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Pathogenesis of acute respiratory illness caused by human parainfluenza viruses

Henrick Schomacker, Anne Schaap-Nutt, Peter L. Collins, and Alexander C. Schmidt*

Laboratory of Infectious Diseases, RNA Viruses Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services. Bethesda, MD, USA

Abstract

Human parainfluenza viruses (HPIVs) are a common cause of acute respiratory illness throughout life. Infants, children, and the immunocompromised are the most likely to develop severe disease. HPIV1 and HPIV2 are best known to cause croup while HPIV3 is a common cause of bronchiolitis and pneumonia. HPIVs replicate productively in respiratory epithelial cells and do not spread systemically unless the host is severely immunocompromised. Molecular studies have delineated how HPIVs evade and block cellular innate immune responses to permit efficient replication, local spread, and host-to-host transmission. Studies using *ex vivo* human airway epithelium have focused on virus tropism, cellular pathology and the epithelial inflammatory response, elucidating how events early in infection shape the adaptive immune response and disease outcome.

Introduction

Human parainfluenza viruses (HPIVs) are a common cause of acute respiratory infections (ARIs) in infants and young children, with 80% of children testing seropositive for HPIV1, 2, and 3 by age five [1]. Re-infection with HPIV of the same serotype can occur throughout life but secondary infections are typically limited to the upper respiratory tract (URT). The majority of HPIV patients are treated in outpatient settings, yet HPIVs are a major cause of hospitalization for lower respiratory illness (LRI) in children under five years of age, second only to respiratory syncytial virus (RSV) [2-5]. In this age group, HPIVs are a more common cause of hospitalization for ARI or fever than influenza viruses, with a hospitalization rate of 1/1000/year, or approximately 23,000 hospitalizations per year in the United States alone [4-6]. Of the HPIVs, HPIV3 is the most frequent cause of hospitalization, followed by HPIV1 and 2 [5]. HPIV4 has been reported to be a much less frequent cause of clinically significant respiratory illness [7,8], although a more recent study found HPIV4 in 10% of all HPIV positive samples in a daycare setting [9]. Although significant progress has been made in the past decade toward the development of vaccines for HPIV1, 2, and 3, a licensed vaccine is not yet available and neither are specific antiviral drugs [3,4,10-14].

*Corresponding author. Mailing address: LID, NIAID, NIH, 50 South Drive, Room 6515, MSC 8007, Bethesda, MD 20892. Phone: (301) 594-1650. Fax: (301) 480-1268. as337y@gmail.com.

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The nature of HPIV disease

HPIV infection usually initiates at the epithelium of the URT following exposure by contact or inhalation. Infection frequently spreads to the paranasal sinuses, larynx and bronchi, and obstruction of the Eustachian tubes can lead to otitis media. Each of the HPIVs has been associated with a similar broad spectrum of respiratory tract disease including the common cold, croup, bronchitis, bronchiolitis, and pneumonia, but certain serotypes are more frequently associated with certain illnesses [3,4,10,11]. For example, HPIV1 and 2 are more frequently associated with laryngotracheobronchitis (croup), and HPIV3 is more likely than HPIV1 or 2 to spread to the smaller airways, causing bronchiolitis and pneumonia resembling that of RSV [2,15,16]. Apnea also is associated with HPIV LRI in the first few months of life [5]. Treatment for HPIV disease is largely supportive except in the case of croup, where effective symptomatic relief can be achieved with corticosteroids and nebulized epinephrine [17,18]. Infections are self-limiting. HPIV infection is rarely fatal in otherwise healthy individuals, but mortality can be high in severely immunosuppressed individuals such as hematopoietic stem cell transplant recipients [19,20]. It has been hypothesized that severe HPIV infections in young infants may sometimes have long-term effects on lung function and immunity, but this remains unclear.

