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Perspective on the host response to human metapneumovirus infection: what can we learn from respiratory syncytial virus infections?

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Abstract

Human metapneumovirus (HMPV) is a recently discovered pathogen first identified in respiratory specimens from young children suffering from clinical respiratory syndromes ranging from mild to severe lower respiratory tract illness. HMPV has worldwide prevalence, and is a leading cause of respiratory tract infection in the first years of life, with a spectrum of disease similar to respiratory syncytial virus (RSV). The disease burden associated with HMPV infection has not been fully elucidated; however, studies indicate that HMPV may cause upper or lower respiratory tract illness in patients between ages 2 months and 87 years, may co-circulate with RSV, and HMPV infection may be associated with asthma exacerbation. The mechanisms and effector pathways contributing to immunity or disease pathogenesis following infection are not fully understood; however, given the clinical significance of HMPV, there is a need for a fundamental understanding of the immune and pathophysiological processes that occur following infection to provide the foundation necessary for the development of effective vaccine or therapeutic intervention strategies. This review provides a current perspective on the processes associated with HMPV infection, immunity, and disease pathogenesis.

Keywords: Respiratory syncytial virus; Human metapneumovirus; Asthma

1. Introduction

Seroprevalence studies in children and adults indicate that human metapneumovirus (HMPV) is not an emerging virus, but a newly recognized pathogen that may mediate serious lower respiratory tract illness in very young children, the elderly and immunocompromised patients [1–3]. The virus has been circulating in the population for at least 50 years [1]. Infectious HMPV can be isolated from nasopharyngeal aspirates [4,5] suggesting that infection is associated with the respiratory epithelium; however, the mechanisms contributing to infection, the cells targeted for infection, the host response to infection, and the pathophysiology associated with infection are not well understood. Based on genetic and clinical similarities between HMPV and respiratory syncytial virus (RSV) [6,7], it is likely that HMPV and RSV proteins have similar functions related to replication, pathophysiology and immunity. The current perspective on these processes and HMPV and RSV proteins is discussed.

1.1. Incidence of HMPV infections

Seroprevalence studies have shown that HMPV has worldwide distribution, is acquired early in life, and by the age of 5 years, approximately 70% of all children develop antibodies to HMPV [1,8–10]. HMPV is often identified in isolates from children hospitalized with acute respiratory tract illness, is associated with clinical diagnosis of pneumonia, asthma exacerbation, and acute bronchiolitis ([1–5,11–17] Nissen, unpublished data). For example, the incidence of
HMPV in the Dutch population during the winter months of 2000 was approximately 10% [1]; however, prospective observation for HMPV disease worldwide has shown a wide range of incidence from 1.5% to 41% [9,11]. HMPV primarily afflicts children younger than 2 years of age, in particular, those younger than 12 months of age ([1,8] Nissen, unpublished data). Several reports of HMPV infection in children have shown a trend toward infection in males compared to females ([1,8] Nissen, unpublished data). HMPV infection in older children and adults has been observed, particularly in immunocompromised and transplant patients, or those having a pre-existing chronic lung condition [16,17]. HMPV has also been identified as a potentially significant pathogen in transplant patients [18]. Little is known regarding the impact of differing socio-economic environments on the age of first infection with HMPV, or about the impact on disease severity.

1.2. Seasonal patterns

The seasonality of HMPV epidemics appears to follow a similar trend to RSV having a seasonal period from winter to spring [19,20]. Although the disease burden of HMPV is not fully understood, it appears that the appearance of HMPV in the community tends to follow the presence of RSV and influenza which occur in early to mid-winter, but precedes that of Parainfluenza virus 3 (PIV 3) disease activity which generally occurs in late spring to early summer (Nissen, unpublished data). Several reports of HMPV infection in children have shown a trend toward infection in males compared to females ([1,8] Nissen, unpublished data). HMPV infection in older children and adults has been observed, particularly in immunocompromised and transplant patients, or those having a pre-existing chronic lung condition [16,17]. HMPV has also been identified as a potentially significant pathogen in transplant patients [18]. Little is known regarding the impact of differing socio-economic environments on the age of first infection with HMPV, or about the impact on disease severity.

