

Review Article

Role of human metapneumovirus and respiratory syncytial virus in asthma exacerbations: where are we now?

Penny A. Rudd^{1,*}, Belinda J. Thomas^{2,3,4,*}, Ali Zaid^{1,*}, Martin MacDonald^{2,3,4}, Keiko Kan-o^{2,3,4}, Michael S. Rolph¹, Anand R. Soorneedi¹, Philip G. Bardin^{2,3,4} and Suresh Mahalingam¹

¹Institute for Glycomics, Griffith University, Gold Coast Campus, QLD, Australia; ²Monash Lung and Sleep, Monash Medical Centre, Melbourne, VIC, Australia; ³Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, VIC, Australia; ⁴School of Clinical Sciences, Monash University, Melbourne, VIC, Australia

Correspondence: Suresh Mahalingam (s.mahalingam@griffith.edu.au)

Since its discovery in 2001, human metapneumovirus (hMPV) has been identified as an important cause of respiratory tract infection in young children, second only to the closely related respiratory syncytial virus (RSV). Clinical evidence suggests that hMPV is associated with acute exacerbations of asthma in both children and adults, and may play a role in initiating asthma development in children. Animal models have demonstrated that airway hyperresponsiveness (AHR) and inflammation are triggered following hMPV infection, and hMPV is able to persist *in vivo* by inhibiting innate immune responses and causing aberrant adaptive responses. In this review, we discuss the prevalence of hMPV infection in pediatric and adult populations and its potential role in asthma exacerbation. We also review recent advances made in animal models to determine immune responses following hMPV infection, and compare to what is known about RSV.

Introduction

First identified in the Netherlands in 2001, human metapneumovirus (hMPV) can still be considered a relatively new virus. Approximately 5–15% of all respiratory tract infections of infants and toddlers are caused by hMPV, making it the second most common cause of hospitalization of young children after respiratory syncytial virus (RSV) [1]. Presenting symptoms are similar to those induced by RSV and include fever, cough, runny nose, tachypnea, and wheezing [2]. There are two main viral groups of hMPV: A and B. In temperate countries, hMPV outbreaks seem to follow a seasonal distribution with a significant increase in cases during winter and spring. Although hMPV mainly targets the young, immunocompromised adults and the elderly can also suffer from hMPV, with serious infections sometimes leading to respiratory failure. It has also been reported that hMPV, as well as other respiratory viruses such as RSV, parainfluenza viruses, influenza viruses, coronaviruses, and adenoviruses can exacerbate asthma across all ages [3]. However, direct evidence for this has not been obtained, and determining the etiology of a complex disease such as asthma is not an easy task. In this review, we will look at progress in hMPV research and what we have learned about its potential involvement in asthma exacerbations, and compare to what is known about RSV.

Clinical evidence of hMPV and asthma exacerbations

Since the recent discovery of hMPV as a respiratory pathogen, limited cross-sectional and longitudinal clinical investigations have been conducted to establish the prevalence of this pathogen in the population and its role in respiratory tract illness. A Finnish study investigating the efficacy of systemic glucocorticosteroids in acute expiratory wheezing detected hMPV in patients aged 3 months to 16 years who were

*These authors contributed equally to this work.

Received: 29 October 2016
Revised: 18 April 2017
Accepted: 02 May 2017

Version of Record published:
30 June 2017

consecutively recruited during a 20-month period when hospitalized for acute wheezing. hMPV was detected in 7.6% of 132 children in the first 8 months of the study [4] and 4% of 293 patients over the 20-month period [5]. A 13-month study of children less than 5 years of age hospitalized with acute respiratory illness in the U.S.A. detected hMPV in 3.9% of patients [6], while a 3-month study over the winter period in South Africa reported hMPV in 5.8% of children referred to hospital for upper or lower respiratory tract infection [7]. Williams et al. utilized samples obtained over a 25-year period from otherwise healthy children up to 5 years of age presenting with acute respiratory illness [8]. hMPV was detected in 20% and 15% of samples associated with lower or upper respiratory infection respectively, which had previously tested negative for other respiratory pathogens [8]. A second study by Williams et al. identified hMPV in 13 of 133 children (9.8%) aged 2 months to 18 years who were hospitalized for wheezing during a 1-year period [9]. Interestingly, 70% of these patients were younger than 3 years old [9]. During a 5-year study in Spain of infants under the age of 2 years hospitalized for acute respiratory infection, hMPV was detected in 9% and 7.6% of children after the 3rd and 5th year of the study respectively [10,11]. Edwards et al. assessed children less than 5 years of age who were hospitalized or seen at an outpatient clinic or emergency department for acute respiratory illness, during seven consecutive winter–spring periods. hMPV was detected in 5.7%, 6.8% and 7.5% respectively [12]. Finally, a Korean study over a 13-month period on 309 children hospitalized with acute lower respiratory tract infection identified hMPV in 22.6% of patients [13]. Therefore, depending on the specimen type, the duration of the study, the season during which samples were collected, and the sensitivity of the analysis, a detection rate of 3.9–22.6% is estimated in the pediatric population, with the highest incidence occurring in infants under 12 months of age [9,10,12]. In contrast, two studies conducted by Widmer et al. [14,15] reported much lower detection rates in adults hospitalized with respiratory symptoms, ranging from 2.6 to 4.5%, with hospitalization rates increasing significantly with age. Higher rates of detection were reported for hospitalizations due to asthma or chronic obstructive pulmonary disease (COPD).

