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The CD8 T Cell Response to Respiratory Virus Infections

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Humans are highly susceptible to infection with respiratory viruses including respiratory syncytial virus (RSV), influenza virus, human metapneumovirus, rhinovirus, coronavirus, and parainfluenza virus. While some viruses simply cause symptoms of the common cold, many respiratory viruses induce severe bronchiolitis, pneumonia, and even death following infection. Despite the immense clinical burden, the majority of the most common pulmonary viruses lack long-lasting efficacious vaccines. Nearly all current vaccination strategies are designed to elicit broadly neutralizing antibodies, which prevent severe disease following a subsequent infection. However, the mucosal antibody response to many respiratory viruses is not long-lasting and declines with age. CD8 T cells are critical for mediating clearance following many acute viral infections in the lung. In addition, memory CD8 T cells are capable of providing protection against secondary infections. Therefore, the combined induction of virus-specific CD8 T cells and antibodies may provide optimal protective immunity. Herein, we review the current literature on CD8 T cell responses induced by respiratory virus infections. Additionally, we explore how this knowledge could be utilized in the development of future vaccines against respiratory viruses, with a special emphasis on RSV vaccination.

Keywords: CD8 T cell, memory T cell, respiratory virus, respiratory syncytial virus, influenza A virus, human metapneumovirus, rhinovirus, coronavirus

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INTRODUCTION

Given its continuous exposure to the outside environment, the respiratory mucosa is highly susceptible to viral infection. The human respiratory tract can be infected with a variety of pulmonary viruses, including respiratory syncytial virus (RSV), influenza virus, human metapneumovirus (HMPV), rhinovirus (RV), coronavirus (CoV), and parainfluenza virus (PIV) (1). The severity of disease associated with respiratory viral infection varies widely depending on the virus strain as well as the age and immune status of the infected individual. Symptoms can range from mild sinusitis or cold-like symptoms to more severe symptoms including bronchitis, pneumonia, and even death. RSV is the leading cause of severe lower respiratory tract infection in children under 5 years of age (2). RSV is commonly associated with severe lower respiratory tract symptoms including bronchiolitis, pneumonia, and bronchitis and is a significant cause of hospitalization and mortality in children, the elderly, and immunocompromised individuals (2–6). Similarly, PIV commonly infects children and is a major cause of croup, pneumonia, and bronchiolitis (7, 8). Seasonal influenza infections, most often of the influenza A virus (IAV) subtype, are responsible for 3–5 million cases of severe infection annually (9). Seasonal IAV infections also result in approximately 290,000–650,000 deaths per year, most commonly in either young children or elderly populations (9–11). However, infection with emerging pandemic IAV strains, such as the 2009 H1N1 pandemic strain, primarily induces severe disease and mortality in otherwise healthy adults younger than 65 years of age (12).

In contrast, respiratory infection with HMPV, RV, and CoV are most commonly associated with symptoms of the common cold (13–15). Two notable exceptions are severe acute respiratory syndrome (SARS) CoV and Middle East respiratory syndrome (MERS) CoV, which cause acute respiratory distress and mortality in infected individuals (16–18).

Despite their profound impact on human health, most common respiratory viruses lack an approved vaccine. The strategy employed most often in vaccine development is the induction of robust neutralizing antibody responses. However, the hallmark of many respiratory viral infections, including RSV, HMPV, and RV, is the ability for reinfections to occur frequently throughout life (19–21). This suggests that the antibody response to these respiratory viruses may wane over time. Indeed, despite a correlation between pre-existing nasal IgA and protection from reinfection, the development of long-lasting RSV- and RV-specific mucosal IgA responses was poor in infected adults (22, 23). Although IAV-specific neutralizing antibodies are elicited efficiently through either infection or vaccination, IAV vaccine formulations must be redeveloped annually to account for the rapid mutations of HA and NA genes in seasonal strains (24). Therefore, vaccinations that solely promote the induction of neutralizing antibodies may not be optimal in providing protection against many respiratory virus infections. The induction of cellular immune responses has thus far received little attention in respiratory virus vaccine development. CD8 T cells play a critical role in mediating viral clearance following many respiratory virus infections including RSV, IAV, and HMPV (25–27). In addition, recent murine studies utilizing CD8 T cell epitope-specific immunization strategies observed significantly reduced lung viral titers following IAV, RSV, or SARS challenges (28–30). Therefore, the induction of virus-specific CD8 T cell responses has the potential to improve upon the efficacy of current vaccination strategies. Here, we review the current literature on CD8 T cell responses following respiratory virus infections and discuss how this knowledge may best be utilized in the development of future vaccines.

