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)1–3. Tcm are highly proliferative and express the lymph node homing molecules CCR7 and CD62L, which promote homing to secondary lymphoid organs. Conversely, Tem express slow levels of CCR7 and CD62L, and instead, express integrins and chemokine receptors that facilitate entry into tissues4,5. Studies using tissue transplantation2,6 and parabiosis6–8 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 subset11 residing in barrier tissues such as the skin2,12,13, lung12,14–18, gut18, nasal tissue19, and reproductive tract20–22, non-barrier tissues including the brain23, liver24, and kidney25, as well as lymphoid tissue26–30. 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 localization1,2,31–33. The co-expression of CD69 and CD103 is a hallmark marker of Trm. The constitutive expression of CD69 on Trm limits tissue egress by antagonizing S1P1-mediated extravasation34–36. Additionally, the expression of CD103 which binds to E-cadherin present on epithelial cells in barrier tissues supports tissue retention37–39. It is important to note that co-expression of CD69 and CD103 does not always mark 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 function43,44 and displays prototypic T-cell effector functions such as cytotoxicity and cytokine production45,46,47. Trm also have innate-like “sensing and alarming” properties that can recruit other immune cells to control antimicrobial infections48–50. Although Trm are effector-like and thus terminally differentiated, recent evidence shows that Trm can proliferate in situ following local antigen re-encounter49,50. 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 humans51,52, whereas lung CD4+ Trm express CD69, with or without CD10353,54,55. Lung Trm additionally express the adhesion molecules CD11a(LFA-1) for entry and CD49a(VLA-1) for retention51,54, with recent evidence supporting a role for CD49a (and not CD103) in facilitating Trm motility52. 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 counterparts55. Moreover, murine airway Trm express a distinct transcriptional and epigenetic profile, displaying a pro-apoptotic genetic signature and increased cellular stress levels54. Functionally, in comparison with CD8+ Trm localized to the airways, CD8+ Trm lodged in the
pneumonia possess greater cytolytic function, expressing elevated levels of granzyme B as well as an increased capacity to synthesize IFNγ and TNFα. 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, 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.

In this review, we will discuss factors that influence respiratory tract Trm development, longevity, and immune surveillance and explore vaccine 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, 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. showed 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. demonstrate a negative correlation between TCR signal strength and lung CD8+ Trm formation, a correlation that was also observed for Trm in other tissues. 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 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, although this dependency can be bypassed with the use of certain stimulants that trigger a very specific inflammatory milieu within the lung microenvironment. The requirement of local antigen recognition for lung CD8+ Trm formation influences the immunodominance hierarchy within the lung Trm pool. 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, 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.

ii) LOCAL CYTOKINE MILIEU

Exposure to TGFβ is essential for lung (as well as skin and intestine) Trm development, as it promotes the expression of CD103+ cells. The source of biologically active TGFβ in the lung has been shown to be derived from Type 1 regulatory T cells and DCs. It is worth noting that nasal CD8+ Trm, in contrast to their lung counterparts, develops independently of TGFβ 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. 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. 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. 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, whereas exposure to IL-7 was crucial for their maintenance in animal models of Klebsiella pneumonia and allergy.

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. More recently, a lung CD4+ T-cell subset that co-exhibits 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. 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. 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, although this may not be the case for human lung CD8+ CD4+ Trm. 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.

