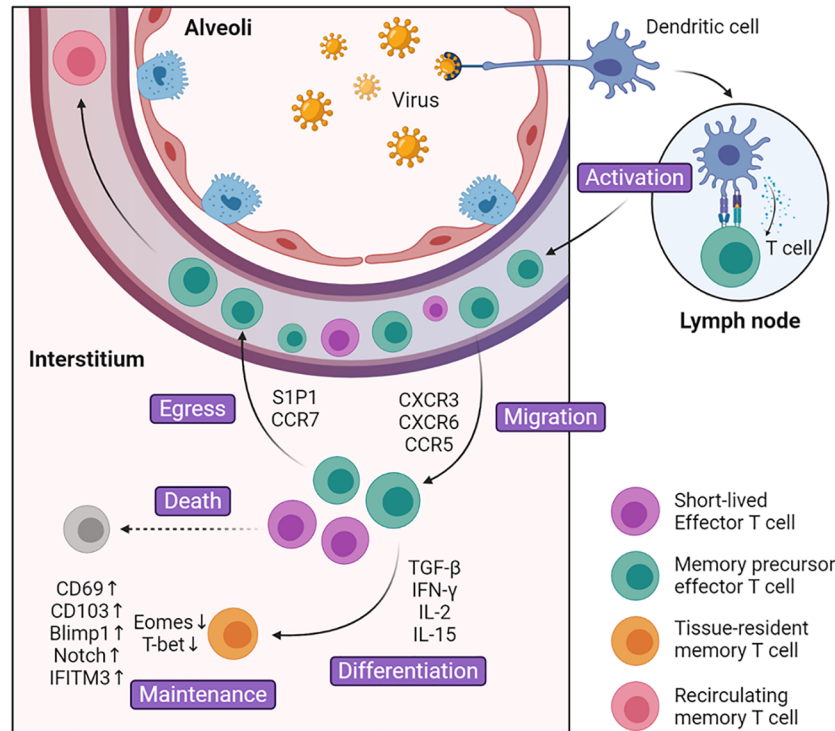


FIGURE 1 | Generation and maintenance of lung T_{RM} cells. During the activated phase of infection, dendritic cells present antigens to activate naïve T cells in the lymph nodes. These cells turn into effector T cells and up-regulate surface marker CXCR3, CXCR6, CCR5, which guide them into inflammatory tissues. After entering lung tissue, part of effector T cells is regulated by environmental signals including cytokines such as TGF-β and cognate antigens, and differentiate into lung T_{RM} cells. The rest of the effector T cells undergoes cell death or egress out of the lung. Compared with T_{eff} cells, lung T_{RM} cells manipulate multiple surface markers and transcription factors that facilitate cell maintenance and survival.

naïve T cells into T_{eff} cells (26). Once activated, the T_{eff} cells up-regulate the expression of CXCR3, CCR5, and CCR4, which specifically guide T_{eff} cells into lung tissue and help control pathogen invasion (27–31). For example, after TB infection, chemokine ligand IP-10 in the lung increases significantly, which binds to CXCR3 and facilitates T cell migration (29). In addition, CD8+ and CD4+ lung T_{eff} cells are regulated differently and tend to localize in different regions. CD8+ T_{eff} cells are inclined to migrate to the collagen IV-rich region and CD4+ T_{eff} cells are more prone to be located in areas abundant in collagen I (32). Compared with CD8+ T cells, CD4+ T cells enter the lung tissues first and direct the localization of CD8+ T cells. CD4+ T cells fine-tune chemokine gradients in the microenvironment such as TGF-β, which promotes the production of CD103 and is crucial for CD8+ T_{RM} cell formation (33).

Differentiation

T_{eff} cells will not transform into lung T_{RM} cells immediately after entering the lung tissues. The tissue microenvironment has an important influence on the development of lung T_{RM} cells. In the early stage of infection, T_{eff} cells that migrate into the infection site will encounter redundant inflammatory signals, which guide T_{eff} cells towards terminal T_{eff} cells (34). They reduce local inflammation, help remold the microenvironment and make it

