Memory T Cell Dynamics in the Lung during Influenza Virus Infection

Angela Pizzolla and Linda M. Wakim

*J Immunol* 2019; 202:374-381; doi: 10.4049/jimmunol.1800979

http://www.jimmunol.org/content/202/2/374

References

This article cites 131 articles, 60 of which you can access for free at:

http://www.jimmunol.org/content/202/2/374.full#ref-list-1

Why The JI? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Memory T Cell Dynamics in the Lung during Influenza Virus Infection

Angela Pizzolla and Linda M. Wakim

Influenza A virus is highly contagious, infecting 5–15% of the global population every year. It causes significant morbidity and mortality, particularly among immunocompromised and at-risk individuals. Influenza virus is constantly evolving, undergoing continuous, rapid, and unpredictable mutation, giving rise to novel viruses that can escape the humoral immunity generated by current influenza virus vaccines. Growing evidence indicates that influenza-specific T cells resident along the respiratory tract are highly effective at providing potent and rapid protection against this inhaled pathogen. As these T cells recognize fragments of the virus that are highly conserved and less prone to mutation, they have the potential to provide cross-strain protection against a wide breadth of influenza viruses, including newly emerging strains. In this review, we will discuss how influenza-specific memory T cells in the lung are established and maintained and how we can harness this knowledge to design broadly protective influenza A virus vaccines.

Influenza A virus (IAV) is an ssRNA virus that infects the respiratory tract. This virus causes the “flu,” a malaise with local inflammation and systemic symptoms that can vary in severity from a runny nose and sore throat to viral pneumonia. Annually, influenza virus infects 3–5 million people worldwide, with an estimated mortality rate of 300,000–600,000 people (1). In addition to the loss of life, annual influenza outbreaks are a major burden on global economies because of the direct cost of healthcare as well as lost productivity due to illness. Currently, the best protection against IAV is annual immunization with an i.m. trivalent or quadrivalent vaccine, which induces neutralizing Abs to the viral surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]). However, as these glycoproteins mutate frequently, it is necessary to reformulate and administer vaccines annually to maintain protective immunity (2). Current vaccines also offer no protection during a pandemic outbreak, in which the emergence of novel viruses from animal reservoirs spreads rapidly worldwide, having a devastating impact on global health.

In contrast to the current vaccines, natural infection with a strain of IAV is able to protect an individual against a secondary infection with a heterologous IAV strain, displaying different HA and NA molecules (3). The T cell compartment is responsible for this heterologous protection (4, 5). This is because T cells recognize internal and more conserved parts of the influenza virus that are far less prone to mutation (6–10). Experiments in animal models clearly highlight the importance of T cell immunity in the protection against influenza virus. The transfer of Ag-experienced IAV-specific CD8+ and CD4+ T cells into naive recipient mice can confer protection against heterologous viruses (11–13). Furthermore, depleting mice that have recovered from a prior IAV infection of memory T cells renders these animals more susceptible to secondary infection (14, 15). Consistent with these animal studies, recently, it was shown that IAV-specific CD4+ and CD8+ memory T cells in humans are also cross-reactive (16), and the presence of IAV-specific CD4+ and CD8+ T cells correlated with better protection from subsequent infection (17, 18). Not all subsets of memory T cells evoked during pulmonary IAV infection are equally protective, with the memory T cells that remain resident in the lung providing the most potent protection against a second heterologous lung IAV infection (15, 19–23). A vaccine that deposits IAV-specific T cells along the respiratory tract has the potential to provide long-lasting immunity against both seasonal circulating influenza strains as well as newly emerging pandemic viruses. Defining parameters that promote influenza-specific lung-resident memory T cell formation and maintenance is a critical step toward the development of such a vaccine.

In this review, we will focus on IAV-specific CD4+ and CD8+ memory T cells that are resident within the lung and will discuss factors influencing their induction, function, and persistence. In addition, we will review exciting new experimental vaccination protocols that are being developed to specifically evoke memory T cells in the lung, which if
proven effective, are likely to save lives, reduce health care costs, and reduce lost economic productivity.

**Priming: the activation of the IAV-specific T cell response**

IAV infection and replication in airway epithelial cells induces the production of cytokines and chemokines. Although these inflammatory agents drive the recruitment of innate immune cells into the lung (neutrophils and monocytes), which are essential to dampen the early stages of IAV infection [reviewed in Ref. (24)], ultimately, it is the adaptive immune response that is responsible for ridding the body of this virus infection (25).

