The Role of Human Parainfluenza Virus Infections in the Immunopathology of the Respiratory Tract

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Abstract Viral infections are leading causes of both upper and lower airway acute illness in all age groups of healthy persons, and have also been implicated in the acute exacerbations of chronic respiratory disorders like asthma and COPD. Human rhinovirus, respiratory syncytial virus, influenza virus and coronavirus have been considered as the most important respiratory pathogens and relatively little attention has been paid to the role of parainfluenza viruses (hPIVs). Human parainfluenza viruses are single-stranded RNA viruses belonging to the paramyxovirus family that may evoke lower respiratory infections in infants, children and immunocompromised individuals. Among non-immune compromised adults, hPIV infection typically causes mild disease manifested as upper respiratory tract symptoms and is infrequently associated with severe croup or pneumonia. Moreover, hPIV infection may be associated with viral exacerbations of chronic airway diseases, asthma or COPD or chronic rhinosinusitis. In this review, we summarized the basic epidemiology and immunology of hPIVs and addressed the more recent data implicating the role of parainfluenza viruses in the exacerbation of chronic airway disorders.

Keywords Parainfluenza viruses · Respiratory viral infections · Asthma exacerbations · Airway diseases · Innate response · Vaccines

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Introduction

Viral infections are the leading causes of upper and lower airway acute illnesses in all age groups of otherwise healthy individuals and have also been implicated in acute exacerbations of chronic respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD). Human rhinovirus (HRV), respiratory syncytial virus (RSV), influenza virus and coronaviruses are considered the most important viral respiratory pathogens. Although parainfluenza viruses (hPIVs) are common respiratory pathogens that cause infections without ethnic, socioeconomic, gender, age, or geographic boundaries [1] and most frequently induce acute respiratory tract diseases in infants and immuno-compromised adults [2, 3], their role in exacerbation of chronic respiratory disorders has received relatively little attention. In non-immune compromised individuals, hPIV infections are typically transient and mild; however, in patients with asthma or COPD, these infections may be associated with severe respiratory symptom exacerbations [4]. Having in mind increasing number of reports on the potential role of parainfluenza viruses in the exacerbation of chronic airway disorders, we aimed to update the potential readers on the epidemiology, immunology and novel clinical aspects of hPIV infections in asthma and COPD.

Epidemiology

After respiratory syncytial virus, hPIVs are the second most common causes of acute respiratory tract infections among children aged <5 years, possibly accounting for up to 17% of hospitalizations [5]. Although age-specific rates of lower respiratory tract infections vary significantly in different populations, the medical burden of hPIVs is similar to that of RSV infection in children [6]. Serological surveys have indicated that 60% of the children are infected with hPIV-3 by 2 years of age, and this

number increases to up to 80% by 4 years of age [7]. Among adults, most infections manifest as mild upper respiratory tract symptoms; however, more severe diseases may also occur, particularly in older adults and the immunocompromised [1].

Although hPIV infections occur throughout the year, several studies have documented distinct temporal trends for hPIV serotypes. A strong relationship has been observed between hPIV serotypes and specific clinical syndromes, the age of the child and the time of year when the infection occurred [1]. The peak seasonal activity for hPIV-1 occurs biennially from late September to December during odd-numbered years. The hPIV-2 season occurs from October through December, and the peak season for hPIV-3 occurs annually each spring from April to June [8] or May to September in England [9]. Frost et al. [10] recently described the year-round prevalence of hPIV-4 infections, with peaks in the autumn of odd-numbered years. Seasonal activity also varied between different years, with higher hPIV-3 activity in years when hPIV-1 was not circulating and conversely, with significantly lower hPIV-3 activity during the years of peak hPIV-1 activity [10]. These phenomena potentially reflect the heterotypic cross protection of antibodies.

Structural organization and viral replication

The human parainfluenza viruses belong to a diverse group of enveloped single-stranded RNA viruses within the *Paramyxoviridae* family, a large and expanding group of viruses that includes metapneumovirus and the mumps, measles and respiratory syncytial viruses. Based on genetic and antigenic analyses, hPIVs have been divided into four major subtypes (hPIV-1 to hPIV-4), classified into *Respirovirus* (hPIV-1 and hPIV-3) and *Rubulavirus* (hPIV-2 and hPIV-4) genera within the *Paramyxovirinae* subfamily (Table 1). hPIVs may be phenotypically distinguished from other members of

Paramyxoviridae based on neuraminidase expression or the presence of thicker nucleocapsids [1, 11].

