Aberrant T cell immunity triggered by human Respiratory Syncytial Virus and human Metapneumovirus infection

Andrea E. González, Margarita K. Lay, Evelyn L. Jara, Janyra A. Espinoza, Roberto S. Gómez, Jorge Soto, Claudia A. Rivera, Katia Abarca, Susan M. Bueno, Claudia A. Riedel, and Alexis M. Kalergis

ABSTRACT

Human Respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV) are the two major etiological viral agents of lower respiratory tract diseases, affecting mainly infants, young children and the elderly. Although the infection of both viruses trigger an antiviral immune response that mediate viral clearance and disease resolution in immunocompetent individuals, the promotion of long-term immunity appears to be deficient and reinfection are common throughout life. A possible explanation for this phenomenon is that hRSV and hMPV, can induce aberrant T cell responses, which leads to exacerbated lung inflammation and poor T and B cell memory immunity. The modulation of immune response exerted by both viruses include different strategies such as, impairment of immunological synapse mediated by viral proteins or soluble factors, and the induction of pro-inflammatory cytokines by epithelial cells, among others. All these viral strategies contribute to the alteration of the adaptive immunity in order to increase the susceptibility to reinfections.

In this review, we discuss current research related to the mechanisms underlying the impairment of T and B cell immune responses induced by hRSV and hMPV infection. In addition, we described the role each virulence factor involved in immune modulation caused by these viruses.

ARTICLE HISTORY

Received 3 February 2016
Revised 22 November 2016
Accepted 23 November 2016

KEYWORDS

adaptive immunity; hMPV; hRSV; immunological synapse; T cells

Introduction

The human Respiratory syncytial virus (hRSV) and human metapneumovirus (hMPV), which belong to the Pneumoviridae family, are the main viral etiological agents of severe lower respiratory tract infection (LRTI), especially in infants, children and the elderly. HRSV and hMPV can lead to bronchiolitis and pneumonia, and have also been implicated in the development of recurrent wheezing and asthma.

HRSV is the second most common etiological pathogen for pneumonia after influenza virus around the world. Furthermore, it has been reported worldwide between 66,000 to 239,000 deaths per year of children less than five years of age, who suffered LRTI caused by hRSV.

HRSV was first isolated in 1956 from a colony of chimpanzees that presented various symptoms, including coughing, sneezing, and purulent nasal discharge. The illness quickly spread from sick chimpanzees to other monkeys, indicating the presence of a highly contagious pathogen that was originally denominated the chimpanzee coryza agent. In 1957, Chanock and Finberg isolated a similar agent from throat swab samples of infants with a severe respiratory disease, which was found to be identical to the one reported by Blount et al in chimpanzees. The isolated pathogen induced syncytia formation, via the virus fusion protein (F) on permissive cell types in cultures. Therefore, this pathogen was renamed as a hRSV.

On the other hand, hMPV was first isolated in 2001 from young Dutch children with a respiratory tract disease. HMPV is a member of the Pneumoviridae family and Metapneumovirus genus. Genomic and phylogenetic analyses suggest that hMPV diverged from avian MPV, a virus that causes serious respiratory diseases in chickens. Infection caused by hMPV presents a
similar symptomatology as other respiratory viruses such as hRSV, parainfluenza virus and influenza, whereby the diagnosis is difficult. Symptoms of hMPV include rhinorrhea, cough, acute otitis media, fever, and, less frequently, conjunctivitis, rash, diarrhea and vomiting. Frequently, risk population, including young children and the elderly, infected with hMPV require hospitalization. Moreover, mortality as a result of hMPV can reach up to 10% in the elderly.

Recurrent infections with hRSV or hMPV are common in children and adults. Studies in healthy young adults subjected to experimental challenge or natural infection, showed mild upper respiratory symptoms for both viruses infection. However, reports performed in humans and mouse dendritic cells (DCs) have described that both viruses infect these cells affecting DCs capacity of promote an adequate immunological memory due to their interference with naïve T cell priming.

The ineffectiveness of the natural infection to induce long-term immunity has hampered vaccine generation and currently there is no licensed vaccine available to prevent the bronchiolitis and pneumonia caused either by hRSV or hMPV. However, several candidate vaccines are in different stages of development for preventing the diseases caused by these viral agents. Candidate vaccines for hRSV and hMPV employ different approaches, including a chimeric virus (hMPV), live attenuated virus (hRSV), and purified proteins (hRSV), among others. Furthermore, recombinant Bacillus Calmette-Guérin (rBCG) vaccines have been developed using hRSV and hMPV antigens to provide a protective T_{H1} response in mice. In addition, antibody-mediated immunity is relevant for protection against both viruses. For example, Palivizumab, an IgG1 humanized anti-F monoclonal antibody, is safe and effective when given in a prophylactic manner to children at risk of severe hRSV infection. Thus, the ideal vaccine for either hRSV or hMPV should provide both antibody and T cell-mediated immune protection.

In this review, we describe the most recent findings relative to the mechanisms employed by hRSV and hMPV to evade the host immune system. Further, we will discuss the virulence factors of each virus that contribute at modulating the adaptive immune response.

**Adaptive immunity triggered by hRSV**

*The Humoral response against hRSV infection*

The humoral immune response plays a major role in protecting humans from hRSV infections. Indeed, high titers of pre-existent mucosal IgG are correlated with reduced viral loads in hRSV-infected infants. Similar correlation have been obtained for nasal IgA levels in naturally infection and in experimental challenges in healthy adults. On the other hand, it has been described in mice that antibodies play a major role during reinfection, even more than in the first infection, giving a principal role to T cells in the clearance of the virus during the first hRSV challenge.

Interestingly, prophylactic treatment with Palivizumab reduces severe hRSV-mediated LRTI, and consequently diminish hRSV-associated hospitalization of premature infants, children with congenital heart disease (CHD) and children with cystic fibrosis (CF), suggesting that a neutralizing antibody of high affinity and titer is enough to confer a clinical protection against hRSV disease. However, in most adults antibody titers are beneath the levels needed to reach a complete airway protection, despite repeated infection occurring throughout life. Experimental challenges in healthy adults have shown that serum and nasal IgA titers increase after infection but those levels are poorly maintained. Similar results have been reported for neutralizing antibodies levels in a birth cohort followed-up over three hRSV epidemics. These antecedents suggest that acute production of short-life antibody-secreting cells (ACS) is not impaired. However, a defect occurs in long-lived plasma cells that arise from the ASC population, which normally should migrate to bone marrow and respiratory mucosa. This phenomenon might explain, at least in part, to why the specific hRSV-IgA generated humoral response is not sufficient to provide protection upon reinfection.

On other hand, several pieces of evidences suggest that the production of virus-specific antibodies plays an important role in the regulation of hRSV-specific T cell responses. The interaction between antibodies and T cell responses was associated with the ratio of neutralizing and non-neutralizing antibodies. Wherein, for hRSV infection, a higher ratio of neutralizing versus non-neutralizing antibodies enhanced the balance of CD4^{+} / CD8^{+} T cells in vitro that respond specifically to the virus in humans PBMC, as well as in vivo assays in mice model. Interestingly, the infection of mice with hRSV immune-complexes increase the immune response against the virus, particularly promoting a TH1 response by CD4^{+} T cells and IgG2c response by B cells. Higher amounts of non-neutralizing antibodies might enhance infection and could cause immune complex deposition, leading to enhanced respiratory disease. Considering the whole body of data described above, it is possible to hypothesize that hRSV infection can modulate the humoral response to impair recurrent reinfection and indirectly affect T cell activation.
The cellular immune response against hRSV infection

Both memory CD4+ and CD8+ T cells contribute significantly at achieving protective immunity upon hRSV infection.57-59 This applies especially in children with defective T cell responses, who exhibit severe hRSV infection and prolonged virus shedding.60 Supporting this observation, T cell depletion assays in BALB/c mice results in higher hRSV replication upon infection, while the adoptive transfer of virus-specific memory T cells enhances virus clearance in recipient mice.61 Furthermore, it has been demonstrated that transfer of hRSV-N-specific T cells also contribute to reduce viral immunopathology.38,39 Moreover, memory T cells appear to be clinically important in protecting from severe diseases caused by hRSV reinfections. This notion is supported by the fact that minor symptoms are observed in populations of older children and young adults infected with hRSV, despite of defective responses in IgA B cell memory and in hRSV-specific serum.65,66

Recently, it has been demonstrated that tissue-resident memory (Trm) T cells are relevant to the capacity of the host to rapidly limiting the spread of pathogens in tissues.63,64 Thus, hRSV-specific CD4+ and CD8+ Trm T cells could provide immediate immunological protection against hRSV infections. In fact, analyses of hRSV-specific CD8+ memory T cells have shown that these cells mostly remain in lungs and a minority of these cells circulates in peripheral blood from healthy individuals.65,66 Moreover, increased activated hRSV-specific airway Trm T cell frequencies were observed in bronchoalveolar lavage fluid (BALF) from healthy adults inoculated with hRSV, which coincided with a reduction in the viral load.59

hRSV-mediated lung pathology in mice is not completely dissected and primary reports attributed this effect to T cells, specially CD8+ T cells67,68 but in humans, it has mostly been associated with a large influx of neutrophils in the lungs of patients with bronchiolitis, as well as in fatal cases of infants.69-71 It is suggested that neutrophils recruitment induced by hRSV infection promote lung damage through the generation of reactive oxygen species and extracellular traps (NETs).72,73

Nevertheless, a recent study using experimental hRSV infection of adults in which a 65% of individuals presented inflammation symptoms, has shown that the virus replicate in the lower respiratory tract, inducing cellular infiltration of CD8+ T cells to the airways.59 Consistent with this notion, there is evidence that CD8+ T cells can cause immunopathology in infants when a high amount of CD8+ T cell encounter a large number of hRSV particles in the tissue.74 However, the drawback of these studies is that no other cell types were evaluated, therefore it is not possible to rule out the neutrophils contribution to the pathology. In addition, another study showed that T cell responses are reduced or absent in exacerbated lungs of fatal cases of infants infected with hRSV, who had a severe LRTI caused by this virus.71 In these tissues a positive staining for macrophages and neutrophils was observed.71 Thus, in more severe cases of infantile viral LRTI caused by hRSV infection, lung inflammation appears to be due to a pronounced infiltration of neutrophils and macrophages.

CD4+ T cell response against hRSV and mechanisms of evasion used by the virus

An adequate CD4+ T cell response can efficiently aid at reducing viral load upon hRSV infection.39 Indeed, it has been reported that adoptive transfer of CD4+ T cells from immunized mice with a prototype vaccine consisting in a recombinant rBCG expressing hRSV N protein (rBCG-N-hRSV), resulted in a significant reduced viral load in the lungs after infection in recipient mice, thus providing a protective Th1 antiviral response.38 These data suggest that CD4+ T cells itself stimulated with the proper antigens in can significantly contribute to hRSV clearance.

However, in infants and children naturally infections, an inefficient adaptive immune response occurs, which is characterized by 1) a skewed Th12 immune response,75,76 2) a deficient anti-viral Th1 response and 3) a low secretion of IFN-γ and TNF-α in peripheral blood mononuclear cells77 and in nasopharyngeal aspirates, respectively. Nevertheless, hRSV-infected infants suffering from bronchiolitis present higher levels of TNF-α in BALF at day 1 of intubation, as compared with controls. This observation suggest that a mixed Th1/Th12 response is generated at the first stage of the disease.79 Consistently with this observation, a mixed Th1/Th12 response was also observed in the lung of hRSV-infected mice, although with a significant increase of IL-13, which is known to induce airway hyperreactivity.80,81

The predominant Th12 immune response observed in infants and children could be due to the capacity of hRSV to polarize the adaptive immune response from a protective Th1 phenotype to a Th12-type response.76 However, whether a pathogenic Th2 immune response significantly contributes to disease in at-risk human groups remains to be demonstrated.

