REVIEW

Tissue resident memory T cells in the respiratory tract

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Owing to their capacity to rapidly spread across the population, airborne pathogens represent a significant risk to global health. Indeed, several of the past major global pandemics have been instigated by respiratory pathogens. A greater understanding of the immune cells tasked with protecting the airways from infection will allow for the development of strategies that curb the spread and impact of these airborne diseases. A specific subset of memory T-cell resident in both the upper and lower respiratory tract, termed tissue-resident memory (Trm), have been shown to play an instrumental role in local immune responses against a wide breadth of both viral and bacterial infections. In this review, we discuss factors that influence respiratory tract Trm development, longevity, and immune surveillance and explore vaccination regimes that harness these cells, such approaches represent exciting new strategies that may be utilized to tackle the next global pandemic.

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MEMORY T-CELL SUBSETS

Immunological memory is defined as the ability of the immune system to respond more rapidly and effectively to pathogens that have been encountered previously. A crucial element of this acquired protective immunity is the generation of memory T cells, which in comparison with their naive counterparts, have increased frequency, effector function, and broader localization. Memory T cells can be divided into three subsets based on localization/trafficking, phenotype, and function; these subsets are termed central memory (Tcm), effector memory (Tem), and tissue-resident memory T cells (Trm)[1–5]. Tcm are highly proliferative and express the lymph node homing molecules CCR7 and CD62L, which promote homing to secondary lymphoid organs. Conversely, Tem expres slow levels of CCR7 and CD62L, and instead, express integrins and chemokine receptors that facilitate entry into tissues[6,7]. Studies using tissue transplantation[2,8] and parabiosis[9–11] demonstrated that an additional non-recirculating, self-sustaining class of memory T-cell persists long term within tissues—these cells were termed Trm.

Trm are the most abundant memory T-cell subset[11] residing in barrier tissues such as the skin[2,12,13], lung[12,14–18], gut[5], nasal tissue[19], and reproductive tract[20–22], non-barrier tissues including the brain[23], liver[24], and kidney[25], as well as lymphoid tissue[26–30]. The positioning of Trm in diverse tissues with distinct microenvironments drives the development of Trm that are phenotypically and functionally different. At most sites, Trm are characterized by the downregulation of CCR7 and CD62L, which serve to prevent tissue egress, and the upregulation of adhesion molecules including CD103, CD69, CXCR3, and the integrin CD49a that collectively act to maintain tissue localization[12,31–33]. The co-expression of CD69 and CD103 are Trm signature markers. The constitutive expression of CD69 on Trm limits tissue egress by antagonizing S1P1-mediated extravasation[34–36], whereas the expression of CD103 which binds to E-cadherin present on epithelial cells in barrier tissues supports tissue retention[37–39]. It is important to note that co-expression of CD69 and CD103 does not always mark all bona fide Trm, and hence there is the requirement for additional techniques including parabiosis, intravascular labeling, and tissue transplantation to validate true tissue residency (reviewed in ref. [40–42]). Trm are transcriptionally programmed for rapid effector function[43,44] and displays prototypic T-cell effector functions such as cytotoxicity and cytokine production[2,9,17,22,24,45]. Trm also have innate-like "sensing and alarming" properties that can recruit other immune cells to control antimicrobial infections[3,46–48]. Although Trm are effector-like and thus terminally differentiated, recent evidence shows that Trm can proliferate in situ following local antigen re-encounter[49,50]. Together, these reports highlight Trm as localized memory T cells that are poised to provide immediate frontline immunity.

Trm can be found in the upper (nasal mucosa) and in the lower (lung) respiratory tract and these cells play a critical role in the local defense against respiratory infections. The co-expression of CD69 and CD103 can be used to identify $CD8^+$ Trm in the lung and nose in both mice and humans[15,19], whereas lung $CD4^+$ Trm express CD69, with or without CD103[44,45,51]. Lung Trm additionally express the adhesion molecules CD11a(LFA-1) for entry and CD49a(VLA-1) for retention[51–54], with recent evidence supporting a role for CD49a (and not CD103) in facilitating Trm motility[32]. Pulmonary Trm can reside in either the airways (epithelium) or parenchyma (interstitium). The cells in these distinct pulmonary compartments have different phenotypic and functional profiles that likely reflect adaption to their local niche. For example, human airway $CD8^+$ T cells have greater expression of CD103 compared with their parenchymal counterparts[55]. Moreover, murine airway Trm express a distinct transcriptional and epigenetic profile, displaying a pro-apoptotic genetic signature and increased cellular stress levels[56]. Functionally, in comparison with $CD8^+$ Trm localized to the airways, $CD8^+$ Trm lodged in the

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parenchyma possess greater cytolytic function, expressing elevated levels of granzyme B as well as an increased capacity to synthesize IFNγ and TNFα[57,58]. Although Trm lodged in the lung parenchyma are poised for activation, in part owing to their constitutive expression of deployment-ready mRNA encoding effector molecules[43,44], the additional expression of inhibitory receptors such as PD-1 on these cells suggests they are also restrained, an approach to potentially minimize unwarranted activation and local immunopathology[43,59].