Paramyxoviruses are enveloped animal viruses with nonsegmented negative-strand RNA genomes (complementary to mRNA), which are identified almost exclusively in nucleocapsid structures. The hPIV genome comprises approximately 15,000 nucleotides and encodes six common structural proteins: 'large' (L) nucleocapsid protein, P and N, which are closely associated with viral RNA, haemagglutinin-neuraminidase (HN), and fusion (F) and membrane (M) proteins (3′-N-P-C-M-F-HN-L-5′) (Table 2). The template for paramyxovirus RNA synthesis is not naked RNA but rather the helical nucleocapsid core of the virus, comprising viral RNA (vRNA) together with the N, P and L proteins. The N protein binds to vRNA, creating a template for the L (RNA-dependent RNA polymerase) and P proteins to transcribe and replicate the hPIV genome. Each nucleocapsid protein (N protein) has been associated with precisely 6 nucleotides (nt). As a consequence of this association, paramyxovirus genomes are most efficiently replicated only when the number of nucleotides in the genome is divisible by 6, described as the 'rule of six' [1, 12].

Human parainfluenza virus type 3 enters cells by directly fusing with the cell membrane. During entry, the viral surface glycoproteins HN, a receptor-binding protein, and F cooperate to mediate fusion upon receptor binding [13]. The first step towards viral replication is the fusion of the virus and host cell membranes. The hPIV nucleocapsid is subsequently expulsed into the cell cytoplasm where its transcription occurs using the L-P RNA polymerase complex. In this first step, the N-encapsidated negative-sense RNA genome is transcribed to produce low levels of viral mRNA. Subsequently, the genome is replicated into a complementary N-encapsidated antigenome, followed by the generation of progeny genomes. Next, the secondary transcription of progeny genomes occurs, generating a large burst of viral mRNA with cellular ribosomes then translating these mRNAs into viral proteins. The negative-sense RNA produced

Table 1 Taxonomic classification of hPIV subtypes 1–4 within the Paramyxoviridae family

Subfamily	Genus	Species
Paramyxovirinae	Respirovirus	Human parainfluenza virus 1 (hPIV-1)
		Human parainfluenza virus 3 (hPIV-3)
	Rubulavirus	Human parainfluenza virus 2 (hPIV-2)
		Human parainfluenza virus 4 (hPIV-4)
		Mumps virus
	Morbillivirus	Measles virus
	Megamyxovirus	Hendra virus
		Nipah virus
Pneumovirinae	Pneumovirus	Human respiratory syncytial virus (hRSV)
	Metapneumovirus	Human metapneumovirus (hMPV)

Table 2 Characterization of hPIV common structural proteins

Localisation	Protein	Function
Associated with vRNA (nucleocapsid formation)	L	RNA-dependent RNA polymerase
	P	Phosphoprotein subunit of the RNA-dependent RNA polymerase
	N	Nucleocapsid protein
Surface glycoproteins	HN	Haemagglutinin-neuraminidase, found on the lipid envelope of hPIV and infected cells, functions in virus-host cell attachment via sialic acid receptors
	F	Fusion protein that allows the viral nucleocapsid to enter and infect a host cell; required for membrane fusion between host cells (syncytial formation)
	M	Matrix protein with a role in attaching nucleocapsids to areas of the infected cell membrane, generating the viral envelope; may be involved in viral budding

is subsequently encapsidated with N and P proteins and may be exported as a new virion [1, 14].

Infections and host defence against hPIV

hPIV effectively replicates in lower and upper airway epithelial cells with or without killing the cells [15, 16•]. hPIV infections of airway epithelial cells induce the upregulation of ICAM-1 expression [17] and the generation of several proinflammatory (IL-1β, IL-6 and TNF-α) and tissue remodelling-related cytokines (PDGF and VEGF) [18••]. This pattern of cytokine release might contribute to the prolonged airway inflammation and airway structural changes typical of asthma. Foetal lung fibroblasts infected with hPIV release an array of cytokines, including the proinflammatory cytokines IL-1β, IL-6 and TNF-α, the anti-inflammatory cytokine IL-1ra, Th1 cytokines (IFN-γ and IL-2), Th2 cytokines (IL-4, IL-5 and IL-10), granulopoiesis-inducing cytokines (granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor), neutrophil recruitment-inducing cytokines (IL-8 and interferon-inducible protein-10) and eosinophil recruitment-inducing cytokines (eotaxin and RANTES) [19••]. A recent study reported that hPIV-3 infection of human airway epithelial cells (RPMI 2650) might induce the expression and release of interferon gamma, potentially contributing to local antiviral defence. Additionally, although the RANTES protein concentration and mRNA expression were significantly increased after infection, no measurable concentrations of TNF-α, IL-10, TSLP, IL-8, GM-CSF or eotaxin were detected in virus-infected cell supernatants [18••].