Virus infections, particularly rhinovirus (RV) and RSV, are commonly associated with acute exacerbations of asthma [16]; however, there are limited data linking asthma exacerbations with hMPV. Most commonly, hMPV in younger patients is associated with bronchiolitis [8,17], recurrent wheezing [9,17–19], pneumonia, and croup [8]. While a study by Rawlinson et al. [20] found no association between hMPV infection and asthma, reports linking hMPV to asthma have started to appear indicating that hMPV may be implicated in up to 14% of asthma exacerbations [8,13]. A study by Kwon et al. [13] reported the average age of patients presenting with asthma exacerbations due to hMPV infection was 60 months, compared with 16 months for bronchiolitis, suggesting exacerbation of asthma by hMPV may be more evident in older children. Infants hospitalized with bronchiolitis or wheezing may also be at increased risk of developing asthma or recurrent wheezing [19,21]. Garcia-Garcia et al. [17] reported that an episode of hMPV bronchiolitis at age less than 2 years resulted in 52% of patients wheezing at 3 years of age and 64% wheezing at 5 years, rates similar to those following RSV infection; therefore, hMPV infection during infancy may be a significant risk factor for development of preschool asthma. Thus, hMPV infections might not only be associated with acute exacerbations in asthmatic children, but also initiate development of asthma in the first 5 years of life, as demonstrated for RV and RSV [22–24].

Whilst there are some studies describing hMPV infection in children, there have been few studies in adult populations. Following hospitalization with an exacerbation of asthma, hMPV was detected in 6.9% of adult patients, with infection generally occurring in individuals with more severe or with less well-controlled asthma [25]. Exacerbations of COPD can also be triggered by virus infection [26], and studies investigating the role of hMPV in acute exacerbations of COPD have reported detection rates of 2–12% [27–30]. In a cohort of 167 patients presenting to a hospital emergency department with a COPD exacerbation, we identified viruses in 26% of patients, with the most frequently identified virus being RV (38.6%). hMPV was detected in 18.2% of patients; this rate of detection was higher than the prevalence of COPD exacerbations caused by RSV infection within the same cohort (13.6%) [31].

Lessons learned from animal models

Until 2001, metapneumoviruses had only been identified in avian species. As previously mentioned, the first clinical case was reported in the Netherlands in children presenting with respiratory signs reminiscent of RSV. Once the causative agent was clearly identified as hMPV, many countries such as Australia, Canada, and France also reported hMPV circulating in the pediatric community [32–34]. Early research into hMPV focused on a variety of aspects including genome organization, genetic analysis, virological features, pathogenesis/clinical manifestations, and development of robust methods for detection. As for all diseases, the development of an adequate animal model is extremely useful in order to gain insight into virus–host interactions and to aid in the development of therapeutics and/or vaccines. A variety of small animals such as mice, hamsters, guinea pigs, ferrets, and cotton rats as well as

non-human primates such as chimpanzees, rhesus macaques, and African green monkeys have all been explored as suitable models for hMPV [35–37]. However, with the exception of chimpanzees, none of these animal models mimic all of the signs of human disease. The degree of hMPV replication in the upper and lower respiratory tracts is variable and many contradictory results have been reported for these animal models. Age and strains of animals and viruses as well as infectious dose all seem to contribute to the heterogeneity seen within the models. Nevertheless, these models have still proved valuable in gaining insight into hMPV pathogenesis and examining the relationship between hMPV and asthma.

Asthma is defined as a chronic inflammatory response accompanied by airway hyperresponsiveness (AHR). It is still unclear if viral infections exacerbate asthma or are involved in etiology or if asthmatics are just more susceptible to viral infections. As previously discussed, many children admitted to hospital at a young age suffering from respiratory illnesses with a viral etiology go on to develop asthma at later stages in life. One explanation for this may lie in viral persistence. Toward resolving the contribution of hMPV to asthma exacerbation, several mouse studies have shown that AHR and inflammation can be triggered by hMPV infection and that hMPV is able to persist *in vivo*.

hMPV-induced immune response and persistence

BALB/c mice are often used in hMPV studies because they are permissive to both RSV and hMPV infections and provide an adequate small animal model. Most research on asthma exacerbations examines the contributions of both of these respiratory viruses due to their clinical relevance. In such studies, mice from 4 weeks of age to adult are usually used and are inoculated intranasally with various strains of hMPV. Mice infected with hMPV can display ruffled fur, lethargy, hunched posture, tachypnea, and weight loss [38,39]. Weight loss appeared to coincide with peak lung viral titers, which exhibited a biphasic growth kinetic, peaking on days 7 and 14 post-infection (p.i.). Furthermore, replicating virus was retrieved up to 60 days p.i. despite the presence of neutralizing antibody [38]. This suggests that hMPV has the ability to circumvent host immune responses and could cause long-term lung damage. In this study, airway remodeling and mucus production were also observed, suggesting that hMPV can cause exacerbated lung disease. Other groups also reported asthma-like signs of disease, including airway obstruction and AHR, following viral challenge in mice [40,41]. In accordance with these results, Hamelin et al. also observed chronic inflammation in the lungs of infected mice for up to 154 days p.i. with peribronchiolitis and perivascularitis accompanied by early mucus hyperproduction and increased respiratory rate. Although they failed to recover live virus after day 21 post inoculation, viral RNA was detected for the duration of the chronic pathology seen in the lungs [42]. Interestingly, AHR could be triggered by methacholine treatment up to 70 days post hMPV infection, thereby indicating that hMPV can be responsible for this type of pathological change at later times after primary infection, which could in turn explain subsequent asthma seen in children following hospitalization with hMPV. Liu et al. suggested that persistence in neuronal cell processes in the lung may explain how hMPV can persist for extended amounts of time. It was shown, via *in situ* hybridization, that this immune privileged area could harbor the virus for extended periods of time, and viral reactivation could be observed following glucocorticoid treatment [43]. So how does the virus circumvent the innate immune response? Several studies have shown that hMPV infection inhibits innate immune responses and causes aberrant adaptive responses. While Th1 responses are detected early during infection, Th2 responses along with increased IL-10 levels are seen at later stages. In addition, low interferon (IFN)- γ production and delayed cytotoxic T-lymphocyte responses have also been reported upon hMPV infection. Together, all of these may be contributing factors to hMPV persistence *in vivo* [44,45]. However, there may be additional factors contributing to persistence. Several other immune components play key roles in hMPV clearance such as NK and T cells, IFN- β production via IRF-3/7, IPS-1, and MDA5 as well as the production of IFN- λ [41,46–48]. Additional in-depth studies need to be undertaken to pinpoint the exact mechanisms that lead to hMPV persistence.

