# Immunity to Respiratory Viruses

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# Immunity to Respiratory Viruses

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## Key Words

lung, T cell, memory, influenza

## Abstract

The respiratory tract is characterized by an extensive surface area that is in direct contact with the environment, posing a significant problem for effective immune surveillance. Yet most respiratory pathogens are quickly recognized and controlled by a coordinated response involving the innate and adaptive arms of the immune system. The investigation of pulmonary immunity to respiratory viruses during a primary infection has demonstrated that multiple innate and adaptive immune mechanisms are necessary for efficient antiviral responses, and the inhibition of any single mechanism can have disastrous consequences for the host. Furthermore, the investigation of recall responses in the lung has shown that protection from a secondary challenge infection is a complex and elegant process that occurs in distinct stages. In this review, we discuss recent advances that describe the roles of individual components during primary and secondary responses to respiratory virus infections and how these discoveries have added to our understanding of antiviral immunity in the lung.

## INTRODUCTION

**RSV:** respiratory syncytial virus

**PRR:** pattern-recognition receptor

**Chemokines:** a family of chemoattractant proteins that direct cell migration

The mucosal surfaces of the respiratory, intestinal, and genital tracts are the primary portals of entry for a wide range of pathogens. The lung in particular is in direct and continuous contact with the surrounding environment, sampling nearly 10 liters of air each minute. Despite this continual exposure to potential antigens, the lung is generally maintained in a quiescent, noninflamed state. However, once a pathogen establishes a productive infection in the lung, an orchestrated process of pathogen recognition, inflammatory cytokine production, and cell migration leads to the generation of robust adaptive immune responses that eradicate the invading organism while limiting collateral damage to the lung tissue.

Respiratory viruses, such as influenza virus, parainfluenza virus, respiratory syncytial virus (RSV), severe acute respiratory syndrome coronavirus (SARS-CoV), rhinovirus, and adenovirus, are important human pathogens that establish acute infections usually localized to the upper respiratory tract. Despite the presence of these viruses in the population at endemic levels, it has taken the emerging threat of potential pandemics to thrust this class of pathogens into the public spotlight. For instance, the emergence of the highly pathogenic H5N1 influenza virus in 1997 has generated substantial public health concern owing to its extreme virulence and its potential to spread rapidly through the human population (1). Currently, there is considerable effort to develop improved vaccines capable of providing broad protection against these different types of viruses. However, achieving the goal of developing safe and effective vaccines to these pathogens has been complicated by our incomplete knowledge of how the immune system recognizes, contains, and eradicates respiratory viruses.

An increasing number of reports have taken advantage of respiratory virus infection models in an attempt to develop a better understanding of mucosal immune responses in general and to provide new insight into specific pathogens that are major causes of morbidity and mortality worldwide. The use of small animal models to investigate immunity to these pathogens has generated a wealth of information regarding the dynamics of immune responses in the lung and has identified many individual components of the response necessary for the successful resolution of infection. Perhaps the best-characterized models are mouse-adapted strains of influenza and murine parainfluenza viruses, which elicit robust T cell and B cell responses similar to infections in humans. The power of these models for understanding antiviral immunity in the lung has been enhanced by the advent of technologies for identifying antigen-specific responses and by the availability of a wide array of genetic tools. Here, we discuss recent advances in our understanding of antiviral immunity in the lung, from the initiation of innate and adaptive responses following primary virus infection to the recall of antigen-specific T cells during a secondary response, with a focus on lessons learned from murine models of influenza and parainfluenza virus infections.

## INITIATION OF IMMUNE RESPONSES IN THE LUNG

### Innate Recognition of Infection

A common feature of respiratory virus infections is that the initial infection is established in epithelial cells lining the respiratory tract. Epithelial cells, as well as alveolar macrophages and dendritic cells (DCs), continually sample the constituents of the airway lumen and detect the presence of an invading virus through pattern-recognition receptors (PRRs) (**Figure 1**) (2). The recognition of pathogen-associated molecular patterns by these receptors initiates a cascade of signals that results in the production of cytokines and chemokines. The release of these inflammatory mediators into the surrounding environment alerts the innate immune system to the presence of infection and establishes a localized antiviral state. In addition, chemokines provide the necessary signals for the recruitment