A powerful first line of extracellular defence against paramyxoviruses within the respiratory tract is complement, which exists at relatively low levels on the mucosal surface but whose concentrations dramatically increase upon cell injury associated with infection. Parainfluenza viruses contain glycoproteins that may activate complement factors via an alternative pathway. The extent of activation is inversely correlated with sialic acid concentrations on either the viral particle or the infected cell, reflecting the presence of HN neuraminidase activity [14].

Specific host defences against hPIV are primarily mediated through humoral immunity directed against two surface glycoproteins, HN and F. The prevalence of antibodies against parainfluenza virus types 1, 2 and 3 increases with age, becoming more than twice as prevalent among older adults (55–73 years old) as among younger individuals (18–34 years old) [20]. In Norway, 1:8 parainfluenza virus antibody titres were more prevalent for type 2 than types 1 and 3 and were detected in over 10 accumulated percentages of the adult general population [20]. Moreover, the immunologic response to hPIV infection is mediated primarily through antibodies belonging to the IgG1 subclass; however, 30% of the infected adults additionally demonstrate increased serum levels of IgG3, IgG4, IgM and IgA. hPIV-3 infects approximately two-thirds of the children during the first year of life, causing symptomatic disease in approximately one-third of them. By the age of 3 years, most children will demonstrate serological evidence of infection. Immunity to hPIV-1 and hPIV-2 develops later than immunity to hPIV-3, increasing more rapidly during the second and third years of life. In contrast, immunity to hPIV-4 develops later, occurring among school-aged children [1].

The cytotoxic T-lymphocyte response during hPIV infections is directed against determinants on the HN, P and NP proteins of hPIV-1 and hPIV-3 and is important for the clearance of hPIV-3 from the lower respiratory tract [1]. Moreover, hPIV-3 inhibits T cell function through the induction of large amounts of IL-10, which reduces the T cell proliferative response and protects these cells from virus-mediated apoptosis. This mechanism has been suggested as a potential explanation for persistent hPIV infections in humans [21, 22].

Paramyxoviruses have developed various strategies to overcome the host innate immunity response. Despite a genome size restricted to only six principal genes, these viruses produce 'accessory' protein isoforms (V or C) within the P gene, which act as interferon antagonists. These proteins target signalling molecules of the IFN system, including melanoma differentiation-associated

factor 5 (MDA5), retinoid acid-inducible gene I (RIG I), IFN regulatory factor (IRF-3) and signal transducers and activators of transcription (STAT1 and STAT2) [11]. Diverse members of the Paramyxovirus family of negative-strand RNA viruses effectively suppress host innate immune responses through V proteins. These proteins mediate interference with the interferon regulatory RNA helicase MDA5 to avoid cellular antiviral responses. The hPIV-2-encoded V protein binds to MDA5 and inhibits IFN induction [23]. Moreover, Lu et al. [24] showed that within all members of the *Rubulavirus* genus, the same V proteins are essential for the inhibition of dsRNA-mediated signalling through interactions with IRF3 kinase that inhibit its phosphorylation. Intriguingly, hPIV-4 is the only paramyxovirus studied to date that cannot evade the IFN-induced antiviral response, because its V proteins do not degrade STATs or affect the localization or phosphorylation of these proteins [25]. STAT targeting by respiroviruses is markedly different from that of other paramyxoviruses, reflecting the expression of another accessory protein (C) from the P gene. This protein is sufficient for IFNα/β antagonism in hPIV-1 viruses [26].

Sensitive to neutralization by complement factors, parainfluenza viruses also delay or inhibit complement activity. As previously described, the viral genome is small, and none of the viral proteins exhibit direct anti-complement activity. Therefore, parainfluenza viruses derive benefit from the normal cellular pathways that block complement activity to protect against potential cellular damage. Parainfluenza viruses incorporate membrane-bound host CD46 (which mediates C3b cleavage) and CD55 (which inhibits C3 complex formation) into the virion envelope during budding, thereby delaying complement-mediated neutralization [14].

