Immune Memory Focus

Local memory CD4 T cell niches in respiratory viral infection

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Respiratory viral infections present a major threat to global health and prosperity. Over the past century, several have developed into crippling pandemics, including the SARS-CoV-2 virus. Although the generation of neutralizing serum antibodies in response to natural immunity and vaccination are considered to be hallmarks of viral immune protection, antibodies from long-lived plasma cells are subject to immune escape from heterologous clades of zoonotic, recombined, or mutated viruses. Local immunity in the lung can be generated through resident memory immune subsets that rapidly respond to secondary infection and protect from heterologous infection. Although many immune cells are required to achieve the phenomenon of resident memory, herein we highlight the pleiotropic functions of CD4 tissue resident memory T cells in the lung and discuss the implications of resident memory for vaccine design.

Introduction

Respiratory virus outbreaks have deleteriously impacted global health and prosperity throughout human history. A lack of immunity to emergent respiratory viral infections is the underlying cause of several global pandemics that have occurred over the past century, including influenza A pandemics (Monto and Fukuda, 2020) and novel coronavirus pandemics, such as the current SARS-CoV-2 pandemic responsible for ~2.5 million deaths (World Health Organization, 2021a). Although neutralizing antibodies produced by B cells in the bone marrow, called long-lived plasma cells (LLPCs), offer excellent protection to previously circulated strains of respiratory viruses (Lam and Baumgarth, 2019), the occasional zoonotic emergence or recombination event results in viral clades with novel surface proteins that are not well-recognized by circulating antibodies or memory lymphocytes, introducing the potential for unrestrained infection or pandemic (Gostic et al., 2016; Horimoto and Kawaoka, 2005). Even when partial immunity in many communities exists, as seen in the seasonal influenza epidemics, significant mortality and loss of productivity remains (World Health Organization, 2021b). Understanding how to elicit immunity to respiratory viruses through vaccination in order to prevent the emergence of disease is therefore a significant focus of ongoing research.

The immune system is rapidly called into action if a respiratory virus is able to productively infect a host. While it takes days to mount a primary adaptive immune response to a previously unperceived virus, memory lymphocytes can become activated in response to a prior or closely related viral infection within hours. Layers of adaptive immune memory have evolved to respond to homologous or heterologous viral antigens through a variety of mechanisms. LLPCs provide the first line of defense by constitutively secreting antibodies. While these antibodies may provide sterilizing immunity to homologous infection, they also exert immune pressure on viral surface antigens, evolutionarily driving the outgrowth of mutated virions. Cross-reactive memory T and B lymphocytes are therefore an important next layer of protection from viruses that may express closely related surface proteins but have mutated to evade the circulating LLPC-derived antibody repertoire. While some memory B cells can rapidly respond to heterologous reinfection by making antibody-secreting cells (Wong et al., 2020), others can reenter a germinal center response for further diversification (Pape et al., 2011; Shlomchik, 2018; Dogan et al., 2009). Memory T cells retain the ability to respond to homologous or heterologous viral antigens both through their ability to bind peptide:MHCs (pMHCs) with a broad range of receptor affinities and through their capacity to respond to intracellular proteins that may have avoided antibody-mediated immune pressure. This is important because many of the intracellular antigens are critical, highly conserved housekeeping proteins.
necessary for viral replication and function. For example, memory CD4 and CD8 T cells elicited by seasonal influenza A infection can be rapidly activated ex vivo in response to elements of pandemic strains of influenza, including H5N1, H3N2, and H1N1 (Chen et al., 2014; Richards et al., 2010), and their presence correlates inversely with disease severity, even in the absence of neutralizing antibodies (Seidhar et al., 2013; Wilkinson et al., 2012). When analyzed in more detail, T cell cross-reactivity to pandemic strains was heavily enriched for clones specific to the internal proteins nucleoprotein (NP) and M1 (Lee et al., 2008), supporting the notion that internal proteins are more highly conserved between seasonal and pandemic strains of virus (Sant et al., 2018).