In this review, we will discuss factors that influence respiratory tract Trm development, longevity, and immune surveillance and explore vaccination strategies aimed at evoking this highly protective tissue-bound memory T-cell subset.

THE DEVELOPMENT OF RESPIRATORY TRACT TRM

Although there are universal requirements for Trm development across different tissues[60], the local microenvironment in which these cells develop heavily influences their differentiation. An intricate assortment of both intrinsic and extrinsic factors, which can act on the T cell either early during priming, or later, during the effector phase, can determine whether selection into the pulmonary Trm pool occurs (Fig. 1).

During T-cell priming, both the subset of dendritic cell (DC) and the strength of the T-cell receptor (TCR) signal can impact pulmonary Trm development. Specific DC subsets preferentially drive Trm development. Iborra et al.[61] show that murine $CD8^+$ T-cell priming in the mediastinal lymph node by cross-presenting $DNGR1^+$ $Batf3^-$ DCs is essential for the establishment of lung $CD8^+$ Trm, however, the development of circulating memory T cells was shown to be less reliant on this DC population. TCR signal strength can also influence selection into the lung Trm pool. Utilizing a panel of influenza A virus strains engineered to express defined epitopes of varying degrees of affinity for a fixed TCR transgenic $CD8^+$ T cell, Fiege et al.[62] demonstrate a negative correlation between TCR signal strength and lung $CD8^+$ Trm formation, a correlation that was also observed for Trm in other tissues[63]. The authors propose that this bias in Trm formation for lower affinity cells helps to ensure a broad TCR diversity in the Trm pool, a characteristic that may prevent escape from $CD8^+$ T cell-mediated pathogen control.

Following T-cell priming, effector $CD8^+$ T cells migrate to the respiratory tract where local conditioning events driven by (i) cognate antigen recognition (ii) the cytokine milieu, and (iii) cellular interactions and co-stimulation are required for optimal antiviral effector $CD8^+$ T-cell responses[64,65] and for the successful development of pulmonary Trm. The following sections will use predominantly mouse studies to expand on how the above-mentioned local conditioning events drive Trm development.

i) Local cognate antigen recognition

In general, lung Trm requires local cognate antigen recognition for their development[16,17,37,66] although, this dependency can be bypassed with the use of certain stimulants that trigger a very specific inflammatory milieu within the lung microenvironment[67]. The requirement of local antigen recognition for lung $CD8^+$ Trm formation influences the immunodominance hierarchy within the lung Trm pool[19]. Using an influenza virus mouse model, it was shown that different specificities of influenza-reactive $CD8^+$ T cells were recruited into the lung Trm pool with varying efficiencies. The relative epitope abundance within the lung over the course of the influenza virus infection was identified as a major factor that modulated the immunodominance hierarchy within the Trm compartment. The dependence on local antigen recognition for lung Trm development may serve as a selection process to induct the most "fit" T cells into the Trm pool in a tissue where space is limited. In contrast, nasal $CD8^+$ Trm form without the need for local antigen recognition[19], and as such, there appears to be no local bias in T-cell selection, with all T-cell specificities in this region having a comparable Trm conversion rate[19].

ii) Local cytokine milieu

Exposure to TGFβ is essential for lung (as well as skin and intestine) Trm development[12,68,69] as it promotes the expression of CD103[25,37,70]. The source of biologically active TGFβ in the lung has been shown to be derived from Type 1 regulatory T cells[71] and DCs[70,72]. It is worth noting that nasal $CD8^+$ Trm, in contrast to their lung counterparts, develops independently of TGFβ[19], and to date, the cytokines driving the emergence of this Trm pool remain undefined. In addition, TGFβ exposure serves to cause the downregulation of the T-box transcription factors T-bet and Eomes, the repression of which is required for Trm development[73]. Though not reported specifically for lung $CD8^+$ Trm, other cytokines such as IL-33 and TNFα have been shown to support the establishment of $CD8^+$ Trm in other tissues[36]. Exposure to these inflammatory cytokines promotes the downregulation of the transcription factor, Krüppel-like factor 2, and the downregulation of the tissue exit receptor S1PR1, which serves to limit tissue egress[36]. In regards to lung $CD4^+$ Trm, the cytokines IL-2 and IL-15 were shown to be important for their generation in mouse models of influenza, LCMV, and asthma[18,74,75], whereas exposure to IL-7 was crucial for their maintenance in animal models of *Klebsiella pneumonia* and allergy[76,77].