CD8 T CELL RESPONSES TO ACUTE RESPIRATORY VIRUS INFECTIONS

Following an acute respiratory infection, dendritic cells (DCs) that have taken up viral antigen stimulate the activation of naive CD8 T cells in the lung draining lymph node to induce robust virus-specific CD8 T cell responses [reviewed in Ref. (31–33)]. Respiratory virus infection in mouse models results in an increase in the frequency and number of total and antigen-specific CD8 T cells in the lungs and airways. RSV-specific CD8 T cell responses typically reach peak numbers in the lung at approximately day 8 following an acute infection (34–37). The kinetics of virus-specific CD8 T cells are slightly more delayed following other respiratory virus infections with peaks occurring at approximately day 10 for IAV, days 10–14 for HMPV, and days 12–14 for pneumonia virus of mice (PVM), a model respiratory virus (38–42). The peak of the antigen-specific CD8 T cell response generally corresponds to lung viral clearance following RSV, IAV, HMPV, and PVM infections (36, 40–43). Human CD8 T cell kinetics following respiratory virus infections are less well known given the lack of identified CD8 T cell epitopes and the difficulty in obtaining respiratory tract samples from children following initial virus exposure. The frequency of total activated CD8 T cells in tracheal aspirates peaked at approximately 10 days after the onset of symptoms in children with RSV, IAV, RV, or CoV infections (44). RSV-specific CD8 T cells were detected in the tracheal aspirates of children; however, the evaluated epitopes were present at very low frequencies, comprising up to only 2% of the total CD8 T cell response (44). Following peak expansion, CD8 T cell contraction occurs and a memory population of virus-specific CD8 T cells remains within the lung. The majority of virus-specific CD8 T cells are located within the lung parenchyma, rather than the pulmonary vasculature, following localized respiratory infections in mice (37). Similarly, human RSV- and IAV-specific CD8 T cells were enriched within the lung compared to the blood (45). RSV-specific CD8 T cells in tracheal aspirates of children remain elevated during convalescence following a severe RSV infection, in contrast to murine studies (46). These studies suggest that CD8 T cell responses in the airways may be more prolonged following viral clearance in humans compared to mice.

Following respiratory virus infection, CD8 T cells become activated and develop the ability to produce inflammatory cytokines. Virus-specific CD8 T cells in the lung and airways of mice upregulate expression of markers associated with activation including CD11a, CD25, NKG2a, and CD44 as well as downregulate expression of the lymphoid homing receptor CD62L (34, 35, 47, 48). Activated CD8 T cells also acquire effector functions following viral infection. Virus-specific CD8 T cells rapidly produce cytokines including IFN-γ and TNF as well as degranulate, as measured by CD107a expression, following *ex vivo* peptide stimulation (35, 38, 41, 48). Human virus-specific CD8 T cells also acquire an activated phenotype and effector functions following a respiratory virus infection. CD8 T cells from the tracheal aspirates of children following RSV, RV, or CoV infections expressed elevated levels of the activation markers CD38 and HLA-DR and the proliferation marker Ki-67 (44). Expression of effector molecules such as granzyme B and perforin were also increased. Similarly, CD8 T cells from bronchiolar lavage (BAL) fluid samples exhibited increased expression of Ki-67, granzyme B, CD38, and HLA-DR following either experimental RSV infection of adults or severe, natural RSV infection of infants (46, 49). Additionally, human virus-specific CD8 T cells produce cytokines following respiratory virus infection, as peripheral blood CD8 T cells secreted IFN-γ, TNF, and IL-2 following stimulation with peptides derived from RSV, IAV, HMPV, or RV (49–53).

Following contraction, a subset of virus-specific CD8 T cells remain in the host to form a long-lasting memory population that provides protection against subsequent infection. CD8 T cell contraction to form long-term memory populations in the lung is regulated in part by inflammatory chemokine signaling (54). Mice deficient in either CXCR3 or CXCR3 and CCR5 exhibit a significant increase in the number of memory CD8 T cells following IAV infection, suggesting that chemokine

signaling through CXCR3 and CCR5 plays a critical role in T cell memory generation (54). Following respiratory viral infections in mice and humans, virus-specific CD8 T cells can be detected up to several months post-infection (47, 49, 55, 56). However, respiratory virus-specific memory CD8 T cell populations decline in magnitude with age in the peripheral blood (57). Interestingly, adult RSV-specific CD8 T cell responses are significantly reduced compared to IAV-specific CD8 T cell responses in the peripheral blood, suggesting that memory CD8 T cell responses to IAV in humans may be more stable than RSV (57). Memory CD8 T cells rapidly expand in the lung following a secondary respiratory virus infection in both mice and humans (35, 38, 39, 44, 49). The observed expansion is primarily due to the migration of circulating CD8 T cells into the lung and airways, rather than proliferation of resident cells (58). The expansion of virus-specific CD8 T cells in the lung and airways following infection corresponds with an increase in CXCR3- and CCR5-binding chemokines, supporting a role for chemokine-mediated migration of CD8 T cells following secondary infection (59). Indeed, CCR5 expression on memory CD8 T cells is required for their early recruitment into the airways after secondary infection, but not to the lung parenchyma (59). Following secondary expansion, memory CD8 T cells rapidly produce effector cytokines such as IFN-γ and TNF (30, 38, 60). Additionally, virus-specific memory CD8 T cells express high levels of CD11a and produce cytolytic molecules, such as granzyme B, after infection (61, 62). These effector functions of respiratory virus-specific memory CD8 T cells are critical for mediating viral clearance and protecting against infection, as discussed below.