Virus-specific memory T cells can behave as sentinels against reinfection due to their localization. Memory T cells persist in various anatomical compartments following respiratory viral infection, including the blood, lymphatic organs, and lungs (Szabo et al., 2019; Jameson and Masopust, 2018). Multiple studies have focused on the ability of resident memory T cells (TRM cells) to facilitate an optimally efficient response to viral reinfection, and they have therefore become an important focus of investigation. Using parabiosis experiments in mice, virus-specific CD4 and CD8 TRM cells have been observed to be retained in tissues across the body following systemic and mucosal viral infections (Steinert et al., 2015; Beura et al., 2019). Following in vivo antigen restimulation, both CD4 and CD8 TRM cells can rapidly secrete cytokines, including IFN-γ, which facilitates the recruitment of circulating immune cells and the activation of other resident cells critical for protection against disease (Schenkel et al., 2014; Beura et al., 2019). For example, in two separate animal models, the activation of viral-specific CD8 and CD4 TRM cells was required to control viral burden during reinfection in the skin and female reproductive tract through the production of IFN-γ (Iijima and Iwasaki, 2014; Schenkel et al., 2014). In studies focused on respiratory infection, the transfer of CD4 lung TRM cells from mice that had previously cleared influenza virus protected unexposed mice from infection, while the transfer of spleen-derived memory T cells provided no better protection than naive T cells (Teijaro et al., 2011). This inability of circulating T cell memory to recapitulate the protection afforded by aspects of resident memory has been reported frequently (Wu et al., 2014; Slüter et al., 2013; Teijaro et al., 2010) and highlights the importance of understanding TRM cell biology for protection against viral infections.

Herein, we will specifically focus on virus-specific CD4 TRM cells and the important niches they occupy within the lung to prevent disease. We highlight the multifaceted roles these key sentinels play in the lung tissue upon reinfection, including direct modes of pathogen control and indirect coordination of immune functions in the tissues. The ability to localize long-lived TRM cells to a given mucosal tissue while simultaneously imparting the characteristics needed to coordinate the elimination of a target pathogen is the holy grail of vaccine development. We therefore also discuss key aspects of CD4 TRM cell differentiation and maintenance, which can be modulated by specific vaccine strategies to harness a coordinated immune response in the lung and prevent future pandemics.

CD4 TRM cells in natural infection

CD4 TRM cells in the lung are critical mediators of protection against respiratory viral infections. Experiments using cell transfers in an animal model have demonstrated that unexposed recipients of influenza-specific CD4 TRM cells are better protected than recipients of splenic-derived memory cells (Teijaro et al., 2010). While in these studies lung CD4 TRM cells were protective in the absence of both CD8 T cells and B cells, recent studies have also demonstrated important roles for CD4 TRM cells in recruiting and maintaining CD8 T cells (Son et al., 2021; Laidlaw et al., 2014) as well as in activating lung resident B cells (Swarnailekha et al., 2021). Here, we discuss these unique attributes of CD4 TRM cells, including the niches they occupy in lung tissue, and the specific effector functions that have been associated with antiviral protection (Fig. 1).