Role of RSV infection in asthma exacerbation and susceptibility

RSV, another prominent paramyxovirus in childhood infections, is closely related to hMPV. RSV causes significant morbidity and mortality in infants worldwide, and accounts for as much as 70% of all childhood respiratory infections, with almost all children infected with RSV by the age of 3 [49,50]. In humans, two distinct serotypes circulate, and while RSV is mostly regarded as a childhood virus, adults are also susceptible to infection [23,49]. In children up to 18 months old, RSV infection is associated with moderate to severe upper and lower respiratory tract infections, with symptoms including acute bronchiolitis, reactive airway disease, and excessive mucus production [49,51]. In infants presenting with RSV-induced bronchiolitis, cellular infiltrates of monocytes, lymphocytes, neutrophils, and

eosinophils dominate the inflammatory microenvironment in both the pulmonary epithelium and lumen, and are concomitant with airway hyperplasia and constriction [49].

The link between RSV infections in early childhood and susceptibility to asthma in later childhood years has been extensively investigated and debated. Reactive airway disease, or wheezing, is a key symptom common to both asthma and RSV infection, though it is widely established that genetic predisposition to atopic (or allergic) asthma is an important factor when drawing parallels between respiratory viral infections and asthma [52]. Reports showing an association between respiratory viral load, wheezing, and atopy suggest that the inflammatory milieu resulting from lung epithelial RSV infection can favor, if not trigger, clinical manifestations of asthma in later years, beyond resolution of infection.

While a direct ‘RSV-asthma’ causative link appears to be speculative, studies have shown that infants who experienced severe RSV bronchiolitis, where wheezing was a dominant feature, went on to experience more wheezing episodes in later childhood, and a significant proportion of these children developed asthma in later years [53–56]. A longitudinal follow-up study by Sigurs et al. showed that while bronchiolitis and pneumonia in general were associated with an increased risk of developing asthma in later years, cohorts of RSV-induced bronchiolitis patients showed a significant increase in asthma frequency, with a prevalence of up to 30% compared with non-RSV groups [23].

Several mechanisms have been proposed to support the idea that childhood RSV infection predisposes to asthma. First, it has been suggested that IgE antibodies abundantly produced following RSV-induced bronchiolitis could be a driving factor for an increase in wheezing episodes during the first 2 years of life, primarily by skewing responses to common allergen exposure at the local tissue level [57]. Second, neonatal RSV infection is associated with a Th2-skewed response, in contrast with a Th1 response in RSV-infected adults, lending weight to the notion that a cytokine microenvironment permissive to asthma can be established upon early childhood RSV infection. This was supported by the enhanced AHR and increased eosinophilia and neutrophilia in RSV-infected mice exposed to ovalbumin (OVA) airway allergen [52,58]. The effector function of eosinophils in airway inflammation has been largely attributed to the coordinated action of IL-5, IL-13 and CCL11 (eotaxin), while the induction and maintenance of a Th2-skewed milieu also requires IL-9 and IL-33 in addition to IL-25 and IL-17 family cytokines [59–61]. Considering the importance of Th17 responses in asthma, the role of Th17 in driving Th2-skewed responses after primary RSV infection may provide a plausible explanation as to how early childhood viral infections can modulate tissue and cellular environments to favor chronic allergic conditions upon challenge [62–64].

The potential for RSV infection to exacerbate allergic asthma has been investigated by examining the response to RSV following allergen sensitization in mice. Mice exposed to OVA (an intranasally delivered sensitizing agent) and subsequently infected with RSV showed enhanced AHR and a significantly higher proportion of Th2 cells, along with increased pulmonary infiltration of leukocytes [58]. By replicating an artificial allergic RSV infection model, studies have shown that a Th2 cytokine milieu can exacerbate the pathophysiology of RSV infection, which is typically Th1-skewed. Further, if the cytokine milieu rich in IL-13 and IL-5, and thus typically representative of allergen-induced asthma, is replicated, RSV infections are known to result in more severe symptoms [49,59,65,66]. This, along with several reports examining other respiratory viruses such as RV, points toward the possibility of increased susceptibility to severe RSV infection in asthmatic individuals, but the question remains: can early childhood RSV infection predispose children to asthma in later years [67,68]?

Host responses to RSV infection

As for hMPV, mouse models of RSV infection have helped our understanding of how host immune responses to RSV infection are coordinated, and how they relate to pathological manifestations.