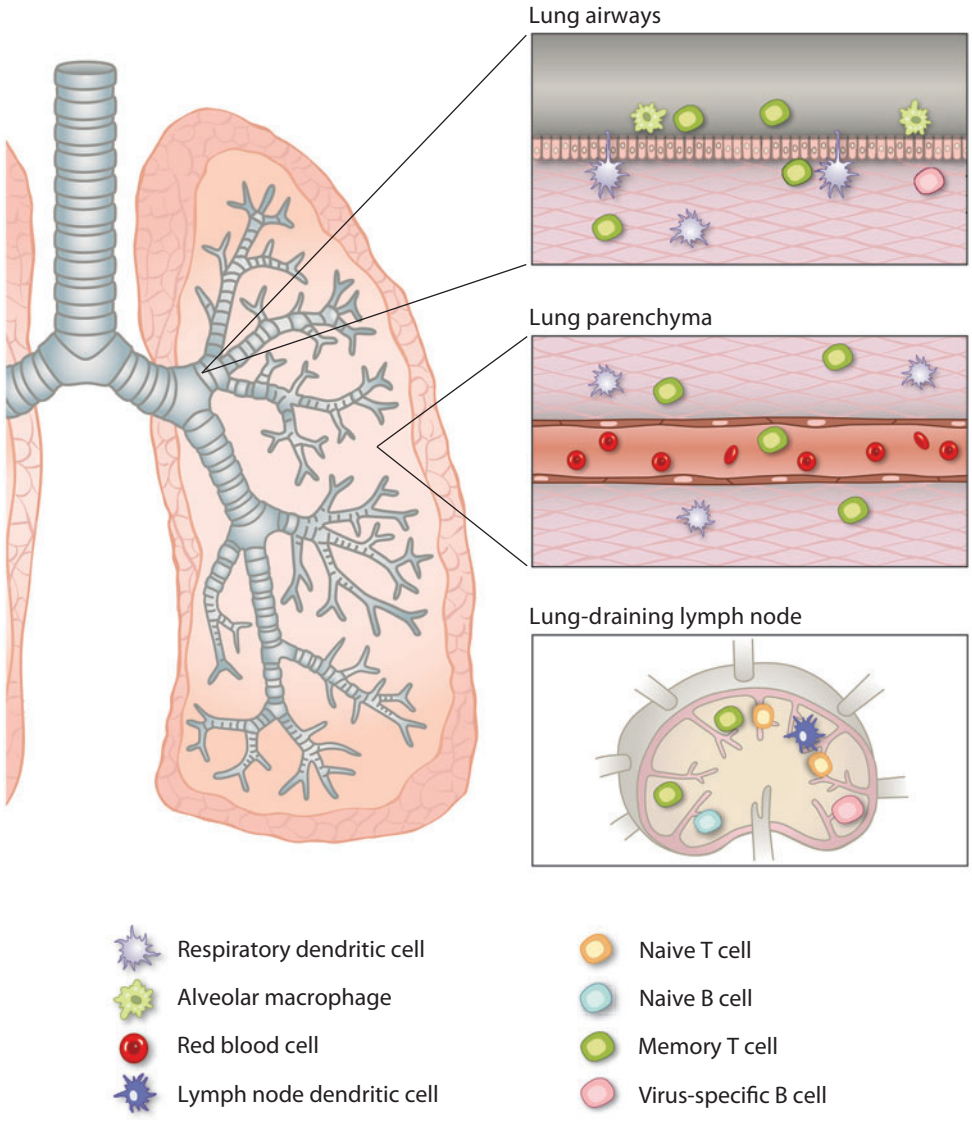


**Figure 1**

The resting pulmonary immune system. The lung airways, lung parenchyma, and lung-draining lymph nodes are three key sites of the antiviral immune response to respiratory viruses. In the absence of infection, alveolar macrophages and dendritic cells sample the constituents of the airway lumen for the presence of invading pathogens. Memory T cells from prior respiratory virus infections are localized to each of these sites, with large numbers of cells present in the airways and parenchyma for several months postinfection. Virus-specific B cells are also localized to each of these sites, with resting memory B cells widely distributed while long-lived plasma cells are localized primarily to lymphoid tissue associated with the respiratory tract and the bone marrow.

of circulating leukocytes to the site of infection. Finally, the combination of inflammatory cytokines and PRRs initiates the process of DC maturation and trafficking that is required for the induction of adaptive immune responses.

The best described of the PRRs are those of the Toll-like receptor (TLR) family, which in

**Type I interferons:** a family of inflammatory cytokines, including IFN-α and IFN-β, produced in response to PRR signaling

**NK cell:** natural killer cell



mammals is composed of up to 15 unique receptors that are expressed by numerous cell types and recognize a wide range of microbial proteins, lipids, and nucleic acids (3). With respect to respiratory viruses, TLR3, 7, and 9 recognize various products of viral replication (dsRNA, ssRNA, and unmethylated CpG DNA, respectively) (4–7), whereas TLR4 recognizes the fusion (F) protein of RSV (8). TLRs that recognize nucleic acids are located in late endosomes. This location optimizes the TLRs' ability to interact with viral nucleic acids while limiting their access to host-derived nucleic acids (9, 10). Although TLRs expressed on the cell surface (TLR4) or within the cell (TLR3, 7, 8, and 9) utilize different signaling pathways, each of these receptors can activate the transcription of interferon (IFN)-inducible genes (11).

In addition, several recent reports have demonstrated that viral RNA is also recognized by several RNA helicases. Retinoic acid-inducible gene I (RIG-I) interacts with 5′-triphosphate RNA and is important for early cytokine production in response to numerous RNA viruses (12–15). Melanoma differentiation-associated gene 5 (MDA5) is a related helicase that recognizes polyinosinic polycytidylic acid and is crucial for innate recognition of picornaviruses (16). Similar to signaling through TLRs, the pathways utilized by RNA helicases ultimately trigger IFN regulatory factor (IRF) and nuclear factor-κB (NF-κB) activation (17). The key difference between these molecules and TLRs is that the RNA helicases are localized throughout the cytosol, rather than being regulated to intracellular compartments. Thus, viruses that infect cells by direct membrane fusion and do not enter endosomes can nevertheless trigger innate immune responses via RNA helicases.

## The Early Inflammatory Response

The innate recognition of viral components through PRRs described above leads to a program of gene expression that promotes a localized antiviral state and elicits the recruitment of inflammatory cells to the site of infection. Foremost among the early cytokines produced following infection are the pleiotropic antiviral cytokines of the type I interferon family, of which IFN-α and IFN-β are most commonly associated with early antiviral responses in the lung. Although nearly all cell types are capable of producing type I IFNs, numerous studies in mice and humans have shown that plasmacytoid DCs (pDCs) are the primary source of IFN-α and IFN-β following infection with a systemic virus (18). With respect to respiratory viruses, a recent study has provided in vivo evidence that alveolar macrophages are the primary producers of IFN-α during a parainfluenza virus infection and necessary for efficient virus clearance (19). Notably, this study demonstrated that although IFN-α production by pDCs was largely TLR-dependent, alveolar macrophages required RIG-I signaling for optimal IFN-α production. However, the importance of alveolar macrophage-derived IFN-α remains uncertain, as subsequent work has demonstrated that alveolar macrophage depletion had no effect on virus clearance during RSV infection (20). Therefore, type I IFN production in the lung appears to employ a level of redundancy, with alveolar macrophages or pDCs predominating depending on the type of virus infection.

Type I IFNs produced following respiratory virus infections form a feedback loop by signaling through the IFN-α/β receptor and act in concert with PRR signaling to promote sustained production of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 from lung-resident innate immune cells (21, 22). These proinflammatory cytokines and PRR-mediated signals also prompt alveolar macrophages, DCs, and epithelial cells to initiate a coordinated program of chemokine production following virus infection. DCs secrete successive waves of chemokines following influenza virus infection, beginning with those capable of recruiting inflammatory cells such as neutrophils and NK cells, and followed by chemokines associated with the recruitment of monocytes and memory T cells (23). Epithelial cells and alveolar macrophages also contribute

to early chemokine production following infection and/or inflammation, particularly those chemokines capable of recruiting monocytes and memory T cells (24, 25).