1.3. Strain variation

Little is known regarding strain variation and disease pathogenesis associated with HMPV infection. Mounting evidence suggests that both strain A and B HMPV cause annual outbreaks of infection [16,21], and this may be mediated by one or more of the four known sub-lineages (A1, A2, B1, B2) of HMPV [22]. Similar to RSV [23–25], the predominance of a HMPV strain circulating in the community may be related to amino acid sequence variation in surface protein genes; however, the mechanisms affecting immunity and virus replication are not fully understood. There is some evidence that HMPV does not elicit a cross-protective immune response [26], and can persist as infectious virus in the lungs of mice despite the presence of neutralizing antibodies [27].

1.4. Route of transmission

The route of transmission of HMPV infection in the community has yet to be determined, but is likely similar to the pattern described for RSV where the infection may be introduced via an older family member [28] via respiratory droplets as occurs with RSV [29], or by hand-to-mouth or hand-to-eye contact with contaminated surfaces [30].

1.5. Clinical features

The spectrum and severity of acute clinical disease associated with HMPV infection compared to RSV has been investigated in small groups of patients. In the original report by van den Hoogen et al., the clinical symptoms described were largely similar to those caused by RSV which included a range of clinical features from mild respiratory problems to bronchiolitis, severe cough, high fever, myalgia, and pneumonia [1]. Other studies have confirmed this initial description, and shown that HMPV as an important lower respiratory tract pathogen in children that is difficult to distinguish clinically from RSV ([1,16,31] Nissen, unpublished data). In general, acute HMPV infection appears to be less severe than that caused by RSV; however, HMPV infection has been associated with several cases of severe pneumonitis leading to death ([16–18] Nissen, unpublished data).

Some of the clinical features of HMPV disease in young hospitalized children are compared with the clinical features of young children hospitalized with RSV disease (Table 1). Common clinical features for HMPV infection may include bronchiolitis (27–59%), pneumonia (8–27%), virus associated asthma exacerbation (5–27%), laryngotracheobronchitis (croup) (3–18%), apnoea (12%), and otitis media (12%) ([8,22] Nissen, unpublished data). When a chest radiograph was performed, abnormalities were noted in 39–84% of HMPV cases, with bilateral pneumatic infiltrates and hyperinflation the most frequent ([8,31] Nissen, unpublished data). Acute complications associated with HMPV infection led to oxygen therapy (32–54%), intensive care admission (8–15%), or mechanical ventilation (5–8%) ([8] Nissen, unpublished data). HMPV re-infection has been observed [17,32]; however little is known regarding the outcome of re-infection. Similar to RSV infection, a wide spectrum of potential risk factors for HMPV infection has been examined, but none have been conclusively linked with disease [8]. There is mounting evidence that HMPV infection may lead to asthma or chronic lung disease [12,32–34], and it has been suggested that there may be an increased risk of post-infectious bronchial hyperreactivity following HMPV infection (Nissen, unpublished data). However, there are conflicting reports to the contrary [31,35], thus it remains unclear if HMPV may cause or precipitate conditions related to asthma pathogenesis.

RSV infection is the most common cause of viral nosocomial infection in pediatric wards, and represents a significant additional burden on health care resources [36,37]. Although

| Symptom                  | HMPV cases | Percentage |
|--------------------------|------------|------------|
| Cough                    | 46–92      | 97–100     |
| Fever                    | 50–92      | 45–65      |
| Respiratory distress     | 43–83      | 36–78      |
| Rhinitis/rhinorhoea      | 57–83      | 56–82      |
| Respiratory crackles/tâles | 57       | 27–72      |
| Wheezing/khonchi         | 45–50      | 45–78      |
| Pharyngitis              | 19–43      | 45–54      |
| Otitis media             | 16         | 31         |
| Conjunctivitis           | 14         | 9          |

Table 1
Symptoms and signs of HMPV infection compared with human RSV infection in hospitalized children from cited published studies
the disease burden of HMPV is unclear, recent evidence suggests that nosocomial infections with HMPV may be similar to those caused by RSV (Nissen, unpublished data). Given similar mode of transmission, it is likely that similar infection control practices should be applied for HMPV as for RSV, particularly in neonatal health care settings.

