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Pulmonary immunity and immunopathology: lessons from respiratory syncytial virus

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Abstract

Respiratory syncytial virus (RSV) is the leading cause of severe respiratory disease in infants and is an important source of morbidity and mortality in the elderly and immunocompromised. This review will discuss the humoral and cellular adaptive immune responses to RSV infection and how these responses are shaped in the immature immune system of the infant and the aged environment of the elderly. Furthermore, we will provide an overview of our current understanding of the role the various arms of the adaptive immune response play in mediating the delicate balance between the successful elimination of the virus from the host and the induction of immunopathology. Efficacious immunization against RSV remains a high priority within the field and we will highlight recent advances made in vaccine design.

Keywords

chemokine; cytokine; eosinophil; respiratory syncytial virus; T cell; vaccine

Respiratory syncytial virus immunobiology

Respiratory syncytial virus (RSV) is an enveloped, negative-sense, ssRNA virus that belongs to the *Paramyxoviridae* family of viruses. RSV contains ten genes that encode 11 proteins owing to a translational termination–reinitiation mechanism that occurs within the M2 mRNA that results in the production of two separate proteins [1].

The respiratory syncytial virus genome encodes multiple proteins that exhibit immunomodulatory activity. The nonstructural genes, *NS1* and *NS2*, have been reported to alter the type I interferon (IFN) pathway by inhibiting signal transducer and activator of transcription (STAT) signaling molecules that are downstream of the IFN- α/β receptors [2–6]. Although the NS1 and NS2 proteins are not essential for virus replication *in vitro*, recombinant RSVs that contain mutations in the genes that encode the NS1 and NS2 proteins replicate very poorly in humans [7]. All of the RSV-encoded transmembrane surface glycoproteins have also been reported to modulate the adaptive immune system. The RSV attachment (G) protein is a highly glycosylated type II transmembrane protein that mediates

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virus attachment to the host cell via cell surface glycosaminoglycans, such as heparan sulfate [8,9]. Furthermore, the G protein has been shown to bind to the chemokine receptor CX₃CR1 and function as a mimic of the chemokine CX₃CL1 (fractalkine) [10]. By imitating the function of CX₃CL1, RSV may influence inflammation during infection by promoting the recruitment of particular cell types, such as macrophages, that may not directly facilitate virus clearance [11-13]. Furthermore, humans with polymorphisms in the *CX₃CR1* gene exhibit an increased risk for the development of severe RSV-induced respiratory disease [14].

The fusion (F) protein mediates virus entry by directing the fusion of the virion envelope with the plasma membrane of the host cell. The F protein has been demonstrated to activate Toll-like receptor (TLR)4 [15-17]. Human studies have shown a correlation between the TLR4 genotype and susceptibility to developing severe RSV-induced respiratory disease [18,19]. In these studies, epidemiological data have linked the relative severity of RSV-induced respiratory disease with missense polymorphisms at the TLR4 locus that result in an aspartic acid to glycine substitution at position 299 (Asp²⁹⁹Gly) and a threonine to isoleucine substitution at position 399 (Thr³⁹⁹Ile) in the receptor protein [19-22]. Studies in mice demonstrate that TLR4- or CD14- (the lipopolysaccharide [LPS]-binding partner of TLR4) deficient mice exhibit delayed clearance of RSV compared with wild-type mice after RSV infection [15]. Furthermore, human airway epithelial cells or peripheral blood mononuclear cells (PBMCs) isolated from children expressing TLR4 missense polymorphisms produced reduced levels of inflammatory cytokines compared with wild-type controls [23]. These results may be explained by the inability of RSV to activate inflammatory cells in the lung (i.e., alveolar macrophages) via a TLR4 and nuclear factor- κ B-dependent pathway [17].

The small hydrophobic glycoprotein (SH) has been demonstrated to alter the immune response in mice by decreasing TNF- α signaling and thus inhibiting cell death after infection [24]. More recent work has suggested that the SH protein forms ion channels in infected cells [25]; however, it remains unclear if this pore formation is required to mediate the antiapoptotic ability of the SH protein. Thus, despite its relatively small genome, RSV has evolved several mechanisms to influence the host's response to infection.

Humoral immunity

Humoral responses predominantly target viral surface proteins in order to facilitate opsonization or neutralization of extracellular virions and play a critical role in the control and resolution of viral infections. Studies in humans examining humoral immunity following vaccination against vaccinia, measles, mumps or varicella zoster viruses revealed that the neutralizing antibody titers in vaccinees remain stable throughout the life of the individual, with the half-life of the antibodies estimated to range between 50 and 200 years [26,27]. Humoral responses against RSV also appear to remain stable for life in humans [28-30] and play an important role in protection from RSV infection. This has been substantiated in the BALB/c mouse model of RSV infection where, as with humans, individuals exhibiting decreased titers of RSV-specific IgG in the serum correlated with decreased protection from secondary infection [31]. Depletion of B cells from mice does not alter the clearance of virus in the primary infection but significantly decreases the rate of viral clearance after secondary infection [32]. This section will focus on the protective capacity of RSV-neutralizing antibodies and their role in providing protective immunity.

IgA

IgA is largely associated with defense of the mucosal surfaces and because RSV replication is thought to occur primarily in ciliated respiratory epithelial cells that line the lung airways, IgA probably plays an important role in protection from initial infection [33]. RSV-specific IgA is secreted rapidly in the upper airways following primary RSV infection of mice [34]. Despite

a rapid increase in IgA-secreting plasma cells, titers of RSV-specific IgA wane over time and are largely undetected by 8 weeks postinfection in mice [35]. In agreement with this, decreased RSV-specific IgA titers in nasal wash correlated with increased infection in human adults [30]. Murine studies also showed that intranasal administration of RSV-specific IgA prior to RSV infection resulted in enhanced protection compared with untreated controls [36]. However, intranasal administration of RSV-specific IgA was not as efficient as administration of IgG in decreasing overall pulmonary RSV titers in mice [37]. These data, taken together, suggest that IgA plays an important role in preventing an initial RSV infection or re-infection of the upper airways.

IgG

RSV-specific IgG antibodies are found at high titers in human serum and remain relatively stable throughout life [28,29,38]. Although IgG antibody does not effectively reach the upper airways, it is believed to play an important role in the clearance of virus from the lower airways [39,40]. Several human studies indicate that high RSV-neutralizing IgG titers correlate with decreased incidence of severe disease associated with lower-airway infection in both the young and elderly [30,41,42]. Owing to the potent neutralizing capacity of IgG antibodies, an ideal RSV vaccine would induce a potent RSV-specific IgG antibody response directed against one or more of the viral glycoproteins. However, passive immunization with IgG antibodies specific to the G or F protein has led to viral antibody escape mutants in both humans and mice [43,44]. These data suggest that vaccines that elicit humoral immunity to RSV should target the induction of a broad spectrum of RSV-specific antibodies in order to limit the likelihood of the development of RSV antibody-escape mutants.

