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Human lung tissue resident memory T cells in health and disease

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The human lung contains a heterogeneous population of immune cells which mediate protective responses, maintain tissue homeostasis, but can also promote immunopathology in disease. The majority of T cells in the human lung are tissue resident memory T cells (T_{RM}) which have been shown in mouse models to provide vital roles in the protection against multiple respiratory pathogens, and contribute to heterosubtypic protection in the context of vaccination. In this review, we will discuss recent studies in humans identifying lung TRM, their role in maintaining tissue homeostasis, and emerging evidence implicating T_{RM} in anti-tumor immunity and immune surveillance as well as their potential for immunopathology in chronic airway inflammation.

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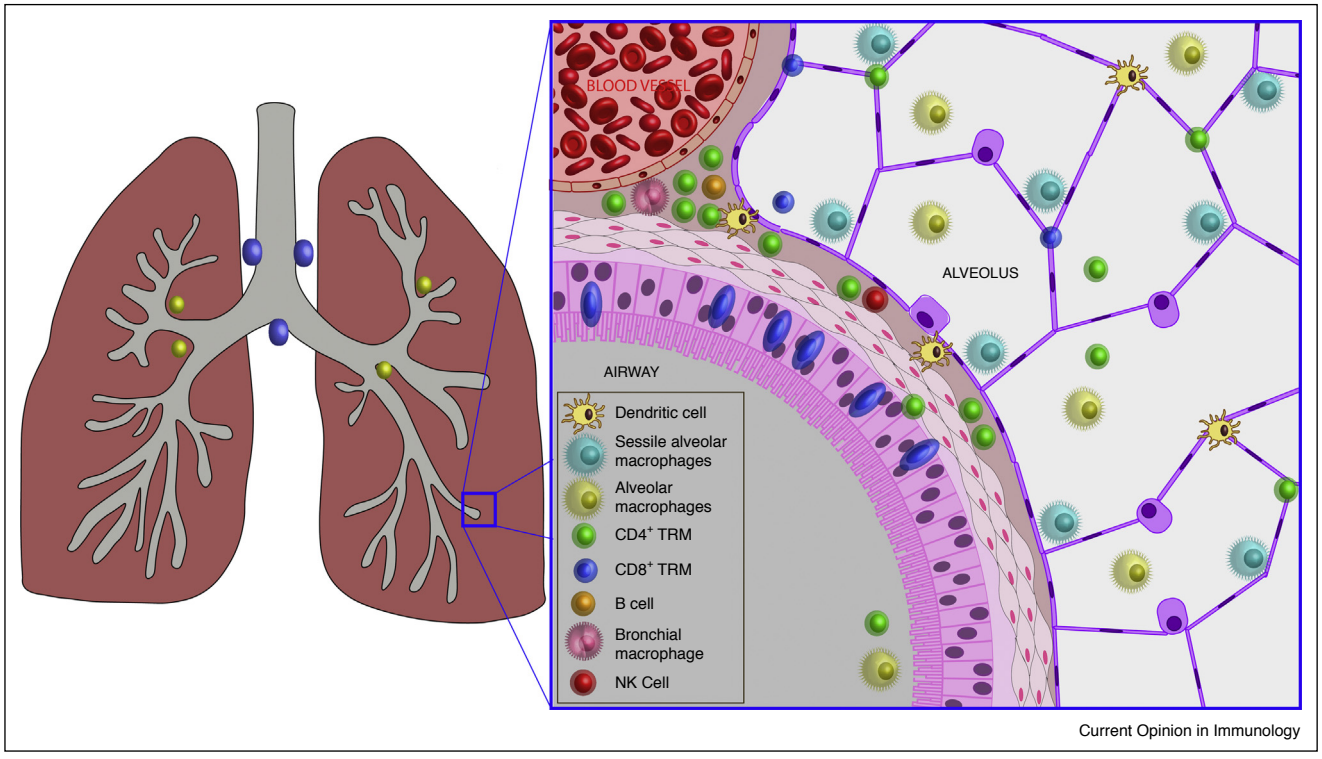
Introduction