The incidence of co-infections with HMPV has not been adequately defined, but co-infections with other viral respiratory pathogens may occur, e.g. RSV, influenza virus and SARS-coronavirus [5,16,38,39]. Boivin et al. [16] reported that in Canada, 24% (nine of 38) of HMPV-positive isolates were co-infected with other viruses; however, it is not known if viral co-infections affect the severity of HMPV disease [38]. Similarly, in a study examining the overall impact of HMPV infection in 1505 children, of 42 HMPV-positive samples tested, there was RSV or influenza virus co-infection rate of 16.7% (39). The symptoms associated with HMPV co-infection were presentation of fever that was more frequent in the HMPV- and influenza-positive children and wheezing with bronchiolitis or asthma exacerbation that was more frequent among HMPV- and RSV-positive cases. Significant clinical respiratory bacterial infections associated with HMPV detection have also been reported, though less frequently than clinical respiratory bacterial infections associated with HMPV. van den Hoogen et al. [1] noted that the virus was not detected in respiratory samples taken from 400 asymptomatic children younger than 2 years of age.

1.6. Immune response to HMPV infection

The agents of immunity provide host resistance to infection, and deficiencies or absence of aspects of the immune response may lead to a prolonged period of recovery, subsequent re-infection, or potentially persistence [30]. To date, the majority of studies examining the immune response to HMPV infection have been restricted to animal models, and only limited data are available regarding the immune response to HMPV infection in the human host. Preliminary human studies from our laboratory indicate that both serum and secretory antibodies are produced in response to infection with HMPV; however, the role of these immune components in long-term protection against re-infection with HMPV is unknown but the subject of ongoing studies.

Studies in animal models have provided the foundation for understanding aspects of immunity and disease pathogenesis associated with human viral pathogens. Given the clinical and genomic similarities between RSV and HMPV (Table 1 and Fig. 1), it is important to consider what has been learned from RSV studies in animal models to guide prospective HMPV studies, and important to note that none of the HMPV proteins have yet been fully biochemically characterized. RSV has two major surface proteins important for infection, i.e. the attachment (G) and fusion (F) proteins. RSV infects respiratory epithelium by interaction of heparin-binding domains on these surface proteins with glycosaminoglycans (GAGs) on the cell surface [40,41]; however, G protein contributes to the majority of virus binding [42,43]. In addition, the non-glycosylated, central conserved region of the G protein contains a CX3C chemokine motif at amino acid positions 182–186 that is capable of interacting with the CX3C chemokine receptor, CX3CR1, and facilitating infection [44]. The putative HMPV G protein does not appear to have a CX3C chemokine motif; however similar to RSV, appears to consist of a type II mucin-like glycoprotein based on the distribution of hydrophobic and hydrophilic regions along the predicted amino acid sequence [21]. The putative HMPV F protein, like RSV, has two heptad repeats that for RSV are required for viral fusion [45,46], thus the RSV and HMPV G and F proteins appear to have similar features necessary for infection. The cell types in the lung targeted by HMPV are not known. RSV primarily infects type II respiratory epithelial cells, and in human primary airway epithelial cell cultures, specifically targets ciliated columnar cells [47]. There is mounting evidence that RSV may cause persistent or latent infection, as RSV protein and genomic RNA have been detected in mouse lungs for at least 180 days post-infection, as well as in lung alveolar macrophages in guinea pigs, in murine macrophage-like cells and macrophage cultures, and in B cells following infection with bovine RSV [30,48]. These features allude to the possibility that RSV persistence may serve as a reservoir for transmission, reinfection, or contribute to the pathogenesis of chronic wheezing and asthma in children who have experienced bronchiolitis. It is not known if HMPV persists in humans; however, studies from our laboratories have shown that infectious HMPV persists in the lungs of BALB/c mice up to day 60 post-infection (p.i.) despite the presence of neutralizing antibodies, and genomic RNA can be detected in the lungs for ≥180 days p.i. by RT-PCR [27]. Consistent with attenuated responses and virus persistence [49], HMPV infection in young children appears to induce a restricted chemokine-mediated inflammatory response, particularly compared to RSV infection [10]. Our recent studies from examining features of the innate and adaptive immune response to HMPV infection in a BALB/c mouse model have shown that primary HMPV infection elicits weak innate and aberrant adaptive immune response (Alvarez et al., in press). These responses are characterized by induction of a Th2-type cytokine response at later stages of infection that coincides with increased IL-10 expression and persistent virus replication in the lung. Examination of the CTL and antibody response to HMPV infection revealed a delayed response, but passive transfer of HMPV-specific antibodies provided considerable protection (Alvarez et al., in press). These features are consistent with virus persistence, the existence of complex immune interactions in response to HMPV infection, and that the immune response to HMPV is unique compared to RSV infection in BALB/c mice.