Based on the expression of activation marker CD45RA and lymphoid homing receptor CCR7, human memory CD8 T cells have been broadly separated into four major subsets: (1) naive ($CD45RA^+CCR7^+$), (2) central memory (T_{CM}; $CD45RA^-CCR7^+$), (3) effector memory (T_{EM}; $CD45RA^-CCR7^-$), and (4) late effector memory (T_{EMRA}; $CD45RA^+CCR7^-$) (63). Due to their expression of CCR7, T_{CM} home primarily to secondary lymphoid organs, while T_{EM} migrate to peripheral tissues and rapidly exert effector functions. T_{EMRA} are a subset of T_{EM} cells that have re-expressed CD45RA. They exhibit reduced proliferative and functional capacity, and thus are considered to be terminally differentiated cells. Human virus-specific memory CD8 T cell populations are typically composed of a combination of T_{EM} and T_{EMRA} within the peripheral blood (44, 46, 50, 52, 55). Alternatively, RSV-specific memory CD8 T cells located in the airways in both adults and infants are primarily of T_{EM} phenotype and also express high levels of CD27, CD28, and CCR5 and low levels of CD62L (46, 49). Together, these studies indicate that T_{EM} CD8 T cells are dominant following respiratory virus infection in humans. Given the frequent exposure to viruses in the respiratory tract, T_{EM} cells may be critical for the rapid employment of CD8 T cell effector mechanisms following reinfection.

Recently, an additional population of memory CD8 T cells that persist within peripheral tissues has been identified, termed tissue-resident memory CD8 T cells (T_{RM}) (64). T_{RM} have been observed within several peripheral organs including the intestine, skin, female reproductive tract, and lung. T_{RM} generated following a respiratory virus infection represent a non-circulating population of memory CD8 T cells that are maintained within the lung parenchyma (65). Virus-specific T_{RM} are located along the wall of large airways and within pulmonary tissue surrounding bronchioles and alveoli (66, 67). Respiratory virus infection also induces T_{RM} within the airway lumen (68, 69). Airway T_{RM} downregulate CD11a expression and can be distinguished from recently trafficked CD8 T cells that express high levels of CD11a (70, 71). The localization of lung and airway T_{RM} following respiratory virus infection is distinctly different from that of T_{CM}, which traffic through the pulmonary vasculature and accumulate in the lung-draining lymph node (72, 73). Virus-specific T_{EM} are also differentially located from T_{RM} residing primarily in the pulmonary vasculature or within the lung tissue near blood vessels, spacially distinct from regions that contain T_{RM} (67, 73). Following either RSV or IAV infection in mice, lung and airway T_{RM} are induced and can be identified by their expression of the canonical resident memory markers CD69 and CD103, which promote their migration to and retention within the lung tissue (37, 65, 74–76). IAV- and RSV-specific T_{RM} are also generated in the lungs of mice that have been locally vaccinated via an intranasal route, but not mice that have been immunized systemically (30, 77–79). Importantly, IAV-specific T_{RM} expressing CD69 were also detected in human lung tissue sections but were absent from the spleen (65). Similarly, RSV-specific T_{RM} expressing both CD69 and CD103 were identified in the human BAL but were not present in the peripheral blood (49). Following secondary viral infection, T_{RM} expand prior to the recruitment of circulating memory CD8 T cell populations from the peripheral blood and rapidly produce IFN-γ (60, 66). Thus, T_{RM} provide a crucial first-line of defense for protecting the host from re-infection with a respiratory virus. However, in contrast to other memory CD8 T cell subsets that remain stable for long periods of time, IAV-specific T_{RM} exhibit limited longevity and enhanced apoptosis with time following infection (66, 80). The loss of IAV-specific T_{RM} corresponds to an increase in viral titers and weight loss following a heterosubtypic IAV infection (66, 80). Interestingly, infant mice generate fewer lung T_{RM} following IAV infection or vaccination and exhibit reduced heterosubtypic protection compared to mice initially infected as adults (79). Given the role of lung T_{RM} in providing protection against respiratory virus infections, identifying strategies to promote the generation of long-lived T_{RM} will be critical for future vaccines, particularly for infant populations.

MECHANISMS OF CD8 T CELL-MEDIATED VIRAL CLEARANCE

It has been well established that CD8 T cells are critical for viral clearance following an acute respiratory virus infection in mice. Adoptive transfer of CD8 T cell clones resulted in significantly reduced viral titers in the lung following RSV, IAV, or HMPV infections (25, 27, 81–84). Similarly, the transfer of either RSV- or IAV-immune splenic CD8 T cells accelerated viral clearance in the lung following infection (85–89).

Accordingly, RSV infection of mice depleted of CD8 T cells resulted in significantly increased lung viral titers at day 7 post-infection, although the virus was ultimately cleared by day 11 (26). In contrast, depletion of CD8 T cells alone did not alter clearance of HMPV (40). Instead, depletion of both CD4 and CD8 T cells together elevated lung virus titers at day 7 following infection with HMPV. Importantly, CD8 T cells have been shown to be sufficient to mediate viral clearance in the lung following acute respiratory infections (87, 88). Athymic nude mice, which lack T cells, fail to clear either RSV or IAV resulting in persistent infections (87, 90). However, the transfer of either RSV- or IAV-immune splenic CD8 T cells into athymic mice resulted in significantly reduced lung viral titers by day 15 and 21, respectively (87, 88). Together, these studies indicate that CD8 T cells play a critical role in mediating viral clearance following acute respiratory infections in mice.