The human lung experiences continuous and direct exposure to environmental and microbial antigens – both innocuous and pathogenic – through inhalation and oropharyngeal aspiration. Consequently, acute and chronic lower respiratory tract infections, including influenza and mycobacterial tuberculosis, remain the leading cause of death in developing countries and contribute to over five million global deaths annually [1]. To maintain tissue homeostasis and protection from pathogens in the context of these recurrent challenges, the lung contains heterogeneous populations of innate and adaptive immune cells, many of which are tissue resident. Deficiencies in the immunologic response to inhaled pathogens can result in life threatening infections, sepsis, and even cardiovascular shock. Conversely, dysregulation in the interactions between the resident immune cells and cellular components of the lung can lead to acute respiratory distress syndrome [2], or more insidious chronic inflammatory diseases such as pulmonary fibrosis [3] and asthma [4]. Understanding the mechanisms by which lung immune cells interact with and function in health and disease is essential to begin to develop therapies to modulate these interactions *in situ*.

The major immune cell populations in the lungs include macrophages and dendritic cells as the prevalent innate cells, and $CD4^+$ and $CD8^+$ T lymphocytes as the predominant adaptive immune cells (Figure 1). Alveolar macrophages (AM) comprise the majority of lung macrophages [5]. Mouse AM are critical for protection from infection and are implicated in dysregulations involved in lung inflammation and fibrosis [6,7]. Mouse and human AM can be delineated into subsets based on their location and migration properties (Figure 1); intravital imaging in murine studies detail how sessile macrophages remain adherent to the epithelial layer of the alveoli and work to attenuate immune responses, providing much of the regulatory signals preventing lung injury during an inflammatory response to antigen, while non-sessile alveolar macrophages which continually surveil the airspace and initiate inflammatory responses to inhaled or aspirated pathogens [8,9•,10,11]. Therefore, resident innate cells in the lung are essential for tissue homeostasis and are involved in its breakdown in disease.

T cells become primed and activated in lymphoid tissues and migrate to the lung during responses to respiratory pathogens. A subset are found to persist as memory T cells after pathogen clearance. The lung was shown some time ago to contain predominant effector memory T cell (T_{EM}) populations ($CD45RA^-$, $CD45RO^+$, $CCR7^-$) [12–14], that were initially thought to represent a migrating, surveilling population. However, it is now clear that the majority of lung T cells persist as non-circulating tissue-resident memory T cells (T_{RM}). T_{RM} are now recognized as a distinct subset of T_{EM}, present in multiple mucosal, barrier, lymphoid and peripheral tissues, and that T_{RM} are difined by upregulation of CD69 and CD103

Figure 1



The major immune cell populations in the lung and their tissue localization. The most abundant innate cell found in the human lung are alveolar macrophages (AM), including sessile AM remain adherent to the epithelium and non-sessile AM that surveil the alveolar space. Tissue resident memory T cells (T_{RM}), the most numerous adaptive immune cells in the lung, are predominantly found in and around the airways and can also persist in the parenchyma.

expression promoting tissue retention; notably, T_{RM} have a unique transcription profile distinguishing them from circulating T_{EM} [15,16]. Lung T_{RM} were initially identified as $CD4^+$T cells which were retained specifically in the lung in parabiosis studies, occupied specific niches around airways, and mediated optimal protective responses to influenza challenge [17,18•]. Lung $CD8^+$ T_{RM} are also generated following influenza infection and both $CD4^+$ and $CD8^+$ T_{RM} can be established in intranasal vaccines and mediate cross-strain protection to heterosubtypic strains of influenza virus [19,20•]. Conversely, lung $CD4^+T_{RM}$ generated in response to allergen exposure can mediate immunopathology and promote reactive airway disease [21,22•]. Mouse studies have therefore revealed a central role for T_{RM} in multiple aspects of lung immunity.

The importance of T_{RM} in lung immunity in mouse models has prompted extensive study into their potential role in human lung homeostasis and disease. Studying lung tissue in humans is challenging due to limited sampling opportunities. From patients, one can obtain surgical explants that have been removed due to lung cancer or other diseases, or biopsies obtained in disease diagnosis or monitoring. Bronchoalveolar lavage (BAL) are bronchoscope-directed washes of the airway and alveoli obtained both for clinical monitoring in infection and transplantation. Finally, obtaining lungs from organ donors that are not used for transplantation can provide a rich source of healthy tissue for studying all aspects of lung biology, from cells to genes to tissue organization [23].