Cellular immunity

Both CD4 and CD8 T cells play a critical role in achieving protective immunity after viral infection. The effector functions of CD4 T cells are mediated primarily through their production of cytokines, such as IL-2, IL-4 and IFN- γ , that orchestrate the adaptive immune response, including aiding both CD8 T-cell-mediated cytotoxicity as well as directing antibody production by B cells. CD8 T cells can kill virus-infected host cells by one of several mechanisms, including via the release of perforin and granzymes or through the upregulation of the death-inducing molecule Fas ligand (FasL). The role of adaptive cellular immunity in reducing RSV viral titers is especially evident in children with defective T-cell responses. These children often suffer more severe RSV disease and demonstrate prolonged virus shedding [45,46]. Additionally, more recent data suggest that severe RSV-induced respiratory disease in infants correlates with a decreased frequency of T cells in the lungs of these children [47].

CD4 T cells

Most of what is known about RSV-specific CD4 T-cell responses comes from the BALB/c mouse model of infection. In BALB/c mice, at least 16 CD4 T-cell epitopes have been identified (Table 1) [48-54]. Of these, the G₁₈₃₋₁₉₅ and F₅₁₋₆₆ epitopes have been studied in the greatest detail. The majority of CD4 T cells that recognize the I-E^d-restricted G₁₈₃₋₁₉₅ epitope express the V β 14 chain as a component of their T-cell receptor (TCR) [50]. G₁₈₃₋₁₉₅-specific CD4 T cells are not reproducibly detected after an acute RSV infection [50]. However, after RSV challenge of mice previously immunized with a recombinant vaccinia virus (vacv) that expresses the RSV G protein (vacvG), G₁₈₃₋₁₉₅-specific CD4 T cells robustly expand and comprise 20–40% of the CD4 T cells present in the lungs between days 5 and 7 postinfection [50,55,56]. Cytokine analysis following *in vitro* stimulation of the G₁₈₃₋₁₉₅-specific CD4 T cells demonstrates that the G₁₈₃₋₁₉₅-specific memory CD4 T-cell response comprises primarily Th1 cells, with Th2 cells representing a small proportion of the RSV-specific CD4

T-cell population [50,57]. The F_{51–66} epitope is I-E^d-restricted and generates a CD4 T-cell response that utilizes a diverse TCR repertoire [48]. Similar to the G_{183–195}-specific CD4 T-cell response, the F_{51–66}-specific CD4 response cannot be reproducibly detected after acute RSV infection [48]. However, F_{51–66}-specific memory CD4 T cells comprise 3–5% of the total CD4 T cells in the lung after RSV challenge of vacvF-immunized mice [48]. However, unlike G_{183–195}-specific memory CD4 T cells, F_{51–66}-specific memory CD4 T cells appear to be almost exclusively Th1 cells [48,57].

Mice immunized with either vacvG or vacvF exhibit accelerated viral clearance compared with nonimmunized controls [56,58,59]. Despite mounting robust G_{183–195}- and F_{51–66}-specific memory CD4 T-cell responses after RSV challenge of vacvG- and vacvF-immunized mice, respectively, it appears that RSV-specific memory CD4 T cells are not required for protection. Antibody-mediated depletion of CD4 T cells in either vacvG- or vacvF-immunized mice did not delay viral clearance after RSV challenge, suggesting that the protective effect of either vacvG- or vacvF-immunization is dependent on either CD8 T cells or antibodies and not the direct antiviral actions of virus-specific CD4 T cells [56]. These data were further substantiated in that antibody-mediated depletion of Vβ14⁺ CD4 T cells, which constitute the majority of the G_{183–195}-specific memory CD4 T-cell population, also did not alter the rate of viral clearance in vacvG-immunized mice undergoing challenge RSV infection [60]. However, in the absence of pre-existing RSV-specific antibodies, RSV-specific CD4 T cells can mediate antiviral activity that is best exhibited by the enhanced protection against RSV infection in naive mice that receive an adoptive transfer of CD4 T-cell lines generated from vacvG-immunized mice [61,62]. Although these data suggest that CD4 T cells can play a direct role in viral clearance, it remains to be proven in physiological settings that these cells are a key contributor to eliminating virus from the host. In addition to their role in mediating protective immunity, RSV-specific memory CD4 T cells have also been demonstrated to play a major role in the development of RSV-induced immunopathology.

Very little is known regarding RSV-specific CD4 T-cell responses in humans [63]. However, there has been extensive mapping of human CD4 T-cell epitopes to the RSV F protein (Table 2) [64,65]. There appears to be significantly fewer circulating RSV-specific CD4 T cells in healthy adults compared with influenza virus-specific CD4 T cells [66]. Furthermore, RSV-specific CD4 T cells in humans produce Th1, Th2 and regulatory cytokines (e.g., IL-10) upon restimulation *in vitro* [63,67]. These data indicate that RSV-specific CD4 T cells with very diverse effector functions are elicited following acute RSV infection in humans.

CD4 T-cell-mediated immunopathology

In the 1960s, a formalin-inactivated form of RSV (FI-RSV) was utilized in a series of vaccine trials. In these trials, approximately 80% of the vaccinees immunized with FI-RSV were hospitalized and two children tragically died after contracting a natural RSV infection [68–71]. Histological analysis of the lungs from the deceased revealed extensive mononuclear cell infiltration as well as numerous eosinophils [68]. Much of our current understanding regarding the underlying mechanisms that mediate the development of RSV vaccine-enhanced disease stems from studies utilizing the BALB/c mouse model. In this model, RSV challenge of mice previously immunized with either FI-RSV or vacvG develop mononuclear cell infiltration into the lung and pulmonary eosinophilia, mimicking what was observed in the children from the FI-RSV vaccine trial [57,72,73]. Mice immunized with either FI-RSV or vacvG also lose significant amounts of weight, a sign of systemic illness, after RSV challenge [55,74].

CD4 T cells appear to be the critical mediators of both the local pulmonary injury and the systemic disease. Antibody-mediated depletion of CD4 T cells from FI-RSV-immunized mice significantly reduced immunopathology in the lung [75]. In addition, adoptive transfer of CD4

T-cell lines derived from vacvG-immunized mice results in extensive pulmonary eosinophilia following RSV challenge [61,62]. Furthermore, antibody-mediated depletion of either $V\beta 14^+$ or $T1ST2^+$ (a molecule expressed on the cell surface of Th2 cells) CD4 T cells from vacvG-immunized mice resulted in a decrease in both pulmonary eosinophilia and reduced weight loss [57,76]. Depletion of these cell types probably inhibits pulmonary eosinophilia by reducing the production of Th2 cytokines. The Th2-associated cytokine IL-13 has been recently demonstrated to be required for the development of pulmonary eosinophilia in vacvG-immunized mice, whereas IL-4 and IL-13 are both required for development of pulmonary eosinophilia in FI-RSV-immunized mice [55,77]. Whereas cytokines produced by Th2 cells have been implicated in the development of pulmonary immunopathology, the inflammatory cytokine TNF- α has been shown to be required for the weight loss and the development of systemic disease [74]. Depletion of TNF- α in vacvG-immunized mice challenged with RSV results in almost complete abrogation of weight loss [74]. However, because depletion of TNF- α in vacvG-immunized mice also significantly reduces pulmonary eosinophilia following RSV challenge, it is unclear whether TNF- α directly causes systemic disease or indirectly by contributing to the development of pulmonary immunopathology.