iii) Local cellular interactions and co-stimulation

A diverse network of local cellular interactions within the tissue further supports the differentiation of lung Trm. Early work demonstrated that the presence of IFNγ-producing $CD4^+$ T cells in the lung was necessary for efficient lung airway $CD8^+$ Trm development[78]. More recently, a lung $CD4^+$ T-cell subset that co-exhibited phenotypic and transcriptional profiles of follicular helper T cells and Trm cells, termed tissue-resident T helper (Trh) cells, were also shown to support the generation of local $CD8^+$ T cells via IL-21-dependent mechanisms[79,80]. Pulmonary monocytes also promote the differentiation and persistence of lung $CD8^+$ Trm through their interaction with effector T cells and their capacity to locally present antigen[81,82]. In contrast to the beneficial role of pulmonary monocytes in lung Trm development, tissue-resident alveolar macrophages were reported to be negative regulators of murine $CD8^+$ Trm differentiation[83], although this may not be the case for human lung $CD8^+$ and $CD4^+$ Trm[84]. Work compiled by the Watts group further supports a role for local co-stimulation by inflammatory antigen-presenting cells (APCs) in the generation of lung Trm—a phenomenon they refer to as signal 4. They show that signaling via the TNF receptor family members, 4-1BB (CD137) and GITR are required for the optimal accumulation of lung $CD4^+$ and $CD8^+$ Trm[85–87].

The local conditioning events that act on Trm within the local microenvironment of the lung drives the expression of a transcriptional signature within these T cells that encourages tissue residency. Although the transcription factors Hobit and Blimp1, which cooperatively act to suppress the expression of proteins involved in tissue egress (CCR7 and S1PR1), control the generation of $CD8^+$ Trm across different tissues including the skin, liver, kidney, and small intestine[88], in the lung, it has been shown that Blimp1 alone regulates $CD8^+$ Trm formation[89]. Downregulation of T-bet and Eomes is also required for lung Trm development[73]. Elegant work by the Farber group studying the kinetics of T-bet expression in lung Trm development across different age groups further highlights T-bet as a rheostat for the regulation of effector and Trm cell generation. Investigating in an infant mouse model of influenza and in pediatric patients screened for viral respiratory tract infections (mostly respiratory syncytial virus (RSV)), they show infant T cells expressed greater levels of T-bet compared with adult T cells, and this promoted effector memory T-cell generation but inhibited Trm