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, in the lung, it has been shown that Blimp1 alone regulates CD8+ Trm formation. Downregulation of T-bet and Eomes is also required for lung Trm development. 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), they show infant T cells expressed greater levels of T-bet compared with adult T cells, and this promoted effector memory T-cell gene expression but inhibited Trm
formation\textsuperscript{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 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.\textsuperscript{17} was the first to highlight the indispensable role of local tissue-bound memory CD8\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} Trm\textsuperscript{17,19,92}. Influenza-reactive CD8\textsuperscript{+} 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\textsuperscript{19}. Pulmonary Trm deposit around bronchus-associated lymphoid tissues in the lung parenchyma\textsuperscript{18} and confer protection via rapid and robust IFN\gamma and TNF\alpha cytokine production upon reactivation\textsuperscript{17,92}. 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...
presentation by nonhematopoietic APCs-regulating proliferation. 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\(^{51,93-95}\). These cells were shown to be highly proliferative and polyfunctional, composed of a diverse TCR repertoire, and a proportion were cross-reactive against multiple influenza strains\(^{93,94}\). Influenza virus infection can also be attenuated by lung CD4\(^+\) T cells which are positioned within inducible bronchus-associated lymphoid tissues\(^{51,68}\). Experiments that utilized a transgenic RSV against secondary RSV infection\(^{100,101}\), and importantly persisted for at least 10 months in parenchymal CD8\(^+\) T cells, as similar findings are observed following Sendai virus\(^{116,118}\) and RSV\(^{101}\) infections in mice. 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). 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\(^{71,116,117}\). 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\(^{119}\).

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\(^{43,44,73}\). Airway and lung parenchymal CD8\(^+\) Trm can stabilize CXCL5 transcripts and induce bystander 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.

**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 Ifgae; 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 (Sipr1, 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 Ifgae locus inhibiting CD103 expression | Human 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 |

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). 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\(^{71,116,117}\). 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\(^{119}\).
lymphatic vessels to the draining mediastinal LN. These cells recently explained by the repositioning of lung CD8+ Tem cells without recruitment from the circulating T-cell pool. The maintenance of both airway and interstitial CD8+ Trm was shown in studies with respiratory tract pathogens. Instead, these studies show that lung interstitial CD8+ Trm do not replace the airway CD8+ Trm, and the resolution of the inflammation within the lung was proposed to contribute to the gradual loss of lung Trm. 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. Instead, these studies show that lung interstitial CD8+ Trm are maintained in situ by homeostatic proliferation, and it is these local T cells that replenish the airway CD8+ Trm compartment. 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. 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. 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 pulmonary Trm pool. In line with this, studies delivering antigen to the lung improves the durability and survival of CD8+ Trm. In contrast to this model, other studies report that lung interstitial 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 longevity of these cells. Utilizing a consecutive adoptive transfer model to generate CD8+ Trm cells with varying levels of antigen exposure, Van Braechkel-Budimir et al. 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. 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 2. Trm responses elicited by respiratory tract pathogens.

| Type       | Pathogen        | Tissue | Trm markers                                                                 | Ref.                  |
|------------|-----------------|--------|-----------------------------------------------------------------------------|-----------------------|
| Virus      | Influenza       | LI     | Mouse CD8+, CD69+/−, CD103+/− (PD-1hi, IFITM3+, CD11a+, CD49a+, Ly6C−)        | 383                   |
|            |                 |        | Mouse CD4+, CD69+/− (CD11a+, PD-1hi, FR4hi, PSGL1hi)                       |                       |
|            |                 |        | Human CD8+, CD69+/−, CD103+/− (PD-1hi, HLA-DRhi, NKG2A+), CD11a+           | 16,17,19,37,45,47,51,52,56,58,59,66,68,74,76–81,85–87,89,90,92–97,125,128,135,146 |
|            |                 | LA     | Mouse CD8+, CD69+/−, CD103+/− (CD49a−)                                    | 52,55–58,66,84,125    |
|            |                 |        | Human CD8+, CD69+/−, CD103+/− (PD-1hi, CD49a+, CD11a+)                    |                       |
|            |                 | Nasal  | Mouse CD8+, CD69+/−, CD103+/− (Ly6C+)                                    | 19                    |
|            |                 | mLN    | Mouse CD8+, CD69+/−, CD103+/− (Ly6C−)                                    | 29,94                 |
| Bacteria   | Streptococcus   | LI     | Mouse CD4−, CD69+/− (CD11a−)                                             | 99,100,147            |
|            | pneumoniae      |        | Human CD8+, CD69+/−, CD103+/− (CD11a−)                                    |                       |
|            |                 |        | Human CD4+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            | 100,101               |
|            | Bordetella      | LI     | Mouse CD8+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            | 104–106               |
|            | pertussis       |        | Mouse CD4+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            |                       |
| Fungi      | Aspergillus     | LI     | Mouse CD8+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            | 82,126                |
|            | fumigatus       |        | Mouse CD4+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            |                       |
| Parasite   | Nippostrongylus| LI     | Mouse CD8+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            | 126                   |
|            | brasiliensis    |        | Mouse CD4+, CD69+/−, CD103+/− (CD49a−, CD11a+)                            |                       |