Sentinel responses at sites of reinfection

TRM cells act as sentinels that prevent respiratory disease through their ability to localize to tissues, reactivate in response to reinfection, and rapidly express effector molecules to limit viral replication. Although naive and resting memory T cells share the expression of ~95% of their transcriptome, memory T cells selectively possess an epigenetic landscape that retains open accessibility to genes that were expressed during their effector phase (Akondy et al., 2017; Araki et al., 2009). Demethylated chromatin at sites of effector genes, such as CXCRI, CXCR4, CCR5, IL-2Ra, IL-18Ra, IFN-γ, granzyme B, and perforin, have been detected in memory T cells, allowing for rapid transcription and accelerated effector protein production in response to TCR stimulation (Akondy et al., 2017; Weng et al., 2012). Using in vitro ovalbumin peptide simulations, ovalbumin-specific CD4 memory T cells have been shown to display an open chromatin landscape at the IFNG locus, allowing for rapid production of IFN-γ within 2 h (Lai et al., 2011). In stark contrast, a naive ovalbumin-specific CD4 T cell migrating through lymphoid organs must find an APC expressing its pMHCII in the context of costimulation and cytokines, which would lead to the upregulation of T-bet, bearing a delay of up to 24 h before it gained the ability to open the IFNG locus (Lai et al., 2011). Additionally, in response to ex vivo restimulation, CD4 TRM cells isolated from human airways produced the effector cytokines IFN-γ and TNF-α faster and in larger quantities compared with circulating CD4 memory cells from the blood, suggesting that TRM cells represent the memory T cell compartment that is the most epigenetically poised to respond rapidly to viral reinfection (Oja et al., 2018).

Cytokines produced rapidly by memory CD4 TRM cells are essential for the accelerated recruitment, localization, and activation of innate immune cells only hours after reinfection, a process that takes up to 1 d in mice without CD4 TRM cells (Soudja et al., 2014). In a model of secondary influenza infection, upregulation of the secondary effector molecules IL-1α, IL-1β, TNF-α, IL-6, and the chemokines CXCL9, CXCL10, and CCL2 by tissue-residing innate cells depended on the presence of CD4 TRM cells and their interactions with CD11c+ cells presenting viral antigen on pMHCII (Strutt et al., 2010; Soudja et al., 2014). In these settings, innate cells also facilitated the recruitment of
Ly6C+ monocytes from the bloodstream to further amplify rapid antiviral activity (Soudja et al., 2014).

Importantly, through their immediate production of IFN-\(\gamma\), CD4 TRM cells can modulate the localization of key effector cells to sites of active viral replication in the respiratory tract. Since most respiratory viruses propagate by infecting cells within the airways and not the parenchyma, many viral-specific CD4 TRM cells constitutively express the chemokine receptor CXCR3 and integrin CD49a (VLA-1) and can rapidly migrate to subepithelial or bronchoalveolar spaces in response to the CXCR3 ligands CXCL9 and CXCL10 produced by infected airway epithelial cells (Guvenel et al., 2020; Chapman et al., 2011; Chapman and Topham, 2010). Unlike what is seen in certain bacterial infections like Mycobacterium tuberculosis (Moguche et al., 2015), viral-specific CD4 T cells localized to the airways produce the largest amount of cytokines, including IFN-\(\gamma\), IL-10, and IL-2. In a model of SARS-CoV vaccination, the blockade of IFN-\(\gamma\) either by administration of IFN-\(\gamma\)-blocking antibody or depletion of CD4 TRM cells in the airway led to a loss in protection from viral challenge (Zhao et al., 2016). In this study, it was determined that IFN-\(\gamma\) from CD4 TRM cells was needed to activate resident dendritic cells (DCs) and recruit CXCR3+ memory CD8 TRM cells to the airway through the IFN-\(\gamma\)-dependent upregulation of CXCL9 and CXCL10. Notably, only the blockade of IFN-\(\gamma\) during a secondary, but not a primary, infection with influenza restricted viral clearance (Teijaro et al., 2010), underscoring its select role in secondary responses.

In addition to IFN-\(\gamma\), CD4 TRM cells can also rapidly produce the cytokines TNF-\(\alpha\) and IL-10, although these cytokines have been shown to be either beneficial or detrimental to the host.
depending on the model of infection studied. In a model of respiratory syncytial virus (RSV) infection, mice that received TNF-α-blocking antibodies developed reduced tissue pathology and clinical disease. In this study, mice treated with anti-TNF-α also produced lower levels of IFN-γ from their CD4 T cell compartment, suggesting that TNF-α production may cause T cell-dependent pathology in the lung (Hussell et al., 2001). Conversely, TNF-α KO mice during primary influenza infection displayed reduced viral burden and weight loss and heightened levels of both IFN-γ-producing virus-specific CD8 T cells in the lungs and hemagglutinin (HA)-specific antibody in the serum, suggesting that TNF-α production was actually suppressing a productive adaptive immune response (Damjanovic et al., 2011). Additionally, in coronavirus infection, the i.n. administration of recombinant TNF-α to naive mice 12 h before challenge with SARS-CoV led to decreased survival (Zhao et al., 2016). These conflicting reports and the relatively small body of detailed research on the mechanism of TNF-α during viral infection highlights the need to study this important cytokine, especially as it is often used as a proxy of productive antiviral immunity, and its blockade is a common therapy for diseases such as rheumatoid arthritis and inflammatory bowel disease.