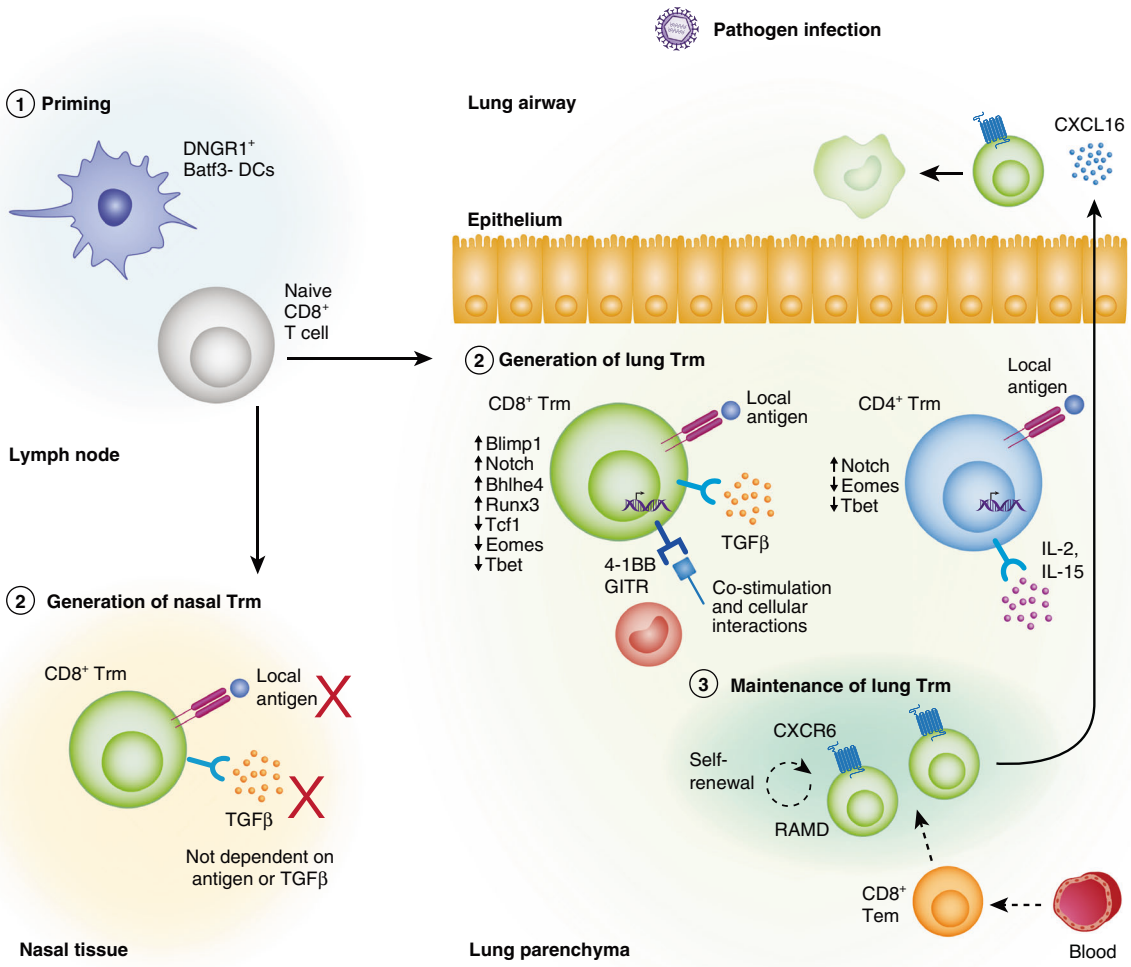

Fig. 1 Respiratory tract Trm generation and maintenance. 1) Upon respiratory pathogen encounter, dendritic cells (DCs) migrate to the mediastinal lymph node to activate naive $CD8^+$ T cells for which effector $CD8^+$ T cells then migrate into the nasal or lung tissue for their conversion into Trm. 2) In the lung, local tissue factors including antigen, cytokines, co-stimulation, and cellular interactions together with a tissue-resident transcriptional profile drive the formation of Trm. The development of nasal $CD8^+$ Trm is independent of local antigen or TGFβ cytokine production. 3) The maintenance of parenchyma lung $CD8^+$ Trm has been proposed to be dependent on replenishment from circulating $CD8^+$ Tem cells or via in situ homeostatic proliferation of lung $CD8^+$ Trm in sites of tissue regeneration called repair-associated memory depots (RAMDs). $CD8^+$ Trm in such sites were shown to replenish the pro-apoptotic airway $CD8^+$ Trm compartment via a CXCR6-CXCL16 axis. The maintenance of lung $CD4^+$ Trm is less studied, though IL-7 has been shown to be required for their maintenance.

formation[90,91]. Additional transcription factors, which are important in lung Trm development have been identified (Table 1), and these collectively regulate the expression of proteins that promote tissue retention and survival.

THE PROTECTIVE CAPACITY OF RESPIRATORY TRACT TRM

The respiratory tract represents an entry point into the body for an array of pathogens. Many studies highlight the importance of respiratory tract Trm in the protection against respiratory pathogens (Table 2). The following sections will highlight studies that show a protective role for respiratory tract Trm against a range of clinically relevant viral and bacterial pulmonary infections.

Respiratory viral infections

It is well characterized that respiratory tract Trm have a critical role in the protection against influenza virus infection. An elegant study by Wu et al.[17] was the first to highlight the indispensable role of local tissue-bound memory $CD8^+$ T cells in mediating cross-protective immunity against the influenza virus. Using a mouse model, they show that heterosubtypic immunity against influenza virus is lost 6–7 months after primary infection, even though a large population of influenza-specific $CD8^+$ Tem and Tcm remained within the circulation. The waning of protective immunity against secondary influenza challenge tightly correlated with a loss of lung influenza-specific $CD8^+$ Trm[17,19,92]. Influenza-reactive $CD8^+$ Trm are also present in the upper airways of mice, where they are ideally situated to block the transmission of inhaled influenza virus into the lower respiratory tract, and in doing so, can prevent severe pulmonary disease[19]. Pulmonary Trm deposit around bronchus-associated lymphoid tissues in the lung parenchyma[68] and confer protection via rapid and robust IFNγ and TNFα cytokine production upon reactivation[57,93]. Interestingly, recent evidence suggests the quality of the Trm re-call response can be influenced by the identity of the APC which triggers their reactivation, with presentation by hematopoietic APCs-regulating chemokine/cytokine production, and

Table 1. Transcription factors (TF) that regulate respiratory tract Trm.