Innate immune responses provide the first line of defense against infection, and affect the magnitude and quality of the
acquired immune response. Conserved structural motifs on pathogens trigger pattern recognition receptors, e.g. Toll-like receptors (TLRs), which initiate signaling cascades, activation of the transcription factor NF-κB, and expression of proinflammatory and effector cytokines required to direct the acquired immune response [50,51]. It is not known if HMPV proteins interact with TLRs; however, the RSV F protein has been shown to stimulate innate immunity through activation of CD14 and TLR4 [52], and TLR4-deficient mice challenged with RSV exhibit impaired pulmonary NK and CD14+ cell trafficking, deficient NK cell function, impaired interleukin IL-12 expression, and impaired virus clearance [53]. Airway epithelial cells express a variety of functionally active TLRs including TLR4 [54], and RSV infection of respiratory epithelial cells has been shown to induce increased TLR4 mRNA expression and TLR4 membrane localization [55]. In addition, children experiencing RSV bronchiolitis have significantly higher TLR4 expression on peripheral blood monocytes compared to controls which remains elevated for 4-6 weeks as the children enter the convalescent phase of RSV bronchiolitis [56]. These results suggest an important role for TLR4 in the immune response to RSV infection and associated disease pathogenesis. The motif on the RSV F protein that triggers TLR4 activation is unknown, but there is 30–43% amino acid identity between RSV and HMPV F protein [45], thus there is a possibility that HMPV F protein may have similar features. Since TLRs are important initiators of inflammation and immunity, one consequence of RSV-mediated TLR4 activation may be initiation of pathophysiologic processes that exacerbate obstructive airways. The innate immune response to RSV infection is also modified by G protein expression. Studies in mice have shown that RSV G protein...
expression is associated with aberrant T cell responses [57], altered pulmonary NK cell and PMN trafficking [58], modified CC and CXC chemokine mRNA expression by broncho-alveolar leukocytes [59], and CX3C chemokine mimicry that affects leukocyte chemotaxis and fractalkine-mediated responses [44]. It is possible that HMPV G protein may also mediate immune modulatory effects; however, the putative HMPV G protein does not appear to contain a CX3C chemokine motif, and its deduced amino acid sequence analysis suggests it is highly divergent from RSV G protein [6,21,60].

Little is known about the acquired immune response to HMPV infection in either animal models or humans. In contrast, the protective antibody response to RSV infection is directed against the two major viral proteins, i.e. F and G, and serum neutralizing antibody responses are primarily directed against the F protein [61]. Although the neutralization and protective antigens of HMPV remain to be determined, it is likely that the high degree of divergence of the G protein between and within HMPV subgroups [21,60] will contribute to limited cross-neutralizing and cross-protective antibody responses as has been observed for RSV [61]. The T cell response to HMPV infection in humans has not been characterized; however recent studies from our laboratories have shown that antibody-mediated depletion of T-cells or DXS+ cells in BALB/c mice results in increased HMPV titers in the lungs of infected mice suggesting that cytotoxic cells control aspects of replication and may control HMPV persistence [27].