Importantly, in vitro studies have described that hRSV-infected mouse DCs are unable to properly activate T cells,27 due to an impairment of immunological synapse formation. This study evaluated the formation and functionality of the immunological synapse between
OT-II CD4+ T cells with hRSV-infected DCs, pulsed with OVA peptide, showing a lack of sustained immunological synapse as well as reduced secretion of cytokines from OT-II cells, as compared with uninfected controls. This phenomenon was observed by using naive T cells, in contrast, it has been described that memory/effector T cells have different responses, which is less affected by hRSV-infected DCs. This phenomenon, could explain why healthy adults infected with hRSV can clear the virus and not in young children who have no memory T cells.

TH2 polarization also is induced by Tymic-stromal lymphoprotein (TSLP), an epithelial cell-derived cytokine that signals through the TSLP receptor (TSLPR). This cytokine potently activates myeloid DCs (mDCs), since these cells are known to express high levels of the TSLPR. Then, TSLP-stimulated DCs upregulate OX40 ligand (L) cell surface expression and produce T112 cell-attracting chemokines, including CCL17 and CCL22. Indeed, TSLP from in vitro hRSV-stimulated rat AECs induced the functional maturation of mDCs and enhanced the surface expression of the thymus-activation-regulated chemokine (TARC) and OX40L on DCs, which induce mainly inflammatory T112-polarized immune responses. Experiments performed in BALB/c mice infected by hRSV shown elevated levels of TSLP protein in lungs compare with uninfected control. The role of TSLP in the promotion of Th2 response under hRSV infection was evaluated using a TSLPR-deficient (TSLPR−/−) mice. These studies shown a decrease hRSV-mediated immunopathology in this model after infection. In addition, analysis of supernatant fluids of re-stimulated mediastinal lymph nodes (MLN) of TSLPR−/− mice infected with hRSV show a significant decrease of IL-13 and IL-5 production, as compare with WT mice, but both mice produced equivalent levels of IFN-γ and IL-17A. These results support, the notion that TSLP is required to TH2 polarization.

In other studies, it has been shown that the TSLP-OX40L -OX40 axis contributes to the hRSV-induced airway hyperresponsiveness (AHR) and inflammation after infection of mice that were initially infected as neonates. Further, administration of an anti-OX 40L antibody treatment during primary infection as neonates prevented the enhancement of the AHR upon re-infection 5 weeks later. Moreover, treatment with the anti-OX40L during primary infection in newborn BALB/c mice reduced the TH2 cytokines IL-5 and IL-13 in BALF upon re-infection.

The pathogenic role of CD4+ T during re-infection with hRSV in adult mice, after being infected as neonates, can be explained by the induction of an exaggerated T112 response, demonstrated by the cytokine profile observed in these animals. This could be explained, at least in part, by the upregulation of the IL-4Ra. Specific deletion of the IL-4Ra gene in CD4+ T cells abolished hRSV-induced airway AHR and lung damage upon re-infection with hRSV. However, these results are controversial because several reports have shown that CD4+ T cells are required for the clearance of the virus and to promote antibody response against hRSV. In this sense, it seems that dysregulation of CD4+ T cells promote harmful Th profiles but these cells are still required for hRSV clearance.

Additionally, an IL-17-mediated T117 response is a possible third type of immune response associated with the respiratory pathogenesis induced by CD4+ T cells during hRSV infection. Indeed, binding of IL-17 to its receptor promotes an inflammatory response and increased viral loads in the lungs of infected BALB/c mice. Stimulation of T cells with hRSV-infected human bronchial epithelial cells (HBEC) induced the production of IFN-γ, IL-4, and IL-17, suggesting that hRSV can activate these three T112 cell subsets. Consistent with the latter, stimulation of PBMC from healthy donors with hRSV infected A549 cells induced the production of IFN-γ and IL-4. In other studies, the concentration of IL-17 in nasopharyngeal aspirates of children during hRSV infection was higher at the moment of discharge of the hospital. However, the data are controversial since the latter was only valid for infants that do not require ventilation.

In addition to these mechanisms used by hRSV to avoid the CD4+ T other studies have demonstrated that IL-25 and IL-17RB are expressed in lungs of hRSV-infected BALB/c mice and their expressions correlate with potentially pathogenic cytokines, such as IL-13 (T112), IL-5 (T112) and IFN-γ (T111), promoting the hRSV-mediated lung disease.

Finally, using a mouse model, it has been demonstrated that antibody-mediated depletion of neutrophils decreased the number of IL-13 producing CD4+ T cells, as well as TNF-α and mucin production, when compare with the isotype-control treated group upon hRSV infection, suggesting that an interaction of neutrophils and CD4+ T cells occurs during hRSV infection. On the other hand, has been described that neutrophils can have a APC-like phenotype expressing costimulatory molecules and activating CD4+ T cells polarizing to a TH1/TH17 phenotype in inflammatory mouse model, but for hRSV infections this phenomenon has not been demonstrated yet. This could be an important unexplored area because has been described that hRSV can infect human neutrophils. Indeed, it has been possible to measure F, G and N proteins and viral RNA on those cells, suggesting that neutrophils can uptake virus in the
airways of infected children and then present antigens to T cells, but further evidence is necessary to support this idea.

Taken together, data derived from in vivo and in vitro studies suggest that hRSV infection in the lung can induce different mechanisms to prevent an efficient CD4⁺ T cell proliferation and differentiation into protective antiviral memory or effector cells of the host.

**CD8⁺ T cell response against hRSV and mechanisms of evasion used by the virus**

It is well documented that CD8⁺ T cells are pivotal at controlling respiratory viral infections, such as the one caused by hRSV.⁶⁷ Indeed, data from an adoptive transfer model demonstrated that transfused hRSV H2-K₄ restricts Cytotoxic T lymphocytes (CTLs) specific for the M₂₈₂₉₀ (KdM₂₈₂) epitope, the most predominant one of the M₂ protein recognized in vivo (dominant epitope)¹⁰⁰ rapidly clearing the virus from the lungs of recipient BALB/c mice. However, a significant lung pathology was also observed.⁶⁷ Consistent with that observation, BALB/c mice immunized with a DNA vaccine expressing the KdM₂₈₂ epitope linked to human HLA class I-restricted Cytotoxic T lymphocytes (CTLs) specific for the subdominant epitope (KdF₈₅) developed an enhanced pulmonary inflammation and illness than the KdM₂₈₂ specific CD8⁺ T cell response.¹⁰¹ Likewise, intranasal administration of the KdM₂₈₂ epitope combined with Escherichia coli heat-labile toxin (LT)/LT-K63 elicited a strong antiviral CD8⁺ T-cell response in BALB/c mice, but also enhanced lung pathology.¹⁰² This data indicates that the KdM₂₈₂ epitope-specific CD8⁺ T-cell response is associated with enhanced disease. In addition, Ruckwardt et al. demonstrated that infection of CB6F1/J mice with a recombinant hRSV containing a mutation in the dominant KdM₂₈₂ epitope resulted in an increased response of CD8⁺ T cells specific for the subdominant epitope D₈₂₉₀ (M₁₈₇₁₉₅) with significantly less clinical disease. In contrast, hRSV containing mutations in this subdominant epitope induced an augmented KdM₂₈₂-specific CD8⁺ T-cell response and increased severity of illness.¹⁰³ Consistent with the latter, in CB6F1 hybrid mice, which recognize multiple MHC class I-restricted epitopes, it was described that D₈₂₉₀ specific CD8⁺ T cells control hRSV replication more efficiently with less pulmonary inflammation and illness than the KdM₂₈₂ specific CD8⁺ T cells.¹⁰⁴ In addition, another study has shown that after immunization with a recombinant PR8 influenza virus carrying the subdominant hRSV KdF₈₅ epitope, KdF₈₅ specific CTLs were induced in BALB/c mice with a significant reduction of the viral load in the lungs upon hRSV challenge,¹⁰⁵ indicating a protective effect against this virus. Taken together, these results from the mouse models suggest that a subdominant epitope-specific CD8⁺ T cell response could be more beneficial to the host by promoting an effective anti-viral immune response and reduced lung disease, than the dominant ones, which promote a robust anti-viral immune response but also an enhanced lung pathology upon hRSV infection.

Likewise, when CD8⁺ T cells from rBCG-hRSV-N immunized-BALB/c mice are stimulated with hRSV N peptides, these cells produce significant amounts of IFN-γ, partially protecting recipient mice of hRSV infection.³⁸ The efficiency of the CD8⁺ T cell-mediated virus control could depend on how the hRSV antigen is presented by DCs, since less IFN-γ secretion was observed when BALB/c mice were immunized with purified hRSV N or M₂ in alum, compare with when mice immunized with rBCG-N-hRSV and rBCG-M₂-hRSV.³⁸ Furthermore, CD8⁺ T cell-mediated virus control and immunopathology is dependent on IFN-γ production during early infection.⁶⁷ In human, has been described lesser about single epitope immune-modulation, in fact the majority only describe hRSV-derived peptides that induce IFN-γ secretion by stimulated T cells,⁵⁹,¹⁰⁶,¹⁰⁷ but they do not address their possible role during pathology. Table 1 summarizes the hRSV epitopes described so far for HLAs. It is possible that these epitopes are implicated in CD8⁺ T cells response but further studies are required to demonstrate if these specific T cells response is favorable or harmful for an infected person.

The impairment of CTL function appears to be another immune evasion mechanism evolved by hRSV.⁵⁷ How specific hRSV proteins affect these cells will be discussed below, but here additional mechanisms that have not been related yet to specific hRSV proteins. For instance, the upregulation of the programmed-death ligand 1 (PD-L1), which binds PD-1 on CD8⁺ T cell-mediated virus control and immunopathology, occurs after hRSV infection and it has been shown to cause a

**Table 1. hRSV epitopes described for HLA.**

| Protein | Epitopes | HLA          | Reference |
|--------|---------|--------------|-----------|
| N      | N peptides (NPKASLLSL [NPK], QVMRLWGVL [QVM]) | HLA-A*02'01 and HLA-B*08 | 106 |
| M₂     | M₂₁₈₇₁₉₅ (NSDSTMNTY [NSD]) | HLA-A*01'01 | 59 |
| NS₁    | NS₂₄₆₄₉₉ | HLA-B₅₁ | 107 |
| L      | L₁₇₅₈₅₂₃ | HLA-B*07'02 | 107 |
| M      | M₁₉₅₂₃ | HLA-A*01'01 | 59 |
| G      | KPNIRTLT [KPN] | HLA-B*07'02 | 59 |
functional impairment of CD8\(^+\) T cells.\(^{150}\) Indeed, blocking PD-L1 with a specific-antibody in hRSV-infected BECs, co-cultured with CD8\(^+\) T cells, enhances CD8\(^+\) T cell effector functions and decreases hRSV gene expression in BECs.\(^{108}\)

In addition, CD8\(^+\) T cells may not only be directly influenced by the impairment of the T cell priming by DCs, but also be indirectly affected by the modulation of cytokine environment, as NK cell-derived IFN-\(\gamma\) production precedes lung CD8\(^+\) T cell recruitment,\(^{109}\) in a mechanism similar to the Influenza A virus.\(^{110}\) Also, temporally association has been made between CD8\(^+\) T cells and neutrophils, where an important influx of neutrophils to the airways in infants occurs before CD8\(^+\) T cells activation. This initial neutrophil influx correlates to the expression of the most severe hRSV symptoms suggesting that the inflammatory environmental potentiated by neutrophil influx could modulate CD8\(^+\) T cell response.\(^{111}\)

In summary, CD8\(^+\) T cell function appears to be impaired by hRSV through different mechanisms. Nonetheless, these different strategies used by this virus in targeting the CD8\(^+\) T cell response may not be that relevant in populations of older children and young adults, since most of them have mild symptoms or are asymptomatic when undergoing hRSV viral infection.