more appropriate for the differentiation of lung T_{RM} cells. In the later stage, CD8+ T cells are recruited into tissue damage sites, which later developed into regenerative tissues termed as repair-associated memory depots (RAMDs). RAMDs provide environmental cues that help drive CD8+ T_{eff} cells into CD8+ T_{RM} cells and later become niches for CD8+ T_{RM} cells (35, 36). Predominant environmental cues include cytokines such as TGF-β, IL-33, TNF, IFN-γ, IL-15, and cognate antigens (18, 33, 37). TGF-β plays an important role in promoting the expression of T_{RM} cell marker CD103 and CD69. Together with IL-33 and TNF, TGF-β can provoke KLF2 down-regulation, which further down-regulates its target protein S1P1 and increases expression of CD69 (18). Furthermore, TGF-β down-regulate T-box transcriptional factor and promote the expression of CD103. T-box transcriptional factors are composed of eomesodermin (Eomes) and T-bet, and they vary in the degree of decline. While Eomes is effectively removed, T_{RM} cells maintain residual levels of T-bet which is important for T_{RM} cell survival (37). The decrease in production of T-box transcriptional factor is demonstrated in mature lung CD8+CD103+ T_{RM} cells (33, 37). Unlike CD8+ T_{RM} cells in other tissues like skin and vagina, where they can be generated with only local inflammatory signals (38), lung CD8+ T_{RM} cells must interact with cognate antigen before

differentiation. After the exposure to cognate antigen, CD8+ T_{eff} cells increase the expression of CD69, CD103, and collagen-binding integrin VLA-1 (39). T cell receptor (TCR) signaling can also induce Blimp-1 expression, which biased CD8+ T_{eff} cell differentiation towards T_{RM} cells rather than T_{CM} cells (40). It is surprising that pulmonary monocytes and type 1 regulatory T (T_{reg}) cells also contribute to the differentiation. Pulmonary monocytes are the major cells to present pathogen antigens, while type 1 T_{reg} cells promote the bioavailability of TGF- β (41, 42). As mentioned above, CD4+ T_{RM} cells have different development pathways compared with CD8+ T_{RM} cells. CD4+ T_{RM} cells express different cell markers and are affected by different cytokines (43). They have low expression of CD103, and their generation is not interfered by TGF- β , which has a great impact on the generation of CD8+ T_{RM} cell (44, 45). Beyond that, IL-2 and IL-15 were found to affect the differentiation of CD4+ T_{eff} cells in different subsets, respectively (44). Researches on differentiation of CD4+ T_{RM} cells are not as thorough as those on CD8+ T_{RM} cells, and there are still many points to be clarified.

Maintenance

While persisting in lung tissues, CD8+ and CD4+ T_{RM} cells will construct different structures that contribute to long-term survival. Most CD8+ T_{RM} cells reside in specific niches we refer to as RAMDs, which are constructed by tissue regeneration after tissue damage. These niches are significant for lung CD8+ T_{RM} cells. They may present cytokines that help lung CD8+ T_{RM} cell maintenance. Considering that the recovery of tissue damage takes a long time, the lung CD8+ T_{RM} cells may protect this vulnerable part from secondary infection (35, 36). Unlike CD8+ T_{RM} cells, lung CD4+ T_{RM} cells combine with B cells and other cells to form ectopic lymphoid tissue called inducible bronchus-associated lymphoid tissue (iBALT) that benefits cell survival. In iBALT, CD4+ T_{RM} cells surround B cell follicles, which facilitate rapid interaction with each other and provide a recall response toward potential infection (43, 46). Compared with circulating T_{EM} cells, lung T_{RM} cells displayed different patterns of genes and transcription factors that regulate the expression of cytokine receptors and adhesion molecules, most of which have been mentioned above. Single-cell sequencing found an important transcription factor Notch, which controls the expression of CD103 and the basic metabolic function of lung T_{RM} cells (47). The absence of Notch greatly reduces the population of lung T_{RM} cells. Another study indicated that lung T_{RM} cells were programmed to express IFITM3, which can protect them from secondary infection and improve survival (48). Except for cytokines and surface molecules, M1^{hot} tumor-associated macrophages can also contribute to the maintenance of lung T_{RM} cells in tumor, possibly due to reduction in nutrition competition (49). In comparison with other tissue T_{RM} cells that may persist for a long time or even a lifetime, lung T_{RM} cells gradually disappear 4–5 months after infection. Lung T_{RM} cells that reside in the airway quickly decline due to the harsh environment, where amino acid starvation triggers the integrated stress response,