2. Links between respiratory viral infections and asthma exacerbations

Asthma is a chronic, episodic inflammatory disease of the airways where inflammation is the cause of airway hyperreactivity with associated narrowing of the small airways and variable airflow in response to triggering events that include allergens, viral infections, and exposure to airway irritants [62–66]. Asthma is a major cause of morbidity and mortality affecting >14 million people in the United States, and is the most common chronic disease of childhood, affecting >4.8 million children [67–69]. Our understanding of the multifactorial nature of asthma is incomplete, and little is known regarding viral mechanisms that may contribute to asthma disease pathogenesis. Consistent with asthma being the most common chronic disease of childhood [68], RSV is recognized as the primary cause of morbidity and life-threatening lower respiratory tract disease in infants and young children worldwide, and numerous studies suggest linkages between RSV infection in early childhood, respiratory sequelae, and subsequent manifestations of asthma [64,69–71]. Several studies now indicate that HMPV infection may contribute to aspects of asthma pathogenesis [12,32–34]. Similar to RSV infection in infants and young children, it is likely that HMPV does not engender a fully protective immune response [72], as studies in mice have shown HMPV may cause persistent infection [27].

2.1. RSV and asthma pathogenesis

Several cross-sectional and prospective studies indicate that RSV infection in early childhood is linked to subsequent manifestations of asthma. For example, in school children, 80% of wheezing episodes have been associated with viral respiratory tract infections [73]. In infants and toddlers under the age of 2 years, RSV is the virus most frequently isolated during wheezing episodes [74]. In addition, epidemiological evidence suggests that RSV may also contribute to early allergen sensitization and subsequent development of childhood asthma. For example, Frick and colleagues observed a coincidence of respiratory infections and the onset of allergic sensitization to aeroallergens in infants with a positive family history of allergy [75]. In a prospective cohort study with matched controls, Sigurs and colleagues identified RSV bronchiolitis in infancy as the most important risk factor for the subsequent development of asthma and sensitization to common allergens by the age of 3 years [76]. This risk further increased if there was a family history of asthma or atopy. A follow-up report by the same group showed that by the age of 7 years, allergic sensitization and asthma were still more prevalent in the group of children who had had RSV bronchiolitis in the first year of life than in the controls [77]. Recently, Schauer et al. confirmed the increased risk for allergen sensitization and asthma symptoms in 12-month-old infants following RSV bronchiolitis [78]. Interestingly, sensitization was almost exclusively linked to food allergens, suggesting that pulmonary infection with RSV has a systemic impact on sensitization. However, several studies which have shown a link between RSV bronchiolitis and asthma do not find an association with allergen sensitization [79–81]. It is likely that the studies showing RSV infection with allergen sensitization reflect the relatively small minority of RSV-infected children who require hospitalization, thus selecting for those with a predisposition to severe RSV disease. Perhaps the most compelling study because of large birth cohort examined is from the Tucson children’s respiratory study group [76]. In this study all children with RSV infection during the first 3 years of life were followed, and the results showed that these children had an increased risk to wheeze at age 6, but this risk did not persist beyond age 13 years. In this study, RSV infection was not a risk factor for allergen sensitization indicating that only severe RSV bronchiolitis may be associated with early allergen sensitization.