Respiratory dendritic cells (DCs) play an important role in orchestrating this adaptive immune response. In mice, lung conventional DCs can be subdivided into two subsets termed CD103+ DCs and CD11b+ DCs (26). IAV infection of the airways induces the maturation of these respiratory DCs, which migrate from the lung to the mediastinal lymph nodes (LN) carrying IAV Ags, which are necessary to initiate an IAV-specific T cell response (27). Both DC subsets are important in the development of an optimal T cell response; the CD103+ DCs subset is essential and most efficient at priming of IAV-specific CD4+ and CD8+ T cells within the first few days postinfection, whereas the CD11b+ DC subset is the dominant APC at later time points (28). In addition to lung-migratory DCs, LN-resident DCs, specifically the CD8α+ XCR1+ subset, are also involved in priming naive CD8+ T cells via cross-presentation following IAV infection (29–32).

Upon reaching these lymphoid organs, DCs develop into a mature state, which is characterized by high levels of expression of MHC and T cell costimulatory molecules (CD80/CD86/CD70) and the ability to present Ag captured in the periphery to naive T cells. Naive T cells encounter their cognate Ag presented by activated DCs loaded on MHC class I, in the case of CD8+ T cells, or MHC class II (MHC-II) for CD4+ T cells. The T cells activated following IAV infection recognize viral Ags derived from several internal and external viral proteins. In both mice and humans, the CD8+ T cell response is focused toward internal proteins (nucleoprotein [NP] and polymerases [PA and PB1] and matrix protein M1) (6, 33, 34). In contrast, the CD4+ T cell response is directed toward both external proteins, HA and NA, as well as internal proteins, NP, PA, PB1 (35–39). As T cells recognize peptides derived from more conserved influenza virus proteins (8, 39–42), they have the potential to mediate cross-reactive immunity toward distinct IAV strains.

Following their activation within lymphoid organs, IAV-specific effector CD8+ and CD4+ T cells upregulate adhesion molecules and chemokine receptors that guide their migration to the infected respiratory tract. Activation by lung-migratory DCs drives IAV-specific CD4+ T cells to upregulate expression ofCCR4 (43), and this facilitates homing into the infected lung tissue, which expresses elevated levels of MIP-1α, MCP-1, and CCL5 (RANTES) (44). IAV-specific effector CD8+ T cells upregulate CXCR4 and are guided by a trail of CXCL12 left behind by neutrophils, which infiltrate the IAV-infected respiratory tract (45). Once in the inflamed IAV-infected lung tissue, effector CD4+ and CD8+ T cells need to see their cognate Ag again, either on infected cells or presented on lung DCs, to gain full effector function, become cytotoxic, as well as produce proinflammatory cytokines (46, 47). This secondary antigenic interaction is directed by the chemokine CXCL10, which recruits CXCR3+ effector T cells to Ag depots (48).

Within the IAV-infected lung tissue, CD4+ and CD8+ T cells produce both proinflammatory cytokines (TNF-α, IFN-γ, and IL-2), as well as anti-inflammatory cytokines (such as IL-10) (49, 50), which allow these cells to clear the virus infection and moderate tissue damage. Although effector CD4+ T cells after IAV infection predominately adopt a Th1 phenotype, in the absence of IL-10, these cells can be persuaded to differentiate into IL-17–producing Th17 cells, which are also protective during a high-dose influenza virus challenge (51). Additionally, CD4+ T cells can also differentiate into T follicular helper cells (Thf), which are pivotal for the activation of B cells, and cytotoxic CD4+ T cells, which can kill viral-infected cells by direct cytolysis. CD4+ T cell differentiation is directed by several factors, including cytokine environment (i.e., IL-21 sways the cells toward a Th1 phenotype, whereas IL-2 promotes differentiation of cytotoxic CD4+ T cells) and TCR–MHC-II affinity (i.e., high–Ag affinity and TCR signal strength favors Th1 differentiation) (52, 53). IAV-specific cytotoxic CD4+ T cells in the lung can kill via a perforin-dependent MHC-II–specific mechanism (49, 54). These cells express granzyme B and IFN-γ and are characterized by the expression of NK markers like NKG2C/E and CRTAM (MHC class I–restricted T cell–associated molecule) (49, 55, 56). These killer CD4+ T cells have also been identified in humans after IAV challenge (17).