Using these different sampling approaches, it is now established that the majority of both $CD4^+$ and $CD8^+$ T cells in the human lung are memory phenotype, and exhibit T_{RM} phenotypes and transcriptional profiles [18•,24••,25•,26,27]. Human lung T_{RM} are found throughout all regions of the respiratory tract including in the parenchyma, airways and associated lymph nodes [28•,29,30••] (Figure 1), suggesting roles in protection, immunosurveillance, and homeostasis. Here, we review the different properties of human lung T_{RM} and recent studies in humans that reveal specific roles for human lung T_{RM} in health and disease.

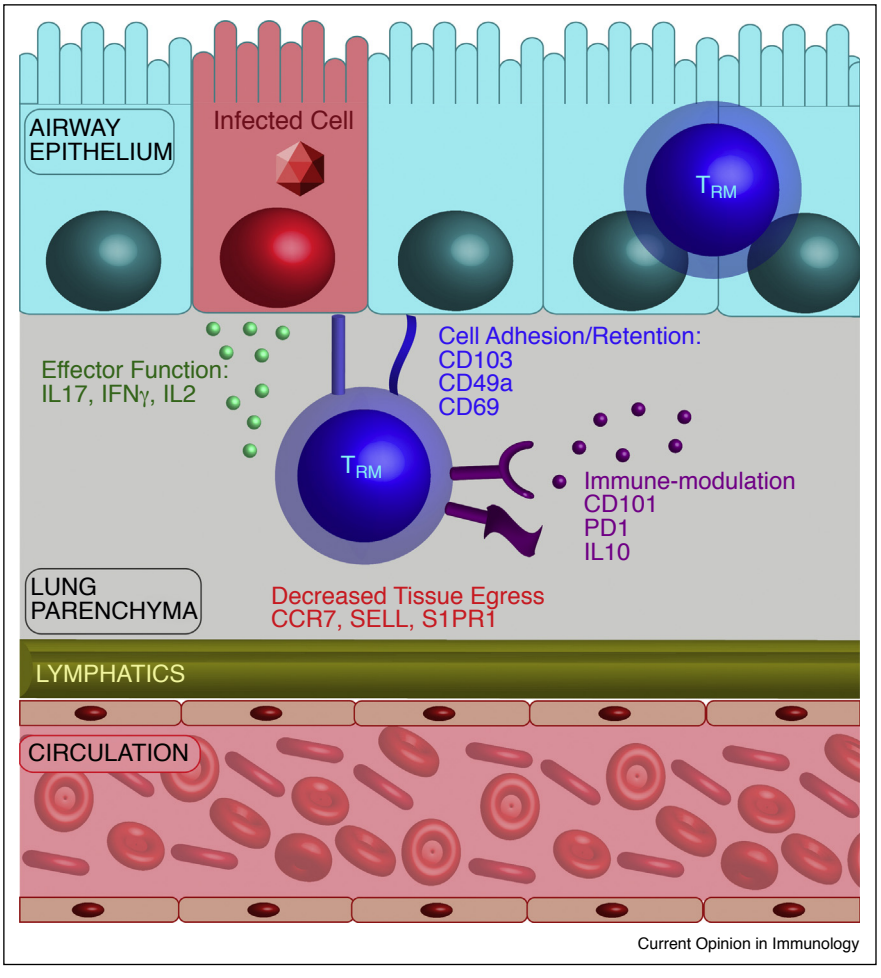
Defining human lung T_{RM}

The vast majority of $CD4^+$ and $CD8^+$ T cells in the human lung have a T_{EM} phenotype, but have a unique transcriptinal profile from circulating T_{EM}, and

substantial differences in protein expression and effector function. Notably, the majority of lung $CD4^+$ and $CD8^+$ T_{EM} phenotype cells express the canonical T_{RM} marker CD69 [24••,31]. CD69 is an early activation marker for T cell receptor signaling, but also exhibits retention functions through coordinated downregulation of sphingosine-1-phosphate receptor 1 (S1PR1) by sequestration, thereby preventing cells from responding to sphingosine-1-phosphate (S1P) required for tissue egress [32,33,34•,35]. The importance of CD69 expression in maintaining tissue residence was demonstrated in mouse models of infection using parabiosis to show lack of recirculation by virus-specific T cells [34•]. A subset of lung $CD69^+CD8^+$ T cells, and to a lesser extent $CD69^+CD4^+$ T cells, also express the integrin, CD103 ($\alpha E\beta 7$) [24••,25•,28•,36••], which mediates binding to E-cadherin on epithelial cells. CD103 expression is upregulated by TGF-β, and can promote T_{RM} retention *in vivo* in mice [37]. In the human lung, $CD8^+$ T cells from the airway and BAL had higher CD103 expression compared to $CD8^+$ T cells from the tissue parenchyma [30••], suggesting that CD103 expression may promote localization in airways due to interactions with epithelial cells (Figure 2).