IL-10, a key regulatory cytokine in the lung, has been shown to be produced by T-bet+ FOXP3+ effector (Sun et al., 2009) and memory (Zhao et al., 2016) CD4 T cells in the lung. To prevent lethal immunopathology during the late stages of influenza infection, CD4 T cells are required to orchestrate a contraction of the inflammatory immune response through the upregulation of IL-10 in activated effector T cells (Sun et al., 2011; O’Garra and Vieira, 2007). Although necessary for restraining lethal inflammation, IL-10 has also been shown to diminish the antibody response to influenza virus (Sun et al., 2010), and its deletion led to survival in a model of lethal influenza infection (McKinstry et al., 2009). Therefore, as is likely the case for TNF-α, the role of IL-10 and the relative production of IL-10 by CD4 T RM cells may vary depending on the timing, nature, and severity of the infection. For example, compared with a primary response, lower levels of IL-10 are produced during the secondary response to influenza infection (Strutt et al., 2012). This phenomenon is likely explained by the accelerated antiviral response to secondary infection when local T cell memory is present. Since viral burden is kept in check, the immune system does not employ a highly inflammatory response to clear infection and perhaps does not need large quantities of anti-inflammatory mediators, such as IL-10, to dampen inflammation.

In addition to cytokine production, some CD4 T cells have been shown to engage in pMHCII-restricted lysis of virus-infected cells, a mechanism of viral control previously thought to be restricted to CD8 CTLs (Brown et al., 2012). CD4 CTLs have been described clustered around infected cells in the airways and produce the effector molecules granzyme B and perforin (Marshall et al., 2017). CD4 CTLs depend on IL-2, Blimp-1, and Eomes for their generation (Qui et al., 2011) as well as type I IFN (Hua et al., 2013), which is produced by infected airway epithelial cells (Ioanidis et al., 2013). Intriguingly, CD4 T cell differentiation to a CD8 CTL-like fate is mediated by repression of the transcription factor ThPOK (Mucida et al., 2013), which alternatively is needed to repress CD8-associated molecules in thymic and peripheral differentiation of CD4 T cells (He et al., 2005; Wang et al., 2008). More research is needed to determine the fate of these cells following viral clearance as CD4 T RM cells are not detected in the airways at memory time points (Turner et al., 2014), and ThPOK is necessary for the generation of functional central memory CD4 T cells and their production of IL-2 (Ciucci et al., 2019).

**Helper mechanisms within ectopic lymphocyte clusters**

Although many virus-specific CD4 T RM cells migrate to the airways in order to combat active viral replication (Zhao et al., 2016), there is also a functionally distinct population of CD4 T RM cells that remains in the parenchymal lung tissue and provides important helper functions to other virus-specific immune cells. Following contraction of a primary immune response, two distinct populations of CD4 T RM cells form in the lung that can be detected by their reciprocal expression of folate receptor 4 (FR4) and P-selectin glycoprotein ligand 1 (PSGL1; Swarnalekha et al., 2021; Son et al., 2021). PSGL1+FR4- CD4 T RM cells express higher levels of T-bet and CXCR6, resembling a sentinel CD4 T RM cell subset that rapidly migrates to areas of infection and engages in direct viral clearance. In contrast, FR4+PSGL1- CD4 T RM cells selectively express PD-1, CXCXR5, CXCXR4, and ICOS; depend on BCL-6, MHCII, and B cells for their development; and colocalize with B cells within the parenchymal lung tissue (Swarnalekha et al., 2021; Son et al., 2021). During influenza reinfection, mice that did not develop CD4 T RM cells with the capability to cluster with B cells had an inability to produce large amounts of HA- or NP-specific B cells or antibodies and displayed reduced survival (Son et al., 2021).