TF	↑/↓	Function	Lung Trm population	Ref.
Notch	↑	Regulates CD103 expression, controls metabolic functions in Trm; required for maintenance of Trm	Human $CD8^+CD103^+$ Human $CD4^+CD103^+$	43,44
Bhlhe4	↑	Survival and function of Trm; CD103 regulation via acetylation of *Itgae*; required for Runx3 TF expression	Mouse $CD8^+CD69^+CD103^+$ Human $CD8^+CD103^+$	43,142
Runx3	↑	Required for Trm formation; overexpression enhances lung Trm differentiation; suppresses tissue egress genes (*S1pr1, Ccr7*)	Mouse $CD8^+CD69^+CD103^+$	143
Blimp1	↑	Suppress expression of tissue egress proteins (CCR7/S1PR1); suppresses Tcf1 TF	Mouse $CD8^+CD69^+CD103^+$	89
Tcf1	↓	Binds to the *Itgae* locus inhibiting CD103 expression	Mouse $CD8^+CD103^+$	144
T-bet	↓	Downregulation required for TGFb signaling and CD103 expression; residual expression necessary for survival (IL-15)	Mouse $CD8^+CD69^{-/+}CD103^+$ Mouse $CD4^+CD69^+$ Human $CD8^+CD103^+$ Human $CD4^+CD103^+$	43,44,73,90,145
Eomes	↓	Downregulation required for TGFb signaling	Mouse $CD8^+CD103^+$ Human $CD8^+CD103^+$ Human $CD4^+CD103^+$	43,44,73

presentation by nonhematopoietic APCs-regulating proliferation[94]. Studies characterizing the immune cell landscape in lung tissue from human organ donors revealed that the human lung harbors a large pool of influenza-specific $CD8^+$ Trm[51,93,95–97]. These cells were shown to be highly proliferative and polyfunctional, composed of a diverse TCRαβ repertoire, and a proportion were cross-reactive against multiple influenza strains[93,98]. Influenza virus infection can also be attenuated by lung $CD4^+$ Trm which are positioned within inducible bronchus-associated lymphoid tissues[51,68]. Experiments that utilized a transgenic $CD4^+$ T cell-specific for influenza hemagglutinin demonstrated the generation of influenza-specific $CD4^+$ Trm and their protective capacity in preventing weight loss and promoting rapid viral clearance in mice[45].

Lung Trm have also been shown to protect against RSV infection. Using an elegant experimental human challenge model, Jozwik et al.[99] identified RSV-specific $CD8^+$ Trm in the airways and showed that high numbers of virus specific airway $CD8^+$ Trm, but not virus specific circulating blood T cells, correlated with less-severe lower respiratory tract symptoms and reduced viral loads post RSV challenge. Subsequent murine studies corroborate this data and show that airway and parenchyma $CD8^+$ T cells protect against secondary RSV infection[100,101].

Of great relevance, pulmonary Trm limit the severity of SARS-coronavirus infection. Early work in mouse models established that the deposition of IFNγ-producing airway $CD4^+$ Trm and parenchymal $CD8^+$ Trm could provide protection against SARS-CoV-1 infection[102,103]. More recently it has been demonstrated that lung $CD4^+$ and $CD8^+$ Trm were present in SARS-CoV-2-infected patients and reduced disease severity was shown to be associated with increased numbers of airway $CD4^+$ and $CD8^+$ Trm[104,105]. These Trm cells produced IFNγ upon in vitro stimulation and importantly persisted for at least 10 months in convalescent patients[104,106]. Although less is known about SARS-CoV-2-specific Trm, it has been reported that the depletion of $CD8^+$ T cells in non-human primates prior to re-challenge with SARS-CoV-2 resulted in elevated viral titers in the nasal tissue, which may indicate a role for nasal $CD8^+$ Trm in protection[107]. Collectively, this emerging body of work suggests $CD4^+$ and $CD8^+$ respiratory tract Trm may play a key role in the protection against SARS-CoV-2 infection.

Respiratory bacterial infections

In bacterial infection models of *Streptococcus pneumoniae*[108,109], *Bordetella pertussis*[110], *Klebsiella pneumoniae*[76], and *Mycobacterium tuberculosis*[111,112] respiratory tract $CD4^+$ Trm have been shown to contribute to bacterial clearance, typically via the production of the pro-inflammatory cytokines IL-17 and IFNγ. An interesting report by Shenoy et al.[113] provides insight into how mouse $CD4^+$ Trm improves bacterial clearance from the lung. They show that IL-17-producing $CD4^+$ Trm can stabilize CXCL5 transcripts produced by lung epithelial cells, which in turn enhanced neutrophil recruitment and pneumococcal clearance. Bystander activation of nonspecific lung Trm cells triggered by a local bacterial infection in mice was also shown to boost neutrophil recruitment into the airways, which attenuated the severity of the *S. aureus* bacterial pneumonia[47]. This work highlights the protective role of anti-bacterial Trm through their involvement in accelerating innate immune responses.

THE PERSISTENCE OF RESPIRATORY TRACT TRM

The capacity to persevere long-term within tissues is an important characteristic of Trm. Although the stable long-term persistence of Trm has been documented in a variety of tissues including the nose, skin, liver, and intestinal mucosal[19,21,24,92,114,115], pulmonary $CD8^+$ Trm possess an unusually short half-life (12 days in mice)[19,67]. This attrition is consequential as animal studies clearly show that a loss of influenza-specific Trm in the parenchyma and airways correlates with waning cross-protective immunity[17,116,117]. Similarly, influenza-specific $CD8^+$ Trm in human lung tissue wane with advanced age, and this resulted in a lag in the development of an antiviral response following influenza exposure[95]. The attrition of lung $CD8^+$ Trm is not restricted to influenza-specific Trm cells, as similar findings are observed following Sendai virus[116,118] and RSV[101] infections in mice. Although murine lung $CD4^+CD69^+$ Trm also decline, their decay is less rapid relative to $CD8^+$ Trm[18,51,119].