Although studies are limited, a role for CD8 T cells in the elimination of respiratory viruses has also been established in humans. Early studies demonstrated that immunocompromised children with T cell defects experienced prolonged viral shedding following RSV, IAV, or PIV infections compared to immunologically normal children (3, 91, 92). Following bone marrow transplantation of an RSV-infected child with severe combined immunodeficiency, a marked reduction in nasal viral load was observed that correlated with an elevation of CD8 T cell counts (92). Recently, it has been demonstrated that the number of pre-existing virus-specific CD8 T cells in the airway of adults experimentally infected with RSV correlated with reduced overall viral load in the nasal cavity and bronchial brushings (49). In addition to pre-existing CD8 T cell numbers, CD8 T cell effector functions also correlate with reduced viral load. CD8 T cell target cell lysis activity measured by chromium-release assay correlated with a lack of viral shedding in the nasal washes of adults experimentally infected with H1N1 IAV (93). Additionally, individuals with the lowest frequencies of IFN-γ^+ CD8 T cells exhibited the highest viral titers following natural H7N9 IAV infection (94). These studies support the role of CD8 T cells in respiratory virus clearance in humans, consistent with the numerous murine studies.

CD8 T cells mediate viral clearance by utilizing a variety of effector mechanisms to induce the apoptosis of virus-infected cells (95). CD8 T cells can use direct cell–cell contact to eliminate target cells through the interactions of surface molecules such as Fas (CD95) and FasL (CD95L). Additionally, TRAIL expressed on CD8 T cells can interact with its receptors DR4 and/or DR5 to induce the destruction of infected cells. CD8 T cells can also secrete perforin and granzymes to cause membrane pore formation and induce apoptosis. Lastly, CD8 T cells produce inflammatory cytokines, such as IFN-γ and TNF, which may either directly or indirectly promote the cell death of virus-infected cells. While the exact mechanism utilized is unclear, many of these effector functions have been associated with CD8 T cell-mediated clearance of respiratory viruses. Fas/FasL interactions and the perforin pathway have been established as the primary mechanisms by which CD8 T cells eliminate infected cells following an IAV infection (96, 97). Studies utilizing TRAIL-deficient mice and antibody-mediated TRAIL blockade have also demonstrated a role for TRAIL in CD8 T cell-mediated clearance of IAV (98, 99). Similarly, Fas/FasL and perforin pathways have also been associated with virus elimination following RSV infection. Perforin-deficient and FasL-deficient *gld* mice exhibit significantly delayed viral clearance (100, 101). However, both perforin-deficient and *gld* mice achieve complete viral clearance by day 10 post-infection, suggesting that CD8 T cells compensate for those deficiencies through alternative mechanisms. One such mechanism is likely TNF production, as neutralization of TNF in perforin-deficient and *gld* mice significantly increased viral titers compared to IgG-treated controls (101). This is in contrast to studies following PVM and IAV infections, where viral clearance occurs independently of TNF (102, 103). IFN-γ does not appear to play a prominent role in CD8 T cell-mediated viral clearance, as both IFN-γ-deficient mice and mice that received IFN-γ-deficient CD8 T cells exhibit equivalent viral titers to wild-type mice following RSV, PVM, or IAV infections (101, 103–105). Together, these studies demonstrate that CD8 T cells use multiple complementary mechanisms to eliminate virally infected cells following a respiratory virus infection.

CD8 T CELLS PROTECT AGAINST A SECONDARY INFECTION

Given the ability of CD8 T cells to mediate viral clearance following an acute viral infection, it is no surprise that memory CD8 T cells also play a critical role in protecting against secondary respiratory virus infections. The adoptive transfer of airway IAV-specific memory CD8 T cells resulted in significantly reduced lung titers following IAV challenge compared to PBS transfer controls (60). Similarly, transfer of airway RSV-specific memory CD8 T cells reduced lung viral load and weight loss following subsequent RSV infection (76). These studies indicate that transferred memory CD8 T cells are capable of providing protection against secondary respiratory virus challenge.

Memory CD8 T cell-mediated protection against secondary infection has been shown more convincingly in mouse models through the use of vaccination strategies to generate virus-specific memory CD8 T cells. Recombinant baculovirus or murine cytomegalovirus (MCMV) vectors expressing the RSV M2 protein induced M2-specific CD8 T cells that mediated the reduction of lung viral titers following RSV challenge (78, 106). Whole protein vaccination with HMPV virus-like particles containing F and M proteins elicited HMPV-specific CD8 T cells that reduced viral titers in μMT mice, which lack antibodies (107). CD8 T cell epitope vaccines against either RSV or HMPV have also demonstrated CD8 T cell-mediated protection following challenge by reducing lung viral load and histopathology compared to unimmunized controls (108, 109). A similar strategy utilizing DC-peptide vaccination to generate pre-existing PVM-specific memory CD8 T cells also resulted in enhanced viral control following PVM infection (42). Recently, several studies have utilized DC-prime, recombinant *Listeria monocytogenes*- (DC-LM) or vaccinia virus-boost (DC-VV) vaccination protocols to generate a high frequency

of pre-existing antigen-specific memory CD8 T cells in the absence of virus-specific CD4 T cell memory and antibodies (28–30). Prime-boosted mice exhibited significantly reduced lung viral titers following RSV, IAV, or SARS infections compared to controls lacking virus-specific memory CD8 T cells. Additionally, memory CD8 T cells were able to reduce weight loss and mortality following lethal challenges with either IAV or SARS. Overall, these studies provide clear evidence that memory CD8 T cells provide protection against secondary respiratory virus infection by reducing viral titers.