The chemokines produced during the innate immune response in the lung are capable of recruiting a wide range of innate and adaptive immune cell types. However, during a primary respiratory virus infection, the innate immune cells recruited to the lung are predominantly neutrophils and NK cells (**Figure 2**). The role that neutrophils play in respiratory virus clearance has not been well defined, despite the large number of these cells present in the lung following infection. In fact, it has recently been shown that inhibition of neutrophil recruitment to the lung following influenza virus infection had no effect on the course of infection, suggesting that these cells do not play an essential role in virus clearance (26). In contrast, NK cells directly recognize influenza virus– and parainfluenza virus–infected cells through the interaction of the activating receptor NCR1 (NKp46 in humans) with hemagglutinin glycoprotein (27). NK cell recognition of virus-infected cells in coordination with proinflammatory cytokines resulted in enhanced cytolysis and IFN-$\gamma$ production by NK cells (28–30). A recent definitive study investigating the importance of NK cells for protection against influenza virus showed a significant increase in the number of NK cells in the lung beginning around day 3 postinfection. Importantly, this study also demonstrated that influenza virus infection was lethal in mice lacking the NK cell–activating receptor NCR1 (31).

In addition to establishing a localized inflammatory environment and recruiting innate immune cells, PRR- and cytokine-mediated signals are important for the maturation and trafficking of DCs to the draining lymph nodes preceding the initiation of the adaptive immune response. Under steady-state (i.e., noninflammatory) conditions, the trafficking of DCs from the lung compartment to the draining lymph nodes is a continuous process that is dependent on the chemokine receptor CCR7 (32). The DCs that enter the lymph nodes under these conditions are not fully mature, and it is believed that this process plays a role in the establishment of immune tolerance in the lung. Following influenza virus infection, lung-resident DCs increase expression of molecules involved in antigen presentation such as MHC class II, CD80, CD86, and CD40. Beginning as early as 6 h postinfection, there is an increase in the trafficking of DCs from the lung to the draining lymph nodes that is maintained for several days (33, 34). In addition, the trafficking of respiratory DCs to the lymphoid tissues is essential for the generation of adaptive immunity, as blocking DC migration abrogates antigen-specific T cell responses. Curiously, the trafficking of DCs to the draining lymph nodes during both steady-state and inflammatory conditions is dependent on CCR7, suggesting that the accelerated recruitment following infection is not mediated by different chemokine receptors. However, investigators (35, 36) recently demonstrated that the interaction of the chemokine CCL5 with its receptor CCR5 is indirectly responsible for this enhanced trafficking following infection by increasing the expression of CCR7 expression on DCs and enhancing migration across high endothelial venules. Thus, the innate recognition of infection leading to inflammatory cytokine and chemokine expression results in both the maturation and accelerated trafficking of DCs, enabling more efficient antigen presentation to T cells in the draining lymph nodes.

**BALT:** bronchus-associated lymphoid tissue

A unique aspect of antiviral immunity in the lung is the potential for adaptive responses to be generated in local lymphoid structures such as nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) (37). These structures exhibit similar organization to encapsulated lymph nodes with distinct T and B cell zones, high endothelial venules, and the expression of homeostatic chemokines important for DC and naive T cell migration (38). Importantly, these structures can significantly contribute to the antiviral response, as mice devoid of secondary lymphoid tissues are able to mount effective, albeit delayed, virus-specific T cell and B cell responses that are