Studies in mouse models have given rise to several theories to explain the attrition of lung Trm, put simply, it has been suggested that lung Trm wane because they either die, leave, or fail to be replenished. Initial work revealed that memory $CD8^+$ T cells located in the airways lose sensitivity to the cytokines IL-7 and IL-15[120], a defect that was likely driven by the airway microenvironment as transfer of cells from the spleen into airways resulted in the downregulation of these pro-survival cytokine receptors[121]. Airway and lung parenchymal $CD8^+$ Trm also display a pro-apoptotic phenotype and express elevated levels of active caspases 3/7 and reduced levels of the anti-apoptotic molecule Bcl-2[56,92]. This then raises the question as to why pulmonary $CD8^+$ Trm are prone to cell death. Evidence suggests that the local microenvironment of the lung, which is oxygen-rich and

Table 2. Trm responses elicited by respiratory tract pathogens.

Type	Pathogen	Tissue	Trm markers	Ref.
Virus	Influenza	LI	Mouse $CD8^+CD69^{+/-}CD103^{+/-}$($PD\text{-}1^{hi}$, $IFITM3^+$, $CD11a^+$, $CD49a^+$, $Ly6C^-$) Mouse $CD4^+CD69^+$($CD11a^+$, $PD\text{-}1^+$, $FR4^{lo/hi}$, $PSGL1^{lo/hi}$) Human $CD8^+CD69^{+/-}CD103^{+/-}$($HLA\text{-}DR^+$, $NKG2A^+$, $CD11a^+$)	16,17,19,37,45,47,51,52,56,58,59,66,68,74,78–81,85–87,89,90,92–97,125,128,135,146
		LA	Mouse $CD8^+CD69^+CD103^+$($CD49a^+$) Human $CD8^+CD69^+CD103^+$($CD49a^+$, $CD101^+$, $PD\text{-}1^{hi}$) Human $CD4^+CD69^+CD103^{+/-}$($PD\text{-}1^{hi}$, $CD49a^+$, $CD101^+$)	52,55–58,66,84,125
		Nasal	Mouse $CD8^+CD69^+CD103^+$	19
		mLN	Mouse $CD8^+CD69^+CD103^+$($Ly6C^-$)	29,94
	Sendai	LA	Mouse $CD8^+$	57
	RSV	LA	Mouse $CD8^+CD69^+CD103^+$ Human $CD8^+CD69^+CD103^+$ Human $CD4^+CD69^+CD103^{+/-}$	99,100,147
		LI	Mouse $CD8^+CD69^+CD103^+$ Mouse $CD4^+CD69^+CD103^-$($CD49d^+CD11a^{hi}$)	100,101
	SARS-CoV-2	LA	Human $CD8^+CD69^+CD103^{+/-}$($HLA\text{-}DR^+$, $PD\text{-}1^+$) Human $CD4^+CD69^+CD103^{+/-}$($HLA\text{-}DR^+$, $PD\text{-}1^+$)	104–106
	Vaccina virus	LI	Mouse $CD8^+CD69^+CD103^{+/-}$($CXCR3^{lo/hi}$)	82,126
		LA	Mouse $CD8^+CXCR3^{hi}$	126
Bacteria	*Streptococcus pneumoniae*	LI	Mouse $CD4^+CD69^+$($CD11a^{hi}$)	108,113
		Nasal	Mouse $CD4^+CD69^+$($CD11a^{hi}$)	109
	Bordetella pertussis	LI	Mouse $CD4^+CD69^+$	110,148
		Nasal	Mouse $CD4^+CD69^+$	109,110,148
	Klebsiella pneumoniae	LA	Mouse $CD4^+CD69^+CD103^-$	76
	Mycobacterium tuberculosis	LI	Mouse $CD4^+CD69^+$($CXCR3^{hi}$, $PD\text{-}1^{hi}$)	111
		LA	Human $CD4^+$($CD45RO^+$)	112
Fungi	*Aspergillus fumigatus*	LI	Mouse $CD4^+CD69^{hi}CD103^{lo}$	149
Parasite	*Nippostrongylus brasiliensis*	LI	Mouse $CD4^+$($CD44^+CD62L^-$)	150

LI lung Interstitium, *LA* lung airway, *mLn* mediastinal lymph node. Text within brackets indicates additional markers expressed by respiratory tract Trm.

characterized by cellular stress and amino acid starvation is not suitable for long term T-cell survival or optimal functional capacity[56,122], and this is perhaps designed to stop the excessive accumulation of T cells in a delicate site, vital for respiration[123].