**Role of hRSV Proteins in Immunomodulation**

The hRSV genome consists of a 15.2 kb long, negative sense RNA, which contains 10 genes encoding for 11 proteins, with two overlapping open reading frames encoding two proteins: M2-1 and M2-2 (Fig. 1).\(^{112,113}\) Several hRSV proteins play a role in evading the immune system of the host (see Table 2). The hRSV glycoproteins, located in the viral envelope, that have been involved in interfering the immune response of the host are:

- HRSV Glycoprotein protein (G): this protein is involved in virus attachment\(^{114}\) and it also exists as a secreted form, which prevents opsonization and neutralization of hRSV by anti-G specific antibodies.\(^{115}\)

![Figure 1. Viral Structure and Genome Organization of hRSV and hMPV. Schematic representations of hRSV and hMPV structures are shown. Both are negative single-stranded RNA (3' to 5'), enveloped viruses that mainly differ in the number and order of genes in their genomes. These genes encode for P, N, SH, G, F, L, M, and M2 proteins, which are similar for both viruses. The M2 gene has an open reading frame that encodes for the M2-1 and M2-2 proteins. The hRSV genome also contains the non-structural proteins NS1 and NS2, which are absent in hMPV.](image-url)
Moreover, the secreted form of the G protein has a chemokine-like motif (CX3C) that competes with fractalkine (CX3CL1) in binding to its receptor CX3CR1, thus reducing the CX3CR1+ T cell response.116

HRSV Fusion (F) protein: this viral protein is required for the fusion of viral particle with the host cells4 and also play a direct role in the ability of hRSV to decrease the proliferation of these cells by contact.117 In fact, when Vero cells express the F protein or are infected with a version of hRSV that only have F protein on its surface, they reduce the proliferation and response to mitogen stimulus.117

HRSV small hydrophobic (SH) protein: it is thought that this protein works as an important viroporin during hRSV pathogenesis.118 This protein also inhibits apoptosis in order to promote viral replication, as recombinant hRSV lacking the SH protein induced a significant cytopathic effect in different cell lines, compare with WT hRSV.119 In addition, the hRSV SH has been shown to inhibit the NF-κB pathway through a decrease in the TNF-α production in mouse fibroblastic cells.119

Another studies have demonstrated that glycoproteins affect the innate and adaptive immune response to hRSV. In this study, immunization of BALB/c mice with a recombinant strain of hRSV lacking both G and SH (CP52) increased the number of pulmonary natural killer (NK) cells, as well as the levels of IFN-γ and TNF-α at day 3 p.i. with a concordant reduced expression of the T<sub>H</sub>2 related cytokines (IL-4 and IL-6) when compare with the parental RSV strain (B1).120 However, in the same study it was also shown that during primary infection, RSV-specific MHC II CTL precursor frequencies were delayed in CP52-immunized mice compare with B1-immunized mice in BALF, cervical lymph nodes and spleen at day 5 p.i.120 Furthermore, during secondary infection, both RSV-specific MHC I and MHC II CTL precursor frequencies were delayed in spleen at day 3 p.i in CP52-immunized mice, as compare with mice immunized with B1 or control Vero cell lysate.120 Altogether these data suggest that the hRSV G and/or SH proteins play a role in: a) downregulation of specific NK cell response, as mutants lacking these proteins increase pulmonary NK cells; b) polarization toward a T<sub>H</sub>2 immune response, as RSV strains deficient in these proteins decrease T<sub>H</sub>2 related cytokines.

Beside glycoproteins, hRSV also expresses other proteins involved in the impairment of the host’s immune response, which are described below.

HRSV Non-structural (NS) proteins 1 and 2: these proteins impair the type I IFN pathway by targeting the Table 2. Function of hRSV and hMPV proteins and their roles modulation of immune response.

| Protein/Gene | Protein Function | Role in Immunomodulation | References |
|--------------|-----------------|--------------------------|------------|
| G            | Attachment to host cells | -Evasion of the anti-G antibody functions. | 114-116,155,156 |
| F            | Fusion of viral particle with host cells | -Impairment of CX3CR1+ T cell migration and function (CX3C motif). | N/A 4,117 |
| SH           | Virooporin   | -Inhibition of apoptosis. | 118,119,157 |
| NS1          | N/A           | -Inhibition of TNF-α mediated NF-κB signaling and NF-κB activation. | N/A 121-127 |
| NS2          | N/A           | Impairment of the type I IFN pathway. (IRF-3) Pathways | N/A 121-127 |
| M2.1         | Transcription anti-terminator factor | Activation of the NF-κB pathway | N/A 52,53,145,146,161 |
| M2.2         | Regulatory factor in replication and in transcription | -Interference with the immune synapse assembly. | N/A 112,124,162,163 |
| N            | Assembly of the nucleocapsid and protection of the viral RNA | -Inhibition of the Type I IFN pathway by MAVS interaction | N/A 129-131 |
| M            | Viral particle assembly | N/A | 158,159 |

Moreover, the secreted form of the G protein has a chemokine-like motif (CX3C) that competes with fractalkine (CX3CL1) in binding to its receptor CX3CR1, thus reducing the CX3CR1+ T cell response.116

HRSV Fusion (F) protein: this viral protein is required for the fusion of viral particle with the host cells4 and also play a direct role in the ability of hRSV to decrease the proliferation of these cells by contact.117 In fact, when Vero cells express the F protein or are infected with a version of hRSV that only have F protein on its surface, they reduce the proliferation and response to mitogen stimulus.117

HRSV small hydrophobic (SH) protein: it is thought that this protein works as an important viroporin during hRSV pathogenesis.118 This protein also inhibits apoptosis in order to promote viral replication, as recombinant hRSV lacking the SH protein induced a significant cytopathic effect in different cell lines, compare with WT hRSV.119 In addition, the hRSV SH has been shown to inhibit the NF-κB pathway through a decrease in the TNF-α production in mouse fibroblastic cells.119

Another studies have demonstrated that glycoproteins affect the innate and adaptive immune response to hRSV. In this study, immunization of BALB/c mice with a recombinant strain of hRSV lacking both G and SH (CP52) increased the number of pulmonary natural killer (NK) cells, as well as the levels of IFN-γ and TNF-α at day 3 p.i. with a concordant reduced expression of the T<sub>H</sub>2 related cytokines (IL-4 and IL-6) when compare with the parental RSV strain (B1).120 However, in the same study it was also shown that during primary infection, RSV-specific MHC II CTL precursor frequencies were delayed in CP52-immunized mice compare with B1-immunized mice in BALF, cervical lymph nodes and spleen at day 5 p.i.120 Furthermore, during secondary infection, both RSV-specific MHC I and MHC II CTL precursor frequencies were delayed in spleen at day 3 p.i in CP52-immunized mice, as compare with mice immunized with B1 or control Vero cell lysate.120 Altogether these data suggest that the hRSV G and/or SH proteins play a role in: a) downregulation of specific NK cell response, as mutants lacking these proteins increase pulmonary NK cells; b) polarization toward a T<sub>H</sub>2 immune response, as RSV strains deficient in these proteins decrease T<sub>H</sub>2 related cytokines.

Beside glycoproteins, hRSV also expresses other proteins involved in the impairment of the host’s immune response, which are described below.

HRSV Non-structural (NS) proteins 1 and 2: these proteins impair the type I IFN pathway by targeting the Table 2. Function of hRSV and hMPV proteins and their roles modulation of immune response.

| Protein/Gene | Protein Function | Role in Immunomodulation | References |
|--------------|-----------------|--------------------------|------------|
| G            | Attachment to host cells | -Evasion of the anti-G antibody functions. | 114-116,155,156 |
| F            | Fusion of viral particle with host cells | -Impairment of CX3CR1+ T cell migration and function (CX3C motif). | N/A 4,117 |
| SH           | Virooporin   | -Inhibition of apoptosis. | 118,119,157 |
| NS1          | N/A           | -Inhibition of TNF-α mediated NF-κB signaling and NF-κB activation. | N/A 121-127 |
| NS2          | N/A           | Impairment of the type I IFN pathway. (IRF-3) Pathways | N/A 121-127 |
| M2.1         | Transcription anti-terminator factor | Activation of the NF-κB pathway | N/A 52,53,145,146,161 |
| M2.2         | Regulatory factor in replication and in transcription | -Interference with the immune synapse assembly. | N/A 112,124,162,163 |
| N            | Assembly of the nucleocapsid and protection of the viral RNA | -Inhibition of the Type I IFN pathway by MAVS interaction | N/A 129-131 |
| M            | Viral particle assembly | N/A | 158,159 |
impairment of the immunological synapse assembly by full-length viral mRNAs. However, M2 needed for a proper immunological synapse assembly, is shown to suppress the CD8+ T cell anti-viral function of CD8+ T cells at the mucosal epithelium of the respiratory tract. In addition, NS1 was shown to suppress the CD8+ T cell anti-viral response, as there were increased levels of IFN-γ in the supernatant of lymphocytes co-cultured with human DCs infected with hRSV ΔNS1 as compare with lymphocytes co-cultured with DCs infected with WT RSV.

Therefore, the hRSV NS1 protein can impair the efficient anti-viral function of CD8+ T cell in different manners. Further, in vivo suppression of the CTL response by hRSV is mediated by NS2, as BALB/c mice infected with a hRSV mutant deficient in the NS2 gene (ΔNS2) showed increased pulmonary hRSV-specific CTL responses compare with those of mice infected with WT hRSV or with the virus lacking NS1 (ΔNS1).

HRSV M2–1 protein: This is a transcription anti-termination factor important for the efficient synthesis of full-length viral mRNAs. However, M2–1 has been shown to activate the NFκB pathway as demonstrated by translocation to the nuclei of the NFκB factor in A549 cell transfected with a M2–1 encoding vector.

HRSV N protein: This protein is critical for the assembly of the hRSV nucleocapsid and protection of the viral RNA (Fig. 1). Furthermore, the hRSV N protein interfere with the type I IFN pathway by targeting the mitochondrial antiviral signaling protein (MAVS), thus inhibiting the MAVS-dependent antiviral pathway. Moreover, hRSV-infected DCs are unable to activate naïve CD4+ T cells in vitro probably due to the impairment of the immunological synapse assembly by the hRSV N protein expressed at the host cell membrane (Fig. 2, upper left box), rendering T cells unresponsive to subsequent TCR engagement. Specifically, the Golgi apparatus polarization within T cells, an event needed for a proper immunological synapse assembly, is barely detectable in T cells co-cultured with hRSV-infected DCs, in contrast to T cells co-cultured with mock-inoculated DCs. Moreover, impairing this signaling event significantly decreases tyrosine phosphorylation of TCR-associated CD3ζ-chain tyrosine-based activation motifs (ITAM) by LCK, in naïve T cells stimulated with a cognate antigen. Furthermore, immune synapse assembly inhibition is accompanied by a reduced binding of ICAM-1, suggesting that the N protein interferes with receptor-ligand interactions at the immunological synapse, reducing the TCR clusters that are usually observed within a mature immunological synapse. A putative viral mechanism of this inhibitory effect could be the interaction of the hRSV N protein with an element of the TCR complex, since central clustering of this protein occurs alongside the TCR, even in the absence of pMHC. Further studies are required to define the molecular mechanisms underlying the immune synapse inhibition by RSV infection.