leading to cell apoptosis (50). And those retained in the parenchyma decrease along with the shrink of RAMDs. After full regeneration, most of the RAMDs will disappear, and only a minority of lung CD8+ T_{RM} cells may survive in iBALTs (35, 36). In order to compensate for the constant loss, airway T_{RM} cells are replaced primarily by recruitment from lung interstitium (51), and T_{RM} cells in interstitium receive continuous replenishment from circulating T_{EM} cells. T_{EM} cells are recruited and transformed into lung T_{RM} cells under the influence of TGF- β , IL-33, and TNF but antigen-independently. However, T_{EM} cells gradually lose their ability to migrate and convert into lung T_{RM} cells after infection (52). All in all, T_{RM} cells can only provide a short period of protection, which leaves the lung much more susceptible to further infection. However, this may be a designed mechanism for the prevention of pathological immune response.

LUNG T_{RM} CELLS AGAINST INFECTION

The lungs and respiratory tract, as part of direct access to the outside world, are easily exposed to various pathogens. Common pulmonary pathogens include influenza virus, respiratory syncytial virus (RSV), as well as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Bordetella pertussis*, and *Mycobacterium tuberculosis*. Under normal circumstances, the first infection caused by these pathogens will not only be cleared by the body's immune system but also induce memory T cells, some of which settle in the lungs as T_{RM} cells (Figure 2).

A large aggregation of studies has shown that the lung is rich in T_{RM} cells specific to a variety of pathogens such as viruses and bacteria. These T_{RM} cells have the potential to mediate immunity against different pathogens and protect the body from re-infection. It has been demonstrated that influenza-specific T_{RM} cells exhibited rapid and robust IFN- γ and TNF- α responses after restimulation *in vitro* (53, 54). In human RSV challenge model, cells with T_{RM} phenotype can be detected in BALFs, and the higher frequency of RSV-specific CD8+ T_{RM} is related to the decrease in the severity of disease and the viral load (55). CD4+ T_{RM} cells accumulate in the lungs after *Bordetella pertussis* infection. These cells are pathogen-specific and can secrete IL-17 and/or IFN- γ . A research observed that mice treated with the S1P antagonist Fingolimod (FTY720) to prevent lymphocyte migration into the lungs before initial infection with *Bordetella pertussis* were significantly more severely affected in the later stages of infection. However, in the case of re-infection, because the tissue-infiltrated T_{EM} cells have partially transformed into T_{RM} cells in the lung, they are not affected by Fingolimod treatment and can still quickly clear the bacillus. At the same time, the adoptive transfer of CD4+ T_{RM} cells from the lungs of mice in convalescence to uninfected mice can protect the latter from pathogens attack (56). All these evidences indicate that T_{RM} cells act as a pivotal role in the rapid response of secondary infection.

However, while T_{RM} cells eliminate invasive pathogens, the released proinflammatory factors such as IFN- γ or perforin and granzymes may damage normal cells, cause lung injury and lead

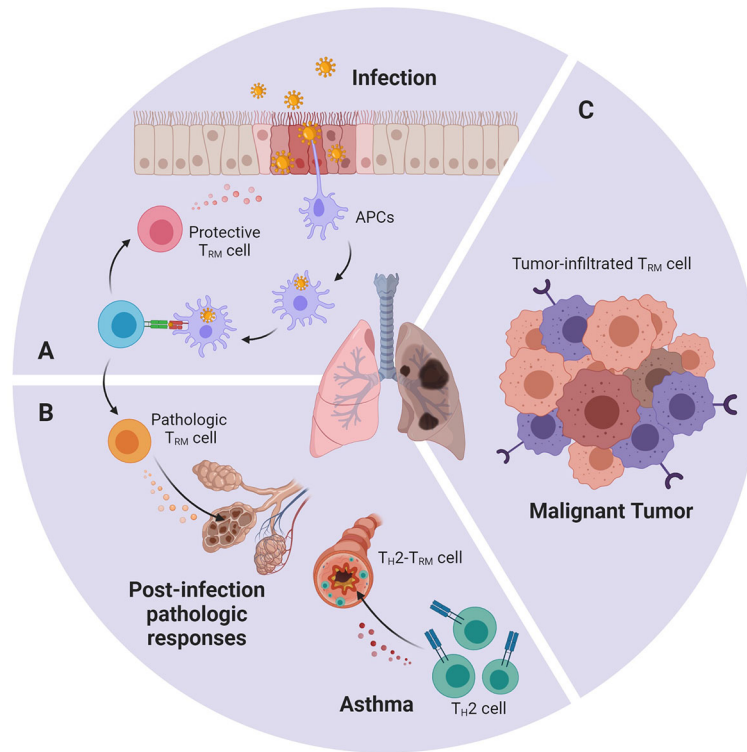


FIGURE 2 | An abstract figure of the role of T_{RM} cells in various lung diseases. Lung T_{RM} cells can: **(A)** rapidly respond towards invasive pathogens during re-infection, **(B)** cause pathologic immune response after overactivated by environmental stimuli or allergen **(C)** infiltrate in lung tumor and express cytotoxic molecules and effector cytokines.