An alternative explanation for the decay of lung $CD8^+$ Trm was recently explained by the repositioning of lung $CD8^+$ Trm via lymphatic vessels to the draining mediastinal LN[29]. These cells committed to the residency profile as they did not equilibrate among immunized parabiotic mice and continued to express Trm signature markers CD69 and CD103[29]. Although this mobility blurs the definition of Trm as sentinels permanently residing in tissues, the authors suggest this feature of Trm serves to provide protection in the draining LN and/or acts to repopulate the downstream non-lymphoid tissue after reinfection, thus serving as a mechanism by which local T cells can contribute to systemic protection[26].

While $CD8^+$ Trm in most tissues exist independently of the circulating memory T-cell pool[2,5,42,51], it has been proposed that the maintenance of both airway and interstitial $CD8^+$ Trm is reliant on continual replenishment by circulating $CD8^+$ Tem cells[92,116,124]. With time, the frequency of circulating Trm precursors waned, this, coupled with the resolution of the inflammatory signature within the lung, was proposed to contribute to the gradual loss of lung Trm[92]. In contrast to this model, other studies report that lung interstitial and airway $CD8^+$ Trm are essentially maintained without recruitment from the circulating T-cell pool[56,58,125]. Instead, these studies show that lung interstitial $CD8^+$ Trm are maintained in sites of tissue regeneration by homeostatic proliferation, and it is these local T cells that replenish the airway $CD8^+$ Trm compartment[58,66,125,126]. Migration into the airways was dependent on the expression of the chemokine receptor CXCR6 on interstitial $CD8^+$ Trm (and not $CD8^+$ Tem), which mobilized these cells in a process driven by an airway epithelial cell generated CXCL16 gradient[58]. Although it remains unclear whether local or circulating memory T cells reseed the pulmonary Trm pool (Fig. 1), this necessity for continued supplementation together with a time-dependent loss of Trm precursors and the resolution of pulmonary inflammation may underlie the impermanence of the lung Trm compartment[17,92,125,127].

To compensate for the transient nature of lung $CD8^+$ Trm, researchers have investigated different approaches that can extend the longevity of these cells. Utilizing a consecutive adoptive transfer model to generate $CD8^+$ T cells with varying levels of antigen exposure, Van Braechkel-Budimir et al.[128] demonstrated that quaternary-boosted influenza-specific $CD8^+$ Trm persisted significantly longer in the lung tissue compared with primary-boosted lung Trm. This improved stability in the lung Trm compartment due to multiple antigenic exposures extended the duration of cross-protective immunity against influenza challenge[128]. This work highlights that repeated antigen exposure improves the durability and survival of $CD8^+$ Trm within the microenvironment of the lung. In line with this, studies delivering into the lung a replication-defective adenovirus expressing the influenza virus nucleoprotein resulted in persistent local antigen exposure and this evoked a population of influenza-specific $CD8^+$ Trm in the respiratory tract that remained stable for at least 1 year

Table 3. Vaccine strategies that generate respiratory tract Trm.

Strategy	Type	Respiratory pathogen
Vaccine vectors	Adenovirus	Influenza[129,151], SARS-CoV-2[133], *Mycobacterium tuberculosis*[152]
	MCMV	Influenza[153], RSV[131,154]
	MVA	Influenza[155]
	Influenza A virus	*Mycobacterium tuberculosis*[156]
	CMV	*Mycobacterium tuberculosis*[157]
	Sendai virus	*Mycobacterium tuberculosis*[158]
	Vaccina virus	SARS-CoV[102]
Particulate vaccines	Nanoparticles	Influenza[137,139,159,160], *Mycobacterium tuberculosis*[161]
	Virus-like particles	Influenza[162], RSV[163]
Other	Attenuation	Influenza[130], *Brucella abortus*[164], *Mycobacterium tuberculosis*[132], *Streptococcus pneumoniae*[109]
	Antibody-targeted vaccination	Influenza[70]
	Chitosan-hydrogel	Influenza[140]
	Outer membrane vesicles	*Bordetella pertussis*[165]
	Virus replicon particle	SARS-CoV/MERS-CoV[103]

following vaccination[129]. Importantly, these cells did not become exhausted and could provide long-term protection against secondary influenza virus infection. In humans, this repeated stimulation or cross-reactivity from a lifetime of infections and/or annual vaccinations may also account for the observation of donor-derived $CD8^+$ Trm persisting for over a year post-transplantation in the airways of lung transplant recipients[55]. Since the size of the lung Trm compartment often correlates with protection against respiratory pathogens[17,99], it is important to understand mechanisms that account for lung Trm decay and identify approaches that can circumvent this attrition.