The involvement of the innate immune system has been demonstrated by the key role of innate recognition mechanisms, such as toll-like receptors (TLR), which are essential for regulation of antiviral innate immune responses [69]. TLR3, which recognizes double-stranded RNA, is required for sensing RSV viral RNA and promotes the production of proinflammatory cytokines CCL5, CXCL10, CXCL8, and type I IFN upon RSV infection in mice [70]. TLR4, which recognizes bacterial lipopolysaccharide (LPS), recognizes RSV by interacting with CD14 upon viral fusion, resulting in the activation of proinflammatory transcription factors such as NFκB [69,71]. Though TLR3 is likely to be important in viral clearance due to its high specificity for RNA viruses, the majority of studies characterizing its role have been performed in mice, and its function in humans during RSV infection remains unclear. In contrast, distinct TLR4 polymorphisms in humans have been associated with an increased risk of severe RSV bronchiolitis [70]. Another RNA-sensing mechanism, TLR7, is activated after RSV infection, leading to the production of IL-23, an important differentiation factor required in Th17 differentiation [70,72].

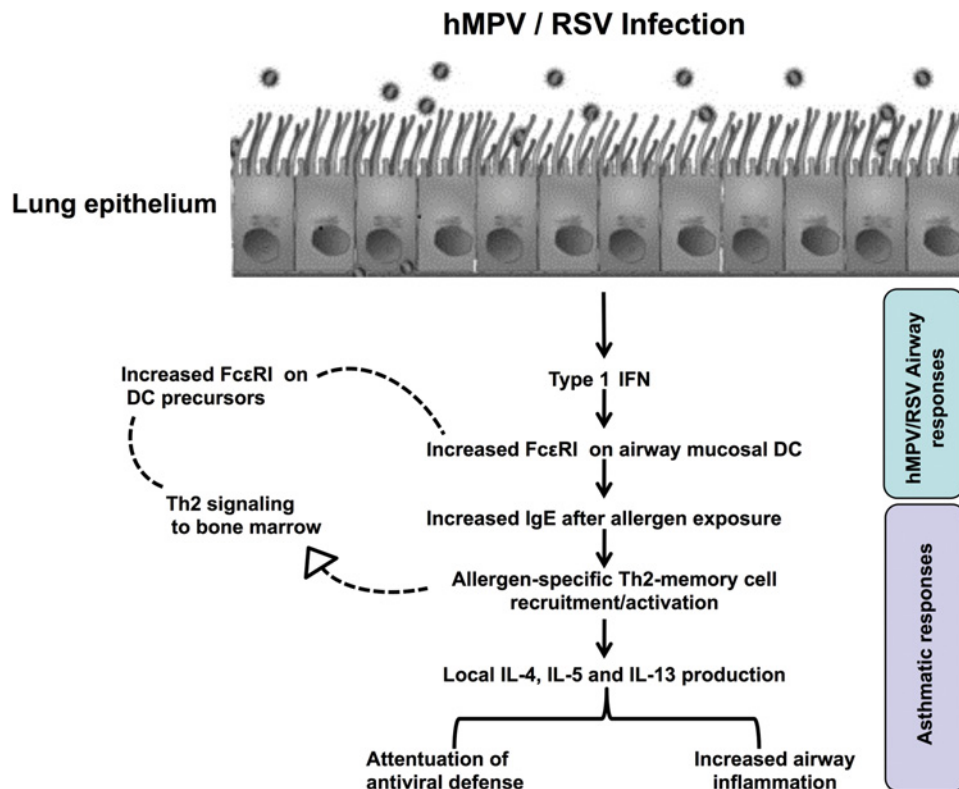


Figure 1. Model of how hMPV and RSV may be exacerbating asthma.

hMPV and RSV infection of pulmonary epithelial cells lead to potent antiviral responses and type I IFN production. Mucosal dendritic cell (DC) activation allows the up-regulation of FcεRI and a subsequent systemic Th2-biased signaling cascade. In asthmatic patients infected with hMPV or RSV, allergen exposure could further promote the secretion of IgE leading to Th2 T-cell recruitment and activation to the airways, stimulating local IL-4, IL-5, and IL-13 production. As a result of this Th2-biased response profile, such patients are at increased risk of severe airway inflammation and impaired viral clearance.

While the adaptive immune system has been recognized as essential in viral clearance—requiring both CD4⁺ and CD8⁺ T cells—there is a growing body of evidence supporting the notion that Th17 responses are important in RSV infection [49,58,73]. CD4⁺ and CD8⁺ T cells were previously understood to dominate T-cell responses and mediate efficient viral clearance; however, it is now apparent that a balance of Th1, Th2, regulatory T cells (T_{reg}), and Th17 signaling microenvironments is necessary for effective resolution [49,73]. Th17 cells are generated via IL-23, IL-6, and TGF-β induction, and are characterized by a subset of IL-17-producing CD4⁺RORγt⁺ T cells that have been implicated in a range of inflammatory conditions [72]. Elevated numbers of Th17 cells have been reported in infants with RSV infection, and CD4⁺-derived IL-17 was found to induce airway epithelial IL-8 following RSV infection. In mice, IL-17 production in the lung of RSV-infected mice was associated with mucus hypersecretion [74–76] and depletion of IL-17 during RSV infection resulted in enhanced CD8⁺ T cell-mediated viral clearance and reduced mucus production and lung inflammation [73]. Importantly, and relevant to the idea of balance, studies have shown that Th17 stimulates neutrophil infiltration into lung tissue, and skews the response toward a Th2-like cytokine production profile while attenuating CD8⁺ T cell-mediated viral clearance [76].