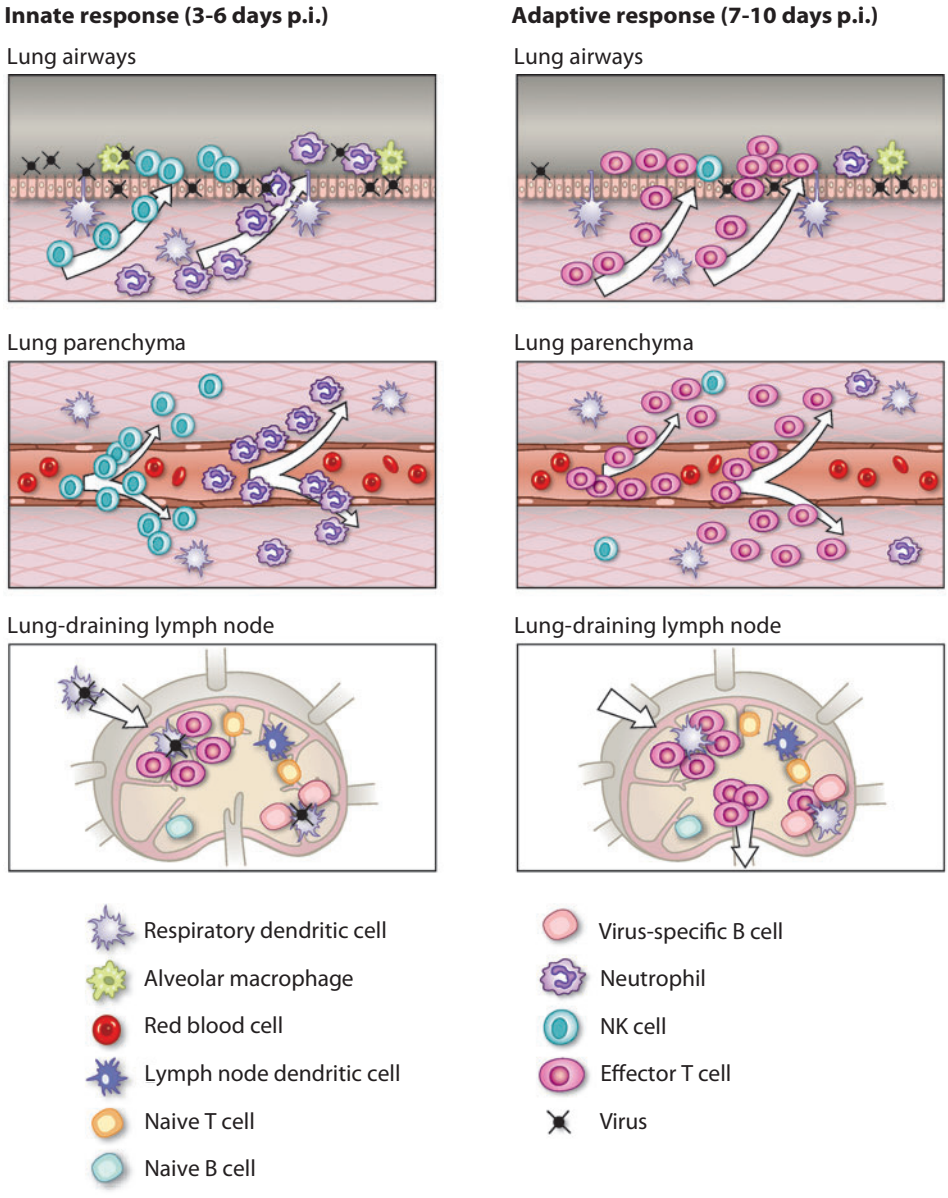


**Figure 2**

Innate and adaptive immune responses during a primary respiratory virus infection. During the innate response, virus detection in the lung by PRRs initiates a program of cytokine and chemokine production that leads to the recruitment of neutrophils and NK cells from the circulation to the lung airways and lung parenchyma. The influx of innate immune cells and the production of cytokines limits early virus replication prior to the adaptive response. Concurrently, activated, antigen-bearing DCs migrate to the lung-draining lymph node, where they interact with antigen-specific naive T cells and generate a population of differentiated effector T cells. During the adaptive response, effector $CD4^+$ T cells provide help to virus-specific B cells within the lymph node, and effector $CD4^+$ and $CD8^+$ T cells exit the lymph node and migrate to the lung. Large numbers of effector T cells accumulate in the lung airways and lung parenchyma, and through the production of cytokines and the lysis of infected epithelial cells, virus is cleared around 10 days postinfection. It is important to note that virus-specific antibody, which is not illustrated, also plays a critical role in virus clearance.

originated and propagated within the BALT (39). Although the contribution of these localized lymphoid structures to the overall immune response in mice with normal secondary lymphoid tissues is difficult to dissect, the proximity of these structures to the site of virus replication and their ability to support a robust and diverse adaptive response suggest that they may play an important role in antiviral immunity.

## ADAPTIVE IMMUNITY DURING PRIMARY RESPONSES

### T Cell Responses

Naive $CD4^+$ and $CD8^+$ T cells within lymphoid tissues continually scan the surface of DCs for the presence of cognate antigen/MHC complexes (40, 41). This dynamic process, combined with the protrusion of dendrites that increases the DC surface area and the circulation of naive T cells through the lymph node, enhances the probability that extremely small numbers of antigen-specific naive T cell precursors, which range from 20–1200 cells of a given specificity in mice, will come into contact with their cognate antigen and enter into a program of proliferation and differentiation (42–44). As antigen-bearing mature DCs enter the lung-draining lymph nodes following respiratory virus infection, naive T cells specific for that antigen form stable interactions with the DCs, and the signals delivered by antigen recognition through the T cell receptor in addition to accessory signals delivered through costimulatory molecules result in T cell priming (45, 46). The initial priming of naive antigen-specific $CD4^+$ and $CD8^+$ T cells occurs within 72 h following influenza virus infection and initiates a program of sustained proliferation resulting in the accumulation of large numbers of virus-specific effector T cells (47–49). The instructions delivered by DCs during this initial expansion phase can have a dramatic impact on the survival and function of the responding T cells. For example, expression of FasL on DCs following influenza infection has been shown to regulate the magnitude of the $CD8^+$ T cell response (50). In addition, factors such as TCR avidity, costimulation, and the local inflammatory milieu all contribute to the generation of differentiated effector T cells prior to their exit from the lymph node and subsequent trafficking to the lung (51–54).

The appearance of antigen-specific effector T cells at the site of virus infection (i.e., the lung airways and lung parenchyma) is first observed around days 6–7 postinfection with influenza and parainfluenza viruses (**Figure 2**). Chemokines expressed in the lung are recognized by blood-borne effector T cells, leading to changes in integrin affinity that allow for tight binding to the blood vessel wall and extravisation into the surrounding tissue (55, 56). Endothelial selectins are also important for this process, as mice lacking expression of the molecules or their receptors showed a dramatic decrease in the trafficking of $CD4^+$ and $CD8^+$ T cells to the lung (57, 58). Having migrated from the circulation into the lung tissue, effector T cell–expressed adhesion molecules are important for movement and survival within the interstitial spaces and airways of the lung. In addition, the expression of β1 integrins has been shown to control the localization of effector T cells to distinct compartments of the lungs. For example, cells expressing the integrin α1β1 (VLA-1) are predominantly associated with collagen Type IV–rich areas surrounding the airways and blood vessels, whereas cells expressing α2β1 (VLA-2) are predominantly associated with the collagen Type I–rich areas of the interstitial spaces (59).

Analyses of chemokine and chemokine receptor expression in the lung during the adaptive phase of the immune response have shown elevated expression of numerous molecules associated with effector T cell trafficking (60, 61). Surprisingly, very few published reports have directly investigated the role of specific chemokine receptors in the trafficking of effector T cells during acute respiratory virus infections. One reason for this gap may be that the presence of multiple chemotactic signals in the lung at this stage of infection and the redundant nature of the chemokine system have made

dissecting the importance of individual receptors in effector T cell recruitment a difficult task (62). Nevertheless, several studies have identified roles for specific chemokine receptors in effector T cell trafficking to the lung under resting or inflammatory conditions using different (i.e., nonviral) models that may provide some insight for future studies employing respiratory virus infections. For example, effector $CD8^+$ T cells require CCR5 for migration from the pulmonary vasculature into the lung parenchyma in naive, uninfected mice (63). Also, an analysis of $CD4^+$ T cells to the lung airways in asthmatic humans has shown that CCR6 and CXCR3 may be important for their trafficking to this site (64). With regard to respiratory viruses, the trafficking of effector T cells during RSV infection is at least partially dependent on CX3CR1, suggesting a potential role for this chemokine receptor in other paramyxovirus infections (65). To elucidate a role for specific chemokine receptors during acute respiratory virus infections requires future studies focused on receptors that are known to contribute to recruitment during inflammation, combined with studies already conducted that have characterized the expression of chemokine receptors on respiratory virus–specific T cells (66, 67).