The majority of effector CD8+ T cells within IAV-infected lungs produce proinflammatory cytokines (TNF-α, IFN-γ, and IL-2); however, a small proportion produce IL-4 and IL-5 or IL-17, which are classified as Th2 and Th17 cytokines (57, 58). Although cytokine production by effector CD8+ T cells aids in IAV control, the main function of these cells during IAV infection is to kill virus-infected cells (59). This cytotoxicity is mediated by granymes and proapoptotic proteases, which penetrate the target cells through perforin pores (60, 61) and by the interaction of the cell surface death receptors FAS–FASL and TRAIL–DR5 (61, 62). CD8+ T cell function is regulated by TCR signal strength, with cytolytic activity being triggered by weak TCR signaling and cytokine production being favored following stronger TCR signaling interactions (63–65).

**Development of IAV-specific memory T cell responses**

Following the clearance of IAV from the lung, the vast majority of effector CD4+ and CD8+ T cells perish, leaving behind a small pool of long-lived, Ag-experienced memory T cells. Whether an effector T cell transitions into a memory cell is influenced by early events occurring during T cell priming (66), which see KLRG1lo CD127(−IL-7Ra)+ effector T cells segregating into either terminally differentiated short-lived effector T cells (KLRG1hi and CD127lo) or memory precursors T cells (KLRG1lo CD127hi) (67). This differentiation process is driven by a panel of transcription factors; effector T cells highly express T-bet, ID2, Blimp-1, and STAT4, whereas memory precursor effector T cells express Eomes, ID3, Bcl-6, STAT3, Foxo1, and Tcf1, Hobit, and Runx3 (68, 69).

Other signaling events, including TCR stimulation, cytokine/chemokine exposure, and costimulatory molecule signaling, all impact on the ability of an effector T cell to transition into a long-lived memory cell. In particular, the
an inflammatory environment within the host over the course of an infection heavily influences effector T cell survival and memory T cell development (70). High levels of IFN-γ in the lung during an IAV infection help to dampen inflammation and promote T cell contraction (71, 72), and blocking this cytokine during IAV infection increased the accumulation of effector and memory CD8+ T cells in the lung, which leads to an accelerated clearance of virus from this tissue (72). The inflammatory milieu during the later stages of IAV infection favors memory T cells differentiation (73). Consistent with this, when naive CD4+ T cells were transferred into an IAV-infected mouse during the early stages of infection, these cells developed into both effector and memory T cells; however, if the transfer was postponed until the later stages of infection, naive T cells preferentially differentiated into memory cells (74).

Optimal transition of effector CD4+ and CD8+ T cells into long-lived memory cells requires a secondary conditioning event in which these cells see their cognate Ag and receive costimulatory signaling from DCs (75). Costimulation, in the form of CD27–CD70 interaction, TCR–MHC-II ligation, and IL-2 signaling is required 5–7 d after priming of CD4+ T cells for optimal CD4+ T cell memory development (76). Similarly, CD8+ T cells require costimulatory signaling from CD27, 4-1BB, and OX40, to promote their survival and differentiation into quality memory cells capable of robust secondary expansion (77, 78). CD4+ and CD8+ memory T cells require IL-7 and IL-15 for their long-term survival and renewal (79–82). Both IL-15 and IL-7 influence T cell metabolism by promoting fatty acids oxidation, the main source of energy for memory cells, via increasing storage of triglyceride (83) or mitochondrial biogenesis (84).

Both memory CD4+ and CD8+ T cells are able to protect mice from a lethal IAV infection in the absence of any other immune cell population (14, 23, 85), and the size of the memory T cell compartment is a good correlate of protection against a secondary heterologous infection (86, 87). In humans, survival from a new influenza strain depends on the presence of memory CD8+ T cells (17, 18), and in a rechallenge study, the numbers of CD4+ T cells directed against internal proteins correlated with lower viral titers and weaker symptoms (17).

Subsets of memory T cells. Memory CD4+ and CD8+ T cells can be subdivided into three subsets termed central memory, effector memory, and tissue-resident memory T cells (Trm). These cells reside in different anatomical locations and differ both phenotypically and functionally. Central memory T cells (Tcm) express high levels of CD62L and CCR7 and are found in the spleen, LNs, and blood. Effector memory T cells (Tem) do not express CD62L or CCR7, and in addition to being present in the spleen, lymphoid organs, and circulation, can also traffic through peripheral tissues (88–90). Trm represent a self-sustaining, nonmigratory population of memory T cells that are deposited within peripheral tissues, commonly at sites of prior infection (91).