to emphysema or fibrosis, even result in ARDS. Hence, an effective immune response to these infections requires precise immune regulation to eliminate pathogens while protecting the function of normal lung tissue. Many mechanisms exist in the lung to restrict the inflammatory response to acute infection, including inhibitory receptors, immunomodulatory molecules and cells like FOXP3+CD4+ T_{reg} cells (57). Under stable conditions, a large number of T_{reg} cells is reserved in the lung and IL-10 expression is significantly increased after influenza infection (58). In RSV-infected mice, the TCR of T_{reg} cells can specifically recognize the viral epitope-MHC II complex. Immunization of mice with this epitope can reduce clinical manifestations and immunopathology without virus clearance defects (59). In addition, PD-L1 and PD-L2 are expressed in alveolar epithelial cells and are significantly up-regulated to control inflammation in RSV infection (60). However, some studies held that this may limit the formation and development of T_{RM} cells and cause negative effects (61). The detailed mechanisms of lung T_{RM} cell function and immune homeostasis are not yet fully understood, and future improvement in the number and stability of T_{RM} cell population must be carried out on the premise that prevents re-infection of the virus and does not impair the respiratory health of the host.

LUNG T_{RM} CELLS IN PATHOLOGIC IMMUNE RESPONSE

As mentioned above, sometimes T_{RM} cells may cease to be the protector and become part of the destructor, and thus attack normal tissue and induce chronic inflammatory diseases (13) (**Figure 2**). After acute influenza infection, antigen deposits in the lung for 2–3 months. In young mice, the persistent presentation of the antigens may induce part of the T_{RM} cells to exhibit exhausted-like phenotype. This phenotype is thought to help maintain lung's immune balance and prevent damage. If PD-L1 antibody is used to blockade PD-L1 and PD-1 interaction, exhausted-like T_{RM} cells would rejuvenate, express more cytokines, and enhance their heterogeneous protection against infection. But they would also cause pulmonary pathological change and fibrosis (62). In elderly mice, increased expression of TGF- β in the environment led to accumulation of T_{RM} cells in the lungs. However, these T_{RM} cells have low effector activity due to intrinsic defects and fail to enhance the protective function, but can instead lead to chronic inflammation and fibrotic sequela (63). Also, it has been discovered that T_{H2} - T_{RM} cells are closely related to asthma (64). They release specific cytokines that recruit eosinophils and maintain mast cells in the airway, which result in

the inflammatory response. Using a mouse model exposed to house dust mite (HDM), T_{H2} - T_{RM} cells that specifically respond to HDM are identified. These T_{H2} - T_{RM} cells are developed from HDM-specific $CD4^+$ T_{eff} cells and are mediated by IL-2 signaling. IL-2 up-regulates chemokine receptors such as CCR4 and CXCR3 that improve migration into the lung, as well as programs related to tissue intention (64). A recently published paper further reports that these T_{H2} - T_{RM} cells highly express CD44 and ST2, and can reside in lung tissue and maintain their memory towards allergen for the whole life of a mouse (65). Once re-exposed to allergen, T_{H2} - T_{RM} cells robustly proliferate near airways, produce type 2 cytokines, enhance eosinophil activation, and promote peribronchial inflammation. They together with circulating memory T_{H2} cells perform nonredundant function in the induction of asthma (66, 67).