The continual migration of effector T cells from lymphoid tissues during an acute infection results in a massive increase in the numbers of antigen-specific cells in the lung airways and lung parenchyma from days 7–10 postinfection (68). The arrival of effector T cells has an immediate and dramatic impact on the viral load through the expression of cytokines and the direct lysis of infected cells. Influenza-specific $CD4^+$ and $CD8^+$ effector T cells in the lung predominantly produce IFN-$\gamma$ and TNF-$\alpha$, and $CD4^+$ effector T cells also produce IL-2 and IL-10 (69–72). $CD8^+$ effector T cells localize to the respiratory epithelium and induce apoptosis of infected epithelial cells through Fas-FasL interactions or the exocytosis of cytolytic granules containing perforin and granzymes (73, 74). Together, these effector mechanisms contribute to the rapid decline in viral load beginning around day 7 postinfection and result in virus clearance around day 10 postinfection (75, 76).

## B Cell Responses

B cell responses and virus-specific antibody play an important role in the clearance of influenza virus during primary infection, especially during infection with a highly pathogenic virus (77). Studies with B cell–deficient mice have shown that, although early virus control (days 3–6 postinfection) is not impaired, these mice fail to clear the virus and ultimately succumb to infection (78, 79). The protective effect of B cells in these studies appears to be mediated at least in part through the production of virus-specific IgM because mice lacking only this isotype had delayed virus clearance and increased mortality (80, 81). Also, virus-specific IgM has been shown to provide protection from influenza-induced pathology in the presence of T cells (82). Together, these studies clearly define a role for the early production of virus-specific IgM in B cell–mediated protection during influenza virus infection. However, it is important to note that a direct comparison of B cell–deficient and IgM-deficient mice found that B cell–deficient mice were more susceptible to influenza virus infection (81). Therefore, the production of neutralizing isotype-switched, virus-specific antibody during the later stages of the primary response is required for optimal virus clearance and antibody-mediated protection (83).

The conventional model of isotype-switching involves direct contact between antigen-specific $CD4^+$ T cells and antigen-presenting B cells in lymphoid tissues. This antigen-dependent interaction, in addition to CD40-CD40L interactions and cytokine signaling, drives B cell proliferation and antibody isotype switching (84). Although this model of B cell activation and antibody production is the primary mechanism for virus-specific antibody production during influenza virus infection, several studies have demonstrated that these interactions are not absolutely required for virus-specific IgA and IgG antibodies. For

example, influenza virus–specific IgA production during the early stages of infection is $CD4^+$ T cell dependent but does not require cognate T-B cell interactions (85). In addition, CD4 T cell–deficient mice can generate virus-specific IgG, although the influenza-specific antibody titers are considerably decreased in these mice (79). Taken together, these studies demonstrate that both T cell–dependent and –independent mechanisms contribute to influenza-specific antibody production.

## T CELL AND B CELL MEMORY TO RESPIRATORY VIRUSES

### T Cell Memory

The peak of effector T cell numbers in the lung following influenza and parainfluenza virus infections generally occurs around 10 days postinfection and coincides with virus clearance. The resolution of infection and waning inflammation has a dramatic impact on the virus-specific T cell population, initiating a program of contraction in which 90–95% of effector T cells are deleted by apoptosis (86). The outcome of this process is the establishment of a stable pool of memory T cells that persists in both peripheral and lymphoid tissues (87–89). Considerable progress has been made in recent years identifying the cues that instruct antigen-specific effector T cells to develop into long-lived memory T cells, the factors that maintain the memory T cell population over time, the anatomical location and trafficking patterns of different memory T cell subsets, and the relationship between different memory T cell subsets and the efficacy of the recall response.

In the initial months following a respiratory virus infection, antigen-specific $CD4^+$ and $CD8^+$ T cells can be found throughout lymphoid and nonlymphoid tissues in mice and humans, with the highest frequency of these cells located in the lung airways and lung parenchyma (90–93). However, the number of antigen-specific T cells in the lung wanes over time, and by one year postinfection the frequency and number of antigen-specific T cells is similar between the lung and other peripheral or lymphoid tissues (90, 94). Importantly, the decline in memory T cell numbers in the lung over time correlates with a decline in the ability of antigen-specific T cells to control viral load during a secondary challenge (95). The large number of antigen-specific T cells in the lung following respiratory virus infection is believed to be maintained by persistent depots of influenza antigens that are presented within the draining lymph nodes for several months following virus clearance (96, 97). This antigen depot is capable of inducing T cell activation (as measured by CD69 expression) and low levels of proliferation (as measured by CFSE dilution) for up to 60 days postinfection. However, the low level of proliferation supported by this antigen depot may be sufficient to account for the higher number of antigen-specific T cells found in the lung for several months postinfection. In support of this hypothesis, the waning antigen-specific T cell numbers observed beginning several months postinfection coincides with the disappearance of prolonged antigen presentation. However, although antigen-specific T cell numbers at sites such as the lung airways decline for several months postinfection, this population of cells stabilizes at a low level and is maintained indefinitely (94, 98, 99). Thus, once the depot of persistent antigen has been cleared, the low number of antigen-specific memory T cells found in the lung airways is maintained by a background level of recruitment from the circulation (100).

Memory T cells generated by respiratory viruses are heterogeneous in terms of their phenotype and function. This heterogeneity has led to the classification of memory T cells into two subsets based on their preferential migration of peripheral (effector memory T cells, $T_{EM}$) or lymphoid (central memory T cells, $T_{CM}$) tissues (101). These subsets can be delineated on the basis of expression of CD62L and CCR7, which direct entry into lymphoid tissues. The majority of virus-specific memory T cells present throughout the body 1–6 months postinfection express a $T_{EM}$ phenotype ($CD62L^{lo}$ and $CCR7^-$) and preferentially

**$T_{EM}$:** effector memory T cell

**$T_{CM}$:** central memory T cell

migrate to nonlymphoid sites (67, 102). Over time, the systemic memory T cell pool undergoes a gradual conversion to a $T_{CM}$ phenotype ($CD62L^{hi}$ and $CCR7^{+}$) that results in their localization to the lymph nodes and also to the bone marrow (103–105). Although there is conflicting evidence regarding the relationship between the $T_{EM}$ and $T_{CM}$ lineages, current evidence suggests that these two subsets are distinct populations generated during the initial infection, and the outgrowth of the $T_{CM}$ population over time is due to increased homeostatic turnover (106, 107). Regardless of the relationship between $T_{EM}$ and $T_{CM}$, the passage of time leads to a shift in the preferential localization of virus-specific memory T cells, and in turn alters the dynamics and efficacy of the recall response. For several months following virus clearance, large numbers of $T_{EM}$ are present in the lung and are able to provide immediate antiviral effector functions upon secondary infection. Over time, however, the number of virus-specific $T_{EM}$ able to provide this immediate response in the lung dramatically declines. Instead, the majority of virus-specific cells exist as $T_{CM}$ present in lymphoid tissues capable of rapid proliferation and the generation of new effector T cells following a secondary challenge. Therefore, the ability of antigen-specific memory T cells to immediately recognize and respond to a secondary virus challenge at the site of infection is lost over time.