T_{reg}, which finely balance pro- and anti-inflammatory responses by dampening excessive effector T-cell proliferation, also play a role in RSV infection [73,77]. For example, depletion of T_{reg} cells was associated with delayed CD8⁺ T-cell expansion and viral clearance, followed by excessive T cell and eosinophil recruitment and inflammation [78–80]. This fine balance is demonstrated by the functional redundancies within differentiation mechanisms: specifically, IL-21 is required for Th17 differentiation, but suppresses FoxP3 transcription and T_{reg} differentiation [73,81]. IL-10 is a cytokine that is produced by T_{reg} cells, but also promotes T_{reg} differentiation; interestingly, IL-10 is known to inhibit the production of Th17 cytokines [73,82]. In infectious inflammatory conditions like *Borrelia burgdorferi*-induced arthritis, IL-10 was found inhibit IL-17 production and IL-17-induced synovial inflammation

[83]. It is therefore highly likely that a feed-forward signaling mechanisms where T_{reg} and Th17 cells both operate during RSV infection drives the type of inflammatory response seen in humans and mouse models of RSV infection.

With a growing and increasingly complex network of signaling pathways and differentiated subsets, our understanding of how the host immune system shapes its response to RSV infection has vastly improved. A closely orchestrated process requiring innate recognition of viral RNA and finely balanced helper T-cell responses are keys for viral clearance and resolution, yet much remains to be understood about how best such responses can be coordinated to promote more efficient clearance of this virus, and to minimize physiological damage to airway tissue following localized inflammatory responses.

Conclusions

There is still no direct clinical or laboratory evidence that hMPV leads to exacerbation of asthma. However, there is sufficient evidence to reinforce the notion that such an association is plausible, especially when considering the correlations observed with RSV. A model of our current understanding is outlined in Figure 1. Asthma and COPD are serious diseases and frequent virus exacerbations increase morbidity and contribute to increased mortality. Taken together, this indicates that investigations into the role of hMPV as a causative agent for disease initiation and/or as a trigger for respiratory disease exacerbation is of considerable importance.

Funding

This work was supported by the Victorian Government's Operational Infrastructure Support Program; the Australian National Health and Medical Research Council [APP1047250 (to S.M.)]; Banyu Life Science Foundation International Fellowship; and the NHMRC Senior Research Fellowship [grant number APP1059167 (to S.M.)].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AHR, airway hyperresponsiveness; COPD, chronic obstructive pulmonary disease; hMPV, human metapneumovirus; IFN, interferon; LPS, lipopolysaccharide; OVA, ovalbumin; p.i., post-infection; RSV, respiratory syncytial virus; RV, rhinovirus; TLR, toll-like receptor.

References

- Boivin, G., De Serres, G., Côté, S., Gilca, R., Abed, Y., Rochette, L. et al. (2003) Human metapneumovirus infections in hospitalized children. *Emerg. Infect. Dis.* **9**, 634–640
- Esper, F., Martinello, R.A., Boucher, D., Weibel, C., Ferguson, D., Landry, M.L. et al. (2004) A 1-year experience with human metapneumovirus in children aged <5 years. *J. Infect. Dis.* **189**, 1388–1396
- Gern, J.E. (2004) Viral respiratory infection and the link to asthma. *Pediatr. Infect. Dis. J.* **23**, S78–S86
- Jartti, T., van den Hoogen, B., Garofalo, R.P., Osterhaus, A.D.M.E. and Ruuskanen, O. (2002) Metapneumovirus and acute wheezing in children. *Lancet Lond. Engl.* **360**, 1393–1394
- Jartti, T., Lehtinen, P., Vuorinen, T., Osterback, R., van den Hoogen, B., Osterhaus, A.D.M.E. et al. (2004) Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg. Infect. Dis.* **10**, 1095–1101
- Mullins, J.A., Erdman, D.D., Weinberg, G.A., Edwards, K., Hall, C.B., Walker, F.J. et al. (2004) Human metapneumovirus infection among children hospitalized with acute respiratory illness. *Emerg. Infect. Dis.* **10**, 700–705
- Ijpm, F.F.A., Beekhuis, D., Cotton, M.F., Pieper, C.H., Kimpen, J.L.L., van den Hoogen, B.G. et al. (2004) Human metapneumovirus infection in hospital referred South African children. *J. Med. Virol.* **73**, 486–493
- Williams, J.V., Harris, P.A., Tollefson, S.J., Halburnt-Rush, L.L., Pingsterhaus, J.M., Edwards, K.M. et al. (2004) Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N. Engl. J. Med.* **350**, 443–450
- Williams, J.V., Tollefson, S.J., Heymann, P.W., Carper, H.T., Patrie, J. and Crowe, J.E. (2005) Human metapneumovirus infection in children hospitalized for wheezing. *J. Allergy Clin. Immunol.* **115**, 1311–1312
- García-García, M.L., Calvo, C., Martín, F., Pérez-Breña, P., Acosta, B. and Casas, I. (2006) Human metapneumovirus infections in hospitalised infants in Spain. *Arch. Dis. Child* **91**, 290–295
- García-García, M.L., Calvo, C., Pérez-Breña, P., De Cea, J.M., Acosta, B. and Casas, I. (2006) Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. *Pediatr. Pulmonol.* **41**, 863–871
- Edwards, K.M., Zhu, Y., Griffin, M.R., Weinberg, G.A., Hall, C.B., Szilagyi, P.G. et al. (2013) Burden of human metapneumovirus infection in young children. *N. Engl. J. Med.* **368**, 633–643
- Kwon, J.-M., Shim, J.W., Kim, D.S., Jung, H.L., Park, M.S. and Shim, J.Y. (2014) Prevalence of respiratory viral infection in children hospitalized for acute lower respiratory tract diseases, and association of rhinovirus and influenza virus with asthma exacerbations. *Korean J. Pediatr.* **57**, 29–34