Adaptive Immunity Triggered by hMPV

The humoral response against hMPV infection

It has been described that hMPV-specific antibodies are produced after hMPV infection in the childhood. The most predominant antibodies detected are anti-F antibodies after the first infection and can be detected over the time at least for 20 y evidenced by the fact that 95% of 20 y old individuals are seropositive for the F protein. Likewise, it has been described that individuals with low levels of anti-hMPV antibodies are more susceptible to hMPV infection. Studies in BALB/c mice have demonstrated that passive transfer of hyper-immune hMPV-specific mouse sera to naïve mice decreased virus titer, seven days post-infection, suggesting that hMPV-specific antibodies provide a level of protection from viral challenges. However, the hMPV-specific antibody response appears to be inefficient in mediating viral clearance, since hMPV persists in lungs of infected mice despite the presence of neutralizing antibodies. In fact, it has been observed that in humans hMPV can persist in immunocompromised patients, suggesting a principal role of immune response for control hMPV infection. This could be explained by a waning effect on protective hMPV-specific antibodies over time, or the levels of these antibodies may not be sufficient to protect from a re-exposure to hMPV infection. Moreover, in hMPV-infected BALB/c mice, seroconversion and the development of neutralizing anti-hMPV specific antibodies (IgG, IgG1, and IgG2a) are observed post-challenge, but these antibodies do not prevent the persistence of infectious hMPV. In humans, from 257 hMPV-positive individuals, only a 25% remained asymptomatic, whereas 75%
presented symptoms, despite their severity.\textsuperscript{134} hMPV-infected individuals presented low IgA and IgG titer compared with non-infected individuals, and also lower neutralizing capacity, consistent to what is observed in mice.\textsuperscript{134} Therefore, the humoral responses raised against hMPV following natural infection might not be sufficient to reduce re-infection episodes.\textsuperscript{22}

The cellular response against hMPV infection

For hMPV, it was recently shown that the presence of virus-specific T cells in airways and in lungs of BALB/c mice, are associated with an effective anti-viral immune response.\textsuperscript{139} These data suggest that Trm T cells could also be important for controlling hMPV infection. However, further studies are still required, especially in humans.

CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells are required for the clearance of hMPV from infected lungs in hMPV-infected BALB/c mice.\textsuperscript{140} Likewise, an efficient anti-viral immune response based on IFN-\(\gamma\)-secreting CD4\textsuperscript{+} and CD8\textsuperscript{+} effector and memory T cells is necessary for preventing the spreading of hMPV in airways and to avoiding the development of bronchiolitis and pneumonitis. This is supported by a study, that used a candidate vaccine based on rBCG
expressing hMPV antigens, where vaccination with this hMPV vaccine expressing the M2–1 or P protein protects BALB/c mice from hMPV-mediated lung pathology and reduced viral load in lungs. However, this virus also evades these effective anti-viral immune responses. Moreover, naïve BALB/c mice were shown to develop a biphasic immune response in vivo after hMPV infection. During the first week, a T<sub>1</sub>1 response initially controls virus replication before polarizing toward a T<sub>1</sub>2 immune response that facilitates viral persistence. A recent in vivo study, though, showed mixed T<sub>1</sub>1 and T<sub>1</sub>2 responses during the first week of hMPV infection in BALB/c mice. Consistent with the notion observed in the mouse model, in adults undergoing hMPV infection, it has been described that PBMCs from these patients presented cytolytic activity, as evaluated by aChrome-release assay, which suggest a T<sub>1</sub>1 response. However, PBMCs, from healthy adults, stimulated with heat-inactivated hMPV promotes the induction of high levels IL-6 (a T<sub>1</sub>2 polarizing cytokine that prevents T<sub>1</sub>1 differentiation) and low levels of IFN-γ and CCR5 (T<sub>1</sub>1 cytokines) when compared with hRSV-stimulated PBMCs, thus suggesting a polarization toward a T<sub>1</sub>2 response. In contrast, other studies demonstrate that infants undergoing hMPV infection presented lower levels of pro-inflammatory cytokines compared with infants undergoing hRSV or influenza infection, including TNF-α and IL-1β, two cytokines related to the chemotaxis of neutrophils in lungs, as well as IL-12, IL-6 and IL-8. Furthermore, in infants undergoing hMPV infection it was observed that a predominant T<sub>1</sub>1 response is generated, since it was detected an increase in the IFN-γ/IL-4 ratio in nasal airway secretions. Taken together, this data indicate that hMPV induces a complex immune response, which might include a mixed T<sub>1</sub>1/T<sub>1</sub>2 response, similar to what was observed in mice, though further studies are required to evaluate the T cell response in humans upon hMPV infection.

**CD4<sup>+</sup> T cell response against hMPV and mechanisms of evasion used by the virus**

hMPV has also evasion mechanisms to interfere specifically with CD4<sup>+</sup> T cell function that do not promote an efficient antiviral immune response and enhances lung pathology. Specifically, depletion of CD4<sup>+</sup> T cells in hMPV-infected BALB/c mice reduces lung pathology and airway obstruction, without affecting viral loads. These data demonstrate that the subset of CD4<sup>+</sup> T cells contributes to lung pathology but they are not critical for viral clearance.

As has been mentioned above, hMPV can induce a mixed TH1/TH2 response and this type of response is dependent of activation of TSLP pathway, which favors TH2 response over TH1 response that is known is necessary for hMPV clearance. Related to this, hMPV infection has been proven: to promote TSLP expression in both human AECs and mouse lungs; to stimulate OX40L+CD11b+DCs lung infiltration; and to increase the levels of pro-inflammatory TH2 cytokines-producing T cells, including TARC, IL-5 and IL-13, but also TNF-α in BALB/c mice, as previously reported for OX40L on TSLP-activated DCs. Moreover, TSLPR<sup>−/−</sup> mice showed decreased lung inflammation and hMPV replication, as well as a higher frequency of CD8<sup>+</sup> and CD4<sup>+</sup> T cells.

These findings highlight the possibility that a repertoire of virus-specific T<sub>H</sub> and CTLs may incompletely eliminate infected cells within the airways following primary infection, leading to an exacerbated inflammatory response, mainly mediated by the TSLP pathway, thus inducing an aberrant T cell response.

Moreover, hMPV-infected DCs, similarly to hRSV-infected DCs, also impair the activation of CD4<sup>+</sup> T cells. Upon stimulation with hMPV-infected and antigen-loaded DCs, naïve antigen-specific CD4<sup>+</sup> T cells displayed significantly reduced proliferation, expression of surface activation markers, such as CD25 (IL-2 receptor/IL-2Rα), CD69 and CD71 (transferrin receptor) and IL-2 secretion, as compared with T cells stimulated with uninfected control DCs. However, this inefficiency is not due to a deterioration of the immune synapse assembly since both TCR cluster formation and Golgi polarization still occur (Fig. 2, upper left box). This impairment may contribute to a delayed T<sub>1</sub>1 response. Indeed, CD4<sup>+</sup> T cells elicit a poor IFN-γ response when activated with hMPV-infected human peripheral blood mononuclear cells in vitro, suggesting that these hMPV-infected cells can also inhibit the function of CD4<sup>+</sup> T cells to stimulate an efficient antiviral T<sub>1</sub>1 immune response in humans. The impairment of T cell immunity could be the result of impaired DC functions by hMPV, possibly through of a soluble factor derived from hMPV-infected DCs, as supernatants from these infected cells impairs T cells activation when stimulated by plate-bound anti-CD3ε and anti-CD28. Thus, hMPV impairs the TCR signaling, without disturbing the immune synapse formation and Golgi polarization in T cells (Fig. 2, upper right box). Consistent with the latter, the SH and/or G proteins reduced CD4<sup>+</sup> T cell proliferation in a co-culture assay of hMPV-infected MDDC with CD4<sup>+</sup> T cells when compared with T cells co-cultured with ΔSH/G hMPV-infected DCs. Likewise, in vitro studies show that human and mouse hMPV-infected DCs lose the capacity to activate and expand naïve T cells, although to a lesser degree than hRSV-infected DCs.

Furthermore, the neutralizing antibodies detected in mice after hMPV infection, appear to be dependent on
CD8+ T cells, because no detectable neutralizing antibodies were observed in hMPV-infected mice depleted of CD4+ T cells.140

**CD8+ T cell response against hMPV and mechanisms of evasion used by the virus**

In a similar manner as for hRSV, CD8+ T cell response specific to hMPV antigens is critical for an effective viral clearance, as demonstrated in studies using vaccine candidates against hMPV, such as virus-like particles harboring hMPV F or hMPV M antigens.149 Supporting this notion, hMPV-specific effector CD8+ T cells, in BALB/c mice that lack protective anti-hMPV antibodies and in the absence of CD4+ T cells, no detection of virus load and reduction of lung disease were found upon hMPV infection, suggesting that the cytotxic activity of CD8+ T cells alone can confer protection against hMPV140 and highlighting the importance of CD8+ T cells in controlling hMPV infection. Moreover, when specific hMPV M2 CTLs were transferred into RAG-1−/− mice, these lymphocytes protected the host of hMPV challenge.150 Furthermore, hMPV-specific IFN-γ-producing CD8+ T cells can be found in the mucosa of the airways and in lungs, but not in the lymph nodes or in spleen of hMPV-infected BALB/c mice at 7 d p.i., suggesting the activation of the Trm T cells specific for hMPV epitopes.139 Nevertheless, hMPV-virus specific CTLs were also induced 21 d p.i. in spleen.139 These data underscore the importance of Trm CD8+ T cells in controlling rapidly hMPV infections. Recently, in human has been described that memory CD8+ T cells are reactive to the majority of hMPV proteins (M, F, G, M2-1, N and SH) and particularly, CD8+ T cells that recognize M and F protein can secrete IFN-γ after 21 months post-infection.142 Nevertheless, further studies are required in humans to clarify if this CD8+ T cell populations are protective or not, because independently of the presence of this population, hMPV keeps its capacity to generate re-infection episodes.

Recent studies associate the lack of the type I IFN pathway with CD8+ T cell impairment.151 In fact, hMPV infected-IFNAR−/− mice had a higher peak of early viral replication, less airway dysfunction and lung inflammation, but cleared the virus with the same kinetics as observed in WT mice.151 Likewise, CD8+ T cells from IFNRA−/− mice expressed similar levels of PD-L1 when compared with CD8+ T cells from WT mice. However, these cells showed an upregulation of the inhibitory receptor TIM-3, thus impairing the CD8+ T cell function.151 Additionally, CD8+ T cells can be impaired by hMPV in a PD-1 dependent manner, similar to hRSV, as lung CD8+ T cells are impaired in HLA B7.2 transgenic (B7tg) mice and had upregulated PD-1.152 Conversely, blocking of PD-1 by administration of monoclonal specific antibodies in B7tg mice prevented the CD8+ T cells impairment. Similarly, impairment of CD8+ T cells was prevented on hMPV-infected PD-1−/− mice.152

In other studies, an impairment of lung hMPV-specific memory CD8+ T cells was observed in μMT mice, which lack B-cells and are used as a model for hMPV reinfection, which suggest the importance of memory CD8+ T cells in viral clearance during a second infection.153 Specifically, during reinfection, CD8+ T cells had upregulated several inhibitory receptors, including PD-1.153 Similarly to it is observed in B7tg mice, blockade of PD-1 in μMT mice restored lung CD8+ T cell effector functions (i.e., degranulation and cytokine production) and enhanced viral clearance.153 In other studies, immunization of μMT mice with virus-like particles encoding the hMPV F and M proteins, generates hMPV F-specific and M-specific CD8+ T cells in lungs, but their function is impaired, as inhibitory receptors are upregulated on these cells, similar to what is seen in WT C57BL/6 mice under a second hMPV infection.149

On the other hand, the depletion of CD8+ T cells in hMPV-infected BALB/c mice resulted in a lower lung pathological score, although to a lesser degree than depletion of only CD4+ T cells.140 By other part, mice that was depleted of neutrophils showed a significant reduction of TNF-α and IL-13 secreted by CD8+ T cells suggesting that neutrophils modulate the production of these cytokines by CD8+ T cells.141 In this manner, CD8+ T cells that infiltrate in the airways and secrete TNF-α and IL13 contribute to the lung pathology triggered by hMPV infection in mice.140,141

Taken together, CD8+ T cells confer protection against hMPV infection through cytotoxic activities, but these cells may also contribute to lung pathology.140 Furthermore, the function of these cells can be hampered by evasion mechanisms of the virus. However, further studies in humans are still required.