### B Cell Memory

Similar to the kinetics of T cell memory, influenza-specific memory B cells are rapidly established in multiple tissues following virus clearance. However, it is evident that there are profound differences between T cell and B cell memory with regard to their generation, trafficking, and maintenance. B cell memory is characterized by two distinct populations of cells: long-lived plasma cells that continually secrete antibody and memory B cells that persist in a quiescent state (108, 109). The generation of B cell memory, particularly the generation of long-lived plasma cells, is dependent on cognate T-B cell interactions and CD40 signaling that occurs in the germinal center (110, 111). In support of this finding, investigators demonstrated that the influenza virus–specific IgG response generated in CD40-deficient mice rapidly wanes and is undetectable by 60 days postinfection (79). In addition, it has recently been demonstrated that invariant natural killer T cells can also provide B cell help and enhance IgG responses (112). Therefore, although the data demonstrate that contact-dependent interactions are required for the generation of long-lived B cell memory, these interactions can involve different cell types.

Following influenza virus clearance, plasma cells leave the germinal centers and migrate to the bone marrow, where they continue to secrete virus-specific antibody. In addition, long-lived plasma cells secreting influenza virus–specific IgA are localized and maintained in lymphoid tissues lining the respiratory tract (113). In contrast, resting memory B cells are widely dispersed to many tissues, where they can remain for many months. Interestingly, these cells localize at a higher frequency in the lung tissue following influenza virus infection, suggesting that the nature of the infection may alter the migratory capacity of these cells (114). Although it is unclear whether specific adhesion molecules or chemokine receptors play a role in the tissue-specific migration of memory B cells, the localization of these cells to the lung would allow them to rapidly recognize and respond to a secondary influenza virus challenge.

## RECALL RESPONSES TO SECONDARY CHALLENGE

It is well accepted that neutralizing, virus-specific antibodies (humoral immunity) provide optimal protection against most respiratory viruses by blocking the ability of the virus to establish infection. However, many viruses have evolved mechanisms to circumvent antibody-mediated immunity, allowing for secondary infections of closely related virus strains. For example, variability in the coat proteins of influenza virus enables the virus to evade

neutralizing antibody and allows yearly influenza epidemics (115). Therefore, antibody-mediated protection alone, while effective against secondary infection with a homologous virus, does not protect against variant viruses that arise through mutation. In contrast, the internal proteins of influenza virus, where many T cell epitopes are located, are highly conserved across many strains. Therefore, memory T cells specific for internal virus antigens are able to mount recall responses against heterologous virus strains (cellular immunity) and can provide broader protection against secondary challenge. In addition, non-neutralizing antibody to conserved internal proteins can enhance the memory T cell response to an influenza challenge (116). This section focuses on the experimental evidence that has been generated describing the cellular immune response to secondary virus infection in the absence of neutralizing antibody.

## Lung Conditioning

Much has been learned regarding the factors that influence memory T cell recall responses in animal models. However, a key consideration when interpreting the data from animal models is that these studies are often performed on specific pathogen-free mice. Although this limits the number of variables that could impact the results of an experiment, the immune response in a completely naive lung may not reflect the normal situation (117, 118). For example, the lungs of naive mice are devoid of lymphoid structures, such as BALT, that are present after respiratory virus clearance (119). Another issue is that prior influenza virus infections alter the responses to unrelated pathogens (120). Infection history can dramatically alter the clinical outcome of new infections depending on both the type of pathogen and order in which different pathogens are encountered (121). These studies have suggested a role for prior infections or inflammation in modifying the lung environment, thereby altering the manner in which the innate immune system reacts to subsequent inflammatory cues (122).

## T Cell Recall Responses in the Lung

As stated previously, the resolution of a primary respiratory virus infection generates a substantial number of antigen-specific memory T cells that are localized to both lymphoid and peripheral sites, such as the lung airways and the lung parenchyma. Several seminal studies have demonstrated that these respiratory virus–specific memory T cells mediate accelerated virus clearance and enhance survival following secondary challenge with related viruses (95, 123). More recently, evidence has emerged that memory T cell responses are far more complex than previously appreciated and that distinct populations of memory T cells segregated by their anatomical location and migratory capacity mediate different stages of the recall response. Thus, the recall response can be divided temporally, based on when these populations encounter virus-infected cells in the lung, and functionally, based on the steps required for their accumulation at this site (**Figure 3**). Importantly, it is the combination of these stages (discussed below) that results in the enhanced speed and magnitude of the recall response.