The clustering of CD4 T RM cells with other immune cells, including B cells, CD21+ follicular DCs, and CD8 T cells, in peribronchial areas of the lung was previously described and is sometimes referred to as inducible bronchus-associated lymphoid tissue.
CXCR5+ B cells to the lung parenchyma, which were necessary for infection, the release of type I IFN drove the production of cytokines required multiple factors, although in one model of influenza pulmonary inflammation of ectopic sources of CXCL13/CCL19 is complex and likely requires multiple factors, although in one model of influenza infection, the release of type I IFN drove the production of cytokines. 

Thus, CD4 TRM cells aid in local production of antibodies by BRM cells in other models of inflammation (Rao et al., 2017). Further, respiratory viral infection in μMT mice, which lack B cells, drove more virus-specific T cells to the lung parenchyma during effector phases of the infection but led to reduced numbers of long-lived memory CD4 T_{RM} cells compared with WT mice, suggesting that B cells are essential for the survival and maintenance of CD4 T_{RM} cells in the lung in the memory phase (Hondowicz et al., 2018). Therefore, iBALT represents an important niche for the maintenance of these virus-specific CD4 T_{RM} cell populations in the lung, as has been shown in ectopic memory lymphocyte clusters in other tissues (Iijima and Iwasaki, 2014) and in an allergic airway model of pulmonary inflammation (Shinoda et al., 2016). Upon reactivation in iBALT, CD4 T_{RM} cells have been shown to colocalize with B_{RM} cells, providing a rapid burst of virus-specific antibodies from local plasmablasts (Allie et al., 2019; Swarnalekha et al., 2021) while also leading to further affinity maturation of lung B_{RM} cells toward broadly neutralizing, cross-reactive antibodies that are highly protective in heterologous infections (Adachi et al., 2015; Onodera et al., 2012; Son et al., 2021). In fact, CD4 T_{RM} cells are still detected in the lung parenchymal tissue, and B cells are still found in the lung parenchymal tissue during effector phases of the infection. However, when analyzed in more detail, it was shown that IL-17 induction of CD8 CTLs, B cells, and activated CCR2+ and CCR7+CD86+ DCs in response to secondary stimulation with sterile antigen in vivo (Beura et al., 2019). Therefore, even in the absence of distinct iBALT structures, helper functions of CD4 T_{RM} cells still persist, although more research is needed to determine the importance of these structures in secondary viral infection.

**Type 17 and type 2 immune responses to respiratory viral infection**

During the first days of a primary response to respiratory viral infection, APCs present a combination of viral antigen and cytokines to naive T cells to prime their proliferation and maturation into virus-specific effector cells. Depending on the milieu of cytokines produced by innate cells during priming, naive CD4 T cells divert into distinct effector modes, which have been canonically defined by the effector cytokines they produce (Ruterbusch et al., 2020). Viral infections of the respiratory tract, such as influenza virus, coronavirus, and rhinovirus, primarily induce the production of type I IFN and IL-12 from APCs, priming virus-specific naive T cells to become Th1 helper 1 (Th1) cells that express the transcription factor T-bet. Indeed, the sentinel functions discussed above are performed by Th1 cells, although Th17 and Th2 effector modes are elicited in some viral infections and are therefore discussed below.