- 14 Widmer, K., Griffin, M.R., Zhu, Y., Williams, J.V. and Talbot, H.K. (2014) Respiratory syncytial virus- and human metapneumovirus-associated emergency department and hospital burden in adults. *Influenza Other Respir. Viruses* **8**, 347–352
- 15 Widmer, K., Zhu, Y., Williams, J.V., Griffin, M.R., Edwards, K.M. and Talbot, H.K. (2012) Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J. Infect. Dis.* **206**, 56–62
- 16 Dulek, D.E. and Peebles, R.S. (2011) Viruses and asthma. *Biochim. Biophys. Acta* **1810**, 1080–1090
- 17 García-García, M.L., Calvo, C., Casas, I., Bracamonte, T., Rellán, A., Gozalo, F. et al. (2007) Human metapneumovirus bronchiolitis in infancy is an important risk factor for asthma at age 5. *Pediatr. Pulmonol.* **42**, 458–464
- 18 Laham, F.R., Israele, V., Casellas, J.M., Garcia, A.M., Lac Prugent, C.M., Hoffman, S.J. et al. (2004) Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. *J. Infect. Dis.* **189**, 2047–2056
- 19 Tsukagoshi, H., Ishioka, T., Noda, M., Kozawa, K. and Kimura, H. (2013) Molecular epidemiology of respiratory viruses in virus-induced asthma. *Front. Microbiol.* **4**, 278
- 20 Rawlinson, W.D., Waliuzzaman, Z., Carter, I.W., Belessis, Y.C., Gilbert, K.M. and Morton, J.R. (2003) Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. *J. Infect. Dis.* **187**, 1314–1318
- 21 Singh, A.M., Moore, P.E., Gern, J.E., Lemanske, R.F. and Hartert, T.V. (2007) Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. *Am. J. Respir. Crit. Care Med.* **175**, 108–119
- 22 Kusel, M.M.H., de Klerk, N.H., Kebadze, T., Vohma, V., Holt, P.G., Johnston, S.L. et al. (2007) Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J. Allergy Clin. Immunol.* **119**, 1105–1110
- 23 Sigurs, N., Bjarnason, R., Sigurbergsson, F. and Kjellman, B. (2000) Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am. J. Respir. Crit. Care Med.* **161**, 1501–1507
- 24 Wu, P. and Hartert, T.V. (2011) Evidence for a causal relationship between respiratory syncytial virus infection and asthma. *Expert Rev. Anti Infect. Ther.* **9**, 731–745
- 25 Williams, J.V., Crowe, J.E., Enriquez, R., Minton, P., Peebles, R.S., Hamilton, R.G. et al. (2005) Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. *J. Infect. Dis.* **192**, 1149–1153
- 26 Seemungal, T., Harper-Owen, R., Bhowmik, A., Moric, I., Sanderson, G., Message, S. et al. (2001) Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **164**, 1618–1623
- 27 Martinello, R.A., Esper, F., Weibel, C., Ferguson, D., Landry, M.L. and Kahn, J.S. (2006) Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. *J. Infect.* **53**, 248–254
- 28 Rohde, G., Borg, I., Arinir, U., Kronsbein, J., Rausse, R., Bauer, T. et al. (2005) Relevance of human metapneumovirus in exacerbations of COPD. *Respir. Res.* **6**, 150
- 29 Vicente, D., Montes, M., Cilla, G. and Pérez-Trallero, E. (2004) Human metapneumovirus and chronic obstructive pulmonary disease. *Emerg. Infect. Dis.* **10**, 1338–1339
- 30 Zwaans, W.a.R., Mallia, P., van Winden, M.E.C. and Rohde, G.G.U. (2014) The relevance of respiratory viral infections in the exacerbations of chronic obstructive pulmonary disease—a systematic review. *J. Clin. Virol Off Publ. Pan. Am. Soc. Clin. Virol.* **61**, 181–188
- 31 Kan-O, K., Ramirez, R., MacDonald, M.I., Rolph, M., Rudd, P.A., Spann, K.M. et al. (2017) Human metapneumovirus infection in chronic obstructive pulmonary disease: impact of glucocorticosteroids and interferon. *J. Infect. Dis.* **215**, 1536–1545, doi:10.1093/infdis/jix167
- 32 Nissen, M.D., Siebert, D.J., Mackay, I.M., Sloots, T.P. and Withers, S.J. (2002) Evidence of human metapneumovirus in Australian children. *Med. J. Aust.* **176**, 188
- 33 Peret, T.C.T., Boivin, G., Li, Y., Couillard, M., Humphrey, C., Osterhaus, A.D.M.E. et al. (2002) Characterization of human metapneumoviruses isolated from patients in North America. *J. Infect. Dis.* **185**, 1660–1663
- 34 Freymouth, F., Vabret, A., Legrand, L., Etteradossi, N., Lafay-Delaire, F., Brouard, J. et al. (2003) Presence of the new human metapneumovirus in French children with bronchiolitis. *Pediatr. Infect. Dis. J.* **22**, 92–94
- 35 Zhang, Y., Niewiesk, S. and Li, J. (2014) Small animal models for human metapneumovirus: cotton rat is more permissive than hamster and mouse. *Pathog. Basel Switz* **3**, 633–655
- 36 MacPhail, M., Schickli, J.H., Tang, R.S., Kaur, J., Robinson, C., Fouchier, R.A.M. et al. (2004) Identification of small-animal and primate models for evaluation of vaccine candidates for human metapneumovirus (hMPV) and implications for hMPV vaccine design. *J. Gen. Virol.* **85**, 1655–1663
- 37 Skiadopoulos, M.H., Biacchesi, S., Buchholz, U.J., Riggs, J.M., Surman, S.R., Amaro-Carambot, E. et al. (2004) The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. *J. Virol.* **78**, 6927–6937
- 38 Alvarez, R., Harrod, K.S., Shieh, W.-J., Zaki, S. and Tripp, R.A. (2004) Human metapneumovirus persists in BALB/c mice despite the presence of neutralizing antibodies. *J. Virol.* **78**, 14003–14011
- 39 Darniot, M., Petrella, T., Aho, S., Pothier, P. and Manoha, C. (2005) Immune response and alteration of pulmonary function after primary human metapneumovirus (hMPV) infection of BALB/c mice. *Vaccine* **23**, 4473–4480
- 40 Lai, S.-H., Liao, S.-L., Wong, K.-S. and Lin, T.-Y. (2014) Defective innate immune responses to respiratory syncytial virus infection in ovalbumin-sensitized mice. *J. Microbiol. Immunol. Infect. Wei Mian Yu Gan Ran Za Zhi* **50**, 17–25
- 41 Kolli, D., Bataki, E.L., Spetch, L., Guerrero-Plata, A., Jewell, A.M., Piedra, P.A. et al. (2008) T lymphocytes contribute to antiviral immunity and pathogenesis in experimental human metapneumovirus infection. *J. Virol.* **82**, 8560–8569
- 42 Hamelin, M.-Ç., Prince, G.A., Gomez, A.M., Kinkead, R. and Boivin, G. (2006) Human metapneumovirus infection induces long-term pulmonary inflammation associated with airway obstruction and hyperresponsiveness in mice. *J. Infect. Dis.* **193**, 1634–1642
- 43 Liu, Y., Haas, D.L., Poore, S., Isakovic, S., Gahan, M., Mahalingam, S. et al. (2009) Human metapneumovirus establishes persistent infection in the lungs of mice and is reactivated by glucocorticoid treatment. *J. Virol.* **83**, 6837–6848