Virus-specific memory T cells in the lung airways are believed to provide an initial line of defense against a secondary infection because these cells would be the first to encounter antigen (124). Virus-specific memory T cells are present in considerable numbers for at least several months postinfection, despite their proximity to the harsh external environment and presence of mucus and surfactants (94, 125, 126). However, likely owing to the harsh environment, this population of cells is highly dynamic and is maintained by a process of continual recruitment from the circulation (100). Although lung airway memory T cells lack effector functions such as cytolytic activity (127), these cells are able to produce cytokines in response to antigen or inflammatory cytokines (98). Importantly, a direct role for these cells in protective immunity was demonstrated by the transfer of antigen-specific memory T cells to the lung airways of naive mice, which resulted in significantly reduced viral titers following infection (124). Therefore, the first stage of the

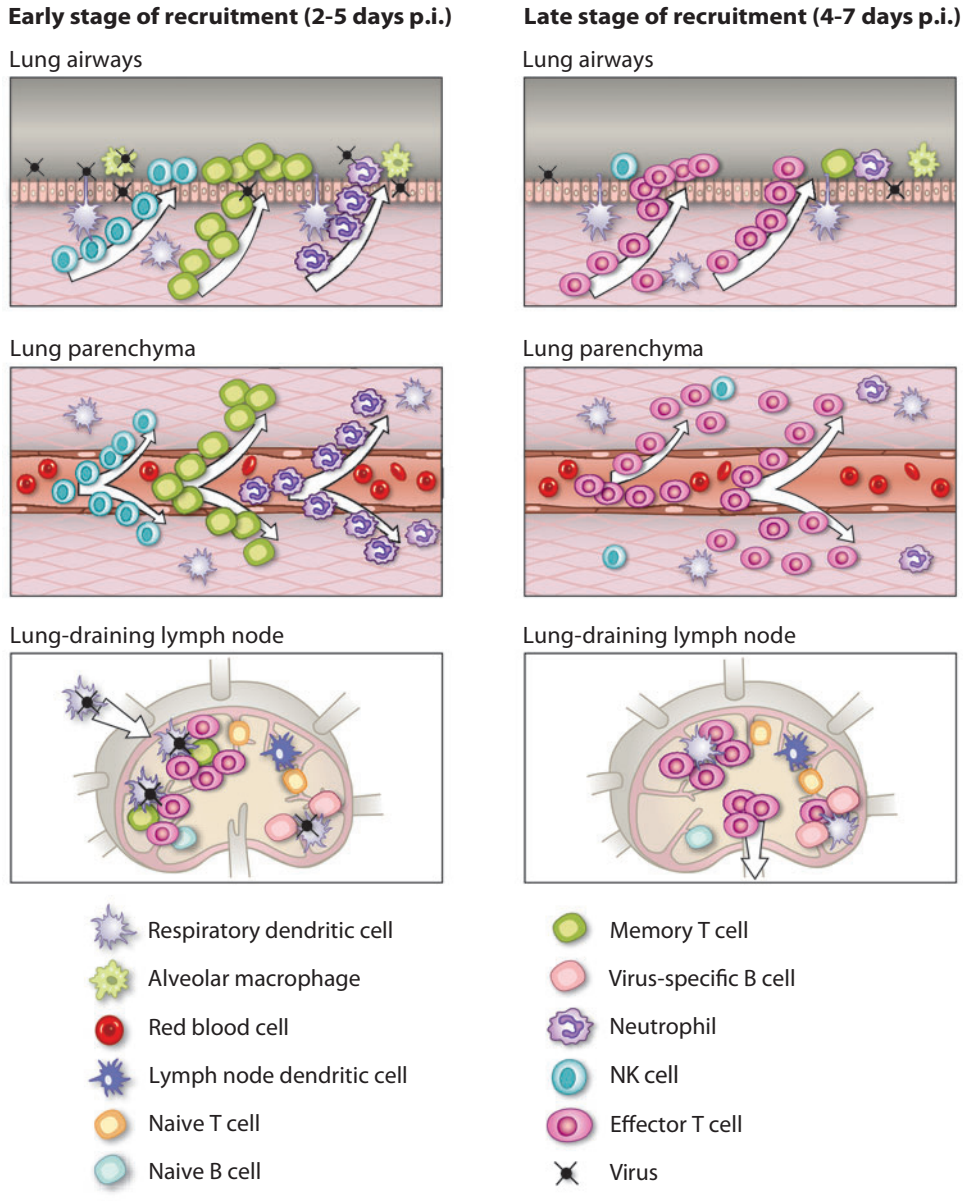


**Figure 3**

Dynamics of the T cell recall response to secondary virus challenge. Similar to a primary response, neutrophils and NK cells are recruited to the lung in response to localized inflammation. In addition, circulating memory $CD8^+$ T cells are also recruited from the circulation to the lung in a CCR5-dependent manner during the early phase of infection, thereby increasing the number of antigen-specific cells at the site of infection and limiting virus replication. Memory T cells in lymphoid tissues are activated by antigen-bearing DCs from the lung, and, owing to their increased precursor frequency and activation status compared with naive T cells, they are able to rapidly generate large numbers of secondary effector T cells. Secondary effector T cells migrate to the lung airways and lung parenchyma and mediate rapid virus clearance. Although not illustrated, the generation of secondary adaptive immune responses can also occur in lymphoid tissues (such as BALT) within the lung.

recall response involves cells present at the site of infection that limit viral replication during the first several days of infection while antigen-bearing DCs are trafficking to lymphoid tissues.

An established paradigm of secondary T cell responses is the more rapid generation of effector T cells compared with a primary T cell response. Despite this more rapid generation, in respiratory virus infections this still allows for approximately four days between initial virus infection and the appearance of secondary effector T cells in the lung. However, several groups have demonstrated a dramatic increase in the number of virus-specific $CD8^+$ T cells in the lung airways prior to the appearance of secondary effector T cells (128, 129). This increase in virus-specific $CD8^+$ T cells could not be accounted for by localized proliferation within the airways, as these cells had not recently divided and the increase in cell number did not require cognate antigen stimulation. Rather, these studies showed that increased numbers of virus-specific $CD8^+$ T cells in the airways was due to the inflammation-dependent recruitment of nondividing memory T cells from the circulation (130). Thus, the recruitment of circulating memory $CD8^+$ T cells to the lung airways in response to inflammation serves to increase the number of antigen-specific memory T cells at the site of virus replication prior to the secondary effector T cell response.

Although these studies had clearly demonstrated that the recruitment of memory $CD8^+$ T cells occurs in response to localized inflammation, the mechanism that directs circulating memory $CD8^+$ T cells to the lung airways and the importance of this process during secondary respiratory virus challenge had not been determined. Recently, we demonstrated that CCR5 expressed on memory $CD8^+$ T cells was required for their recruitment to the airways during the early stages of the recall response (131). The role for CCR5 in memory $CD8^+$ T cell recruitment is unique for the lung airways, as no defect in recruitment was observed in the lung parenchyma. Importantly, inhibiting memory $CD8^+$ T cell recruitment to the airways resulted in significantly higher virus titers during the early phase of infection. Therefore, the second stage of the recall response involves the CCR5-dependent recruitment of circulating memory $CD8^+$ T cells to the lung airways, resulting in increased numbers of antigen-specific cells at the site of virus replication and a further decrease in viral load.