The most well studied example of memory CD8 T cell-mediated protection from a secondary respiratory virus infection is heterosubtypic immunity to IAV subtypes. IAV-specific neutralizing antibody responses recognize the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), which vary between subtypes as a result of genetic reassortment, known as antigenic drift. However, the internal proteins of the virus are often conserved between IAV subtypes. Therefore, memory CD8 T cells that recognize epitopes within conserved viral proteins may be capable of providing cross-protection between IAV viruses of differing subtypes. Evidence of heterosubtypic immunity was first demonstrated by the protection of H1N1 IAV-immune mice from a lethal H2N2 IAV challenge without the induction of a neutralizing antibody response (110). Since then, a memory CD8 T cell-mediated role in accelerating clearance of a heterosubtypic IAV strain has been well-established in mouse, chicken and non-human primate models (66, 111–114). Recently, it has been demonstrated that T_{RM} are essential in providing cross-protection against secondary IAV infection with a heterosubtypic strain (60, 66, 80). Mice with $CD103^+$ T_{RM} in the lung exhibit more efficient viral clearance and reduced weight loss following heterosubtypic challenge than mice lacking a T_{RM} response (66). Importantly, the protection was provided solely by lung-resident memory CD8 T cells, as blocking the ability of recently proliferated T_{CM} cells from trafficking to the lung did not impact protection (66). Consistent with the limited lifespan of IAV-specific T_{RM}, heterosubtypic protection by memory CD8 T cells wanes over time, with a decline observed as early as 60 days following the initial infection (80, 111). Interestingly, systemic immunization with cognate antigen is capable of boosting the T_{RM} pool by expanding the circulating T_{EM} population that seeds the lungs (80). Therefore, it is possible that T_{RM}-mediated heterosubtypic protection could be re-established by vaccination after a waning of the protective T_{RM} population in the lung.

While protection in mouse models is well established, whether memory CD8 T cells play a critical role in protection following secondary respiratory infection in humans is currently unclear. Similar to studies in murine models, evidence for heterosubtypic immunity mediated by memory CD8 T cells has also been demonstrated in humans. Individuals lacking H1N1-specific neutralizing antibody titers exhibited an inverse correlation between memory CD8 T cell activity and viral shedding following their first exposure with H1N1 IAV (93). More recently, it was demonstrated that the frequencies of pre-existing cross-reactive memory CD8 T cells correlated with reduced symptoms, including fewer patients with fever, sore throat, and cough, following infection with the 2009 pandemic H1N1 IAV strain (50). Similarly, a correlation between pre-existing H3N2-specific memory CD8 T cells and reduced risk of viral shedding following 2009 pandemic H1N1 IAV infection was observed (115). Thus, memory CD8 T cell-mediated heterosubtypic protection is also likely to be critical in humans. Following experimental RSV infection in humans, the frequency of pre-existing RSV-specific memory CD8 T cells in the airways correlates with a reduction in both cumulative and lower respiratory tract symptom scores, suggesting a possible role for memory CD8 T cells in protection against RSV in humans (49). However, evidence has also been provided suggesting that memory CD8 T cells may not contribute to protection following respiratory virus infections in humans. Natural reinfection of infants with RSV did not result in a boosting of the CD8 T cell response (116). Similarly, the frequency of RSV-specific memory CD8 T cells in the peripheral blood of healthy adults is significantly reduced compared to IAV-specific memory CD8 T cells (57). Therefore, the extent to which memory CD8 T cells play a role in providing protection against RSV infection in humans remains unclear.

CD8 T CELL-MEDIATED IMMUNOPATHOLOGY FOLLOWING RESPIRATORY VIRUS INFECTION

Despite their beneficial role in mediating viral clearance and protecting against secondary infection, CD8 T cells have also been associated with the induction of immunopathology following respiratory virus infection. Although mice depleted of CD8 T cells exhibited elevated lung viral titers, weight loss and symptom illness scores were significantly reduced in CD8 T cell depleted mice following acute RSV infection (26). Similarly, the adoptive transfer of CD8 T cell lines exacerbated weight loss following an acute RSV infection, despite accelerating viral clearance (82–84). Similar reduction in disease severity was also demonstrated following either HMPV or PVM infection of CD8 T cell depleted mice or mice genetically deficient in CD8 T cells, respectively (40, 103). In addition to the induction of immunopathology following acute respiratory virus infections, we recently demonstrated that memory CD8 T cells also mediate severe immunopathology following secondary RSV infection (30). Large frequencies of systemic, pre-existing RSV-specific memory CD8 T cells generated through DC-LM immunization induced significant weight loss, pulmonary dysfunction, and mortality following RSV challenge, despite a significant reduction in lung viral titers. This result was in contrast to studies using similar immunization strategies to either IAV or SARS, in which memory CD8 T cells mediated protection against lethal viral challenge in the absence of immunopathology (28, 29). Interestingly, the immunopathology induced by RSV-specific memory CD8 T cells occurred only in the context of an RSV infection, as mice challenged with a recombinant IAV strain expressing an RSV-derived CD8 T cell epitope exhibited significantly reduced morbidity and were protected from mortality (30). This result is consistent with several studies that demonstrate CD8 T cells enhance viral clearance while preventing mortality following IAV infection (25, 81,

85, 87, 117). Together, these studies demonstrate a clear role for CD8 T cells in the development of immunopathology following primary and secondary infections with some respiratory virus infections, particularly RSV.