Transcriptome profiling has established that CD69 expressionby human lung T cells defines a T_{RM} subset. The transcriptome profile of $CD69^+$ memory $CD4^+$ and $CD8^+$ T cells from human lung and spleen was distinct from that of $CD69^-$ counterparts from tissue and blood, and exhibited a core gene signature homologous to mouse T_{RM} from skin and intestines [28•,38]. This core gene signature includes the upregulation of genes encoding integrins and chemokine receptors (ITGAE (CD103), ITGA1(CD49a), CXCR6), downregulation of

Figure 2



Properties and localization of Lung T_{RM}. Schematic shows localization of T_{RM} in the lung including in the parenchyma around airways and in the intraepithelial space. Putative interactions with epithelial cells and collagen through upregulation of cell surface proteins promoting cell adhesion (CD103, CD49a) are shown. Downregulation of proteins promoting tissue egress (CCR7, SELL, S1PR1) contributes to tissue retention. Also shown are the dual functional capacities of lung T_{RM} including rapid production of pro-inflammatory cytokines (IL17, IFNγ production) and regulatory cytokines (IL-10), and expression of inhibitory molecules (PD1, CD101) that may act to limit tissue damage during *in situ* recall.

markers associated with tissue egress (CCR7, KLF2, S1PR1, SELL), and upregulation of genes encoding cytokines (IL2, IFNG, IL17) and immunoregulatory molecules (IL10, CD101, PD-1, TIGIT, CTLA4) (Figure 2) [28•]. The role of these inhibitory molecules in lung T_{RM} function is not yet defined; they may regulate inflammatory responses *in situ*, thus limiting tissue damage. A similar T_{RM} gene signature was obtained from lung $CD69^+CD103^+CD8^+$memory T cells isolated from surgical explants [25•,36••], and from T_{RM}-phenotype cells in other tissues including spleen, BM, and lymph nodes [28•,29].

Human lung T_{RM} are heterogeneous in surface phenotype and functional capacities. CD103 is expressed on a subset of lung $CD8^+$ T_{RM} and CD103-expressing T_{RM} have increased effector function potential when compared to $CD103^-$ T cells from the same location [39••]. Human T_{RM} in the lung and other sites can also be fractionated into subsets based on their ability to efflux mitochondrial dyes [40,41]—a feature of stem cells that is associated with cellular quiescence [42]. Lung T_{RM} persist as a relatively quiescent subset with lower effector function, and a second subset with higher effector function [40]. More studies are needed to establish how these complementary subsets persist over time, and whether the stem-cell like subset is more prevalent with age.

Human lung T cell heterogeneity with age

The composition and distribution of T cells in the lung vary with age; the most dynamic changes occur in early life, with relatively stable T cell subset frequencies over most of adult life as in other sites [43]. During infancy, naïve T cells ($CCR7^+CD45RA^+$) represent the predominant population in circulation and lungs along with substantial frequencies of regulatory T cells (Tregs, $CD4^+CD25^{hi}CD127^{lo}FOXP3^+$), which provide a key role in promoting tolerance toward the numerous antigens encountered in the early years of life [44]. With the accumulation of repeated pathogen exposures through inhalation and aspiration over time, T_{EM} become the majority population of T cells in the lung by late childhood, along with coincident expression of T_{RM} phenotypes [24••,44,45]. This preponderance of T_{RM} among T cells in the human lung persists throughout the many decades of adulthood [24••].

The location of T cells within the lung similarly changes with age. At infancy, the lung contains a higher proportion of T and B cell aggregates adjacent to the airways, called bronchus associated lymphoid tissue (BALT) [46]. BALT tissue diminishes with age, to the point that it is a rarely found in non-diseased human lungs by adulthood [46]. Despite this reduction in BALT tissue, immunohistochemistry analysis of non-diseased, human lung section clearly shows that T cells within adult lungs are enriched in areas close to the airways [39••]; however, more detailed spatial analysis of T_{RM} is needed to fully define how T_{RM} are organized within the complex structures of the lung.