Viral genome and proteins

The HPIVs are enveloped, non-segmented, negative-strand RNA viruses of subfamily *Paramyxovirinae*, family *Paramyxoviridae* [15,21]. HPIV1 and 3 belong to genus *Respirovirus*, while HPIV2 and 4 belong to genus *Rubulavirus* [21]. The genomes of HPIV1, 2, and 3 are similar in size (15.5-15.7 kb), whereas that of HPIV4 is somewhat larger (17.4 kb) [8]. They share the same order of 6 genes: 3'-N-P-M-F-HN-L, which are transcribed sequentially into separate mRNAs. There are two viral surface proteins: the hemagglutinin-neuraminidase (HN) protein, which mediates attachment to sialic acid residues on host cell membranes and cleavage of these residues during release, and the fusion (F) protein, which mediates the fusion of the viral envelope with the host cell membrane. These are the viral neutralization antigens and major protective antigens. The N protein coats the genomic RNA, forming a highly stable nucleocapsid. The phosphoprotein (P) and the large polymerase protein (L) are associated with the nucleocapsid, while the matrix protein (M) coats the inner surface of the envelope [15].

The P gene also encodes additional proteins that vary among viruses. These are called accessory proteins because they are not essential for virus replication *in vitro*. The most notable are the C and V proteins, which suppress host innate immune responses as described below. C proteins are expressed by a separate ORF that overlaps the P ORF and are encoded by HPIV1 and 3, but not by HPIV2 and 4, as is characteristic of their respective genera. The sequence of the C protein is not highly conserved and lacks evident motifs.

Expression of the V protein depends on a mechanism called "RNA editing", whereby the polymerase stutters at a specific motif during transcription of the P gene to create mRNA species with frameshifts that link the upstream half of the P ORF to an internal V ORF encoding a protein domain with a characteristic zinc finger motif containing seven cysteine residues. Thus, V is a chimeric protein with an N-terminal domain encoded by the P ORF and a C-terminal domain encoded by the V ORF. All members of *Paramyxovirinae* express a V protein with the curious exceptions of HPIV1 and 3, which contain a relic V ORF that is not expressed in the case of HPIV1 [22] and is either not expressed or is expressed at a low level by HPIV3 [23,24]. The zinc finger motif of the V protein is conserved among all members of *Paramyxovirinae* and is necessary for suppression of both IFN induction and IFN signaling.

Contributions of the HPIV C and V accessory proteins to pathogenesis

For cytoplasmic RNA viruses such as the HPIVs, the most potent stimulus for innate immunity is viral RNA synthesis, which can strongly activate host cytoplasmic sensors such as MDA5, RIG-I, and PKR. Downstream effects include: signal transduction that activates transcription factors IRF3 and NF- κ B and induces IFN- β and pro-inflammatory cytokines; suppression of cellular translation by PKR; and IFN-induced signaling to induce an antiviral state [25-29]. These responses can strongly restrict HPIV replication but are inhibited in multiple ways by the viral V and C proteins [30,31].

The C ORF of HPIV1 encodes four C proteins called C', C, Y1, and Y2 that originate from different translational start sites but share a common C-terminus [32,33]. HPIV1 C proteins have been shown to prevent IFN induction [34-36], IFN signaling [34], and apoptosis [35]. The HPIV1 C proteins do not appear to interact with or directly inhibit the signal transduction pathways leading to activation of IRF3 and NF- κ B: instead, they modulate and reduce viral RNA synthesis to prevent the accumulation of dsRNA, thus avoiding activation of MDA5 and PKR [36]. With regard to IFN signaling, the HPIV1 C proteins have been shown to bind to STAT1 (but not STAT2), but not to interfere significantly with phosphorylation or stability of either STAT1 or 2. Rather, they sequester STAT1 in complexes that accumulate in the cytoplasm associated with late endosomes, thus preventing STAT1 translocation to the nucleus [37]. The mechanism by which the HPIV1 C proteins inhibit apoptosis remains unclear. Infection of respiratory epithelial cells with an HPIV1 mutant engineered to not express the C proteins resulted in changes in the expression of hundreds of cellular genes controlled by IRF3, NF- κ B, and other factors, illustrating that C normally has a broad impact on the host response [38].

HPIV3 encodes a single C protein that is important for efficient replication in experimental animal models [23], but is less well characterized than that of HPIV1. The HPIV3 C protein down-regulates viral RNA synthesis [39], which may reduce the innate response. HPIV3 C inhibits IFN signaling by binding to STAT1 and inhibiting its phosphorylation [40,41]. Interestingly, the HPIV3 C protein enhances activation of the MAPK/ERK pathway, which promotes the cellular response to growth factors and is necessary for efficient viral replication [40].