LI: lung interstitium, LA: lung airway, mLN: mediastinal lymph node. Text within brackets indicates additional markers expressed by respiratory tract Trm.
following vaccination. 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+ Trrm persisting for over a year post-transplantation in the airways of lung transplant recipients. Since the size of the lung Trrm compartment often correlates with protection against respiratory pathogens, it is important to understand mechanisms that account for lung Trrm decay and identify approaches that can circumvent this attrition.

**VACCINE STRATEGIES TO GENERATE RESPIRATORY TRACT TRM**

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

Several types of vaccine formulations have been tested for their capacity to trigger respiratory tract Trrm (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 Trrm 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 Trrm differentiation. Furthermore, the use of vaccine vectors must additionally factor in pre-existing vector immunity that may impair vaccine efficacy 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 Trrm cells is to directly deposit antigen in the lung through antibody-targeted vaccination (ATV) to lung DCs 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 Trrm development such as antigen persistence. Recent work demonstrated a single intranasal dose of a nanoparticle vaccine incorporating influenza virus nucleoprotein induced numbers of lung CD8+ Trrm cells exceeding natural infection and this vaccination regime conferred protection against lethal influenza challenge.

Studies on respiratory tract Trrm vaccination strategies have predominately focused on lodging Trrm in the lung. However, the uncontrolled deposition of Trrm in this delicate, vital organ, may unintentionally cause lung tissue damage and compromise respiratory function. To mitigate the potential challenges associated with depositing Trrm in the lower airways, we recommend the consideration of the nasal mucosa as an alternative site for induction of airborne pathogen-specific Trrm. We have previously shown that influenza-specific Trrm can be deposited in stable quantities in the nasal tissue of mice, these cells do not decline as aggressive as lung Trrm, and in turn, offer long-term protective immunity. In a proof of principle study, we further demonstrate that nasal CD8+ Trrm 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.

Collectively, mouse studies showcase a range of immunization approaches to evoke respiratory tract Trrm with a common requirement of delivering the immunization agent into the respiratory tract to facilitate optimal Trrm formation. The appropriate selection of adjuvant, delivered by the correct route, can also greatly improve vaccines that aim to elicit lung Trrm development. 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 Trrm development. A greater understanding of the appropriate local inflammatory milieu triggered by these Trrm promoting adjuvants will allow for the

**Table 3.** Vaccine strategies that generate respiratory tract Trrm.

| Strategy          | Type             | Respiratory pathogen                                      |
|-------------------|------------------|-----------------------------------------------------------|
| **Vaccine vectors** | Adenovirus       | Influenza129,151, SARS-CoV-2, M. tuberculosis152          |
| MCMV              | Influenza153, RSV131,154 |
| MVA               | Influenza155     |
| Influenza A virus | M. tuberculosis156 |
| CMV               | M. tuberculosis157 |
| Sendai virus      | M. tuberculosis158 |
| Vaccinia virus    | SARS-CoV102      |
| **Particulate vaccines** | Nanoparticles | Influenza137,139,159,160, M. tuberculosis161 |
|                   | Virus-like particles | Influenza162, RSV163                                      |
| **Other**         | Attenuation      | Influenza164, Brucella abortus164, M. tuberculosis152, S. pneumoniae109 |
|                   | Antibody-targeted vaccination | Influenza160 |
|                   | Chitosan-hydrogel | Influenza140                                           |
|                   | Outer membrane vesicles | Bordetella pertussis165                                 |
|                   | Virus replicon particle | SARS-CoV/MERS-CoV103                                     |
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.