CD8 T cells

In contrast to CD4 T cells, more work has been performed examining RSV-specific CD8 T cells in humans after RSV infection. A number of RSV-specific CD8 T-cell epitopes have been identified across several genetic backgrounds (Table 2) [78-83]. As mentioned earlier, children with defective T-cell responses exhibit increased susceptibility to RSV infections [45,46]. Furthermore, enhanced frequencies of RSV-specific CD8 T cells in infants correlated with a decreased risk against secondary infection with RSV [84]. Recent studies have also demonstrated RSV-specific CD8 T cells in the bronchial alveolar lavage and blood of RSV-infected infants [85]. Together, these data suggest that RSV-specific CD8 T cells are not only detectable after primary infection of infants but may also play an important role in protection from secondary infection.

Several RSV CD8 T-cell epitopes have also been identified in mice (Table 1) [59,86-88]. In BALB/c mice, the acute RSV-specific CD8 T-cell response is focused on the immunodominant M2₈₂₋₉₀ epitope, with 30–50% of the CD8 T cells in the lungs at the peak of the acute infection recognizing this epitope (Table 1) [89]. Mice immunized with a recombinant vacv expressing the RSV M2 protein (vacvM2) generate a robust memory CD8 T-cell response after RSV challenge [56,89]. Furthermore, depletion of CD8 T cells from these mice results in a significant delay in viral clearance compared with nondepleted controls [90]. Another subdominant CD8 T-cell epitope has recently been described in the M2 protein between amino acids 127–135 [91]. The M2₁₂₇₋₁₃₅-specific CD8 T-cell response is approximately tenfold less compared with that of the dominant M2₈₂₋₉₀-specific CD8 T-cell response after primary and secondary infection with RSV [91]. A subdominant epitope in the F protein (F₈₅₋₉₃) is also recognized by approximately 1–5% of CD8 T cells at the peak of the acute T-cell response [49]. Interestingly, in vacvF-immunized mice the total number of pulmonary F₈₅₋₉₃-specific CD8 T cells is similar to that of M2₈₂₋₉₀-specific CD8 T cells in vacvM2-immunized mice at the same time point (OLSON M, VARGAS, UNPUBLISHED DATA). Despite generating similar secondary responses after RSV challenge, F₈₅₋₉₃-specific memory CD8 T cells appear to have little effect on the clearance of virus. Mice immunized with vacvF, as with vacvM2-immunization, exhibit decreased viral loads compared with mock-immunized mice. However, depletion of CD8 T cells from vacvF-immunized mice does not delay the clearance of virus [90]. These data suggest that, similar to mice immunized with vacvG, protection induced by immunization with the F protein is primarily due to antibodies. Some evidence exists that F-specific CD8 T cells can mediate viral clearance in the absence of antibodies. In these studies, mice immunized with a recombinant Sendai virus expressing the RSV F protein (SevF) mounted a robust F-specific

CD8 T-cell response [92]. B-cell-deficient mice immunized with SevF cleared virus with similar kinetics to SevF-immunized wild-type mice [92]. However, it is still unclear if RSV F-specific CD8 T cells significantly contribute to viral clearance in the presence of an intact F-specific antibody response.

M2₈₂₋₉₀-specific CD8 T cells have been demonstrated to mediate viral clearance via multiple effector mechanisms. Adoptive transfer of *in vitro*-restimulated spleen cells from wild-type, perforin- deficient or FasL-deficient vacvM2-immunized mice all resulted in accelerated clearance of virus from otherwise naive recipients after RSV challenge [93]. Interestingly, spleen cells from IFN- γ -deficient vacvM2-immunized mice exhibited reduced cytolytic activity both *in vitro* and after transfer into naive recipients. Moreover, these cells failed to protect from a subsequent RSV challenge [93]. These data suggest that IFN- γ production by RSV-specific CD8 T cells is a critical determinant for their ability to eliminate virus. However, these experiments were conducted using the transfer of more than 1×10^7 *in vitro*-stimulated effector CD8 T cells that may not mimic the tempo and kinetics of a RSV-specific CD8 T-cell response during a natural infection. It is currently unclear how the lack of IFN- γ would reduce the cytolytic capacity of the RSV-specific CD8 T cells. Data from other studies suggest that IFN- γ may not be required for CD8 T-cell-mediated viral clearance. In these studies, wild-type or anti-IFN- γ monoclonal antibody-depleted BALB/c mice were immunized with M2₈₂₋₉₀ peptide in adjuvant and later challenged with RSV. Despite exhibiting significantly reduced levels of IFN- γ , both IFN- γ -depleted and wild-type mice exhibited similar kinetics of viral clearance [94,95]. Data from other studies suggest that FasL may also play a role in viral clearance during acute RSV infection. FasL-deficient mice exhibit delayed clearance of RSV compared with wild-type controls [94]. However, since the mouse is deficient in FasL, it is currently unclear if the effect of FasL-deficiency is solely due to the lack of FasL on CD8 T cells. It is clear that CD8 T cells contribute to the control and elimination of acute RSV infection but more work needs to be done to determine what effector molecules are required under physiological circumstances.

CD8 T-cell inhibition of RSV vaccine-enhanced pulmonary eosinophilia

CD8 T cells not only play a critical role in clearance of acute RSV infection but also play an equally important role in inhibiting RSV vaccine-enhanced pulmonary eosinophilia. As discussed above, vacvG- or FI-RSV-immunized mice develop extensive pulmonary eosinophilia after RSV challenge [73,77,96-99]. Additionally, adoptive transfer of Th2 cell lines derived from vacvG-immunized mice also induces robust eosinophilia after RSV challenge [61,62]. Cotransfer of CD4 Th2 cell lines together with CD8 T-cell lines derived from vacvM2-immunized mice reduced the levels of pulmonary eosinophilia after RSV challenge compared with mice that received only the CD4 Th2 cell line [61,62]. Further studies have also demonstrated that mice immunized with a recombinant vacv engineered to express the M2₈₂₋₉₀ CD8 T-cell epitope within the G protein (vacvG/M2) also exhibit reduced eosinophilia compared with vacvG-immunized mice after RSV challenge [100]. We have recently demonstrated that CD8 T-cell inhibition of RSV vaccine-enhanced pulmonary eosinophilia requires memory CD8 T cells to reduce the numbers of Th2 cells in the lung and is independent of IFN- γ [56].

BALB/c mice immunized with vacvF generate a robust memory CD4 and CD8 T-cell response and, as with vacvG/M2-immunized mice, do not develop pulmonary eosinophilia after RSV challenge [101]. Interestingly, antibody-mediated depletion or genetic deficiency of IFN- γ in vacvF-immunized mice results in pulmonary eosinophilia after RSV challenge [48,101]. Similarly, antibody-mediated depletion of CD8 T cells from vacvF-immunized mice also leads to the development of pulmonary eosinophilia after RSV challenge. Taken together, these

observations are consistent with the notion that IFN- γ -production by F₈₅₋₉₃-specific memory CD8 T cells is important for their inhibition of pulmonary eosinophilia.