**HMPV molecular characteristics and the role of its viral proteins in immunomodulation**

The hMPV genome is comprised of a negative, 13 kb single-stranded RNA with the following eight genes: N-P-M-F-M2-SH-G-L (3’ to 5’).18 Furthermore, the hMPV mRNA transcribed by the M2 gene contains two overlapping open reading frames that give rise to the M2–1 and M2–2 proteins, in similar manner than RSV (Fig. 1).14 In contrast to RSV, hMPV lacks the NS1 and NS2 genes, thus the inhibition of the type I IFN pathway is less robust than the one is observed in RSV.154 The G protein, a glycoprotein involved in the attachment of the viral particle,155 inhibits the type I IFN pathway by...
targeting the retinoic-inducible gene 1 (RIG-I), thus it contributes in inhibiting the antiviral innate response of the host.\textsuperscript{156} Likewise, the SH protein, a type 2 transmembrane protein with properties as a viroporin, has been shown to inhibit the NFκB pathway, as infection with a recombinant virus lacking the SH gene increased the NFκB-dependent transcription pathway in BALB/c mice.\textsuperscript{157} The M protein, which participates in virus assembly and packaging,\textsuperscript{158} stimulates the inflammatory response \textit{in vitro} by inducing the maturation of monocyte-derived DCs and the secretion of inflammatory cytokines by these cells, including IL-8, IL-6, IL-1β and TNF-α (Table 2).\textsuperscript{159}

In addition, the M2–1 protein is critical for hMPV replication and pathogenesis through its Zinc binding activity, since a recombinant hMPV carrying mutations in the zinc binging motif was highly attenuated in cotton rats.\textsuperscript{160} This is consistent with studies that demonstrate that hMPV lacking the M2–1 gene could not replicate in hamsters.\textsuperscript{161} However, is not essential for hMPV infectivity and growth \textit{in vitro} as deletion of the ORF of the M2–1 protein is dispensable for viral replication in VERO cells.\textsuperscript{161} In contrast, the hMPV M2–2 protein, defined as a terminator factor, since it inhibits viral replication and transcription,\textsuperscript{162} interact with MAVS,\textsuperscript{163} a protein that links the cytoplasmic viral sensors RIG-I and the melanoma differentiation associated protein 5 (MDA5) to the downstream TNF receptor-associated factors (TRAFs) and IκB kinases (IKKs), which are known to induce the type I IFN-pathway through the activation of IRF-3 and NFκB.\textsuperscript{163} Thus, hMPV M2–2 protein contributes in decreasing efficiently the anti-viral response.

**Concluding remarks**

Adequate T cell priming is critical for establishing effective anti-viral immune responses.\textsuperscript{164,165} Due to the importance of this process several respiratory viruses, including hRSV\textsuperscript{27} and hMPV\textsuperscript{26} have evolved different mechanisms to impair T cell activation. Indeed, both of these viruses impair the T cell response, causing, at least in part, an aberrant adaptive immune response and poor immunological memory against hRSV\textsuperscript{71,166} and hMPV.\textsuperscript{136} However, in both cases, memory T cells are produced after infection but the role of these cells are still controversial because reinfection episodes are recurrent in both viruses. By the other hand, the viral mechanism underlying the inhibition of T cell functions for both viruses is different.\textsuperscript{26,131} hRSV impairs T cell activation by preventing a mature immune synapse assembly, possibly through the hRSV N protein at the DC plasma membrane.\textsuperscript{131} Conversely, hMPV prevents T cell activation,\textsuperscript{26} likely though a soluble factor(s) without interfering with the formation of a mature immune synapse and Golgi polarization in the T cell, as the inhibition of the TCR signaling is induced with the supernatants of hMPV-infected DCs.\textsuperscript{26}

Interference at the immune synapse level by hRSV and T cell activation by hMPV prevents the Th1 polarization required to induce an efficient anti-viral response.\textsuperscript{26,27} Additionally, hRSV infection triggers detrimental inflammation in the airways that is characterized by an exacerbated Th2 response, thus preventing adequate viral clearance.\textsuperscript{167} This exacerbated Th2-type immune response to hRSV could be mediated by TSLP through activation of the OX40/OX40L interaction, which consequently stimulates an inappropriate subset of T cells.\textsuperscript{80} In addition, IL-25, detected in lungs of infected hRSV mice, can also contribute to the hRSV-mediated lung pathology.\textsuperscript{91}

Similarly to hRSV, hMPV activates the TSLP pathway to induce a mixture of Th12- and Th11-type responses, most likely through OX40/OX40L interactions.\textsuperscript{141} This mixed induction causes pathology and promotes viral replication in mice lungs.\textsuperscript{141}

Similarly, to T cells response, antibody response is not effective to reduce reinfection and probably this is mediated by the interaction between B and T cells during infection. Where T cells that present a polarization to Th2 in case of hRSV or Th1/Th2 in case of hMPV, modulate B cell response generating non-protective antibodies. Contrary, has been showed that neutralizing antibodies are produced after infection with these viruses but the proportion between neutralizing and non-neutralizing could be mediate a better outcome.

The respective evasion mechanisms induced by each virus could synergistically act to prevent the activation of an effective anti-viral T and B cell response during infection periods. In this sense, is important to advance in human studies with these two viruses and the mechanism behind its immune modulation.

**Abbreviations**

- AEC: Airway epithelial cell
- BEC: Bronchial epithelial cell
- BCG: Bacillus Calmette-Guérin
- CTL: Cytotoxic T lymphocyte
- DC: Dendritic cell
- hMPV: Human metapneumovirus
- IFN: Interferon
- M: Matrix
- mDC: Myeloid dendritic cell
- N: Nucleoprotein
- NS: Non-structural
References

[1] Afonso CL, Amarasinghe GK, Banyai K, Bao Y, Basler CF, Bavari S, Bejereman N, Blasdel KR, Briand FX, Briese T, et al. Taxonomy of the order Mononegavirales: update 2016. Arch Virol 2016; 161:2351-60; PMID:27216929; https://doi.org/10.1007/s00705-016-2880-1

[2] Domachowske JB, Rosenberg HF. Respiratory syncytial virus infection: immune response, immunopathogenesis, and treatment. Clin Microbiol Rev 1999; 12:298-309; PMID:10194461

[3] Boivin G, Abed Y, Pelletier G, Ruel L, Moisan D, Cote S, Peret TC, Erdman DD, Anderson LJ. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J Infect Dis 2002; 186:1330-4; PMID:12402203; https://doi.org/10.1086/344319

[4] Collins PL, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. J Virol 2008; 82:2040-55; PMID:17928346; https://doi.org/10.1128/JVI.01625-07

[5] Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O’Brien KL, Roca A, Wright PF, Bruce N, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375:1545-55; PMID:20399493; https://doi.org/10.1016/S0140-6736(10)60206-1

[6] Hansbro PM, Starkey MR, Mattes J, Horvat JC. Pulmonary immunity during respiratory infections in early life and the development of severe asthma. Ann Am Thorac Soc 2014; 11 Suppl 5:S297-302; PMID:25525736; https://doi.org/10.1513/AnnalsATS.201402-086AW

[7] Rudan I, O’Brien KL, Nair H, Liu L, Theodoratou E, Qazi S, Lukšić I, Fischer Walker CL, Black RE, Campbell H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health 2013; 3:010401; PMID:23826505; https://doi.org/10.7189/jogh.03.010101

[8] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380:2095-128; PMID:23245604; https://doi.org/10.1016/S0140-6736(12)61728-0

[9] Beem M, Wright FH, Hamre D, Egerer R, Oehme M. Association of the chimpanzee coryza agent with acute respiratory disease in children. N Engl J Med 1960; 263:523-30; PMID:13798226; https://doi.org/10.1056/NEJM19600915152631101

[10] Blount RE, Jr, Morris JA, Savage RE. Recovery of cytopathogenic agent from chimpanzees with coryza. Proc Soc Exp Biol Med 1956; 92:544-9; PMID:13359460; https://doi.org/10.3181/00379727-92-22538

[11] Chanock R, Finberg L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. Epidemiologic aspects of infection in infants and young children. Am J Hyg 1957; 66:291-300; PMID:13478579

[12] Chanock R, Roizman B, Myers R. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. Am J Hyg 1957; 66:281-90; PMID:13478578

[13] Walsh EE, Hruska J. Monoclonal antibodies to respiratory syncytial virus proteins: identification of the fusion protein. J Virol 1983; 47:171-7; PMID:645804

[14] Kahn JS. Human metapneumovirus, a newly emerging respiratory virus. Pediatr Infect Dis J 2003; 22:923-4; PMID:14551495; https://doi.org/10.1097/01.inf.0000091347.27554.ff

[15] Collins MS, Gough RE. Characterization of a virus associated with turkey rhinotracheitis. J Gen Virol 1988; 69:121-3; PMID:3070438; https://doi.org/10.1099/0022-1317-69-4-909

[16] Schildgen V, van den Hoogen B, Fouchier RA, Holmes EC. Evolutionary dynamics of human and avian metapneumoviruses. J Gen Virol 2008; 89:2933-42; PMID:19008378; https://doi.org/10.1099/vir.0.2008/006957-0

[17] Beem M, Wright FH, Hamre D, Egerer R, Oehme M. Association of the chimpanzee coryza agent with acute respiratory disease in children. N Engl J Med 1960; 263:523-30; PMID:13798226; https://doi.org/10.1056/NEJM19600915152631101

[18] Beem M, Wright FH, Hamre D, Egerer R, Oehme M. Association of the chimpanzee coryza agent with acute respiratory disease in children. N Engl J Med 1960; 263:523-30; PMID:13798226; https://doi.org/10.1056/NEJM19600915152631101

[19] Feuillet F, Lina B, Rosa-Calatrava M, Boivin G. Ten years of human metapneumovirus research. J Clin Virol

Disclosure of potential conflicts of interest

The authors declare no potential conflicts of interest.

Acknowledgments

The authors acknowledge Ms. Virna Salazar for critically reading the manuscript.

Funding

Funding awarded by Grants NO 1158262 and 3150559, from the National Fund for Scientific and Technological Development (FONDECYT) program, Ministry of Education, Chile; Grant NO D11/1080, from the Fund for the Promotion of Scientific and Technological Development (FONDEF), Ministry of Education, Chile; and Grant P09/P016-F, from the Millennium Institute of Immunology and Immunotherapy, Ministry of Economy, Chile.
[20] Boivin G, De Serres G, Hamelin ME, Cote S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, et al. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. Clin Infect Dis 2007; 44:1152-8; PMID:17407031; https://doi.org/10.1086/512204

[21] Bont L, Versteegh J, Swensen WT, Heijnen CJ, Kavelaars A, Brus F, Draaisma JM, Pekelharing-berghuis M, van Diemen-stenvoorde RA, et al. Natural reinfection with respiratory syncytial virus does not boost virus-specific T-cell immunity. Pediatr Res 2002; 52:363-7; PMID:12193668; https://doi.org/10.1203/00006450-200209000-00009

[22] Pavlin JA, Hickey AC, Ulbrandt N, Chan YP, Endy TP, de Graaff PMA, de Jong EC, van Capel TM, van Dijk MEA, Roholl PJM, Bois J, Luytjes W, Kimpen JL, van Bleek GM. Respiratory syncytial virus infection of monocytic-derived dendritic cells decreases their capacity to activate CD4 T cells. J Immunol 2005; 175:5904-11; PMID:16237083; https://doi.org/10.4049/jimmunol.175.9.5904

[23] Cespedes PF, Gonzalez PA, Kaleris AM. Human metapneumovirus in adults. Clin Microbiol Rev 2005; 18:655-8; PMID:19483659; https://doi.org/10.1172/JCI78450

[24] Olsen S, Facchinetti V, Diemen-steenvoorde RA, et al. Natural reinfection with respiratory syncytial virus does not boost virus-specific T-cell immunity. Pediatr Res 2002; 52:363-7; PMID:12193668; https://doi.org/10.1203/00006450-200209000-00009