Antiviral mechanisms utilized by CD8 T cells to mediate viral clearance following respiratory virus infection also contribute to the development of immunopathology. Removal of the Fas/FasL pathway in *gld* mice resulted in significant amelioration of weight loss and symptom illness scores following RSV infection (101). Similarly, RSV-infected perforin-deficient mice exhibited prolonged weight loss and symptom illness scores compared to wild-type mice (100). TNF contributes substantially to immunopathology, as antibody-mediated depletion of TNF prior to either RSV or IAV infection significantly reduced weight loss (101, 102, 118). Additionally, mice that are IFN-γ-deficient, depleted of IFN-γ, or received an adoptive transfer of IFN-γ-deficient CD8 T cells prior to RSV infection lost significantly less weight than controls (89). CD8 T cell production of TNF and IFN-γ following PVM infection also induced pulmonary immunopathology by initiating a cytokine storm (119). In addition to causing disease in acute respiratory infections, IFN-γ produced by memory CD8 T cells mediated the severe and fatal immunopathology following RSV infection of DC-LM prime-boosted mice (30).

The role of CD8 T cells in the development of pathology following respiratory infections in humans remains unclear. The best evidence supporting a pathogenic role for CD8 T cells in humans infected with respiratory viruses comes from a study evaluating an RSV-infected severe-combined immunodeficiency patient after bone marrow transplantation (92). The patient exhibited increased CD8 T cell counts following bone marrow transplant, which corresponded to a sharp reduction in RSV nasal titers. However, the appearance of CD8 T cells also correlated with a marked increase in respiratory rate indicative of reduced pulmonary function. Also supporting a pathogenic role of CD8 T cells is the finding that children requiring mechanical ventilation due to severe RSV infection expressed significantly increased levels of activated granzymes and more CD8 T cells producing granzyme B compared to healthy controls (120). In contrast, a study of infants following either fatal IAV or RSV infections revealed a near absence of CD8 T cells from affected lung regions by immunohistochemical staining (121, 122). Similarly, infants with severe RSV infection exhibited an underexpression of genes related to CD8 T cells in the peripheral blood (123). In support of a protective, rather than pathogenic, role of CD8 T cells, correlations have been identified between increased CD8 T cell cytolytic activity and cytokine production with reduced symptom score, faster recovery, and fewer fatalities following H1N1 or H7N9 IAV infections (93, 94). Therefore, whether CD8 T cells play a primary role in mediating pathology versus protection following human respiratory virus infection remains controversial and is an important topic of future investigation.

REGULATION OF CD8 T CELL EFFECTOR FUNCTIONS

Given the potential of CD8 T cell effector functions to cause immunopathology following respiratory virus infection, the immune system has evolved critical regulatory mechanisms to prevent prolonged CD8 T cell effector activity following viral clearance. CD8 T cell effector functions, including production of IFN-γ and TNF, are suppressed in the lung following the resolution of IAV and RSV infections (124–127). One of the primary mechanisms utilized to limit the CD8 T cell response is through suppression by regulatory CD4 T cells (Tregs). Tregs accumulate in the lungs following either RSV or IAV infection peaking at approximately day 6 post-infection, prior to the peak of the CD8 T cell response (36, 128–130). Antibody-mediated depletion of $CD25^+$ Tregs prior to RSV infection resulted in exacerbated weight loss, pulmonary dysfunction, and lung inflammation (128). This enhanced illness corresponded to an increased frequency of RSV-specific CD8 T cells and elevated levels of IFN-γ and TNF protein in the lung (36, 128). Consistent with the Treg depletion studies, increasing RSV-specific Tregs prior to RSV infection using RSV peptide-immunization resulted in an amelioration of weight loss and a reduction in CD8 T cell numbers in the blood and spleen, but not the lung (131). Tregs also can suppress CD8 T cell effector functions following a secondary infection with IAV (130). Antibody-mediated $CD25^+$ Treg depletion prior to heterosubtypic IAV challenge resulted in enhanced inflammation and pulmonary dysfunction corresponding to an increase in CD8 T cell numbers and IFN-γ production. One mechanism through which Tregs may suppress CD8 T cell responses is through the production of the anti-inflammatory cytokine IL-10. $FoxP3^+$ Tregs secrete IL-10 following primary infection with RSV or IAV (130, 132–134). Infection of either IL-10-deficient mice or mice treated with IL-10 receptor blocking antibody resulted in increased numbers of either IFN-γ^+ or IFN-γ^+TNF$^+$ CD8 T cells, suggesting that IL-10 suppresses CD8 T cell effector functions following respiratory virus infection (132–134). Interestingly, IL-10 production by $FoxP3^-$ CD4 T cells and CD8 T cells following either RSV or IAV infection has also been reported, indicating that effector T cell responses may self-regulate their effector functions (132–135). Together, these studies demonstrate that Tregs and IL-10 production play a critical role in regulating CD8 T cells following primary and secondary respiratory virus infections to prevent immunopathology.