Th17 cells are defined by their expression of the transcription factor RORγT and their ability to produce the effector cytokines IL-17, IL-22, and IL-26 (Mangan et al., 2006; Ivanov et al., 2006). Although their presence during the early stages of viral infection induces severe lung pathology, Th17 cells paradoxically provide important functions in the resolution phase of primary viral respiratory infections. In a mouse model of RSV infection, the blockade of IL-17A reduced mucus production and granulocyte accumulation in the airways, and this correlated with improved recruitment of RSV-specific CD4 and CD8 T cells and a reduced viral load (Mukherjee et al., 2011). In mouse models of lethal H1N1 influenza infection, IL-17 KO mice have been shown not only to survive but also to lose minimal weight during infection (Li et al., 2012), suggesting that Th17 cells may be the drivers of mortality. When analyzed in more detail, it was shown that IL-17 was predominantly produced by γδ-T cells, and the blockade of
IL-17A led to improved survival; a concomitant decrease in the inflammatory cytokines IL-6, IL-8, and G-CSF; and a reduction of neutrophil recruitment into the airways (Crowe et al., 2009). Indeed, neutrophil recruitment to the airways is a hallmark of pathology in severe viral infection and has been known to be directly mediated by IL-17 (Laan et al., 1999; Ye et al., 2001).

Paradoxically, Th17 cells also have multiple beneficial roles during the resolution phase of respiratory viral infection. Histological analysis of tissue samples from the 1918 and 2009 influenza pandemics have revealed that bacterial superinfection causing bacterial pneumonia, in conjunction with viral infection, was the overwhelming cause of death (Morens et al., 2008; Bautista et al., 2010). Interestingly, IL-22, a cytokine almost exclusively produced by Th17 cells, mediates barrier tissue repair and potent mucosal host defense against many forms of bacterial infections (Liang et al., 2006). Indeed, in the late stages of influenza infection, the IL-22R is expressed on lung epithelial cells, and the infection of IL-22 KO mice led to decreased lung tissue function and reduced survival (Pociask et al., 2013). IL-22 has pleiotropic effects on the lung epithelium, inducing proliferation and repair mechanisms while also stimulating the production of antimicrobial peptides. Both these mechanisms are important in resolving potential instances of secondary bacterial superinfection (Aujla et al., 2008). Strikingly, in multiple models of influenza infection, it has been shown that the production of type 1 IFN directly inhibits the ability to develop a Th17 response and limits the ability to clear a subsequent superinfection with Staphylococcus aureus, suggesting that susceptibility to bacterial superinfection may have a direct immunological link and is not exclusively due to damage to the epithelium (Shahangian et al., 2009; Kudva et al., 2011). Finally, IL-17 production from CD4+ cells during resolution of infection is necessary for iBALT formation, specifically highlighting the importance of IL-17 for the generation of immune memory (Rangel-Moreno et al., 2011). Th17 cells in respiratory viral infection are therefore somewhat dichotomous in nature: While they can induce IL-17-dependent hyperresponsiveness in acute viral infection, they can also provide reparative signals to epithelial cells, control occurrence of secondary bacterial superinfections, and induce iBALT (Stockinger and Omenetti, 2017). Additionally, whether Th17 cells persist to the memory phase and what their role is during secondary infection are active topics of discussion, as they have been shown to display plasticity and, therefore, varying longevity in different contexts (Pepper et al., 2010; Lee et al., 2009; Amezcuca Vesely et al., 2019).