[25] Walsh EE, Peterson DR, Fennelly GJ, Eugenin EA, Jacobs WR, Jr. Human metapneumovirus infections in adults: another piece of the puzzle. Arch Intern Med 2008; 168:2489-96; PMID:19064834; https://doi.org/10.1001/archinte.168.22.2489

[26] Cespedes PF, Gonzalez PA, Kaleris AM. Human metapneumovirus keeps dendritic cells from priming antigen-specific naive T cells. Immunology 2013; 139:366-76; PMID:23374037; https://doi.org/10.1111/imn.12083

[27] Gonzalez PA, Prado CE, Leiva ED, Carreno IJ, Bueno SM, Riedel CA, Kaleris AM. Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. Proc Natl Acad Sci U S A 2008; 105:14999-5004; PMID:18818306; https://doi.org/10.1073/pnas.0802555105

[28] de Graaff PMA, de Jong EC, van Capel TM. Human metapneumovirus SH and G glycoproteins inhibit macropinocytosis-mediated entry into human dendritic cells and reduce CD4(+)-T cell activation. J Virol 2014; 88:6453-69; https://doi.org/10.1128/JVI.03261-13

[29] Deffrasnes C, Hamelin ME, Prince GA, Boivin G. Identification and evaluation of a highly effective fusion inhibitor for human metapneumovirus. Antimicrob Agents Chemother 2008; 52:279-87; PMID:17967906; https://doi.org/10.1128/AAC.00793-07

[30] Palavecino CE, Cespedes PF, Gomez RS, Kaleris AM, Bueno SM. Immunization with a Recombinant Bacillus Calmette-Guerin Strain Confers Protective Th1 Immunity against the Human Metapneumovirus. J Immunol 2014; 192:214-23; https://doi.org/10.4049/jimmunol.1300118

[31] Mazur NI, Martinon-Torres F, Baraldi E, Fauboux B, Greenough A, Heikinen T, Manzoni P, Mejias A, Nair H, Papadopoulos NG, et al. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. Lancet Respir Med 2015; 3(11):888-900; PMID:26411809; https://doi.org/10.1016/S2213-2600(15)00255-6

[32] Sun Z, Wang Q, Jia R, Xia S, Li Y, Liu Q, Xu W, Xu J, Du L, Lu L, et al. Intranasal administration of maleic anhydride-modified human serum albumin for pre-exposure prophylaxis of respiratory syncytial virus infection. Viruses 2015; 7:798-819; PMID:25690799; https://doi.org/10.3390/v7020798

[33] Schickli JH, Whitacre DC, Tang RS, Kaur J, Lawlor H, Peters CJ, Jones JE, Peterson DL, McCarthy MP, Van Nest G, et al. Palivizumab epitope-displaying virus-like particles protect rodents from RSV challenge. J Clin Invest 2015; 125:1637-47; PMID:25751145; https://doi.org/10.1172/JCI78450

[34] Pham QN, Biacchesi S, Skiodapoulos MH, Murphy BR, Collins PL, Buchholz UJ. Chimeric recombinant human metapneumoviruses with the nucleoprotein or phosphoprotein open reading frame replaced by that of avian metapneumovirus exhibit improved growth in vitro and attenuation in vivo. J Virol 2005; 79:15114-22; PMID:16306583; https://doi.org/10.1128/JVI.79.24.15114-15122.2005

[35] Gomez M, Muñson MA, Dubovsky F, Knightly C, Zeng W, Losonsky G. Phase-I study MEDI-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. Pediatr Infect Dis J 2009; 28:655-8; PMID:19483659; https://doi.org/10.1097/INF.0b013e318199c3b1

[36] Munoz FM, Piedra PA, Glezen WP. Safety and immunogenicity of respiratory syncytial virus purified fusion protein-2 vaccine in pregnant women. Vaccine 2003; 21:3465-7; PMID:12850361; https://doi.org/10.1016/S2213-2600(15)00252-9

[37] Caua CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guerin promotes virus clearance and protects from infection. J Immunol 2010; 185:7633-45; PMID:21084664; https://doi.org/10.4049/jimmunol.0903452

[38] Bueno SM, Gonzalez PA, Cautivo KM, Bueno SM, Gonzalez PA, Cautivo KM, Bueno SM, Cortes CM, Wozniak A, Riedel CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guerin promotes virus clearance and protects from infection. J Immunol 2010; 185:7633-45; PMID:21084664; https://doi.org/10.4049/jimmunol.0903452

[39] Bueno SM, Gonzalez PA, Caua CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guerin promotes virus clearance and protects from infection. J Immunol 2010; 185:7633-45; PMID:21084664; https://doi.org/10.4049/jimmunol.0903452

[40] Ma X, Endo R, Ebitara T, Ishiguro N, Ishiko H, Kikuta H. Production and characterization of neutralizing monoclonal antibodies against human metapneumovirus F protein.
Hibridoma (Larchmt) 2005; 24:201-5; PMID:16120026; https://doi.org/10.1089/hyb.2005.24.201

[41] Mejias A, Chavez-Bueno S, Rios AM, Aten MF, Raynor B, Peromingo E, Soni P, Olsen KD, Kiener PA, Gomez AM, et al. Comparative effects of two neutralizing anti-respiratory syncytial virus (RSV) monoclonal antibodies in the RSV murine model: time versus potency. Antimicrob Agents Chemother 2005; 49:4700-7; PMID:16251314; https://doi.org/10.1128/AAC.49.11.4700-4707.2005

[42] Subramanian KN, Weisman LE, Rhodes T, Ariagno R, Sanchez PJ, Steichen J, Givner LB, Jennings TL, Top FH, Jr, Carlin D, et al. Safety, tolerance and pharmacokinetics of a humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. MEDI-493 Study Group. Pediatr Infect Dis J 1998; 17:110-5; https://doi.org/10.1097/00006454-199802000-00006

[43] Falsey AR, Hennessy PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005; 352:1749-59; PMID:15858184; https://doi.org/10.1056/NEJMoa043951

[44] Falsey AR, Singh HK, Walsh EE. Serum antibody decay in adults following natural respiratory syncytial virus infection. J Med Virol 2006; 78:1493-7; PMID:16998887; https://doi.org/10.1002/jmv.20724

[45] Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. J Infect Dis 2004; 190:373-8; PMID:15216475; https://doi.org/10.1086/421524

[46] Vissers M, Ahout IM, de Jonge MI, Ferwerda G. Mucosal IgG levels correlate better with respiratory syncytial virus load and inflammation than plasma IgG levels. Clin Vaccine Immunol 2015; 23:243-5; PMID:26656116; https://doi.org/10.1128/CVI.00590-15

[47] Habibi MS, Jozwik A, Makris S, Dunning J, Paras A, DeVincenzo JP, de Haan CA, Wrammert J, Openshaw PJ, Chiu C. Impaired Antibody-mediated Protection in protection from Natural Respiratory Syncytial virus infection. J Med Virol 2015; 87:859-65; PMID:22335218; https://doi.org/10.1002/jmv.202078

[48] Habibi MS, Jozwik A, Makris S, Dunning J, Paras A, DeVincenzo JP, de Haan CA, Wrammert J, Openshaw PJ, Chiu C. Impaired Antibody-mediated Protection in protection from Natural Respiratory Syncytial virus infection. J Med Virol 2015; 87:859-65; PMID:22335218; https://doi.org/10.1002/jmv.202078

[49] Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from Natural Respiratory Syncytial virus infection in adults. J Infect Dis 2004; 190:373-8; PMID:15216475; https://doi.org/10.1086/421524

[50] Graham BS, Bunton LA, Rowland J, Wright PF, Karzon DT. Respiratory syncytial virus infection in anti-mu-treated mice. J Virol 1991; 65:4936-42; PMID:1908028

[51] Homaira N, Rawlinson W, Snelling TL, Jaffe A. Effectiveness of Palivizumab in Preventing RSV hospitalization in high risk children: A real-world perspective. Int J Pediatr 2014; 2014:571609; PMID:25548575; https://doi.org/10.1155/2014/571609

[52] Lambert L, Sagfors AM, Openshaw PJM, Culley FJ, Immunity to RSV in Early-Life. Frontiers Immunol 2014; 5:466; PMID:25324843; https://doi.org/10.3389/fimmu.2014.00466

[53] Sande CJ, Mutungu MN, Okiro EA, Medley GF, Cane PA, Nokes DJ. Kinetics of the neutralizing antibody response to respiratory syncytial virus infections in a birth cohort. J Medical Virol 2013; 85:2020-5; PMID:23983183; https://doi.org/10.1002/jmv.23696

[54] Schuurhuis DH, Ioan-Facsinay A, Nagelkerken B, van der Sluis TC, Kimpen JL, Leusen JH, Coenaerts FE, van Bleek GM. Serum antibodies critically affect virus-specific CD4+ CD8+ T cell balance during respiratory syncytial virus infections. J Immunol 2010; 185:6489-98; PMID:20971927; https://doi.org/10.4049/jimmunol.1002645

[55] Kruijssen D, Bakkers MJ, van uden NO, Viveen MC, van der Sluis TC, Kimpen JL, Leusen JH, Coenaerts FE, van Bleek GM. Intranasal administration of antibody-bound respiratory syncytial virus particles efficiently primes virus-specific immune responses in mice. J Virol 2013; 87:7550-7; PMID:23637394; https://doi.org/10.1128/JVI.00493-13

[56] Polack FP, Teng MN, Collins PL, Prince GA, Exner M, Regele H, Lirman DD, Rabold R, Hoffman SJ, Karp CL, et al. A role for immune complexes in enhanced respiratory syncytial virus disease. The J Exp Med 2002; 196:859-65; PMID:12235218; https://doi.org/10.1084/jem.20020781

[57] Rossey I, Sedeyn K, De Baets S, Scheepens B, Saelens X. CD8+ T cell immunity against human respiratory syncytial virus. Vaccine 2014; 32:6130-7; PMID:25223272; https://doi.org/10.1016/j.vaccine.2014.08.063

[58] Christiaansen AF, Knudj CJ, Weiss KA, Varga SM. The CD4 T cell response to respiratory syncytial virus infection. Immunol Res 2014; 59:109-17; PMID:24838148; https://doi.org/10.1007/s10020-014-8540-1

[59] Jozwik A, Habibi MS, Paras A, Zhu J, Guvenel A, Dhariwal J, Almond M, Wong EH, Sykes A, Maybeno M, et al. RSV-specific airway resident memory CD8+ T cells and differential disease severity after experimental human infection. Nat Commun 2015; 6:61024; PMID:26687547; https://doi.org/10.1038/ncomms10224

[60] Hall CB, Powell KR, MacDonald NE, Gala CL, Menegus ME, Sufin SC, Cohen HJ. Respiratory syncytial viral infection in children with compromised immune function. N Engl J Med 1986; 315:77-81; PMID:3724802; https://doi.org/10.1056/NEJM198607103150201

[61] Cannon MJ, Stott EJ, Taylor G, Askonas BA. Clearance of persistent respiratory syncytial virus infections in immuno deficient mice following transfer of primed T cells. Immunology 1987; 62:133-8; PMID:3496683

[62] Bagga B, Cehelsky JE, Vaishnaw A, Wilkinson T, Meyers R, Harrison LM, Roddam PL, Walsh EE, DeVincenzo JP. Effect of preexisting serum and mucosal antibody on experimental Respiratory Syncytial Virus (RSV) challenge and infection of adults. J Infect Dis 2015; 212:1719-25; PMID:25977264; https://doi.org/10.1093/infdis/jiv281

[63] Jiang X, Clark RA, Liu L, Wagers AJ, Fulbright RC, Kupper TS. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. Nature 2012; 483:227-31; PMID:22388819; https://doi.org/10.1038/nature10851
[64] Schenkel JM, Fraser KA, Vezys V, Masopust D. Sensing and alarm function of resident memory CD8(+) T cells. Nat Immunol 2013; 14:509-13; PMID:23542740; https://doi.org/10.1038/ni.2568