LUNG T_{RM} CELLS IN ANTI-TUMOR IMMUNITY

Accumulating evidence suggests that T_{RM} cells are important in anti-tumor immunity (Figure 2). It is suggested that a part of the tumor-infiltrating lymphocytes (TILs) isolated from several cancers displays a similar transcriptomic and phenotypic feature with T_{RM} cells. Some refer to it as T_{RM} -like TILs (9), but here we still call it “lung tumor T_{RM} cells”, as the consensus in most articles. These lung tumor T_{RM} cells predict a better survival outcome in early-stage non-small-cell lung carcinoma (NSCLC) patients, as well as increased intraepithelial lymphocyte infiltration (68). Single-cell and bulk transcriptomic analysis reveals that lung tumor T_{RM} cells have slightly different transcriptomes compared with other lung T_{RM} cells. They express similar surface marker CD103, CD69, CD49a, and they also up-regulate Notch and Runx3. But lung tumor T_{RM} cells express more cell cycle-related genes, such as CD39, CXCL13, CCL3, and TNFSF4, indicating that they belong to a new subset (22). Comparing samples from different lung cancer patients, the T_{RM} cells of advanced lung cancer are mostly exhausted, while the function of early-stage lung tumor T_{RM} cells is relatively heterogeneous (69). Among them, $CD103^+CD8^+$ T_{RM} cells are found to release more cytokines, proliferate faster, and exhibit better anti-tumor performance (70). It is described that CD103 can connect with E-cadherin on tumor cells, which induces cytotoxic granule polarization at the immune synapses (71, 72). CD103 also facilitates T_{RM} cells to reside near tumor tissues (73). In contrast with previous studies, lung tumor T_{RM} cells show the diffuse expression of inhibitory receptors, but do not exhibit the exhausted phenotype. And instead, transcription factor Eomes is found to negatively correlate with T_{RM} cell function (69, 74). Single-cell analysis even discovered a $PD-1^+TIM-3^+IL-7R^-$ T_{RM} cell subset expresses high levels of inhibitory receptors, but remains the ability to proliferate rapidly *in situ* and displays enhanced capacity to express key cytotoxic molecules and effector cytokines (22). Since $TIM-3^+IL-7R^-$ T_{RM} cells are the major cells expressing PD-1, and $CD103^+CD8^+$ T_{RM} cells show positive responses towards anti-PD-1 and anti-PD-L1 monoclonal antibodies, the researchers believe that these cells may be the

major subset that reacts in anti-PD-1 therapy (22, 68, 70). In combination with the performance of T_{RM} cells in different stages of lung cancer, it has been speculated that T_{eff} cells were influenced by tumor antigens and cytokines such as TGF- β , up-regulate CD39 and CD103, and converted into $CD103^+$ T_{RM} cells. They exercise their anti-tumor function diligently. If, for one reason or another, the tumor is not eliminated, the local microenvironment as well as the repetitive TCR stimulation may trigger their exhaustion program and they finally become hypofunctional T_{RM} cells (69, 75).

VACCINATION STRATEGIES INDUCING LUNG T_{RM} CELLS

The growing literature that considers T_{RM} cells are indispensable in eliminating infectious pathogens and controlling tumor progression has led to increasing interest in the induction of T_{RM} cells by vaccination for disease treatment and prevention. Compared with circulating T cells or B cells, activated T_{RM} cells are more focused in killing virus-infected cells in target tissues, which help complement neutralizing antibodies and reduce antibodies titer threshold needed to control virus (4, 76, 77).

There are two main strategies to establish T_{RM} cell pool within lung tissues. The first approach applies a one-step method to directly induce antigen-specific lung T_{RM} cells by vaccine vectors (78, 79). For this approach, the route of immunization is very important. Direct intranasal or intrapulmonary route provides better protection compared with commonly used intraperitoneal, intramuscular, or subcutaneous administration route (80, 81). Intranasal administration but not injection of live-attenuated influenza virus has shown the capacity to generate long-term $CD4^+$ and $CD8^+$ T_{RM} cells and provide heterosubtypic protection to nonvaccine influenza strains in mice (82). Intratracheal and intranasal rather than subcutaneous inoculation of Bacille Calmette-Guérin (BCG) also results in generation of T_{EM} and T_{RM} cells in the lung, which remedy the low efficacy of parenteral BCG vaccination to prevent pulmonary TB (83). In a preclinical head and neck cancer model, local T_{RM} cells can be induced and tumor growth can be controlled in mice immunized with the cancer vaccine (STxB-E7) by intranasal route (84). Another approach is a two-step method that combines conventional elicitation of systemic T cell response with the recruitment of these cells into target tissues, which are referred to as “prime and pull” (85). Actually, in a very early stage, scientists have discovered that mucosal boosting with the same vaccine after systemic priming can elicit more $CD4^+$ and $CD8^+$ lung T_{RM} cells compared with only mucosal or systemic vaccination (80). There is also evidence indicates that compared with the original “prime and pull” strategy used in genital tract, the pull step applied in lung disease should use pathogen antigens instead of proinflammatory chemokines. This is because only pathogen antigens can maintain the recruited T cells in airway lumen and persevere immune protection over time (86). Intranasal administration of a novel recombinant anti-TB vaccine (SeV85AB) after subcutaneous immunization with BCG uses this way to provide larger immune protection for lungs than either