Th2 cells, defined by their expression of the transcription factor GATA-3 and the production of the cytokines IL-4, IL-5, and IL-13, are very poor at clearing viral infections and are associated with adverse responses to RSV (Dakhama et al., 2005). Seminal work in the field has shown that Th2 cells are generally not associated with protection from type 1-inducing viral infections, as demonstrated by the lack of protection engendered by the transfer of large numbers of influenza-specific Th2 clones into naïve mice (Graham et al., 1994). RSV infection in mice and humans can induce symptomatic airway hyperresponsiveness that depends on the Th2 cytokines IL-4 and IL-13 (Dakhama et al., 2005; You et al., 2013). Studies have suggested that some RSV strains more efficiently polarize a type 2 immune response in the lung (Moore et al., 2009; Lukacs et al., 2006), and this response is dramatically heightened in neonates due to an inability of neonatal plasmacytoid DCs to produce type I IFN before 3 wk of age, leading to an absence in Th1 cells in the lung that produce IFN-γ (Marr et al., 2014; Cormier et al., 2014). In the case of neonatal infection, the administration of type I IFN or the transfer of adult plasmacytoid DCs into neonatal mice rescued them from airway hyperresponsiveness in primary and secondary responses and eliminated the production of Th2 cytokines (Cormier et al., 2014). Recent work has defined type 2 immune niches in the lung that are generated following pulmonary hyperresponsiveness and harbor stromal cells that express the Th2 cytokines IL-33 and thymic stromal lymphopoietin (Dahlgren et al., 2019). The danger of a permanent type 2 environment is apparent in neonatal RSV infection, where even when mice are reinfected in a context capable of driving a Th1 response, Th2 cytokines dominate and induce airway hyperresponsiveness (Culley et al., 2002).

In summary, much is still to be learned concerning the dynamics and diverse functions of CD4 T<sub>RM</sub> cells during natural viral infection of the respiratory tract. For example, it is currently unknown whether the reactivation dynamics of memory CD4 T<sub>RM</sub> cells mirrors the bifurcation events that occur in primary and secondary responses that have been described in lymphoid organs (Pepper et al., 2011; Pepper and Jenkins, 2011; DiToro et al., 2018). Additionally, it will be important to understand the particular effector mechanisms that are required of CD4 T<sub>RM</sub> cells during secondary infections, as most research has focused on primary infection and provides conflicting conclusions on the role of certain cytokines, such as IL-10, TNF-α, and IL-17.

Harnessing virus-specific T<sub>RM</sub> cells by vaccination
Although virus-specific resident memory cells provide numerous benefits during a reinfection event, current vaccination strategies instead often target the development of circulating neutralizing antibodies. Studies are beginning to address the added benefits of T<sub>RM</sub> cells in vaccination. In a mouse model of influenza vaccination, Zens et al. (2016) compared the ability of dead inactivated virus or live, attenuated influenza virus (LAIV) administered either i.m. or as an aerosol i.n. to elicit T<sub>RM</sub> cells. They found that only LAIV administered i.n. drove the development of CD69<sup>+</sup> CD8 and CD4 T<sub>RM</sub> cells that resembled canonical resident memory cells in the lung, suggesting that yearly human tri- or quadrivalent influenza vaccinations, which are administered i.m., do not drive T<sub>RM</sub> cell formation. Further, although i.m. inactivated virus drove a more potent anti-HA antibody response in the serum, only i.n. LAIV vaccination protected mice from disease after infection with a heterologous influenza strain. Studies such as these highlight the urgency for approved vaccination strategies that can drive tissue-resident memory from studies in natural infections, we know that the generation of potent T<sub>RM</sub> cells in vivo requires numerous cellular signals occurring in both the lymphatic organs and the tissues. Although the developmental pathway driving CD4 and CD8 T<sub>RM</sub> cells are not likely identical, studies have revealed...
overarching tissue residency modules in lymphocytes (Mackay et al., 2016), and CD4 and CD8 T cells share many similar molecular axes that can be harnessed through creative vaccination strategies to develop universal vaccines and prevent future pandemics.