- 44 Herd, K.A., Mahalingam, S., Mackay, I.M., Nissen, M., Sloots, T.P. and Tindle, R.W. (2006) Cytotoxic T-lymphocyte epitope vaccination protects against human metapneumovirus infection and disease in mice. *J. Virol.* **80**, 2034–2044
- 45 Alvarez, R. and Tripp, R.A. (2005) The immune response to human metapneumovirus is associated with aberrant immunity and impaired virus clearance in BALB/c mice. *J. Virol.* **79**, 5971–5978
- 46 Baños-Lara, M.D.R., Harvey, L., Mendoza, A., Simms, D., Chouljenko, V.N., Wakamatsu, N. et al. (2015) Impact and regulation of lambda interferon response in human metapneumovirus infection. *J. Virol.* **89**, 730–742
- 47 Spann, K.M., Loh, Z., Lynch, J.P., Ullah, A., Zhang, V., Baturcam, E. et al. (2014) IRF-3, IRF-7, and IPS-1 promote host defense against acute human metapneumovirus infection in neonatal mice. *Am. J. Pathol.* **184**, 1795–1806
- 48 Baños-Lara, M.D.R., Ghosh, A. and Guerrero-Plata, A. (2013) Critical role of MDA5 in the interferon response induced by human metapneumovirus infection in dendritic cells and in vivo. *J. Virol.* **87**, 1242–1251
- 49 Borchers, A.T., Chang, C., Gershwin, M.E. and Gershwin, L.J. (2013) Respiratory syncytial virus—a comprehensive review. *Clin. Rev. Allergy Immunol.* **45**, 331–379
- 50 Rodriguez, R. and Ramilo, O. (2014) Respiratory syncytial virus: how, why and what to do. *J. Infect.* **68**, S115–S118
- 51 Ruckwardt, T.J., Morabito, K.M. and Graham, B.S. (2016) Determinants of early life immune responses to RSV infection. *Curr. Opin. Virol.* **16**, 151–157
- 52 Lotz, M.T., Moore, M.L. and Peebles, R.S. (2013) Respiratory syncytial virus and reactive airway disease. *Curr. Top. Microbiol. Immunol.* **372**, 105–118
- 53 Peebles, R.S. (2004) Viral infections, atopy, and asthma: is there a causal relationship? *J. Allergy Clin. Immunol.* **113**, S15–S18
- 54 Bacharier, L.B., Cohen, R., Schweiger, T., Yin-Declue, H., Christie, C., Zheng, J. et al. (2012) Determinants of asthma after severe respiratory syncytial virus bronchiolitis. *J. Allergy Clin. Immunol.* **130**, 91.e3–100.e3
- 55 Knudson, C.J. and Varga, S.M. (2015) The relationship between respiratory syncytial virus and asthma. *Vet. Pathol.* **52**, 97–106
- 56 Pickles, R.J. and DeVincenzo, J.P. (2015) Respiratory syncytial virus (RSV) and its propensity for causing bronchiolitis. *J. Pathol.* **235**, 266–276
- 57 Sigurs, N., Bjarnason, R., Sigurbergsson, F., Kjellman, B. and Björkstén, B. (1995) Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* **95**, 500–505
- 58 Han, J., Takeda, K., Gelfand, E.W., Han, J., Takeda, K. and Gelfand, E.W. (2011) The role of RSV infection in asthma initiation and progression: findings in a mouse model. *Pulm. Med. Pulm. Med.* **2011**, e748038
- 59 Finkelman, F.D., Hogan, S.P., Hershey, G.K.K., Rothenberg, M.E. and Wills-Karp, M. (2010) Importance of cytokines in murine allergic airway disease and human asthma. *J. Immunol. Baltim. Md. 1950* **184**, 1663–1674
- 60 Phipps, S., Lam, C.E., Mahalingam, S., Newhouse, M., Ramirez, R., Rosenberg, H.F. et al. (2007) Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus. *Blood* **110**, 1578–1586
- 61 Rosenberg, H.F., Dyer, K.D. and Domachowske, J.B. (2009) Respiratory viruses and eosinophils: exploring the connections. *Antiviral Res.* **83**, 1–9
- 62 Bullens, D.M., Truyen, E., Coteur, L., Dilissen, E., Hellings, P.W., Dupont, L.J. et al. (2006) IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res.* **7**, 135
- 63 Chesné, J., Braza, F., Mahay, G., Brouard, S., Aronica, M. and Magnan, A. (2014) IL-17 in Severe asthma. where do we stand? *Am. J. Respir. Crit. Care Med.* **190**, 1094–101
- 64 Chien, J.-W., Lin, C.-Y., Yang, K.D., Lin, C.-H., Kao, J.-K. and Tsai, Y.-G. (2013) Increased IL-17A secreting CD4+ T cells, serum IL-17 levels and exhaled nitric oxide are correlated with childhood asthma severity. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* **43**, 1018–1026