Interactions between inhibitory receptors on CD8 T cells with their ligands represents another important mechanism mediating the inhibition of CD8 T cell effector functions following infection. Regulation of CD8 T cells through the PD-1:PD-L1 pathway is a common inhibitory pathway utilized following respiratory virus infection. Expression of PD-1 on pulmonary CD8 T cells is upregulated following RSV, IAV, or HMPV infection in mice (41, 136, 137). Blockade of PD-L1 in primary RSV, IAV, or HMPV and secondary HMPV infections results in enhanced CD8 T cell effector functions, including IFN-γ, TNF, and granzyme B production (41, 136–138). CD8 T cell effector functions are also enhanced following either HMPV or IAV infections in PD-1-deficient mice (41). Importantly, the PD-1:PD-L1 pathway has also been associated with human CD8 T cell responses. Human CD8 T cells in the nasal cavity significantly upregulated PD-1 following RSV infection compared to CD8 T cells from the blood of either healthy or RSV-infected individuals (137). PD-1 and PD-L1 are also both upregulated in the lung

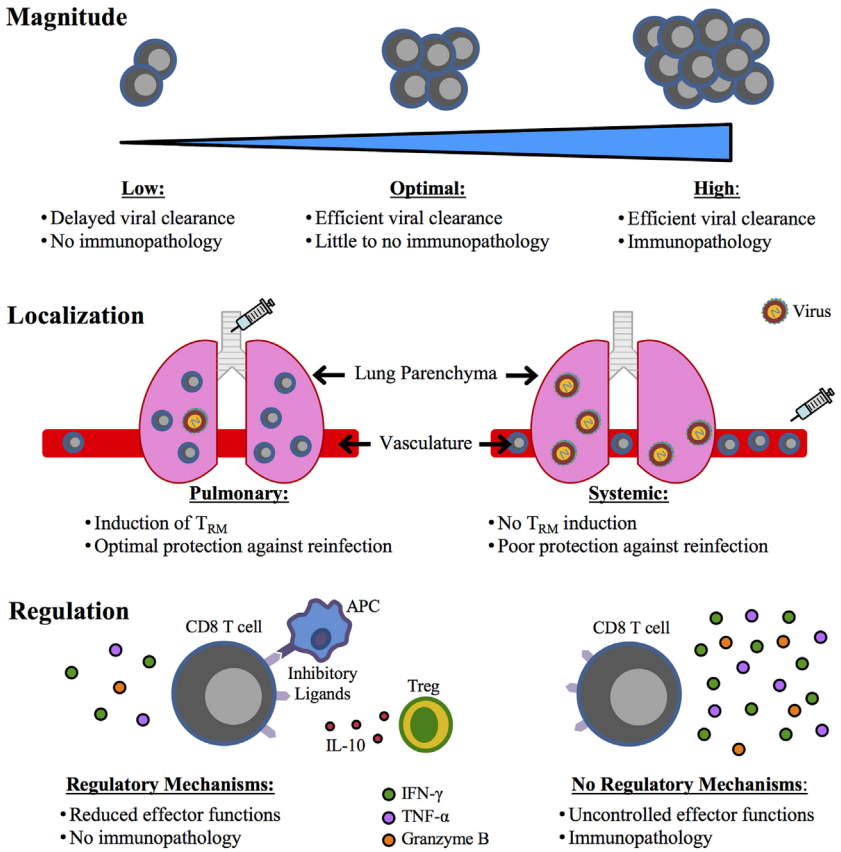

FIGURE 1 | Critical factors for an optimal CD8 T cell-mediated respiratory syncytial virus (RSV) vaccine. A future RSV vaccine designed to elicit a CD8 T cell response will require a balance between CD8 T cell-mediated protection and immunopathology, which may be achieved through the consideration of three important aspects: (1) magnitude, (2) localization, and (3) regulation. An optimal magnitude of the CD8 T cell response will be one that achieves efficient viral clearance in the absence of immunopathology. The vaccination route will be critical in determining the localization of the CD8 T cell response. A pulmonary route of vaccination will induce T_{RM} in the lung that provides superior protection compared to a systemic immunization that would likely not generate protective T_{RM}. Lastly, regulation of the CD8 T cell response generated through vaccination will be crucial, as uncontrolled effector functions, particularly IFN-γ production, can result in immunopathology.

tissue following severe infections with either RSV or the 2009 H1N1 IAV pandemic strain (41). *In vitro* human studies have demonstrated that PD-L1 is constitutively expressed on human airway and bronchial epithelial cells, but expression is significantly upregulated following either IAV or RSV infection (136, 139). Similar to *in vivo* mouse studies, *in vitro* PD-L1 blockade resulted in significantly increased CD8 T cell production of IFN-γ, IL-2, and granzyme B following RSV infection (139). Together, these studies demonstrate a critical role for PD-1 in the suppression of CD8 T cell-mediated immunopathology and cytokine production in both mice and humans. In the absence of PD-1 signaling following HMPV infection, CD8 T cell IFN-γ production remains impaired, suggesting the involvement of compensatory inhibitory pathways (140). Antigen-specific lung CD8 T cells express inhibitory receptors Tim-3, LAG-3, and 2B4 following HMPV infection and exhibit enhanced cytokine production following *in vitro* blockade of each receptor individually (140). *In vivo* blockade of LAG-3 partially restored CD8 T cell IFN-γ production in PD-1-deficient mice following HMPV infection (140). Tim-3 has also been demonstrated to be critical in suppressing CD8 T cell responses *in vivo*, as Tim-3 receptor (Galectin-9)-deficient mice exhibited significantly enhanced CD8 T cell responses following both primary and secondary IAV infections (141). Together, these studies indicate that multiple inhibitory receptor pathways are utilized following pulmonary virus infection to dampen the pathogenic CD8 T cell response and prevent immunopathology.