Mice coimmunized with vacvG plus vacvF, similar to mice coimmunized with vacvG plus vacvM2, also induce both potent memory CD4 and CD8 T-cell responses. Interestingly, vacvG plus vacvF-immunized mice exhibit similar levels of eosinophilia compared with vacvG-immunized mice after RSV challenge (OLSON M, VARGA S, UNPUBLISHED DATA). Both vacvG plus vacvM2 and vacvG plus vacvF-immunized mice mount similar M₂₈₂₋₉₀- and F₈₅₋₉₃-specific memory CD8 T-cell responses at day 7 post-RSV challenge. However, prior to and early after RSV challenge (day 3) there is a dramatic difference in the total number of M₂₈₂₋₉₀- and F₈₅₋₉₃-specific memory CD8 T cells in the lungs of these mice. We have also demonstrated that decreasing the magnitude of the M₂₈₂₋₉₀-specific CD8 T-cell response early after RSV challenge results in pulmonary eosinophilia and increasing the magnitude of the F₈₅₋₉₃-specific memory CD8 T-cell response early after RSV challenge results in decreased eosinophilia (OLSON M, VARGA S, UNPUBLISHED DATA). These data suggest that the absolute number of memory CD8 T cells in the lung early after RSV infection is a critical determinant in their ability to inhibit the development of pulmonary eosinophilia. The mechanism by which memory CD8 T cells alter pulmonary eosinophilia remains unclear. We have demonstrated that M₂₈₂₋₉₀-specific memory CD8 T cells alter the chemokine environment in the lungs of vacvG plus vacvM2-immunized mice, specifically decreasing the protein levels of CCL17 and CCL22 in the lung as compared with vacvG-immunized mice. Such a reduction in chemokines may alter the trafficking of Th2 cells and eosinophils into the lung (OLSON M, VARGA S, UNPUBLISHED DATA). However, it remains unclear how the memory CD8 T cells alter the chemokine environment in the lung.

CD8 T-cell-mediated immunopathology

Similar to CD4 T cells, CD8 T cells can also induce significant immunopathology. This is best demonstrated in either FI-RSV-or vacvG-immunized mice that have also been immunized with vacvM2 [56]. Mice that undergo both a memory CD4 and CD8 T-cell response exhibit enhanced weight loss compared with either mice undergoing only a memory CD4 T-cell response (i.e., vacvG-immunized) or mice undergoing an acute infection (i.e., vacv β -gal-immunized) [56]. Furthermore, immunization of mice with M₂₈₂₋₉₀ peptide in adjuvant induced enhanced immunopathology, including increased weight loss, after RSV challenge [95].

The CD8 T-cell effector molecules that are responsible for this CD8 T-cell-mediated immunopathology in mice remain unclear. Adoptive transfer of *in vitro*-restimulated spleen cells from vacvM2-immunized wild-type mice resulted in enhanced weight loss upon RSV challenge of otherwise naive hosts [93]. Interestingly, adoptive transfer of similarly generated cells from vacvM2-immunized IFN- γ -deficient mice resulted in significantly reduced weight loss [93]. These data suggest that IFN- γ may play a role in both viral clearance and immune-mediated pathology. However, other studies have failed to establish a requirement for IFN- γ in CD8 T-cell-mediated immunopathology. Antibody-mediated depletion of IFN- γ in M₂₈₂₋₉₀ peptide-immunized mice did not result in reduced weight loss after RSV challenge [95]. Furthermore, recent data from our laboratory also suggest that IFN- γ is not required for enhanced weight loss in vacvM2-immunized mice. In these studies, wild-type or IFN- γ -deficient mice were immunized with vacv β -gal or vacvG plus vacvM2 and several weeks later challenged with RSV. Surprisingly, both wild-type and IFN- γ -deficient vacvG plus vacvM2-immunized mice lost an equivalent amount of weight (>25% of starting weight) and with similar kinetics (OLSON M, VARGA S, UNPUBLISHED DATA). Taken together these data suggest that in the absence of IFN- γ , other inflammatory mediators, such as TNF- α or IL-6, may play a compensatory role in causing CD8 T-cell-mediated immunopathology.

Although CD8 T-cell responses appear to play an important role in the development of immunopathology after RSV infection in mice, this may not be absolutely the case in humans. Recent work has demonstrated that there is a decrease in the levels of T-cell-associated cytokines (i.e., IFN- γ) in children with severe RSV- or influenza virus-induced respiratory disease [47]. Furthermore, in autopsy tissue from fatal cases of both human RSV and influenza virus infections, there is a marked absence of CD8 T cells in the proximity of viral antigen-bearing cells [47]. Taken together, these data suggest that in young infants CD8 T cells may be protective against lung pathology rather than the causative agent as suggested by the mouse studies.

Role of chemokines in RSV infection

Chemokines play a critical role in the trafficking of immune cells to sites of inflammation after viral infection. In particular, RSV has been identified as triggering a large array of chemokines in the lung that may alter the balance of the resulting inflammatory response. Of note, RSV is a potent inducer of the Th2-associated chemokine CCL17 after *in vitro* infection of airway epithelial cells [102]. Furthermore, mice previously immunized with vacvG exhibit a dramatic increase in the Th2-associated chemokines CCL11, CCL17 and CCL22 after RSV challenge [55,102,103]. Antibody-mediated depletion or genetic deletion of CCL11 in mice previously immunized with vacvG results in a decrease in pulmonary eosinophilia as well as reduced cellular inflammation [104,105]. Despite the induction of Th2-associated chemokines in vacvG-immunized mice after RSV challenge, there is also a dramatic upregulation of the IFN- γ -inducible chemokine CXCL10 in these mice [102]. These data suggest that RSV challenge of mice previously immunized with vacvG results in an induction of both Th1- and Th2-associated chemokines.

Chemokines that are triggered as early as 24 h after RSV infection of BALB/c mice can remain elevated for several days post-RSV infection [106]. One of the most abundant chemokines produced after acute RSV infection of mice is macrophage inflammatory protein (MIP)-1 α that appears to be produced at high levels by lung epithelial cells [106]. Interestingly, MIP-1 α -deficient mice exhibited reduced pulmonary cellular inflammation after RSV infection, but no significant difference in viral titers at similar time points [106]. In agreement with these studies, mice that exhibit decreased inhibitor of κ B (I κ B) kinase (a key mediator of pulmonary chemokine induction) activity in the lung have reduced levels of CCL1, CCL2, CCL3 and CCL5 early after RSV challenge and also have decreased levels of cellular inflammation compared with untreated controls [107]. Despite the reduced cellular influx into the lung, there was no difference in viral clearance between I κ B kinase-inhibited and -uninhibited mice [107]. Similarly, mice depleted of alveolar macrophages exhibit a sharp decline in the levels of the Th1-associated chemokines CCL3 and CCL5 after acute infection with RSV [108]. Despite the reduction of chemokines and reduced natural killer cell recruitment, there was only a marginal defect in viral clearance in alveolar macrophage-depleted mice compared with controls [108]. However, disruption of chemokines involved in the recruitment of CD8 T cells negatively impacts the rate of virus clearance. For example, antibody-mediated depletion of CXCL10, or its receptor CXCR3 in RSV-infected mice reduces lung pathology and also delays viral clearance [109]. These parameters also correlated with a decrease in the total number of RSV-specific CD8 T cells in the lungs of anti-CXCL10 antibody-depleted mice compared with controls [109].