- 65 Lukacs, N.W., Tekkanat, K.K., Berlin, A., Hogaboam, C.M., Miller, A., Evanoff, H. et al. (2001) Respiratory syncytial virus predisposes mice to augmented allergic airway responses via IL-13-mediated mechanisms. *J. Immunol. Baltim. Md. 1950* **167**, 1060–1065
- 66 Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L. et al. (1998) Interleukin-13: central mediator of allergic asthma. *Science* **282**, 2258–2261
- 67 Regamey, N. and Kaiser, L. (2008) Rhinovirus infections in infants: is respiratory syncytial virus ready for the challenge? *Eur. Respir. J.* **32**, 249–251
- 68 Spann, K.M., Baturcam, E., Schagen, J., Jones, C., Straub, C.P., Preston, F.M. et al. (2014) Viral and host factors determine innate immune responses in airway epithelial cells from children with wheeze and atopy. *Thorax* **69**, 918–925
- 69 Berke, I.C., Li, Y. and Modis, Y. (2013) Structural basis of innate immune recognition of viral RNA. *Cell. Microbiol.* **15**, 386–394
- 70 Mukherjee, S. and Lukacs, N.W. (2013) Innate immune responses to respiratory syncytial virus infection. *Curr. Top. Microbiol. Immunol.* **372**, 139–154
- 71 Kurt-Jones, E.A., Popova, L., Kwinn, L., Haynes, L.M., Jones, L.P., Tripp, R.A. et al. (2000) Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* **1**, 398–401
- 72 Dong, C. (2008) Th17 cells in development: an updated view of their molecular identity and genetic programming. *Nat. Rev. Immunol.* **8**, 337–348
- 73 Mangoldt, T.C., Van Herck, M.A., Nullens, S., Ramet, J., De Dooy, J.J., Jorens, P.G. et al. (2015) The role of Th17 and Treg responses in the pathogenesis of RSV infection. *Pediatr. Res.* **78**, 483–491
- 74 Mukherjee, S., Lindell, D.M., Berlin, A.A., Morris, S.B., Shanley, T.P., Hershenson, M.B. et al. (2011) IL-17-induced pulmonary pathogenesis during respiratory viral infection and exacerbation of allergic disease. *Am. J. Pathol.* **179**, 248–258
- 75 Stoppelenburg, A.J., de Rooij, S., Hennis, M.P., Bont, L. and Boes, M. (2014) Elevated Th17 response in infants undergoing respiratory viral infection. *Am. J. Pathol.* **184**, 1274–1279
- 76 Stoppelenburg, A.J., Salimi, V., Hennis, M., Plantinga, M., in't Veld, R.H., Walk, J. et al. (2013) Local IL-17A potentiates early neutrophil recruitment to the respiratory tract during severe RSV infection. *PLoS ONE* **8**, e78461
- 77 Kimura, A. and Kishimoto, T. (2010) IL-6: regulator of Treg/Th17 balance. *Eur. J. Immunol.* **40**, 1830–1835
- 78 Loebbermann, J., Thornton, H., Durant, L., Sparwasser, T., Webster, K.E., Sprent, J. et al. (2012) Regulatory T cells expressing granzyme B play a critical role in controlling lung inflammation during acute viral infection. *Mucosal Immunol.* **5**, 161–172
- 79 Raiden, S., Pandolfi, J., Payaslian, F., Anderson, M., Rivarola, N., Ferrero, F. et al. (2014) Depletion of circulating regulatory T cells during severe respiratory syncytial virus infection in young children. *Am. J. Respir. Crit. Care Med.* **189**, 865–868

- 80 Ruckwardt, T.J., Bonaparte, K.L., Nason, M.C. and Graham, B.S. (2009) Regulatory T cells promote early influx of CD8+ T cells in the lungs of respiratory syncytial virus-infected mice and diminish immunodominance disparities. *J. Virol.* **83**, 3019–3028
- 81 Bi, Y. and Yang, R. (2012) Direct and indirect regulatory mechanisms in TH17 cell differentiation and functions. *Scand. J. Immunol.* **75**, 543–552
- 82 Gu, Y., Yang, J., Ouyang, X., Liu, W., Li, H., Yang, J. et al. (2008) Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. *Eur. J. Immunol.* **38**, 1807–1813
- 83 Hansen, E.S., Medić, V., Kuo, J., Warner, T.F., Schell, R.F. and Nardelli, D.T. (2013) Interleukin-10 (IL-10) inhibits *Borrelia burgdorferi*-induced IL-17 production and attenuates IL-17-mediated Lyme arthritis. *Infect. Immun.* **81**, 4421–4430