CONCLUDING DISCUSSION

Successful vaccinations against the majority of respiratory viruses remain elusive. The goal of most vaccination strategies is to induce robust virus-specific neutralizing antibody responses. However, the antibody response generated by infection with many respiratory infections, including RSV and RV, wanes over time. Therefore, neutralizing antibody responses as the sole mediator of a vaccine against most respiratory viruses may not provide long-term protection without yearly

vaccination. Vaccination strategies that include the induction of virus-specific CD8 T cell responses, either alone or in combination with humoral immunity, may be advantageous by providing many benefits associated with cellular immune responses. CD8 T cells are critical for the elimination of virus-infected cells, and viral clearance was prolonged in the absence of CD8 T cells following acute respiratory virus infections. Additionally, robust memory CD8 T cell responses efficiently reduced lung viral titers in the absence of neutralizing antibodies following RSV, IAV, or SARS secondary infections. An important property of CD8 T cells is that they often recognize conserved viral proteins, allowing for cross-protection between different virus strains. This is particularly important for heterosubtypic protection of IAV strains, as neutralizing antibodies are not capable of recognizing IAV strains of differing subtypes. Despite their benefits in mediating viral clearance and providing protection against secondary infections, memory CD8 T cell responses have been associated with the induction of immunopathology following respiratory virus infections. The same antiviral mechanisms employed by memory CD8 T cells to accelerate viral clearance also contribute to immunopathology, including the Fas/FasL pathway- and perforin-mediated cytolysis and IFN-γ and TNF cytokine secretion. Thus, the efficient elimination of respiratory viruses by memory CD8 T cells comes at a cost of disease for the host. CD8 T cell-mediated immunopathology appears to be virus-specific. Although high frequency, systemic, antigen-specific memory CD8 T cells induced severe disease and mortality following RSV infection, no pathology was observed using similar systems for IAV and SARS infections. Therefore, induction of memory CD8 T cells as the sole immune mediator may be particularly dangerous for an RSV vaccine, but significantly less so in either an IAV or a SARS vaccine.

To be able to include CD8 T cell responses within a future respiratory virus vaccine, it will be extremely important to determine how to balance CD8 T cell-mediated protection versus immunopathology following respiratory infection. For RSV in particular, three critical aspects to consider in this balance include magnitude, localization, and regulation of the RSV-specific CD8 T cell response (**Figure 1**). DC-LM immunization generated $M2_{82\text{-}90}$-specific CD8 T cells at a frequency of approximately 20% in the peripheral blood, but induced fatal immunopathology following RSV challenge (30). However, DC-prime only and TriVax immunizations generated a much lower frequency of total $M2_{82}$-specific CD8 T cells, and RSV induced significantly reduced disease in these mice (30, 109). Thus, identifying the optimal magnitude of RSV-specific CD8 T cells for protection in the absence of immunopathology is crucial. It is also clear from recent studies in mouse models that localization of RSV-specific CD8 T cells is a significant factor for both their efficacy of mediating viral clearance and their ability to induce immunopathology following infection. Intranasal immunization with MCMV-M generated T_{RM} within the lung tissue that accelerated viral clearance. In contrast, mice administered MCMV-M systemically did not generate T_{RM} and exhibited delayed viral clearance (78). Similar results were observed with local immunization with DC-IAV-$M2_{82}$ (30). $M2_{82}$-specific lung T_{RM} generated by pulmonary immunization did not induce immunopathology following RSV infection, in contrast to systemic DC-LM immunization, which resulted in severe pathology in the absence of T_{RM} cells. Thus, vaccination strategies against RSV will likely be the most effective when administered through a pulmonary route to generate T_{RM} that will provide protection within the lung following reinfection without inducing immunopathology. Lastly, identifying ways to regulate vaccine-generated CD8 T cell responses will likely reduce immunopathology following subsequent infection. IFN-γ produced by CD8 T cells was the primary mediator of immunopathology following RSV infection of DC-LM vaccinated mice (30). However, neutralization of IFN-γ had no effect on lung viral titers, suggesting that CD8 T cells utilize other antiviral mechanisms to mediate viral clearance in this system. Since CD8 T cells are able to reduce viral titers in the absence of IFN-γ, reducing the amount of IFN-γ produced by CD8 T cells would likely result in ameliorated disease following RSV infection. If vaccination strategies can identify mechanisms by which CD8 T cell cytokine production, particularly IFN-γ, can be attenuated without altering their ability to eliminate virus-infected cells, the pathology induced by CD8 T cells would likely also be decreased.

The development of a CD8 T cell-mediated vaccine should be pursued given the limitations of antibody responses to respiratory viruses. It is possible that the ideal vaccine for respiratory virus infections will include the induction of both virus-specific CD8 T cells and neutralizing antibodies. A vaccination approach combining both arms of the adaptive immune response may allow for optimal viral control in the absence of disease symptoms. However, before CD8 T cells can be developed further as a mediator of protective immunity, the balance between protection and pathology must be achieved. Future studies evaluating aspects of memory CD8 T cell magnitude, localization, and regulation will greatly assist in reaching this balance.

AUTHOR CONTRIBUTIONS

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