Although both CD4+ and CD8+ Tcm and Tem are capable of producing cytokines upon antigenic stimulation, can proliferate and swiftly migrate into the lung following IAV re-infection (90), a process dependent on their expression of CCR5 (92), it is the Trm subset located along the respiratory tract that provides a frontline defense, rapidly decreasing viral titers and increasing survival rates following a secondary heterologous IAV infection (15, 20, 21). Trm located along the respiratory tract are indispensable for optimal cross-protection against heterologous pulmonary influenza virus infection (20, 93).

Development of Trm in the lung. Following IAV infection, CD4+ and CD8+ Trm are found in the lung parenchyma, airways, and nasal tissue (15, 20, 22, 94). Lung Trm, similar to Trm characterized in other tissues, are a self-sustaining population that are maintained with minimal need for replenishment by the circulating memory T cell pool (19, 20, 95–97). In the lung, CD4+ and CD8+ Trm congregate either within or around inducible bronchial-associated lymphoid tissue, respectively, and are also enriched in areas where the lung tissue has been remodeled postinfection-associated injury (19, 98, 99). Trm express a variety of adhesion molecules and proteins that facilitate their long-term retention and survival in the lung (19) (Fig. 1). Both CD4+ and CD8+ Trm subsets express CD49a, which complexes with CD29 to form the dimer VLA-1, which binds collagen IV and collagen I and secures these cells in the airways (93, 98, 100). A significant proportion of lung Trm (50–70%) also express CD69 (20, 22), which limits the egress of the cells into the blood stream by antagonizing S1P1 signaling (101). The integrin CD103 is expressed on a proportion of CD69+ CD8+ lung Trm; however, it is absent on the CD4+ lung Trm pool (22, 102). CD103 binds e-cadherin, a junction protein expressed on epithelial cells, which facilitates the retention of the cells within the lung (102–104). Although, CD4+ Trm constitutively express CD11a (22), a component of LFA-1 and ligand of ICAM-1, CD8+ Trm only transiently express this integrin, and as such, the expression of CD11a on CD8+ Trm is commonly used to distinguish recently recruited cells from long-term residents (19, 97). IAV-specific CD4+ and CD8+ Trm have also been identified and characterized in human lung tissue. These cells express the memory marker CD45RO, tissue retention markers CD69, CD49a, and CD103, chemokine receptors CXCR3, 4, and 6 (105–107), and can rapidly produce an array of cytokines (105–107) following TCR stimulation because of their constitutive expression of deployment-ready mRNA encoding effector molecules (108, 109).

Studies in animal models have defined key developmental requirements of lung Trm and identified factors within the lung microenvironment that promote Trm differentiation and maintenance. Optimal development of influenza virus–specific CD8+ Trm in the lung requires the following: 1) local cognate Ag recognition in the tissue (15, 20, 110), 2) exposure to locally produced TGF-β (99, 102, 103), and 3) the presence of IFN-γ–secreting CD4+ T cells (111) (Fig. 1). Because Trm development in the lung is driven by local cognate Ag recognition, changes in epitope availability over the course of a virus infection impacts the selection of different specificities of T cells into the Trm pool (15, 19, 99). As such, the immunodominance hierarchy within the influenza-specific lung Trm compartment does not reflect that of the circulating memory T cell pool.

Once formed, continued exposure to IL-15, which is produced by macrophages and DCs in the lamina propria of the lung, is necessary for the long-term survival of CD8+ CD103+ Trm (112). In addition, lung Trm retain expression of the IFN-induced transmembrane protein IFITM3, which is a
potent antiviral protein that confers broad antiviral resistance (113) and allows these cells to withstand viral infection and better survive within the microenvironment of the lung (113).

Trm have a unique molecular signature that is regulated by a key set of transcription factors. Trm transcriptional profile is characterized by the downregulation of the T-box transcription factors T-bet and Eomes (112, 114), which makes the cells responsive to TGF-β signaling and promotes expression of the IL-15R, which is important for cell survival (112). In addition, exposure of Trm during their development to TGF-β, IL-33, or TNF-α drives the downregulation of the transcription factor KLF2 and its target S1P1R, which limits tissue egress (115). Elevated expression of the transcription factor Runx3 in Trm drives the expression of tissue retention markers CD69 and CD103 (116), whereas elevated expression of the transcription factors Blimp-1 and Hobit also drive the downregulation of molecules associated with T cell egress (117). Although the core transcriptional profile of Trm has been unraveled through extensive interrogation of Trm isolated from mouse tissue, recently it has been shown that CD4+ and CD8+ CD69+ Trm isolated from human tissue exhibit a similar molecular signature and transcriptional program (108, 109, 118).