It is also important to note that the majority of infants infected with RSV do not develop severe disease or asthma. This suggests that genetic predisposition is also important. Consistent with this hypothesis, several studies have shown that the Th2-type cytokine, IL-4, may be linked to the development of severe RSV disease in humans. Infants hospitalized with RSV bronchiolitis and having IL-4 and IL-4Rα gene variants, i.e. IL-4 590T allele and IL-4Rα chain R551 allele, developed more severe disease compared to case-control patients [82], and patients with a common IL-4 haplotype variant, i.e. −589T promoter variant, were shown to be over-
recently, O’Donnell et al. [93] reported the induction of allergen-specific antibodies in mice exposed to these allergens during RSV infection. More specifically, Leibovitz et al. [91] and Freihorst et al. [92] showed increased levels of allergen-specific antibodies and neutrophils, resulting in the development of airway hyperresponsiveness (AHR) [85,86]. AHR and pulmonary granular cell infiltration are hallmark features of bronchial asthma. The pattern of cytokine expression associated with RSV has also been shown to be an important feature that may contribute to disease pathogenesis. In the murine model, primary RSV infection leads to mixed Th1/Th2-type immune responses [30]; however, RSV G protein appears to sensitize for Th2-type responses characterized by IL-4, IL-5, IL-13 and IL-10 expression [30,57–59,87]. In addition, by using depleting antibodies and knock-out mice, Schwarze et al. defined critical roles for IL-5 and for CD8+ T-cells, but not IFN-γ, in the development of AHR in acute RSV infection [88,89]. In addition, IL-13-induced Stat-6 signaling also seems to be important for RSV-induced AHR [90]. To test the hypothesis that RSV infection may trigger and enhance allergic sensitization to inhaled antigens, numerous animal models of infection and concomitant allergen sensitization have been examined. For example, Leibovitz et al. [91] and Freihorst et al. [92] showed increased levels of allergen-specific antibodies to ovalbumin (OVA) and ragweed in serum (IgE, IgG) and in bronchoalveolar lavage (BAL) fluid (IgA, IgG) of mice exposed to these allergens during RSV infection. More recently, O’Donnell et al. [93] reported the induction of anaphylactic antibodies following infection with RSV and concomitant sensitization to OVA-aerosol, but not after sensitization without infection. The ability of RSV and other respiratory viruses to increase production of allergen-specific antibodies has also been shown in guinea pig models of RSV infection where virus infection leads to increased permeability of the respiratory mucosa [94,95]. This may be an important factor for virus-induced enhancement of airway sensitization.

RSV studies in animal models have shown that G protein expression is also associated with the induction of pulmonary Substance P (SP) expression [57–59,96]. SP may exacerbate expression of regulatory mediators, up-regulate cell surface receptors important in leukocyte activation and migration, and mediate pathophysiology associated with mucus production, vascular leakage, and induction of smooth muscle contraction [97–104]. In addition, pulmonary SP expression affects respiratory rates in RSV-infected mice [103]. The cytokine program associated with HMPV infection in humans is not known, but given the clinical similarities between RSV and HMPV infection [67,105] and our recent studies in mice (Alvarez et al., in press) it is likely that HMPV infection may induce similarly aberrant cytokine responses.

HMPV infection is associated with clinical diagnoses of pneumonia, asthma exacerbation, or acute bronchiolitis [12,33]. Emerging evidence suggests that following HMPV infection, wheezing is more likely to represent asthma exacerbation rather than acute bronchiolitis [12]. The disease burden associated with HMPV infection has not been fully defined; however, it is likely that HMPV may contribute to asthma pathogenesis in a fashion similar to RSV and be similarly linked with a similar level of asthma pathogenesis. Thus, there is a critical need to better understand the mechanisms of host–virus interaction and disease pathogenesis that may lead to or exacerbate asthma in order to provide the foundation for intervention strategies.

3. Concluding remarks

A better understanding of the protective and disease causing mechanisms associated with HMPV infection is needed for disease intervention strategies given the substantial disease burden associated with HMPV infection. HMPV predominantly causes respiratory tract infections in patients at the extremes of ages, thus unique HMPV disease intervention strategies may be required for young infants with maternal antibodies and the elderly with waning immunity. The insights revealed from RSV studies should be considered in guiding HMPV studies since these viruses have similar clinical outcomes. With proper translation of RSV findings from animal and human studies, it may be possible to improve our understanding of the host response to HMPV infection, understand the relationship between RSV and HMPV infection, and avoid disease intervention setbacks.

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