VACCINE STRATEGIES TO GENERATE RESPIRATORY TRACT TRM

Trm deposited along the respiratory tract convey protective immunity against respiratory infections. As such, vaccines that evoke respiratory tract Trm are likely to provide potent protection against airborne pathogens. Insights gained from the study of respiratory tract Trm development has revealed key factors that should be considered when developing a vaccine aimed at promoting pulmonary Trm development. For example, as local cognate antigen recognition in the lung significantly improves the induction of pulmonary Trm[16,37,70], vaccines aiming to generate pulmonary Trm should deliver vaccine antigen into the airways. Vaccination strategies tested in mice support this theory, as intranasal but not systemic/parenteral immunizations generated pulmonary Trm against influenza[130], RSV[131], *M. tuberculosis*[132], vaccina virus[126], and SARS-CoV-2[133]. Thus, the route of vaccine administration is a critical factor that determines whether pulmonary Trm develops.

Several types of vaccine formulations have been tested for their capacity to trigger respiratory tract Trm (Table 3) and most have shown excellent efficacy in animal studies. Many studies have investigated the use of vaccine vectors that utilize viruses such as adenovirus, Modified Vaccina Ankara (MVA), and murine cytomegalovirus (MCMV) to deliver antigens intracellularly and to evoke Trm development. However, careful consideration is warranted when selecting these vectors as the route of administration and/or the inflammatory milieu evoked by different vaccine vectors can influence the localization of memory $CD8^+$ T cells and in turn impact lung Trm differentiation[134,135]. Furthermore, the use of vaccine vectors must additionally factor in pre-existing vector immunity that may impair vaccine efficacy[136] and safety concerns of using live replicating vectors in the elderly and immunocompromised will also need to be addressed.

To bypass issues associated with viral vectors, an alternative vaccination approach to generate pulmonary Trm cells is to directly deposit antigen in the lung through antibody-targeted vaccination (ATV) to lung DCs[70] or through the use of particulate vaccines (reviewed in ref. [137]). The latter of which includes nanoparticles that offer the advantage of shielding antigen from degradation compared with naked antigen delivery by ATV. Nanoparticles confer further advantages by engineering properties that promote Trm development such as antigen persistence[138]. Recent work demonstrated a single intranasal dose of a nanoparticle vaccine incorporating influenza virus nucleoprotein induced numbers of lung $CD8^+$ Trm cells exceeding natural infection and this vaccination regime conferred protection against lethal influenza challenge[139].

Studies on respiratory tract Trm vaccination strategies have predominately focused on lodging Trm in the lung. However, the uncontrolled deposition of Trm in this delicate, vital organ, may unintentionally cause lung tissue damage and compromise respiratory function[59]. To mitigate the potential challenges associated with depositing Trm in the lower airways, we recommend the consideration of the nasal mucosa as an alternative site for induction of airborne pathogen-specific Trm. We have previously shown that influenza-specific Trm can be deposited in stable quantities in the nasal tissue of mice, these cells do not decline as aggressive as lung Trm, and in turn, offer long-term protective immunity[19]. In a proof of principle study, we further demonstrate that nasal $CD8^+$ Trm can be induced by a chitosan-hydrogel vaccine that sustains antigen retention in the nasal cavity and that these cells can provide potent protection in a murine influenza virus challenge model[140].

Collectively, mouse studies showcase a range of immunization approaches to evoke respiratory tract Trm with a common requirement of delivering the immunization agent into the respiratory tract to facilitate optimal Trm formation. The appropriate selection of adjuvant, delivered by the correct route, can also greatly improve vaccines that aim to elicit lung Trm development[67,141]. For example, in a proof of concept study, we show that zymosan, an adjuvant derived from yeast cell walls, when co-administered to mice intranasally with influenza vaccines can significantly boost lung Trm development[67]. A greater understanding of the appropriate local inflammatory milieu triggered by these Trm promoting adjuvants will allow for the

generation of refined adjuvants that evoke conditions that favor lung Trm development with minimal side effects.

CONCLUSION

The events of the past 12 months exemplify the catastrophic impact newly emerging respiratory pathogens can have on global health and world economies. Then again, they also demonstrate that vaccination is an extraordinarily effective strategy to curb the spread and impact of airborne diseases. To develop vaccines that protect against respiratory pathogens, it is essential to identify the immune cells that best protect the airways from infection. Trm lodged along the respiratory tract provide exquisite protection against respiratory pathogens. Although vaccines that evoke respiratory tract Trm hold significant therapeutic potential, care should be taken to ensure that elevating these cells does not increase the risk of immunopathology, allergy, or chronic airway inflammation. Vaccines that can safely deposit stable populations of Trm along the respiratory tract represent exciting new approaches that may be utilized to tackle the next global pandemic.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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