Why lung Trm decay is unclear; however, their fate is likely to be heavily influenced by the microenvironment of the lung. It is possible that the abundance of oxygen in the lung environment is not conducive for the long-term survival of lung CD4+ and CD8+ memory T cells. Indeed, blocking the oxygen-sensing capacity of T cells does increase their functionality in a lung cancer model (125). Furthermore, airway CD8+ Trms over time lose expression of IL-7Ra and IL-15R, becoming less responsive to the prosurvival cytokines IL-7 and IL-15, which could be another mechanism underlying the loss of this population of memory T cells (126).

The key to protective pulmonary immunity to IAV is the presence of IAV-specific lung Trm (20). A vaccine that deposits IAV-specific Trm in the lung has the potential to provide long lasting immunity against this respiratory pathogen. Defining parameters that not only promote IAV-specific lung Trm formation but also support their long-term persistence is an important step toward the development of an effective vaccine.

Vaccination strategies that evoke IAV-specific lung Trm

An IAV vaccine that evokes T cell immunity, directed at internal and highly conserved components of IAV, has the potential to provide long-term, cross-strain protection against both seasonal and emerging pandemic influenza strains (127). Influenza vaccines currently in use do not evoke robust T cell immunity but rather rely on the humoral arm of the immune system to confer protection. The injectable IAV vaccine, which is composed of inactivated IAV virions, boosts Th and
B cell responses but does not evoke an IAV-specific CD8\(^+\) T cell response in humans (128). Although the intranasal IAV vaccine, an attenuated influenza virus engineered to replicate only at the lower temperatures (< 33°C) of the upper respiratory tract, should stimulate both humoral and cellular arms of the immune system, the T cell responses evoked by this vaccine are highly variable (129). Vaccine candidates that specifically evoke IAV-specific T cell immunity are in development. Vaccines that combine CD4\(^+\) and CD8\(^+\) T cell epitopes from M1 and NP–IAV proteins that have been integrated into an artificial virus (MVA-NP+M1) or synthesized as synthetic polypeptides (Flu-v, FP-0.01, and Multimeric-001), have been tested in humans and can evoke IAV-specific T cell immunity (130–132). Furthermore, the MVA-NP+M1 vaccine could decrease viral shedding and symptoms in a human study in which volunteers were vaccinated and then challenged with an IAV strain for which they had no prior humoral immunity (131). An effective IAV T cell–based vaccine will not only have to elicit a good quantity memory T cell response but also will need to ensure the memory T cell population is deposited in the right location.

Because the demonstration that lung CD8\(^+\) Tmr are indispensable for optimal cross-protection against pulmonary influenza virus infection, considerable efforts have been made to develop vaccination regimes that specifically evoke this memory T cell population. As the optimal development of respiratory tract Tmr requires local cognate Ag recognition, any effective vaccine intended to elicit respiratory tract Tmr must deliver the vaccine Ag intranasally into the airways. Studies in animal models have shown that intranasal immunization with influenza peptide/protein alone (98, 133–135) or loaded onto DCs (15) or coupled onto mAbs that target Ag to respiratory DCs (119) are able to successfully generate lung CD8\(^+\) Tmr that are highly protective against influenza virus challenge. In addition, the intranasal delivery of a non-replicative influenza virus into the airways or pulmonary vaccination with an adenoviral vector–expressing IAV Ags could also deposit high numbers of influenza-specific lung Tmr (21, 136). Furthermore, animal studies show that the intranasal IAV vaccine but not the inactivated IAV virion can evoke lung Tmr that are highly protective against influenza challenge (21). Whether these vaccination protocols can evoke IAV-specific lung Tmr in humans, which, unlike laboratory mice, have a diverse immunological backdrop to this pathogen, a consequence of prior exposure or vaccination, remains to be determined.

A major obstacle for the development of an intranasal vaccine that evokes lung Tmr is that vaccine delivery into the lower airways presents a risk. The lung is a sensitive environment; excessive inflammation elicited by the pulmonary administration of vaccines or inflammatory agents could damage the lung tissue, a situation that could have a negative impact on respiratory health (137).

Conclusions

Tmr in the lung provide a frontline defense against influenza virus infection. Although able to provide cross-strain protection against a wide breadth of influenza viruses, including newly emerging strains, this protection, because of the short \(t_{1/2}\) of these cells, is only transient. If lung Tmr are to be harnessed in vaccines that protect against influenza virus, then finding ways to safely induce these cells and improve their stability is a challenge that will need to be addressed in coming years.

Disclosures

The authors have no financial conflicts of interest.

References

BRIEF REVIEWS: LUNG IAV-SPECIFIC MEMORY T CELLS