Clinical aspects of RSV infection *Infants*

Respiratory syncytial virus is the leading cause of hospitalization and lower-respiratory tract infection in children under the age of 5 years [110,111]. The risk for severe RSV-induced respiratory disease peaks between 2 and 6 months of age. RSV infection also appears to influence the character of subsequent immune responses for many years [112,113]. Severe

RSV-induced respiratory disease is associated with an increased risk for the development of asthma later in life [112,114]. Owing to the severity of RSV-induced respiratory disease in very young children and the association with the development of asthma later in life, vaccination of infants against RSV is of the utmost importance. However, there are several challenges in creating a RSV vaccine that is both safe and effective in young children.

The neonatal immune system appears to be biased toward a Th2-driven response. PBMCs isolated from children diagnosed with RSV-induced bronchitis predominantly produce Th2 cytokines after restimulation with RSV [115]. This Th2 response is long lasting, as even PBMCs isolated from children 7–8 years of age with a history of severe RSV-induced respiratory disease produce Th2 cytokines after restimulation [113]. These observations have been further substantiated in the mouse model of RSV infection. Adult BALB/c mice infected with RSV mount a predominately Th1 response and lose little weight upon reinfection with RSV 12 weeks later [116]. However, mice that are infected early after birth (i.e., 1–7 days) develop severe weight loss and Th2-driven pulmonary eosinophilia after reinfection with RSV 12 weeks later [116]. This immature immune bias during the initial acute RSV infection correlates with a decrease in the ability to produce IFN- γ early after infection [116]. This Th2 bias in young children may be due to an inability of neonatal dendritic cells to induce a proper Th1 response. Dendritic cells isolated from neonatal mice or human cord blood produce less of the Th1-inducing cytokine IL-12 after stimulation and also exhibit decreased expression of costimulatory molecules after LPS treatment [117–119]. Taken together, these data suggest that early biasing of the neonatal immune system toward a Th2 response may be due to insufficient signals provided by dendritic cells that are necessary to initiate a protective Th1 response. More recent data have suggested that addition of specific adjuvants to vaccines can overcome the inability of neonatal dendritic cells to induce Th1 responses; however, including potent inflammatory adjuvants to vaccines may also reduce their safety profile [120].

Maternal antibodies have also been shown to interfere with the development of early antibody responses after primary infection or immunization [121]. This inhibitory effect of maternal antibodies is largely dependent on the titer of maternal antibodies and has been observed in a wide number of live-attenuated (i.e., measles and oral poliomyelitis) and nonliving (i.e., tetanus and diphtheria) vaccines [120]. This is further substantiated in RSV-infected infants where the generation of neutralizing antibody is significantly delayed compared with older children after RSV infection [122]. Additionally, F-specific IgA titers in nasopharyngeal secretions are significantly decreased in younger individuals compared with older children and inversely correlate with the titers of G-specific IgG present in the maternal serum [123,124]. Evidence suggests that high-dose immunization with a live-attenuated vaccine or mucosal vaccine delivery may help overcome the inhibition of maternal antibodies. However, increasing the vaccine dose also has the potential to induce higher morbidity in vaccine recipients. Interestingly, maternal antibodies do not seem to inhibit T-cell activation in neonatal humans or mice suggesting that T-cell-based immunizations may be more effective in protecting infants from pathogens, such as RSV, that are encountered early in life.

Currently, the only approved prophylactic treatment for RSV susceptible children is passive immunization with palivizumab, a humanized monoclonal antibody specific to the RSV F protein [125]. Palivizumab has little effect on the susceptibility to RSV infection, but rather reduces the frequency of severe RSV-induced respiratory disease as monitored by the development of bronchiolitis, the rate of hospitalization, the length of intensive care unit/hospital stay and the length of intubation [126–129]. Unfortunately, cost-benefit analysis of passive antibody administration to children only proved to be beneficial for children at high risk of suffering from severe RSV-induced disease, whereas treatment of less susceptible children was not cost effective [130]. These data suggest that a more affordable treatment option may benefit a larger group of children.

Elderly

Respiratory syncytial virus has also been demonstrated to cause substantial disease in the elderly and immunocompromised [29]. Primary infection with RSV induces short-lived adaptive immunity, demonstrated by the high frequency of reinfections with RSV throughout life [131,132]. RSV infection of healthy adults often leads to clinical symptoms that are difficult to discern from the common cold. However, elderly and immunocompromised adults suffer more severe RSV-induced respiratory disease that more frequently requires hospitalization. RSV-induced disease is estimated to be responsible for 10,000 deaths each year in individuals over the age of 65 years [133,134]. Numerous studies have demonstrated various defects in innate and adaptive immunity with increasing age [135-138]. Older individuals exhibit reduced virus-specific antibody titers and decreased T-cell numbers compared with younger individuals after inactivated influenza virus immunization [139-141]. Furthermore, thymic involution and T-cell clonal expansions create 'holes' in the naive T-cell repertoire that facilitate more severe viral infections in the elderly [142-144].

Several studies have evaluated the effects of advanced age on the development of adaptive immunity targeted against RSV. In humans, elderly individuals have similar serum neutralizing antibody titers compared with young individuals [28]. Furthermore, RSV challenge of elderly individuals resulted in a similar or greater increase in serum antibody titers compared with young adults [28,38,145]. These data suggest that reduction of protective antibody in the serum is not the cause of the increased risk for the development of severe RSV-induced respiratory disease in the majority of elderly individuals.

Respiratory syncytial virus-specific memory T-cell responses in the elderly have also been examined. Individuals older than 55 years of age had significantly fewer circulating RSV-specific memory CD8 T cells than young adult controls while having similar frequencies of influenza virus-specific memory CD8 T cells [146]. Although the frequency of RSV-specific memory CD8 T cells often fell below the level of detection in elderly individuals, CD8 T-cell lines generated from these individuals were functional as they produced both IFN- γ and granzyme B after RSV peptide stimulation [146]. In agreement with these data, others have reported decreased numbers of RSV-specific memory T cells in the elderly compared with young individuals as determined by ELISPOT analysis [147]. Taken together, these data may not suggest a functional defect in memory T cells in elderly individuals, but instead a defect in the ability to maintain long-lived populations of RSV-specific memory T cells. However, it remains to be conclusively determined if the total numbers and/or function of RSV-specific CD8 T cells is significantly decreased in the elderly compared with younger individuals.