Although there is a consistent proportion of T_{RM} found in the human lung throughout adulthood [24••], the lifespan and tissue retention of T_{RM} are difficult to assess over time *in vivo*. In a recent study, prospective BAL samples were obtained from patients with transplanted lungs from HLA-disparate donors, enabling tracking of donor-derived and recipient-derived T cells over time [30••]. Interestingly, T cells derived from the donor (which by definition originated in the lung) were only detected in the BAL and not peripheral blood of transplant recipients, persisted in the lungs over 15 months post-transplant and uniformly expressed T_{RM} markers [30••]. These findings revealed that lung T_{RM} can remain in the tissue for an extended period *in vivo*. It remains unclear if T_{RM} functional capacity in the lung and associated lymphoid tissue wanes or becomes altered with age.

Lung T_{RM} and protection against respiratory pathogens

Numerous studies in mouse models have established that the lung is enriched for T_{RM} specific to multiple viral and bacterial pathogens generated by respiratory infection or vaccination. Lung $CD4^+$ and $CD8^+$ T_{RM} are associated with heterosubtypic protection from subsequent influenza infection mediated, in part, by rapid IFNγ production following viral re-challenge [20•,47]. The resultant lung T_{RM} accumulate and are maintained around airways [18•,48]. Furthermore, intranasal administration of a live attenuated influenza vaccine leads to increased lung T_{RM} formation and heterosubtypic protection compared to parenteral injection with the inactivated flu vaccine formulation [20•]. Similarly, protective lung T_{RM} can be generated to bacterial pathogens such as *B. pertussis* and *M. tuberculosis* following live infection or vaccination with DTap or BCG, respectively [49–52]. These mouse studies suggest central roles for T_{RM} in protection to diverse respiratory pathogens.

The protective role of human lung T_{RM} *in vivo* is not well defined; however, recent studies indicate that the lung may be enriched for clonally expanded T_{RM} specific for respiratory pathogens. $CD8^+$ T cells specific for influenza and cytomegalovirus (CMV) have been detected in the human lung; however, CMV-specific lung T cells are found within circulating (CD69−) and resident (CD69+) as well as terminal effector (TEMRA) subsets [53], while influenza-specific T cells are preferentially distributed among T_{RM}-phenotype cells [18•,31,39••]. Moreover, influenza-specific lung T_{RM} exhibit polyfunctional profiles [39••] (associated in humans and mice with enhanced protection [54]) and a subset are cross-reactive to multiple influenza

strains [55•], suggesting that T_{RM} could be an important target for universal protection to new and emerging influenza viruses. Clonal analysis of influenza-specific lung T cells following single cell sequencing demonstrated that influenza-specific lung T_{RM} maintain TCR diversity [39••], further indicating their potential for mediating immunity to different strains.

T_{RM} specific for respiratory pathogens are also associated with protection. In an *in vivo* challenge model in humans, healthy volunteers were infected with RSV, and blood and bronchoalveolar lavage (BAL) samples were collected and T cell responses assessed over time. Following infection, a population of RSV-specific $CD8^+$ T cells expressing T_{RM} phenotypes ($CD69^+CD103^+$) was detected and persistence of RSV-specific $CD8^+$ T_{RM} in the BAL was associated with protection from inflammatory effects of future infection with RSV [56•]. In addition to viruses, T_{RM} specific to mycobacterium tuberculosis have been found in the human lung [51]. Airway challenge of tuberculosis-infected individuals with the purified protein derivative (PPD) resulted in appearance of $CD4^+$ T cells in the BAL exhibiting expressing of integrins associated with T_{RM} (although the study was performed before T_{RM} identification) [57]. Together, these challenge studies indicate that human lung T_{RM} can be recalled *in situ*, providing further support for the potential of T_{RM} targeting in vaccines.