[65] Oshansky CM, Zhang W, Moore E, Tripp RA. The host response and molecular pathogenesis associated with respiratory syncytial virus infection. Future Microbiol 2009; 4:279-97; PMID:19327115; https://doi.org/10.2217/fmb.09.91

[66] de Bree GI, van Leeuwen EM, Out TA, Jansen HM, Jonkers RE, van Lier RA. Selective accumulation of differentiated CD8(+) T cells specific for respiratory viruses in the human lung. J Exp Med 2005; 202:1433-42; PMID:16301748; https://doi.org/10.1084/jem.20050136

[67] Ostler T, Davidson W, Ehl S. Virus clearance and immunopathology by CD8(+) T cells during infection with respiratory syncytial virus are mediated by IFN-gamma. Eur J Immunol 2002; 32:2117-23; PMID:12209623; https://doi.org/10.1002/1521-4141 (200208)32:8<3217::AID-IMMU2117330.CO;2-C

[68] Cannon MJ, Openshaw PJ, Askonas BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. J Exp Med 1988; 168:1163-8; PMID:3262705; https://doi.org/10.1084/jem.168.3.1163

[69] Everard ML, Swarbrick A, Wrightham M, McIntyre J, Dunkley C, James PD, Sewell HF, Milner AD. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. Arch Dis Child 1994; 71:428-32; PMID:7826113; https://doi.org/10.1136/adc.71.5.428

[70] McNamara PS, Ritson P, Selby A, Hart CA, Smyth RL. Bronchoalveolar lavage cellularity in infants with severe respiratory syncytial virus bronchiolitis. Arch Dis Child 2003; 88:922-6; PMID:14500316; https://doi.org/10.1136/adc.88.10.922

[71] Welliver TP, Garofalo RP, Hosakote Y, Hintz KH, Avendano L, Sanchez K, Velozo L, Jafari H, Chavez-Bueno S, Ogra PL, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. J Infect Dis 2007; 195:1126-36; PMID:17357048; https://doi.org/10.1086/512615

[72] Hosakote YM, Jantz PD, Esham DL, Spratt H, Kurosky A, Casola A, Garofalo RP. Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis. Am J Respir Crit Care Med 2011; 183:1550-60; PMID:21471094; https://doi.org/10.1164/rcrm.201104-1755OC

[73] Funchal GA, Jaeger N, Czepielewski RS, Machado MS, Muraro SP, Stein RT, Bonorino CB, Porto BN. Respiratory syncytial virus fusion protein promotes TLR-4-dependent neutrophil extracellular trap formation by human neutrophils. PLoS One 2015; 10: e0124082; PMID:25856628; https://doi.org/10.1371/journal.pone.0124082

[74] El Saleeby CM, Suzich J, Conley ME, DeVincenzo JP. Quantitative effects of palivizumab and donor-derived T cells on chronic respiratory syncytial virus infection, lung disease, and fusion glycoprotein amino acid sequences in a patient before and after bone marrow transplantation. Clin Infect Dis 2004; 39:e17-20; PMID:15307047; https://doi.org/10.1086/421779

[75] Roman M, Calhoun WJ, Hinton KL, Avendano LF, Simon V, Escobar AM, Gaggero A, Diaz PV. Respiratory syncytial virus infection in infants is associated with predominant Th-2-like response. Am J Respir Crit Care Med 1997; 156:190-5; PMID:9230746; https://doi.org/10.1164/ajccm.156.1.9611050

[76] Bermejo-Martin JF, Garcia-Arevalo MC, De Lejarazu RO, Ardura J, Eiros JM, Alonso A, Matías V, Pino M, Bernardo D, Arranz E, et al. Predominance of Th2 cytokines, CXCl chemokines and innate immunity mediators at the mucosal level during severe respiratory syncytial virus infection in children. Eur Cytokine Netw 2007; 18:162-7; PMID:17823085

[77] Aberle JH, Aberle SW, Dworzak MN, Mandl CW, Rebhandl W, Vollhofer G, Kundi M, Popow-Kraupp T. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. Am J Respir Crit Care Med 1999; 160:1263-8; PMID:10508817; https://doi.org/10.1164/ajccm.160.4.9812025

[78] Sung RY, Hui SH, Wong CK, Lam CW, Yin J. A comparison of cytokine responses in respiratory syncytial virus and influenza A infections in infants. Eur J Pediatr 2001; 160:117-22; PMID:11271383; https://doi.org/10.1007/s004310000676

[79] McNamara PS, Flanagan BF, Selby AM, Hart CA, Smyth RL. Pro- and anti-inflammatory responses in respiratory syncytial virus bronchiolitis. Eur Respir J 2004; 23:106-12; PMID:14738241; https://doi.org/10.1183/09031936.03.00048103

[80] Lee HC, Headley MB, Loo YM, Berlin A, Gale M, Jr, Deby- ley JS, Lukacs NW, Ziegler SF. Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection. J Allergy Clin Immunol 2012; 130:1187-96 e5; https://doi.org/10.1016/j.jaci.2012.07.031

[81] Lukacs NW, Tekkanat KK, Berlin A, Hogaboam CM, Miller A, Evanoff H, Lincoln P, Maassab H. Respiratory syncytial virus predisposes mice to augmented allergic airway responses via IL-13-mediated mechanisms. J Immunol 2001; 167:1060-5; PMID:11441116; https://doi.org/10.4049/jimmunol.167.2.1060

[82] Rothenfue T, Fischer K, zawatzki S, Schulz V, Popow-Kraupp T. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. Am J Respir Crit Care Med 1999; 160:1263-8; PMID:10508817; https://doi.org/10.1164/ajccm.160.4.9812025

[83] Rehman T, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002; 3:673-80; PMID:12055625; https://doi.org/10.1038/nrn910
Qiao J, Li A, Jin X. TSLP from RSV-stimulated rat airway epithelial cells activates myeloid dendritic cells. Immunol Cell Biol 2011; 89:231-8; PMID:20603637; https://doi.org/10.1038/icb.2010.85

Ito T, Wang YH, Duramad O, Hori T, Delespesse GI, Watanabe N, Qin FX, Yao Z, Cao W, Liu YJ. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. J Exp Med 2005; 202:1213-23; PMID:16275760; https://doi.org/10.1084/jem.20051135

You D, Marr N, Saravia J, Shrestha B, Lee GI, Turvey TL, Wang YH, Duramad O, Hori T, Chojnacki BJ, Miyazaki T, Gallo RL, et al. Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. Blood 2013; 121:1677-89; PMID:23305731; https://doi.org/10.1182/blood-2012-07-445189

Halffide CP, Flanagan BF, Breeaey SP, Hunt JA, Foncetta AM, McNamara PS, Howarth D, Edwards S, Smyth RL. Respiratory syncytial virus binds and undergoes transcription in neutrophils from the blood and airways of infants with severe bronchiolitis. J Infect Dis 2011; 204:451-8; PMID:21742845; https://doi.org/10.1093/infdis/jir280

Kulkarni AB, Morse HC, Bennink JR, Yewdell JW, Murphy BR. Immunization of mice with vaccinia virus-M2 recombinant induces epitope-specific and cross-reactive Kd-restricted CD8+ cytotoxic T cells. J Virol 1993; 67:4086-92; PMID:7685408

Bartholdy C, Olaszewska W, Stryhn A, Thomsen AR, Openshaw PJ. Gene-gun DNA vaccination aggravates respiratory syncytial virus-induced pneumonitis. J Gen Virol 2004; 85:3017-26; PMID:15484365; https://doi.org/10.1099/vir.0.80098-0

Simmons CP, Hussell T, Sparer T, Walzl G, Openshaw P, Dougan G. Mucosal delivery of a respiratory syncytial virus CTL peptide with enterotoxin-based adjuvants elicits protective, immunopathogenic, and immunoregulatory antiviral CD8+ T cell responses. J Immunol 2001; 166:1106-13; PMID:11145691; https://doi.org/10.4049/jimmunol.166.2.1106

Ruckwardt TJ, Luongo C, Malloy AM, Liu J, Chen M, Collins PL, Graham BS. Responses against a subdominant CD8+ T cell epitope protect against immunopathology caused by a dominant epitope. J Immunol 2010; 185:4673-80; PMID:20833834; https://doi.org/10.4049/jimmunol.1001606

Liu J, Haddad EK, Marceau J, Morabito KM, Rao SS, Filali-Mouhim A, Sekaly RP, Graham BS. A Numerically Subdominant CD8+ T Cell response to matrix protein of respiratory syncytial virus controls infection with limited immunopathology. PLoS Pathog 2016; 12: e1005486; PMID:26943673; https://doi.org/10.1371/journal.ppat.1005486

De Baets S, Schepens B, Sedeyn K, Schotsaert M, Roose RP, Braeckmans K, Bogaert P, Fiers W, Saels X. Recombinant influenza virus carrying the respiratory syncytial virus (RSV) F85-93 CTL epitope reduces RSV replication in mice. J Virol 2013; 87:3314-23; PMID:23302879; https://doi.org/10.1128/JVI.03019-12

Vanterm M, Rock M, Puren AJ, Tiemessen CT, Crowe JE Jr. Respiratory syncytial virus nucleoprotein-specific cytotoxic T-cell epitopes in a South African population of diverse HLA types are conserved in circulating field strains. J Virol 2003; 77:7319-29; PMID:12805430; https://doi.org/10.1128/JVI.77.13.7319-7329.2003
Heidema J, de Bree GJ, De Graaff PM, van Maren WW, Hoogerhout P, Out TA, Kimpen JL, van Bleek GM. Human CD8(+) T cell responses against five newly identified respiratory syncytial virus-derived epitopes. J Gen Virol 2004; 85:2365-74; PMID:15269378; https://doi.org/10.1099/vir.0.80131-0

Telpin AG, Laza-Stanca V, Edwards MR, Harker JA, Wang H, Bartlett NW, Mallia P, Zdrengea MT, Kebadze T, Coyle AJ, et al. RSV-induced bronchial epithelial cell PD-L1 expression inhibits CD8(+) T cell non-specific antiviral activity. J Infect Dis 2011; 203:85-94; PMID:21148500; https://doi.org/10.1093/infdis/jiq020

Hussell T, Openshaw PJ. Intracellular IFN-gamma expression in natural killer cells precedes lung CD8(+) T cell recruitment during respiratory syncytial virus infection. J Gen Virol 1998; 79(Pt 11):2593-601; PMID:9820134; https://doi.org/10.1099/0022-1317-79-11-2593

Ge MQ, Ho AW, Tang Y, Wong KH, Chua BY, Gasser S, Kemeny DM. NK cells regulate CD8(+) T cell priming and dendritic cell migration during influenza A infection by IFN-gamma and perforin-dependent mechanisms. J Immunol 2012; 189:2099-109; PMID:22869906; https://doi.org/10.4049/jimmunol.1103474

Lukens MV, van de Pol AC, Coenjaerts FE, Lansen NJ, Kamp VM, Kimpen JL, Rossen JW, Ulfman LH, Tacke CE, Viveen MC, et al. A systemic neutrophil response precedes robust CD8(+) T-cell activation during natural respiratory syncytial virus infection in infants. J Virol 2010; 84:2374-83; PMID:20015982; https://doi.org/10.1128/JVI.01807-09

Fearns R, Collins PL. Role of the M2-1 transcription antitermination protein of respiratory syncytial virus in sequential transcription. J Virol 1999; 73:5852-64; PMID:10364337

Jin H, Cheng X, Zhou HZ, Li S, Seddiqui A. Respiratory syncytial virus that lacks open reading frame 2 of the M2 gene (M2-2) has altered growth characteristics and is attenuated in rodents. J Virol 2000; 74:74-82; PMID:10590093; https://doi.org/10.1128/JVI.74.1.74-82.2000