Aged cotton rat and mouse models of RSV infection also demonstrate a defect in T-cell function and in the ability to maintain RSV-specific memory T cells in aged individuals. Aged cotton rats exhibit a marked delay in viral clearance, as well as inflammatory cytokine production [148]. However, this study was unable to evaluate RSV-specific T-cell function, numbers or antibody titers. Aged mice (>20 months) also exhibit delayed viral clearance after acute RSV infection. Memory CD8 T cells from these aged mice also exhibited a decreased capacity to lyse RSV-infected target cells [149]. Although intriguing, it is unclear if this decreased viral clearance and cytolytic activity is due to decreased T-cell function or simply decreased numbers of RSV-specific memory CD8 T cells. Senescence-accelerated mice strain P1 (SAMP1) has also been used as a model to analyze the effects of aging on RSV immunity. In this model, SAMP1 mice exhibit increased weight loss and delayed viral clearance after acute RSV infection [150]. In agreement with this, SAMP1 mice also have reduced total numbers of CD8 and CD4 T cells in the lungs after acute RSV infection. Correlating with the decreased total number of CD8 T cells, bulk CD8 T-cell cultures from these mice exhibit decreased RSV-specific cytolytic activity *in vitro* compared with wild-type control mice.

To date, both infection of aged rodents and humans with RSV suggest that although RSV-specific antibody responses remain intact, there is a defect in the ability to maintain adequate numbers of RSV-specific memory CD8 T cells. It remains to be established if RSV immunity is compromised because of the decreased total number of RSV-specific memory CD8 T cells and/or the decreased protective capacity of these cells. With the advances of intracellular cytokine and tetramer staining, it is now possible to identify and track epitope-specific subsets in elderly individuals. Future studies using elderly populations should examine T-cell clonal expansions, immunodominance hierarchies and evaluate the functional capacity of RSV-specific memory CD8 T cells to kill and produce cytokines upon appropriate stimulation.

Current approaches to RSV vaccination

As mentioned earlier, passive immunization with RSV F-specific monoclonal antibody provides enhanced protection against developing severe RSV-induced respiratory disease during infancy [125]. Furthermore, serum IgG titers directly correlate with protection from RSV infection in mice [31]. T cells have also been demonstrated to play an important role in elimination of RSV. Children lacking efficient T-cell responses also demonstrated enhanced disease and prolonged viral shedding after RSV infection [45,46]. Taken together, these data suggest that an effective RSV vaccine should induce not only a robust RSV-specific antibody response but also a potent RSV-specific T-cell response. This section will focus on recent approaches to RSV vaccine development that target both humoral and cellular adaptive immunity.

Subunit vaccines

Subunit vaccines are popular vaccine candidates based on their safety profile; however, a number of RSV subunit vaccines provide inadequate protection from RSV infection and several show evidence of vaccine-enhanced disease in cotton rats [151]. Previous subunit vaccines are mostly composed of recombinant RSV F or G proteins or various fusions of these two proteins to each other or to other immunogenic proteins. These subunit vaccines often induce good neutralizing antibody titers and CD4 T-cell responses; however, they generate poor CD8 T-cell responses and do not provide long-term protection from RSV infection. Some subunit vaccines, however, have been demonstrated to induce both humoral and CD8 T-cell-mediated immunity. Recently, a subunit vaccine comprised of a fragment of the RSV G protein (amino acids 12–225) and the M2_{82–90} CD8 T-cell epitope (G1F/M2) was utilized as a vaccine in mice [152]. This vaccine induced a balanced Th1/Th2 cytokine profile as well as balanced G-specific IgG_{2a/b} and IgG₁ response. Furthermore, M2-specific cytotoxic CD8 T cells were identified after immunization [152]. In further studies, this group also demonstrated that G1F/M2-immunized mice exhibited long-term protection from RSV challenge; however, weight loss or other immunopathology was not reported [153]. More recently, a subunit vaccine based on the RSV nucleocapsid (N) protein has been developed. In this system, RSV N protein was formulated in subnucleocapsid nanoparticles or subnucleocapsid ring structures (SRS) that encapsulate immunogenic bacterial DNA [153]. RSV challenge of N SRS-immunized mice resulted in enhanced N-specific IgG and IgA titers, as well as T-cell responses. In agreement with this, N SRS-immunized mice also demonstrated enhanced viral clearance with the absence of both weight loss and pulmonary eosinophilia [153]. These data suggest that the RSV N protein induces a balanced humoral and cellular immune response and may represent a good candidate for continued testing and development as a subunit vaccine.

Viral vectors

Two of the biggest challenges in RSV vaccination are immunizing the very young with highly immature immune systems and maternal antibody interference, as well as immunizing the very old that have pre-existing RSV neutralizing antibody. Viral vectors expressing RSV proteins

offer a means to avoid pre-existing RSV antibodies present in the vaccinees. Viral vector-based vaccines are often extremely immunogenic and can induce a balanced humoral and CD4/CD8 T-cell response in hosts that can often overcome the immunosuppressive environment of the very young and elderly. Sendai virus is a mouse paramyxovirus that causes pneumonia in mice but little to no disease in humans and also induces no cytopathology in human airway epithelial cells [154,155]. Recombinant Sendai virus expressing the RSV G protein induced potent antibody in cotton rats and demonstrated enhanced viral clearance over control-immunized mice without enhanced pulmonary immunopathology [156]. A more recent study has shown that SeVF induces both F-specific CD8 T cells in mice as well as F-specific neutralizing antibodies [92]. In agreement with these data, SeVF-immunized mice also exhibited enhanced viral clearance in the presence and absence of antibody, suggesting that both F-specific antibodies and CD8 T cells play a role in viral clearance. Mice immunized with SeVF also did not undergo significant amounts of weight loss after RSV challenge, suggesting little vaccine-enhanced immunopathology [92].

Other viral vectors have also been described to induce RSV-specific immunity. Modified vaccinia virus Ankara expressing the RSV G or F glycoproteins enhanced viral clearance in immunized cotton rats without development of pulmonary eosinophilia; however, these mice exhibited enhanced weight loss after RSV challenge [92,157]. Adenovirus vectors have also been utilized in RSV vaccination. These adenoviral vectors encoding RSV proteins produce robust neutralizing antibody responses after immunization even in the face of an immature immune system [158,159]. A recent report demonstrated protection of mice from RSV challenge after a single intranasal immunization of a recombinant adenovirus expressing three copies of the RSV G protein [160]. Because a large amount of the host response to adenovirus-based vaccines is targeted at the vector, vector immunity is of great concern. Immunization of previously adenovirus-infected mice only modestly decreased RSV G-specific IgG responses and did not alter G-specific IgA responses, suggesting that in this scenario, previous adenovirus vector immunity did not interfere with inducing RSV-specific antibody responses [160]. Despite these promising studies in mice utilizing adenovirus-based vectors, immunization of chimpanzees with adenoviruses expressing RSV surface glycoproteins did not mediate protection from a subsequent RSV challenge [161].

Newcastle disease virus (NDV) is an avian member of the *Paramyxoviridae* family and has been more recently utilized as a viral vector for a number of pathogens [162]. NDV does not infect humans in high frequency and is dramatically attenuated in nonhuman primates [162]. A NDV virus expressing the RSV F protein has been recently shown to induce robust type I IFN responses after infection and also prime a potent F-specific CD8 T-cell response in BALB/c mice [163]. RSV challenge of NDV-F-immunized mice resulted in an enhanced memory F-specific CD8 T-cell response and decreased RSV viral titers compared with controls [163]. Importantly, there was no sign of vaccine-enhanced disease after RSV challenge of mice previously immunized with NDV-F. These data suggest that use of a closely related paramyxovirus as a viral vector can induce RSV-specific immune responses and protect from future RSV infection.