SeV85AB or BCG alone (87). As opposed to vaccines that directly provide the pathogen antigens like SeV85AB, recent research developed an “antibody-targeted vaccination (ATV)” for the pull step. It connects antigen with antibody that targets lung DC cells, give raise to local antigen presentation, and improve activation of lung T_{RM} cells (88). Pulmonary surfactant-biomimetic liposomes containing stimulator of interferon genes that target alveolar epithelial cells give a new way to recruit CD8⁺ T_{RM} cells and provide long term wide-spectrum protection (89). These methods may also be used in inducing tumor antigen presentation and lung tumor T_{RM} cell function.

In summary, multiple studies have proved that T_{RM} cells can be induced by vaccination to make a difference in preventing pathogens or controlling tumor growth. However, many problems remained to be solved, for example, how to attract T_{eff} cells into target areas not close to mucosal, and how to maintain long-term lung T_{RM} cells (79). Systemic approaches should also be developed to evaluate the safety and efficiency of these vaccines and prevent overactivation of T_{RM} cells resulting in pathologic immune responses (90).

CONCLUDING REMARKS

It is now obvious that lung T_{RM} cells are an important part of the adaptive immune response within lung tissues. Although we have a rudimentary understanding of lung T_{RM} cells, they remain shrouded in mystery, waiting to be discovered more. While mentioning the migration, activation, differentiation, and maintenance of lung T_{RM} cells, main steps are outlined but there are still huge empties in the details. Do lung T_{RM} cells undergo pre-differentiation in lymph nodes before infection (91)? Which cytokines, transcription factors, and surface molecules are more decisive in the migration, formation, and maintenance of lung CD4⁺ or CD8⁺ T_{RM} cells? Are there different subtypes of lung T_{RM} in different lung tissue structures (such as in interstitium and parenchyma)? To answer these questions, more advanced techniques such as single-cell RNA-sequencing that identifies cell-cell interaction and TCR lineage tracking may be used.

A better understanding of these issues will undoubtedly help better manipulate lung T_{RM} cells to prevent or treat disease. Therapy focusing on lung T_{RM} cells in tumor and pathologic immune response is still in a nascent state. Besides direct activation or transmission of tumor-specific T_{RM} cells, currently there are vaccines that activate antiviral lung T_{RM} cells near tumor tissue

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(92), which reverse the immunosuppressive microenvironment, and may pave the way for later cell therapy. Drugs that prevent lung T_{RM} cell formation or function may also be useful in suppressing the immune response to lung transplantations or preventing lung sequela after respiratory infection in the elderly (63). Of course, T_{RM} cells in the lungs are mostly deemed to fight off lung infections. During the COVID-19 pandemic, lung T_{RM} cells are particularly important in the first line of defense against re-infection of SARS-CoV-2. Actually, influenza viruses have never been conquered, not only because of its versatility, but also because the immune memory only lasts for a short time in lung. To fight them, one possible solution is to improve the “width and depth” of the function of vaccines that induce lung T_{RM} cells. The width refers to the prospect that the same vaccine can induce lung T_{RM} cells that resist a wide range of virus strains in response to virus variability (88). The depth hopes that the induced T_{RM} cells can remain in the lungs for nearly lifelong, enhancing the killing effect and duration of protection of the vaccine (79). More insight and precise manipulation of the fate of lung T_{RM} cells will help to better develop novel immunomodulators to treat lung diseases by T_{RM} cells, and thus to exert the rapid and powerful action in critical illnesses such as COVID-19 pandemic.

AUTHOR CONTRIBUTIONS

YQ and YZ contributed to the central idea and coordinated the writing of the manuscript. YQ, YZ, YL, and BL read, discussed, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: BL is a co-founder of Biotheus Inc and the chairman of its scientific advisory board.

The remaining authors declare that the work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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