T_{RM} and anti-tumor immunity

A growing body of evidence suggests that a subset of tumor-associated lymphocytes in lung cancer may comprise T_{RM}. Interestingly, the accumulation of $CD103^+$ $CD4^+$ and $CD8^+$ T cells in primary non-small cell lung cancer was found to be a predictor of more favorable outcome, including increased survival [58•]. $CD8^+$ tumor infiltrating lymphocytes (TIL) expressing $CD103^+$ represent a clonally expanded population of tumor-reactive T cells, and express multiple core T_{RM} markers including CD49a, CXCR6, and PD-1 [58•]. These tumor-associated T cells, when compared to those from adjacent, non-tumor lung, showed enhanced gene expression for many of these classic T_{RM} markers, most notably those normally associated with exhaustion (PDCD1, TIM3, CTLA4) [59•]. However, contrary to exhausted T cells, this population represent a highly activated subpopulation, with increased gene expression of 4-1BB, and enhanced cytotoxicity [59•].

The origin and differentiation of human lung tumor infiltrating lymphocytes (TIL) remain unknown. It is not clear whether T_{RM} associated with the tumor derive directly from T_{RM} maintained in the lung, or infiltrate from the periphery. A recent study has identified T cells specific for common viral pathogens such as EBV, CMV, and influenza within the tumor-associated T cells in lung cancers [60•] and other tumor types [61•]. These findings suggest that T_{RM} within a site may be mobilized during development of a tumor *in situ* raising the possibility that promoting pathogen-specific TRM can improve anti-tumor immunity as recently shown in mouse models [61•].

Lung T_{RM} and immunopathology

T_{RM} have been implicated in both the pathogenesis of and protection from chronic inflammatory diseases involving multiple mucosal surfaces, skin, and the synovium, including inflammatory bowel disease [62•], psoriasis [63], and juvenile arthritis [64•]. In a mouse model of house dust mite (HDM) exposure, mouse airways developed a biased population of HDM-specific $CD4^+$ T_{RM} which had a predominant Th2 phenotype and contributed to airway resistance [21,22•]. In another study, $CD8^+$ T_{RM} generated from influenza infection protected from the later recruitment of $CD4^+$ T cells in response to an allergen challenge [65], suggesting that virus-generated $CD8^+$ T_{RM} may modulate airway inflammation *in situ*. Our understanding of the role of lung T_{RM} in the pathogenesis of human airway disease is based largely on studies on sputum and BAL samples obtained from patients with asthma compared to healthy controls. In the largest cohort study to date, patients with moderate to severe asthma had increased expression of both CD103 and CTLA4 among the BAL $CD4^+$ T cell population, suggesting increased $CD4^+$ T_{RM} [66]. More studies are needed to assess the impact of T_{RM} on asthma disease severity and symptom burden.

T cells have long-been recognized as the primary mediator of acute cellular rejection (ACR) following lung transplantation [67]. Consequently, the main objective of immunosuppressive therapy following lung transplantation is to diminish the functionality of circulating recipient T cells, thereby reducing the risk of developing ACR, while maintaining protective immunity [68,69]. The role of lung T_{RM} in ACR and protection in lung transplantation is an important issue that has been recently been addressed in two studies. A longitudinal analysis of human lung transplant recipients found that allograft-infiltrating, recipient-derived T cells gradually developed T_{RM} phenotypes to levels seen in healthy lungs by six months post-transplantation while donor T cells persisted as T_{RM}; increased proportions of recipient-derived T_{RM} was associated with increased ACR [30••]. In other studies, lung transplant recipients without antecedent ACR were found to have increased aggregates of $FOXP3^+$ T cells within the allograft, [70••]. Together, these data suggest that T_{RM} may impact the inflammatory environment of the lung allograft following transplantation. Larger cohort studies will better elucidate the role that T_{RM} play in allograft tolerance and the impact of immune-depletive and immune-modulatory medicines on lung T_{RM} function and survival.

Conclusions

The human lung predominantly contains $CD4^+$ and $CD8^+$ T_{RM} that persist in stable frequencies for decades of human life. Recent studies in mouse models and in human samples reveal an important role for lung T_{RM} in the defense against inhaled pathogens, in maintaining tissue homeostasis in the face of diverse antigens encountered through respiration, and may also be important in surveillance for tumors and persistent viruses. Lung T_{RM} can also promote pathologic inflammation, inducing chronic inflammatory changes leading to pathogenesis of some types of asthma. Similarly, generation of lung T_{RM} from infiltrating recipient T cells in transplantation may mediate allograft immunopathology and promote lung damage. Advancing our understanding of the factors leading to T_{RM} generation and maintenance may provide insights into the development of lung-targeted immunomodulatory therapies.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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