Live-attenuated RSV

Live-attenuated strains of RSV are attractive vaccines for young individuals lacking previous RSV immunity. First-generation, live-attenuated, cold-passaged temperature-sensitive (cpts) RSV vaccines proved to be under- or overattenuated for use in young children [164-167]. However, more recent generations of these cpts mutants that have been further attenuated by manipulating the RSV genome by inducing mutations or deletions in the *SH*, *NS1*, *NS2*, *M2* or *G* genes. These second-generation mutant viruses have proven to be attenuated compared with wild-type RSV and show promise as potential live-attenuated vaccines [168-171]. However,

the immunogenicity of these viruses has not been extensively examined in children. Only the rA2cp 248/404/1030/ Δ SH vaccine candidate to date has been determined to be properly attenuated in young infants and acceptable for use in further studies [169]. This virus exhibited attenuated viral shedding in both seropositive and seronegative children and induced antibody immunity in almost half of the recipients [169]. Recently this vaccine was demonstrated to induce seroconversion in young recipients and significantly reduced the incidence of disease compared with controls [172]. Importantly, no vaccinees presented with vaccine-enhanced disease. Taken together, these data suggest that live-attenuated RSV strains may be useful for immunizing young children without the risk of enhanced immunopathology.

Expert commentary

Respiratory syncytial virus is the leading cause of hospitalization and lower-respiratory tract infection in young children [110,111,173]. The relative severity of RSV-induced respiratory disease in infants correlates with an increased risk for the development of asthma later in life [174]. Furthermore, RSV has also been shown to induce significant morbidity and mortality in the elderly and immunocompromised adults [29]. Thus RSV has a profound and prolonged impact on human health from early childhood into old age. For these reasons, RSV has become an important target for vaccine development. In the early 1960s, FI-RSV was utilized in a series of vaccine trials. FI-RSV vaccine recipients experienced enhanced disease after contracting a natural RSV infection that included enhanced hospitalization rates, increased pulmonary inflammation, pulmonary eosinophilia and increased mortality [68-71].

The mouse model of RSV immunization has contributed greatly to our understanding of the mechanisms underlying the development of RSV vaccine-enhanced disease. Studies utilizing the mouse model indicate that CD4 T cells, mainly Th2 cells, are the driving factor that led to the development of pulmonary eosinophilia in vaccinated mice. Depletion of Th2 cells results in a decrease in systemic disease (weight loss) as well as pulmonary eosinophilia [57,76]. We now also understand that RSV-specific memory CD8 T cells play an important regulatory role in that these cells are able to inhibit the development of pulmonary Th2 responses and eosinophilia [56,100,101]. However, induction of a robust RSV-specific memory CD8 T-cell response can also lead to severe systemic immunopathology [56,93]. These data suggest that modern RSV vaccines should induce not only virus-specific antibody responses but also a balanced RSV-specific CD4 and CD8 T-cell response to maximize viral clearance as well as inhibit lung pathology.

Respiratory syncytial virus vaccination is challenging due to specific roadblocks in trying to immunize the very young and very old individuals. Young infants exhibit immature immune systems that are skewed to Th2-type responses and are often hard to target owing to the interference of maternal antibody [120]. Older individuals often have pre-existing immunity to potential viral vectors, exhibit decreased numbers of naive T cells due to involution of the thymus, and suffer from T-cell clonal expansion, which leave 'holes' in the naive T-cell repertoire [135]. It is likely that different immunization strategies will have to be utilized for each of these populations. Current promising vaccines include subunit vaccines, viral vectors expressing RSV proteins, as well as live-attenuated versions of the virus. Current studies with the second generation of live-attenuated RSV vaccines suggest that they are sufficiently attenuated in humans and promise to induce RSV-specific immunity without inducing enhanced immunopathology.

Five-year view

In our opinion, the most important issues that are facing the RSV community are:

- Determining the defects in T-cell immunity in aged individuals

- Developing safe and effective vaccines to induce anti-RSV immunity in both the very young and the elderly

Humoral immunity to RSV appears to be life long in the majority of individuals and upon reinfection, elderly individuals often mount more potent memory antibody responses than young individuals [29]. These data suggest that the increased susceptibility of the elderly to RSV infection may lie within the CD8 T-cell arm of the adaptive immune system. In both animal models and human infection, the number of RSV-specific CD8 T cells is decreased in the elderly as compared with younger controls. Several studies indicate that there may also be functional impairments in the ability of aged CD8 T cells to produce cytokines or induce the killing of target cells; however, these results have not been sufficiently separated from the decreased total number of RSV-specific CD8 T cells. Therefore, it is currently unclear if CD8 T cells in elderly individuals are of lesser quality than those in younger individuals. Additionally, influenza virus-specific CD4 T cells generated in elderly individuals are deficient in their ability to proliferate and produce cytokines after recognition of their cognate antigen [175]. To our knowledge, no work has been done to examine the numbers or functionality of RSV-specific CD4 T cells in aged individuals. A better understanding of the quantity and quality of RSV-specific T cells in the elderly will help us better design vaccines and treatments that may overcome the difficulties of protecting the elderly from RSV.

The development of an efficacious RSV vaccine has been a priority since the initial isolation of the virus. Despite numerous progressions in the field, there is still no approved RSV vaccine that has been deemed both safe and effective. Recent advances in RSV vaccinology that include the development of safer live-attenuated strains as well as viral vectors that induce long-lasting RSV-specific immunity hold great promise. These vaccination strategies are particularly attractive for their ability to overcome the Th2 nature of the immature immune system in infants by delivering both viral 'danger' signals that initiate the innate immune response as well as effectively elicit a RSV-specific immune response. Furthermore, and possibly most importantly, neither of these vaccination strategies has led to an incidence of vaccine-enhanced disease that was such a prominent feature of the FI-RSV vaccine trials. We feel that current RSV vaccine design should strongly focus on vaccines that induce a balanced humoral, CD4 and CD8 T-cell immune response. It has also become apparent that the induction of a broad spectrum of both antibodies and T cells with diverse repertoires and epitope recognition protect against both antibody and CD8 T-cell escape mutants. For this reason, it will be important to identify numerous CD4, CD8 and antibody epitopes that are conserved across a large proportion of the population.

Key issues

- Respiratory syncytial virus (RSV) is the leading cause of hospitalization and lower-respiratory tract disease in children under the age of 5 years and causes significant morbidity in the elderly and immunocompromised.
- Humoral responses to RSV consist mainly of IgG and IgA and serum IgG levels of antibody appear to remain stable long-term whereas IgA levels rapidly decline after infection.
- RSV-specific CD8 and CD4 T cells both contribute to viral clearance but can also cause significant immunopathology.
- Unique obstacles must be overcome in order to achieve successful vaccination in both infants and the elderly.
- The most promising vaccination strategies include live-attenuated vaccines and the use of viral vectors to deliver RSV antigens.