REFERENCES

1. Sallusto, F., Lenig, D., Förster, R., Lipp, M. & Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401, 708–712 (1999).
2. Gebhardt, T. et al. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat. Immunol. 10, 524–530 (2009).
3. Schenkel, J. M. & Masopust, D. Tissue-resident memory T cells. Immunity 41, 886–897 (2014).
4. Mueller, S. N. & Mackay, L. K. Tissue-resident memory T cells: local specialists in immune defence. Nat. Rev. Immunol. 16, 79–89 (2016).
5. Masopust, D. et al. Dynamic T cell migration program provides resident memory within intestinal epithelium. J. Exp. Med. 207, 535–564 (2010).
6. Masopust, D., Vezys, V., Marzo, A. L. & Lefrancois, L. Preferential localization of effector memory cells in nonlymphoid tissue. Science 291, 2413–2417 (2001).
7. Reinhardt, R. L., Khoruts, A., Merica, R., Zell, T. & Jenkins, M. K. Visualizing the generation of memory CD4 T cells in the whole body. Nature 410, 101–105 (2001).
8. Wakim, L. M., Waithman, J., van Rooijen, N., Heath, W. R. & Carbone, F. R. Dendritic-cell-induced memory T cell activation in nonlymphoid tissues. Science 319, 198–202 (2008).
9. Jiang, X. et al. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. Nature 483, 227–231 (2012).
10. Iijima, N. & Iwasaki, A. T cell memory. A local macrophage chemokine network 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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22. Shin, H. & Iwasaki, A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. Nature 491, 463–467 (2012).
23. Wakim, L. M., Woodward-Davis, A. & Bevan, M. J. Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. Proc. Natl. Acad. Sci. USA 107, 17872–17879 (2010).
24. Fernandez-Ruiz, D. et al. Liver-resident memory CD8+ T cells form a front-line defense against malaria liver-stage infection. Immunity 45, 889–902 (2016).
25. Casey, K. A. et al. Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues. J. Immunol. 188, 4866–4875 (2012).
26. Beura, L. K. et al. T cells in nonlymphoid tissues give rise to lymph-node-resident memory T cells. Immunity 48, 327–338 e325 (2018).
27. Beura, L. K. et al. CD4+ resident memory T cells dominate immuno-surveillance and orchestrate local recall responses. J. Exp. Med. 216, 1214–1229 (2019).
28. Fonseca, R. et al. Developmental plasticity allows outside-in immune responses by resident memory T cells. Nat. Immunol. 21, 412–420 (2020).
29. Stolley, J. M. et al. Retrograde migration supplies resident memory T cells to lung-draining LN after influenza infection. J. Exp. Med. 217, e20192197 (2020).
30. Kliznık, M. M. et al. Human CD4(-)/CD103(-)/CD69(-) resident cutaneous resident memory T cells are found in the circulation of healthy individuals. Sci. Immunol. 4, eaax995 (2019).
31. Topham, D. J. & Reilly, E. C. Tissue-resident memory CD8+ T cells: from phenotype to function. Front. Immunol. 9, 515 (2018).
32. Reilly, E. C. et al. TRM integrins CD103+CD49a differentially support adherence and metaplasia after resolution of influenza virus infection. Proc. Natl. Acad. Sci. USA 117, 12336–12341 (2020).
33. Kumar, B. V. et al. Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites. Cell Rep. 20, 2921–2934 (2017).