The HPIV2 V protein has been shown to inhibit IFN induction [42], IFN signaling [43], and apoptosis [44]. The HPIV2 V protein inhibits IFN induction in two ways: by binding to and inhibiting the activation of MDA5 [45,46], and by serving as an alternative, competing substrate for cellular kinases in the signal transduction for IRF3 activation [47]. HPIV2 V protein also appears to reduce the production of viral RNA [48], reminiscent of the C proteins noted above. HPIV2 V protein inhibits IFN signaling by binding to and promoting the degradation of STAT2 [49,50]. Overall, inhibition of IFN induction appears to be more important for efficient HPIV2 replication *in vivo* than inhibition of IFN signaling: recombinant HPIV2 viruses carrying V protein point mutations allowing IFN induction are attenuated in non-human primates [51], while HPIV2 viruses carrying V protein point mutations allowing IFN signaling are not attenuated [44]. This difference may reflect the ability of secreted IFN to affect cells beyond those directly infected with virus, while virus-mediated inhibition of IFN signaling operates only in infected cells.

The ability of HPIV4 to interfere with host innate immunity is less well characterized. Surprisingly, HPIV4 does not appear to interfere with IFN signaling [52]. It is possible that this contributes to its reduced virulence. It seems likely that HPIV4 has the ability to inhibit IFN induction, although investigation of this has not been reported.

Tissue tropism and cytopathology

HPIVs replicate to high titer in the epithelial cells that line the respiratory tract [53-55]. In an *in vitro* model of well-differentiated mucociliary human airway epithelium (HAE) [56,57], HPIV1, 2, and 3 infected only the superficial ciliated cells on the apical (luminal) face and did not spread to the underlying basal cells or goblet cells [53-55]. The release of progeny HPIV1, 2, and 3 occurred solely at the apical surface. This selective tropism is consistent with the absence of viremia in otherwise healthy individuals. This tropism also may limit exposure of viral antigen to antigen presenting cells, possibly leading to reduced immune responses that may allow re-infection. Low infectivity of HPIV3 for human dendritic cells may have a similar effect [58].

HPIV infection of non-polarized cell lines *in vitro* is characterized by syncytium formation leading to cytopathology. In contrast, HPIV infection of HAE cultures causes minimal gross cytopathology, with no evidence of cell-to-cell fusion, in contrast to the rapid tissue destruction caused by influenza A virus [53-55,59]. In explanation for the lack of syncytium formation, the HPIV3 F protein was found to be localized solely on the apical surface of these polarized cultures and thus did not contact neighboring cells. In post-mortem studies of human lung tissue, syncytium formation has been observed in the lungs of HPIV-infected individuals with severe immune deficiency or immunosuppression but is uncommon in immunocompetent patients [60-64]. HPIVs also strongly suppress apoptosis, which would reduce cytopathology.

Viremia and infection beyond the superficial respiratory epithelium are rare with HPIV infection. One likely factor is the directional budding of virions noted above. Another important factor is host immunity: systemic spread of HPIV has been detected only in severely immunocompromised patients [19,20,65].

Factors contributing to HPIV pathogenesis

Young age and lack of prior exposure to the infecting HPIV serotype are two major factors associated with HPIV LRI. Young infants are at greater risk in part because their smaller airways are more susceptible to obstruction, and their immune responses are reduced due to immunological immaturity and the presence of HPIV-specific maternal antibodies that suppress antibody responses (reviewed in [15,66]). Host genetic factors, particularly those affecting innate and inflammatory responses, likely also play a role, as has been investigated in greater detail for RSV [15,67].