McLellan JS, Ray WC, Peeples ME. Structure and function of respiratory syncytial virus surface glycoproteins. Curr Top Microbiol Immunol 2013; 372:83-104; PMID:24362685

Bukreyev A, Yang L, Fricke J, Cheng L, Ward JM, Murphy BR, Collins PL. The secreted form of respiratory syncytial virus G glycoprotein helps the virus evade antibody-mediated restriction of replication by acting as an antigen decoy and through effects on Fc receptor-bearing leukocytes. J Virol 2008; 82:12191-204; PMID:18842713; https://doi.org/10.1128/JVI.01604-08

Harcourt J, Alvarez R, Jones LP, Henderson C, Anderson LJ, Tripp RA. Respiratory syncytial virus G protein and G protein CX3C motif adversely affect CX3CR1+ T cell responses. J Immunol 2006; 176:1600-8; PMID:16424189; https://doi.org/10.4049/jimmunol.176.3.1600

Schlender J, Walliser G, Fricke J, Conzelmann KK. Respiratory syncytial virus fusion protein mediates inhibition of mitogen-induced T-cell proliferation by contact. J Virol 2002; 76:1163-70; PMID:11773392; https://doi.org/10.1128/JVI.76.3.1163-1170.2002

Gan SW, Tan E, Lin X, Yu D, Wang J, Tan GM, Vararattanavech A, Yeo CY, Soon CH, Soong TW, et al. The small hydrophobic protein of the human respiratory syncytial virus forms pentameric ion channels. J Biol Chem 2012; 287:24671-89; PMID:22621926; https://doi.org/10.1074/jbc.M111.332791

Fuentes S, Tran KC, Luthra P, Teng MN, He B. Function of the respiratory syncytial virus small hydrophobic protein. J Virol 2007; 81:8361-6; PMID:17494063; https://doi.org/10.1128/JVI.02717-06

Tripp RA, Moore D, Jones L, Sullender W, Winter J, Anderson LJ. Respiratory syncytial virus G and/or SH protein alters TH1 cytokines, natural killer cells, and neutrophils responding to pulmonary infection in BALB/c mice. J Virol 1999; 73:7099-107; PMID:10438795

Spann KM, Tran KC, Collins PL. Effects of nonstructural proteins NS1 and NS2 of human respiratory syncytial virus on interferon regulatory factor 3, NF-kappaB, and proinflammatory cytokines. J Virol 2005; 79:5353-62; PMID:15827150; https://doi.org/10.1128/JVI.79.9.5353-5362.2005

Barik S. Respiratory syncytial virus mechanisms to interfere with type 1 interferons. Curr Top Microbiol Immunol 2013; 372:173-91; PMID:24362690

Elliott J, Lynch OT, Suessmuth Y, Qian P, Boyd CR, Burrows JF, Buick R, Stevenson NJ, Touzelet O, Gadina M, et al. Respiratory syncytial virus NS1 protein degrades STAT2 by using the Elongin-Cullin E3 ligase. J Virol 2007; 81:3428-36; PMID:17251292; https://doi.org/10.1128/JVI.02303-06

Liesman RM, Buchholz UJ, Luongo CL, Yang L, Proia AD, DeVincenzo JP, Collins PL, Pickles R. RSV-encoded NS2 promotes epithelial cell shedding and distal airway obstruction. J Clin Invest 2014; 124:2219-33; PMID:24713657; https://doi.org/10.1172/JCI72948

Munir S, Le Nouen C, Luongo C, Buchholz UJ, Collins PL, Bukreyev A. Nonstructural proteins 1 and 2 of respiratory syncytial virus suppress maturation of human dendritic cells. J Virol 2008; 82:8780-96; PMID:18562519; https://doi.org/10.1128/JVI.00630-08

Munir S, Hillyer P, Le Nouen C, Buchholz UJ, Rabin RL, Collins PL, Bukreyev A. Respiratory syncytial virus interferon antagonist NS1 protein suppresses and skews the human T lymphocyte response. PLoS Pathog 2011; 7:e1001336; PMID:21533073; https://doi.org/10.1371/journal.ppat.1001336

Kotelkin A, Belyakov IM, Yang L, Berzofsky JA, Collins PL, Bukreyev A. The NS2 protein of human respiratory syncytial virus suppresses the cytotoxic T-cell response as a consequence of suppressing the type I interferon response. J Virol 2006; 80:5958-67; PMID:16731934; https://doi.org/10.1128/JVI.00181-06

Reimers K, Buchholz K, Werchau H. Respiratory syncytial virus M2-1 protein induces the activation of nuclear factor kappa B. Virology 2005; 331:260-8; PMID:15629770; https://doi.org/10.1016/j.virol.2004.10.031

Bakker SE, Duquerroy S, Galloux M, Loney C, Conner E, Eleouet JF, Rey FA, Bhella D. The respiratory syncytial virus nucleoprotein-RNA complex forms a left-handed helical nucleocapsid. J Gen Virol 2013;
Falsely AR, Hennessey PA, Formica MA, Criddle MM, Bier JM, Walsh EE. Humoral immunity to human metapneumovirus infection in adults. Vaccine 2010; 28:1477-80; PMID:20003919; https://doi.org/10.1016/j.vaccine.2009.11.063

Alvarez R, Tripp RA. The immune response to human metapneumovirus is associated with aberrant immunity and impaired virus clearance in BALB/c mice. J Virol 2005; 79:5971-8; PMID:15857983; https://doi.org/10.1128/JVI.79.10.5971-5978.2005

Alvarez R, Harrod KS, Shieh WJ, Zaki S, Tripp RA. Human metapneumovirus persists in BALB/c mice despite the presence of neutralizing antibodies. J Virol 2004; 78:14003-11; PMID:15564507; https://doi.org/10.1128/JVI.78.24.14003-14011.2004

Debiaggi M, Canducci F, Sampaolo M, Marinozzi MC, Parea M, Torrella C, Colombo AA, Alessandrinio EP, Bragotti LZ, Arghittu M, et al. Persistent symptomless human metapneumovirus infection in hematopoietic stem cell transplant recipients. J Infect Dis 2006; 194:474-8; PMID:16845630; https://doi.org/10.1086/505881

Abed Y, Boivin G. Human metapneumovirus infection in immunocompromised child. Emerg Infect Dis 2008; 14:854-6; PMID:18439384; https://doi.org/10.3201/eid1405.071459

Herd KA, Nelson M, Mahalingam S, Tindle RW. Pulmonary infection of mice with human metapneumovirus induces local cytotoxic T-cell and immunoregulatory cytokine responses similar to those seen with human respiratory syncytial virus. J Gen Virol 2010; 91:1302-10; PMID:20053825; https://doi.org/10.1099/vir.0.015396-0

Kolli D, Bataki EL, Spetch L, Guerrero-Plata A, Jewell AM, Piedra PA, Milligan GN, Garofalo RP, Casola A. T lymphocytes contribute to antiviral immunity and pathogenesis in experimental human metapneumovirus infection. J Virol 2008; 82:8560-9; PMID:18562525; https://doi.org/10.1128/JVI.00699-08
T-lymphocyte response in mice against human metapneumovirus. J Virol 2007; 81:11461-7; PMID:17670840; https://doi.org/10.1128/JVI.02423-06

[151] Hastings AK, Erickson JJ, Schuster JE, Boyd KL, Tollefson SJ, Johnson M, Gilchuk P, Joyce S, Williams JV. Role of type I interferon signaling in human metapneumovirus pathogenesis and control of viral replication. J Virol 2015; 89:6391-405; PMID:25855728; https://doi.org/10.1128/JVI.03488-14

[152] Erickson JJ, Gilchuk P, Hastings AK, Tollefson SJ, Johnson M, Downing MB, Boyd KL, Johnson JE, Kim AS, Joyce S, et al. Viral acute lower respiratory infections impair CD8^+ T cells through PD-1. J Clin Invest 2012; 122:2967-82; PMID:22797302; https://doi.org/10.1172/JCI62860

[153] Erickson JJ, Rogers MC, Hastings AK, Tollefson SJ, Williams JV. Programmed death-1 impairs secondary effector lung CD8^+ T cells during respiratory virus reinfection. J Immunol 2014; 193:5108-17; PMID:25339663; https://doi.org/10.4049/jimmunol.1302208

[154] Deffrasnes C, Hamelin ME, Boivin G. Human metapneumovirus. Semin Respir Crit Care Med 2007; 28:213-21; PMID:17458775; https://doi.org/10.1055/s-2007-976493

[155] Ishiguro N, Ebihara T, Endo R, Ma X, Kikuta H, Ishikoh H, et al. High genetic diversity of the attachment (G) protein of human metapneumovirus. J Clin Microbiol 2004; 42:3406-14; PMID:15297475; https://doi.org/10.1128/JCM.42.8.3406-3414.2004

[156] Bagnaud-Baule A, Reynard O, Perret M, Berland JL, Maache M, Peyret C, Vernet G, Volchkov V, Paranhos-Baccalá G. The human metapneumovirus matrix protein stimulates the inflammatory immune response in vitro. PLoS One 2011; 6:e17818; PMID:21412439; https://doi.org/10.1371/journal.pone.0017818

[160] Cai H, Zhang Y, Ma Y, Sun J, Liang X, Li J. Zinc binding activity of human metapneumovirus M2-1 protein is indispensable for viral replication and pathogenesis in vivo. J Virol 2015; 89:6391-405; PMID:25855728; https://doi.org/10.1128/JVI.03488-14

[161] Buchholz UJ, Biacchesi S, Pham QN, Tran KC, Yang L, Luongo CL, Skiaidopoulos MH, Murphy BR, Collins PL. Deletion of M2 gene open reading frames 1 and 2 of human metapneumovirus: effects on RNA synthesis, attenuation, and immunogenicity. J Virol 2005; 79:6588-97; PMID:15890897; https://doi.org/10.1128/JVI.79.11.6588-6597.2005

[162] Kitagawa Y, Zhou M, Yamaguchi M, Komatsu T, Takeuchi K, Itoh M, Gotoh B. Human metapneumovirus M2-2 protein inhibits viral transcription and replication. Microbes Infect 2010; 12:135-45; PMID:19913636; https://doi.org/10.1016/j.micinf.2009.11.002

[163] Ren J, Wang Q, Kolli D, Prusak DJ, Tseng CT, Chen ZJ, Li K, Wood TG, Bao X. Human metapneumovirus M2-2 protein inhibits innate cellular signaling by targeting MAVS. J Virol 2012; 86:13049-61; PMID:23015697; https://doi.org/10.1128/JVI.01248-12

[164] Busche A, Jirmo AC, Welten SP, Zischke J, Noack J, Constabel H, Gatzke AK, Keyser KA, Arens R, Behrens GM, et al. Priming of CD8^+ T cells against cytomegalovirus-encoded antigens is dominated by cross-presentation. J Immunol 2013; 190:2767-77; PMID:23390296; https://doi.org/10.4049/jimmunol.1200966

[165] Cox RG, Erickson JJ, Hastings AK, Becker JC, Johnson M, Craven RE, Tollefson SJ, Boyd KL, Williams JV. Human metapneumovirus virus-like particles induce protective B and T cell responses in a mouse model. J Virol 2014; 88:6368-79; PMID:24672031; https://doi.org/10.1128/JVI.00332-14

[166] Singleton R, Etchart N, Hou S, Hyland L. Inability to evoke a long-lasting protective immune response to respiratory syncytial virus infection in mice correlates with ineffective nasal antibody responses. J Virol 2003; 77:11303-11; PMID:14557661; https://doi.org/10.1128/JVI.77.21.11303-11311.2003

[167] Hussell T, Spender LC, Georgiou A, O’Garra A, Openshaw PJ. Th1 and Th2 cytokine induction in pulmonary T cells during infection with respiratory syncytial virus. J Gen Virol 1996; 77(Pt 10):2447-55; PMID:8887477; https://doi.org/10.1099/0022-1317-77-10-2447