34. Showi, L. R. et al. CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature 440, 540–544 (2006).
35. Mackay, L. K. et al. Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. J. Immunol. 194, 2059–2063 (2015).
36. Skon, C. N. et al. Transcriptional downregulation of S1P1 is required for the establishment of resident memory CD8+ T cells. Nat. Immunol. 14, 1285–1293 (2013).
37. Lee, Y. T. et al. Environmental and antigen receptor-derived signals support sustained surveillance of the lungs by pathogen-specific cytotoxic T lymphocytes. J. Virol. 85, 4085–4094 (2011).
38. Hadley, G. A., Bartlett, S. T., Via, C. S., Rostapshova, E. A. & Moainie, S. The epithelial-cell-specific integrin, CD103 (alpha E integrin), defines a novel subset of alveolar CD8+ CTL. J. Immunol. 159, 3748–3756 (1997).
39. Cepek, K. L. et al. Adhesion between epithelial-cells and T-lymphocytes mediated by E-cadherin and the alpha(Ebeta7) integrin. Nature 372, 190–193 (1994).
40. Szabo, P. A., Miron, M. & Farber, D. L. Location, location, location: tissue resident memory T cells in mice and humans. Sci. Immunol. 4, eaas9673 (2019).
41. Reagin, K. L. & Klornowski, K. D. Incomplete memories: the natural suppression of tissue-resident memory CD8+ T cells in the lung. Front. Immunol. 9, 17 (2018).
42. Matopas, D. & Soerens, A. G. Tissue-resident T cells and other resident leukocytes. Annu. Rev. Immunol. 37, 521–546 (2019).
43. Hombrink, P. et al. Programs for the persistence, vigilance and control of human CD8+ lung-resident memory T cells. Nat. Immunol. 17, 1467–1478 (2016).
44. Oja, A. E., Piet, B., Helbig, C., Stark, R., van der Zwan, D. & Blaauwgeers, H. et al. Trigger-happy resident memory CD8+ T cells inhabit the human lungs. Mucosal Immunol. 11, 654–667 (2018).
45. Teijaro, J. R. et al. Cutting edge: tissue-retentive lung memory CD4+ T cells mediate optimal protection to respiratory virus infection. J. Immunol. 187, 5510–5514 (2011).
46. Schenkel, J. M., Fraser, K. A., Vezys, V. & Masopust, D. Sensing and alarm function of resident memory CD8+ T cells. Nat. Immunol. 14, 509–513 (2013).
47. Ge, C. et al. Bystander activation of pulmonary Trm cells attenuates the severity of bacterial pneumonia by enhancing neutrophil recruitment. Cell Rep. 29, 4236–4244 e4233 (2019).
48. Ariotti, S. et al. T cell memory. Skin-resident memory CD8+ T cells trigger a state of tissue-wide pathogen alert. Science 346, 101–105 (2014).
49. Beura, L. K. et al. Intravital mucosal imaging of CD8+ resident memory T cells shows tissue-autonomous cell responses that amplify secondary memory. Nat. Immunol. 19, 173–182 (2018).
50. Park, S. L. et al. Local proliferation maintains a stable pool of tissue-resident memory T cells after antiviral recall responses. Nat. Immunol. 19, 183–191 (2018).
51. Turner, D. L. et al. Lung niches for the generation and maintenance of tissue-resident memory T cells. Mucosal Immunol. 7, 501–510 (2014).
McMaster, S. R. et al. Pulmonary antigen encounter regulates the establishment of tissue-resident CD8 memory T cells in the lung airways and parenchyma. *Mucosal Immunol.* 11, 1071–1078 (2018).