**Priming of antiviral T<sub>RM</sub> cells in the LN**

As described above, the differentiation cascade of a memory T cell begins in the LN. Cues from cellular sources of costimulation and cytokines not only have the potency to drive T cells to distinct effector modes (Th1/Th2/Th17) but also act on virus-specific T cells to modulate their transcriptional and epigenetic landscape, affording them the potential to leave the lymphatics, enter the tissue, and become T<sub>RM</sub> cells. Within 6 h of antigen recognition, and before proliferation, it has been shown that virus-specific T cells that receive heightened TCR signals rapidly upregulate IL-2 and B cell lymphoma 6 (BCL-6) transcripts (DiToro et al., 2018). This increase in BCL-6 represses the ability of these IL-2-producing cells to perceive IL-2 signals through the repression of the IL-2Ra. Conversely, BCL-6<sup>−/−</sup> virus-specific T cells retain the ability to perceive IL-2 signals. Signaling through STAT5 leads to the upregulation of transcripts encoding Blimp-1, KLF2, and Sipri, all associated with exit from the lymphoid tissue (DiToro et al., 2018; He et al., 2020). Indeed, in models of respiratory virus and lung hypersensitivity, the depletion of IL-2Ra on CD4 T cells ablates their ability to migrate to the lung tissue and establish tissue residence (Hondowicz et al., 2016; Hondowicz et al., 2018). Although the administration of recombinant IL-2 in the context of a vaccine would expand T<sub>RM</sub> cells, current strategies of vaccination do not emphasize this platform elicited high numbers of CD8 T<sub>RM</sub> cells that were detected out to 1 year, the farthest time point analyzed (Uddbäck et al., 2020). In addition to antigen, T cells require help from other resident lymphocytes to facilitate their survival. In models of respiratory viral infection, CD4 T<sub>RM</sub> cells required B cells and MHCII for their long-term persistence in the lung tissue (Hondowicz et al., 2018; Swarnalekha et al., 2021). Similarly, during influenza infection, CD8 T<sub>RM</sub> cells require help from the CD4-derived cytokines IFN-γ and IL-21 in order to localize to the airways and develop into T<sub>RM</sub> cells (Laidlaw et al., 2014; Son et al., 2021).

Further, multiple studies have reported attrition of T cells in the lung (Slütter et al., 2017; Wu et al., 2014; Liang et al., 1994) and further research into whether this attrition results in reduced protection over time or, alternatively, is the result of continuous retrograde migration of T<sub>RM</sub> cells to the lung draining LN (Stolley et al., 2020). Therefore, understanding how to induce parenchymal lymphatic niches through vaccination is a promising strategy to promote immune memory niches more efficiently in the tissues.

**Concluding remarks and SARS-COV-2–specific T<sub>RM</sub> cells**

Establishment of T<sub>RM</sub> cells is a dominant and potent mechanism of immune protection from pathogen reinfection at mucosal barrier tissues, but investigation into SARS-COV-2–specific T<sub>RM</sub> cell generation following COVID-19 infection or SARS-CoV-2 vaccination has been limited. Even so, important studies that sampled the bronchoalveolar fluid of severely and moderately infected patients found similar correlates in SARS-CoV-2 to those previously seen in severe influenza, notably the expansion of pathogenic Th17–like T<sub>RM</sub> cells expressing IL-17A and GM-CSF (Zhao et al., 2021). Further, a rigorous longitudinal analysis of T cells in the airway of COVID-19 patients revealed an enrichment in markers of resident memory, including CCR6, JTGAI, and PDCD1, while also expressing higher levels of the effector molecule transcripts IFNG, CCL2, and CCL4 (Szabo et al., 2021), recapitulating data in mice that suggest that T<sub>RM</sub> cells are more potent mediators of viral clearance than blood-derived T cells (Zhao et al., 2016). Current SARS-CoV-2 vaccination strategies aim to drive circulating neutralizing antibodies that bind the receptor-binding domain of SARS-CoV-2 Spike protein. Although circulating IgG antibody is thought to be capable of restricting breakthrough SARS-CoV-2 infection in the lungs of vaccinated individuals, instances of active infection in the nasal tissue have
been detected in previously vaccinated individuals, suggesting that stabilizing immunity is not always achieved (Hacisuleyman et al., 2021). Induction of local immunity at the portal of virus entry may complement vaccine strategies that elicit circulating immune memory, and more research is needed to determine the advantages of inducing local mucosal immunity that may be more protective in the face of already worrisome instances of receptor-binding domain variance and mutation (Starr et al., 2021). Future research into facets of TRM cell binding domain variance and mutation (Starr et al., 2021). Future

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