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•• of considerable interest

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Table 1
Murine CD8 and CD4 T-cell epitopes for respiratory syncytial virus A2 strain.

Mouse strain	RSV protein	Amino acid	Peptide sequence	MHC restriction	Ref.
<i>CD8</i>					
BALB/c (H-2 ^d)	M2	82–90	SYIGSINNI	H-2K ^d	[86,89]
BALB/c	M2	127–135	VYNTVISYI	H-2K ^d	[91]
BALB/c	F	85–93	KYKNAVTEL	H-2K ^d	[49]
BALB/c	F	92–106	ELQLMQSTPTNNR	H-2K ^d	[83]
C57BL/6 (H-2 ^b)	M	187–195	NAITNAKII	H-2D ^b	[87,88]
C57BL/6	G	177–188	SNNPTCWAICKR	H-2D ^b	[87]
C57BL/6	NP	57–64	ANHKTGL	H-2D ^b	[87]
C57BL/6	F	433–442	KTFNSGCDYV	H-2D ^b	[87]
C57BL/6	NP	360–368	NGVINYSVL	H-2D ^b	[87]
C57BL/6	F	250–258	YMLTNSSELL	H-2D ^b	[87]
<i>CD4</i>					
BALB/c (H-2 ^d)	G	183–195	WAICKRIPNKKPG	I-E ^d	[50]
BALB/c	F	51–66	GWYTSVTIELSNIKE	I-E ^d	[48,52]
BALB/c	L	392–406	VWLYNQIALQLKNHA	I-E ^d	[51]
BALB/c	L	790–804	ISLLDLISLKGKESI	I-E ^d	[51]
BALB/c	L	931–945	TMPVYNRQVLYKKQR	I-E ^d	[51]
BALB/c	L	1234–1248	LINIDKIYKKNKHKF	I-E ^d	[51]
BALB/c	L	1364–1378	SIEYILKDLIKDPN	I-E ^d	[51]
BALB/c	L	1650–1664	NEVFSNKLINHHKMN	I-E ^d	[51]
BALB/c	L	1832–1847	INGRWILLSKFLK	I-E ^d	[51]
BALB/c	L	2090–2105	STIASGIIIEKYNV	I-E ^d	[51]
BALB/c	F	146–160	VSVLTSKVLDLKNYI	I-E ^d	[51]
BALB/c	F	184–199	SALASGIASVSKVLH	I-E ^d	[51]
BALB/c	NS2	62–75	LVNYEMKLLHKVGS	I-E ^d	[51]
BALB/c	NS2	67–80	MKLLHKVGS TKYKK	I-E ^d	[51]
BALB/c	M	161–175	PTYLRSISVRNKDLN	I-E ^d	[51]
BALB/c	SH	33–46	IISIMAILN KLCE	I-E ^d	[51]
C57BL/6 (H-2 ^b)	G	168–185	FNFPVPCSISNNPTCWAI	I-A ^b	[53,54]

F: Fusion protein; G: Attachment protein; M2: Proton-selective ion channel protein; NS: Nonstructural protein; RSV: Respiratory syncytial virus; SH: Small hydrophobic glycoprotein. Modified with permission from [59].

Table 2
Human CD8 and CD4 T-cell epitopes for respiratory syncytial virus A2 strain.

RSV protein	Amino acid	Peptide sequence	HLA restriction	Ref.
<i>CD8</i>				
F	118–126	RARRELPRF	HLA-B57	[78]
F	551–559	IAVGLLLYC	HLA-C12	[78]
F	8–17	ANAITTLTA	ND	[80]
F	93–102	LQLLMQSTPA	ND	[80]
F	109–118	RELPRFMNYT	HLA-A1	[80]
F	260–269	LINDMPITND	ND	[80]
F	273–282	LMSNNVQIVR	ND	[80]
F	285–294	SYSIMSIKE	ND	[80]
F	374–383	TLPSEVNLCN	ND	[80]
F	388–397	NPKYDCKIMT	ND	[80]
F	519–528	GKSTINIMIT	ND	[80]
F	521–530	STINIMITTI	ND	[80]
M	229–237	YLEKESIYY	HLA-A1	[82]
M	195–203	IPYSGLLLV	HLA-B51	[82]
M2	151–159	RLPADVLKK	HLA-A3	[82]
M2	64–72	AELDRTEEY	HLA-B44	[82]
NS2	41–49	LAKAVIHTI	HLA-B51	[82]
N	46–59	KLCGMLLITEDANH	ND	[81]
N	232–245	STRGGSRVEGIFAG	ND	[81]
N	250–263	AYGAGQVMLRWGV	ND	[81]
N	253–266	AGQVMLRWGVLA	ND	[81]
N	256–269	VMLRWGVLA	ND	[81]
N	298–311	AGFYHILNNPKASL	ND	[81]
N	306–314	NPKASLLSL	HLA-B7	[79]
<i>CD4</i>				
G	162–175	DFHFEVFNFPVCSI	HLA-DPB1*0401	[63]
F	7–30	KANAITTLTAVTFCFASGQNITE	HLA-DRB1*0101/DRB1*0401	[64]
F	25–42	GQNITEEFYQSTCSAVSK	HLA-DRB1*0401/DRB1*0701	[64]
F	43–60	GYLSALRTGWYTSVITIE	ND	[64]
F	49–72	RTGWYTSVITIELSNIKENKCNGT	ND	[64]
F	55–72	SVITIELSNIKENKCNGT	HLA-DRB1*0701	[64]
F	73–90	DAKVLIKQELDKYKNAV	ND	[64]
F	85–102	KYKNAVTEQLQLMQSTPP	DRB1*0401	[64]
F	109–132	RELPRFMNYTLNNAKKTNTLSKK	ND	[64]
F	175–192	NKAVVSLSNGVSVLT	ND	[64]
F	193–210	LDLKNYIDKQLPIV	ND	[64]
F	229–252	RLLEITREFSVNAGVTPVSTYML	ND	[64]
F	265–288	PITNDQKKLMSNNVQIVRQQSY	HLA-DQB1*03	[64]
F	295–318	EVLAYVVLPLYGVIDTPCWKLHT	HLA-DQB1*05/DQB1*06	[64]
F	337–360	TDRGWYCDNAGSVSFFPQAETCKV	ND	[64,65]
F	391–408	YDCKIMTSKTDVSSSVIT	HLA-DRB1*0401	[64]
F	409–426	SLGAIVSCYGKTKCTASN	HLA-DRB1*0701	[64]
F	427–444	KNRGIKTFSNGCDYVSN	HLA-DRB1*0401	[64]
F	457–486	YYVVKQEGKSLYVKGEPIINFYDPLVFP	HLA-DRB1*0101	[64]
F	493–516	SQVNEKINQSLAFIRKSDELLHNV	ND	[64]
F	517–534	NAGKSTTNIMITTHVI	ND	[64]
F	541–558	LIAVGLLLYCKARSTPVT	ND	[64]

F: Fusion protein; G: Attachment protein; M2: Proton-selective ion channel protein; ND: Not determined; NS: Nonstructural protein; RSV: Respiratory syncytial virus; SH: Small hydrophobic glycoprotein.