The final stage of the recall response involves the activation of memory T cells in the draining lymphoid tissue by antigen-bearing DCs, resulting in the proliferation and expansion of secondary effector T cells that can migrate to the lung and eradicate the infection. Although this process occurs in a similar manner during both primary and secondary infections, the reduced stimulatory requirements, more rapid acquisition of effector functions, and increased precursor frequency of memory T cells compared with naive T cells allows for the accelerated generation of effector T cells (132, 133). Despite the reduced activation requirements of memory T cells, professional antigen-presenting cells (i.e., DCs) are still required for the optimal generation of secondary effector T cells during a recall response (134). The importance of memory T cell priming by DCs during an influenza virus infection was shown when influenza-specific memory $CD8^+$ T cells transferred into mice lacking bone marrow–derived DCs failed to provide protection from virus challenge (135).

The heterogeneity of the memory T cell population can also have a substantial impact on the magnitude of the secondary effector T cell response. We have shown that the ability of memory $CD8^+$ T cells to proliferate and generate effector T cells that accumulate in the lung following secondary virus infection improves over time, and this improvement in recall efficacy was observed in both the $T_{EM}$ and $T_{CM}$ populations (67). However, there have been conflicting reports regarding the relative contributions of the $T_{EM}$ and $T_{CM}$ populations to recall responses (136–140). The discrepancy in these findings suggested that the division of memory $CD8^+$ T cell subsets solely between effector and central memory cells was insufficient to describe their recall potential. A more

thorough examination of the $T_{EM}$ and $T_{CM}$ subsets revealed that both populations could be further delineated based on the expression of activation markers such as CD27, CD43, and killer cell lectin-like receptor G1 (KLRG1) (141). Similar to the expression of CD62L that defines the $T_{EM}$ and $T_{CM}$ subsets, the distribution of the memory $CD8^+$ T cell pool defined by these activation markers changes over time, so that by one year postinfection most cells display a resting ($CD27^{hi}$ $CD43^{lo}$) phenotype. Importantly, the memory T cell subsets defined by activation marker expression showed substantial differences in their ability to mount recall responses to respiratory viruses. Memory $CD8^+$ T cells with the most activated phenotype ($CD27^{lo}$ $CD43^{lo}$) were 5- to 20-fold less efficient at generating secondary effector T cells that could accumulate in the lung than were memory $CD8^+$ T cells with the most resting phenotype ($CD27^{hi}$ $CD43^{lo}$) (141). Therefore, the third and final stage of the recall response depends on the stimulation of memory T cells by DCs in the lymphoid tissues that give rise to a new population of secondary effector T cells. Furthermore, the ability of memory T cells to mount a robust recall response depends on the activation status of these cells when they re-encounter antigen.

## CONCLUDING REMARKS

Our understanding of the mechanisms that the immune system employs to identify and eliminate acute respiratory viruses has grown considerably over the past decade. The discovery of different PRRs and the role they play in the initiation of the immune response has illustrated how the innate immune system distinguishes between host and pathogen. The dissection of the inflammatory response into individual cytokines and chemokines has allowed us to determine the importance of these molecules for protective responses, and to determine the contribution of different cell types to antiviral immunity. The characterization of memory T cell and B cell subsets has demonstrated the importance of these populations for protection from a secondary challenge and shown how different subsets of these cells contribute to the recall response. However, it is apparent that the various facets of antiviral immunity in the lung are far more complex than originally thought, and subtleties discovered so far hint at additional issues that must be resolved. Future studies investigating the innate and adaptive immune responses in ever greater detail will be required to develop a comprehensive picture of antiviral immunity in the lung.

### SUMMARY POINTS

1. Viral nucleic acids recognized by TLR3, 7, and 9 in the endosome, or by RNA helicases in the cytosol, initiate a signaling cascade that activates IRFs and results in the production of type I IFNs.
2. Inflammatory signals trigger the production of chemokines by epithelial cells, alveolar macrophages, and DCs that attract innate immune cells prior to the generation of adaptive immunity. The early recruitment of NK cells to the lung and their recognition of virus-infected cells by the activating receptor NCR1 are essential for protection from an influenza virus infection.
3. The appearance of virus-specific effector T cells expressing cytokines and cytolytic molecules in the lung and the production of virus-specific IgM and IgG by B cells in the lymphoid tissue combine to resolve acute respiratory virus infections within approximately 10 days postinfection.
4. Following virus clearance, memory T cells are established in lymphoid and peripheral tissues, including a large population of antigen-specific cells that is localized to the lung

airways and lung parenchyma. Virus-specific memory B cells are localized to the lymphoid tissue associated with the respiratory tract (primarily IgA-secreting cells) and the bone marrow (primarily IgG-secreting cells).

5. Changes in the systemic memory T cell pool from a predominantly $T_{EM}$ to $T_{CM}$ phenotype over time, coupled with the disappearance of residual antigen in the lymph nodes, results in the redistribution of virus-specific memory T cells from peripheral to lymphoid tissues.
6. The recall response of memory T cells to a secondary virus infection in the lung airways can be separated into three distinct stages. The first phase involves virus-specific memory T cells located in the lung airways, the second phase involves the CCR5-dependent recruitment of circulating memory T cells to the airways, and the third stage involves the appearance of secondary effector T cells that were generated in lymphoid tissue.

### FUTURE ISSUES

1. How do different DC subsets in the lung impact the innate response to virus and influence the quality of the adaptive immune response?
2. Which chemokine receptors, or combination of chemokine receptors, are required for the migration of effector T cells to the various compartments of the lung?
3. Does tissue-specific imprinting occur during a respiratory virus infection that directs the preferential migration of memory T cells and memory B cells to the lung?
4. How is T cell memory generated during a respiratory virus infection, and what are the relationships between memory T cells that express different activation markers?
5. How do previous infections alter the lung environment and what are the consequences of these alterations for innate immunity?
6. By what mechanisms do memory T cells in the lung airways limit early virus replication during a secondary infection, and do these cells influence the development of the secondary immune response by altering the course of infection?

## DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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