It is generally thought that the respiratory pathology observed *in vivo* can be attributed to (i) high levels of virus replication and resulting direct virus-mediated effects, and (ii) the host response to infection. However, the relative contributions of these two factors are unclear and may be situational. While HPIV infection is not rapidly cytopathic in HAE cultures, infection appears to accelerate the normal mechanism of epithelial cell shedding and replacement [53]. The number of mucin-containing cells in HAE cultures increases during HPIV infection, consistent with the increased mucus production seen in HPIV-infected individuals. Also, HPIV infection inhibits ciliary motility in HAE cultures, an effect that *in vivo* would impede clearance of mucus and exfoliated cells from the airways. Airway inflammation and obstruction are certainly important components of HPIV pathogenesis. The histopathology of RSV bronchiolitis and pneumonia, which has been studied in more detail, also is characterized by obstruction of the small airways by inflammatory cell debris, edema, and external compression of the bronchioles from hyperplastic lymphoid follicles [68,69].

Immune responses

HPIV infection induces innate immune responses, serum and mucosal antibody responses, and CD8+ and CD4+ T-cell responses that restrict replication and clear HPIV infection. Neutralizing antibodies that target the HN and F glycoproteins are the most important determinants of long-term protection from HPIV disease [70-72]. Serum IgG antibodies provide the most durable protection against re-infection, but transport to the respiratory lumen is mostly non-specific and inefficient, and protection depends on high serum titers. Maternal IgG is thought to provide protection to infants for the first weeks or months of life. Studies in adults showed that detection of mucosal neutralizing antibody titers was a better correlate of protection than serum titers [70]. Mucosal IgA is transported efficiently through the epithelium to the airway lumen and can neutralize virus within infected epithelial cells, though IgA protection may be relatively short-lived compared with serum neutralizing antibodies. More than one infection is likely needed to maintain protective titers of mucosal IgA. The difficulty in maintaining protective levels of IgA and IgG in the respiratory lumen is an important factor in the ability of the HPIVs to re-infect (especially in the URT) throughout life. However, immunity from prior exposure typically limits re-infections to mild disease [73].

Inflammatory responses

HPIV infection of the airway epithelium causes extensive changes in cellular gene expression and stimulates increased production of numerous cytokines and chemokines that either have antiviral functions themselves or attract and activate cells that mediate an immune response ([38] and Schaap-Nutt et al., unpublished data). Microarray analysis of epithelial cells infected with HPIV1 has indicated that the NF- κ B, IRF3 and type 1 IFN pathways play a major role in regulating the cellular antiviral and inflammatory response to HPIV1 infection, and the HPIV1 C proteins normally suppress activation of these pathways [38]. Correlating with the microarray data, elevated nasal wash concentrations of inflammatory cytokines, including IL-8/CXCL8, MIP1 α +1 β /CCL3+4, RANTES/CCL5, and CXCL9, have been described in case series of children with HPIV disease [74,75]. Patients with primary HPIV infections also develop detectable IFN responses during acute infection [76]. CXCR3 ligands such as IP-10 and I-TAC, which attract activated Th1 cells to the infected epithelium, are the dominant chemokines known to be produced during HPIV infection and are secreted by the airway epithelium as well as by monocytes and neutrophils ([77-79] and Schaap-Nutt et al., unpublished data). High concentrations of IL-8 and IP-10, in particular, have been correlated with more severe HPIV disease [74,75]. It is unclear whether the association between the magnitude of inflammatory responses and disease severity reflects a specific pathogenic feature of these viruses, or the magnitude of virus replication resulting in increased immune responses, or both.

Conclusions

HPIV infections are common throughout life, and disease severity is greatest in the immunologically naïve and the immunocompromised. The magnitude of HPIV replication is a major factor determining severity of illness [80]. The viral C and V accessory proteins are key players in suppression of the innate immune response to HPIV infection, thus enabling efficient virus replication. The response of the airway epithelium to infection is crucial for initiating an inflammatory and innate immune response, but inflammatory cytokine production and subsequent cellular infiltration may also contribute to HPIV pathogenesis. A better understanding of HPIV pathogenesis will aid the design and evaluation of live-attenuated HPIV vaccines and therapeutic drugs.

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Highlights

- HPIVs evade the innate immune response and replicate efficiently in respiratory epithelium
- Cellular pathology in *ex vivo* epithelium is limited
- Previous homotypic HPIV infections protect against severe disease, not against infection
- There are no licensed vaccines or specific antiviral drugs for the HPIVs