Ray, S. J. et al. The collagen binding α1β1 integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. *Immunity* 20, 167–179 (2004).

Galkina, E. et al. Tissue-resident migration of effector CD8+ T cells into the interstitium of the normal lung. *J. Clin. Invest.* 115, 3473–3483 (2005).

Snyder, M. E. et al. Generation and persistence of human tissue-resident memory T cells in lung transplantation. *Sci. Immunol.* 4, eaav5581 (2019).

Hayward, S. L. et al. Environmental cues regulate epigenetic reprogramming of airway-resident memory CD8+ T cells. Nat. Immunol. 21, 309–320 (2020).

McMaster, S. R., Wilson, J. J., Wang, H. & Kohlemeier, J. E. Airway-resident memory CD8 T cells. Cells provide antigen-specific protection against respiratory virus challenge through rapid IFN-γ production. *J. Immunol.* 195, 203–209 (2015).

Wein, A. N. et al. CXCR6 regulates localization of tissue-resident memory CD8 T cells to the airways. *J. Exp. Med.* 216, 2748–2762 (2019).

Wang, Z. et al. PD-1hiCD8+ resident memory T cells balance immunity and fibrotic sequelae. *Sci. Immunol.* 4, eaaw1217 (2019).

Ferreira, C. et al. Type 1 Treg cells promote the generation of CD8+ T cells during respiratory influenza infection. *Immunity* 202, 2482–2492 (2019).

McGill, J., Van Rooijen, N. & Legge, K. L. IL-15 supports the generation of protective lung-resident memory CD8 T cells. Front. Immunol. 10, 2332 (2019).

Mak, S. et al. Developmental regulation of effector and resident memory T cell generation during pediatric viral respiratory tract infection. *J. Immunol.* 201, 432–439 (2018).

Sutter, B. et al. Dynamics of influenza-induced lung-resident memory T cells underlie waning heterosubtypic immunity. *Sci. Immunol.* 2, eaag2031 (2017).

Pizzolla, A. et al. Influenza-specific lung-resident memory T cells are proliferative and polyfunctional and maintain diverse TCR profiles. *J. Clin. Invest.* 128, 721–733 (2018).

Low, J. S. et al. Tissue-resident memory T cell reactivation by diverse antigen-presenting cells imparts distinct functional responses. *J. Exp. Med.* 217, e20192291 (2020).

Nguyen, T. H. et al. Influenza, but not SARS-CoV-2, infection induces a rapid interferon response that wanes with age and diminished tissue-resident memory CD8 T cells. *Clin. Transl. Immunol.* 10, e1242 (2021).

de Bree, G. J. et al. Selective accumulation of differentiated CD8+ T cells specific for respiratory viruses in the human lung. *J. Exp. Med.* 202, 1433–1442 (2005).

Piet, B. et al. CD8+ T cells with an intraepithelial phenotype upregulate cytotoxic function upon influenza infection in human lung. *J. Clin. Invest.* 121, 2254–2263 (2011).

Koutsakos, M. et al. Human CD8+ T cell cross-reactivity across influenza A, B and C viruses. *Nat. Immunol.* 20, 613–625 (2019).

Jozwik, A. et al. RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. *Nat. Commun.* 6, 10224 (2015).

Kinnear, E. et al. Airway T cell responses protect against RSV infection in the absence of antibody. *Mucosal Immunol.* 11, 249–256 (2018).

Luangrath, M. A., Schmidt, M. E., Hartwig, S. M., Varga, S. M. & Tissue-Resident Memory, T. Cells in the lungs protect against acute respiratory syncytial virus infection. *Immunity Horizons* 5, 59–69 (2021).

Channappanavar, R., Fett, C., Zhao, J., Meyerholz, D. K. & Perlman, S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *J. Virol.* 88, 11034–11044 (2014).

Zho, J. et al. Airway memory CD4+ T cells mediate protective immunity against emerging respiratory coronaviruses. *Immunity* 44, 1379–1391 (2016).

Szabo, P. A. et al. Longitudinal profiling of respiratory and systemic immune responses reveals myeloid cell-driven lung inflammation in severe COVID-19. *Immunity* 54, 797–814 e6 (2021).

Liao, M. et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat. Med.* 26, 842–844 (2020).

Grau-Exposito, J. et al. Peripheral and lung resident memory T cell responses against SARS-CoV-2. *Nat. Commun.* 12, 3010 (2021).

McMahan, R. et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* 590, 630–634 (2021).
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The authors declare no competing interests.

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