Clinical features of hPIV-induced respiratory tract infections

Whereas the nasopharynx and oropharynx are primary locations of initial hPIV replication, hPIVs have also been associated with upper and lower respiratory tract illnesses, including the common cold, laryngotracheobronchitis (i.e. croup), tracheobronchitis, bronchiolitis and pneumonia, in both children and adults [1]. Although these infections are rather mild in healthy individuals, they may lead to serious respiratory diseases in children and immunocompromised individuals.

Following experimental infection in adults, the first symptoms manifested 3 to 4 days post-inoculation and lasted up to 17 days, with an average of 4 days for hPIV-1 and 6 to 13 days for hPIV-2 and hPIV-4 [27], respectively. Despite being compatible with a common cold diagnosis, the illnesses were rather severe, manifesting nasal discharge that frequently became mucopurulent and lasted for 1 to 2 weeks. A few adult patients developed mild cough, without signs of the severe lower respiratory tract involvement typically observed in children infected with parainfluenza 2 viruses. Additionally, in patients infected with hPIV-2, no striking predominance of pharyngitis was observed. While all hPIV serotypes share common structures and replication strategies, these viruses target different parts of the respiratory tract and may cause different clinical manifestations (Table 3, based on [16•]). Specific serotypes have been reported to differ in the kinetics of replication and cytokine release, which may reflect differences in the serotype-specific clinical manifestations of infections [16•]. However, a recent large study from China, based on analyses of throat swabs collected from approximately 5000 children and adults with upper or lower respiratory tract infections, challenged the dogma of the hPIV-serotype specificity of airway symptoms. These authors reported that the clinical spectrum of respiratory symptoms associated with hPIV infection did not differ regardless of which of the four hPIV types was detected [28].

Biennial fall epidemics of hPIV-1 constitute a minimum of 50% of croup cases in the USA [29]. The majority of infections occur in children aged 7–36 months, with a peak incidence observed in the second and third year of life. In young infants, hPIV-1 causes lower respiratory illness (LRI); however, the full burden of this virus has not yet been determined in adult and elderly populations [1]. hPIV-2 also leads to LRI, particularly in children younger than 5 years, although this virus has been reported much less frequently than hPIV-1 and hPIV-3, potentially reflecting difficulties in isolation and detection. Unlike the other

Table 3 Kinetics of replication, cytokine release and clinical manifestations of infections with hPIV serotypes 1–3 according to Schaap-Nutt et al. [16•]

Serotype	Locus of replication	Kinetics of replication	Released cytokine upon infection	Clinical manifestations	Remarks
hPIV-1	Upper respiratory tract	Replicates to high titres and does not induce cytokine secretion until late in infection	RANTES IP-10 I-TAC	Upper respiratory tract illnesses, croup	Undetected for several days post infection
hPIV-2	Upper respiratory tract	Replicates less efficiently than hPIV-1 but induces an early cytokine peak	IFN-α IL-6 MCP-1 RANTES IP-10 I-TAC	Upper respiratory tract illnesses, croup	Less able to inhibit an early immune response
hPIV-3	Lower respiratory tract	Replicates to high titres but induces a slower increase in cytokine secretion	IFN-α IL-6 MCP-1 RANTES IP-10 I-TAC	Bronchiolitis, pneumonia	Induces a steadily increasing inflammatory response over several days

hPIV syndromes, nearly half of the hPIV-3 infections manifesting as bronchiolitis and pneumonia occur within the first year of life. Moreover, hPIV-4 did not receive much attention in research studies compared with the other virus subtypes, reflecting isolation and detection difficulties. However, the development of molecular techniques has facilitated the improved characterization of hPIV-4 epidemiology and its disease associations. Frost et al. [10] conducted a unique large-scale study documenting similar clinical presentations of hPIV-4 and hPIV-3 infections. These authors also showed that hPIV-4 is the only hPIV that does not cause croup.

In human hosts, the percentage of hPIV infections that result in persistent infection remains unclear. Among haematopoietic cell transplantation recipients, hPIV appears to represent the most frequent infection (17.9% estimated cumulative incidence); additionally, hPIV was the only virus for which asymptomatic infections were detected [30]. Both HN and F proteins are involved in cell membrane fusion associated with hPIV infection. Paramyxovirus-infected cells have a decreased level of neuraminic acid residues on their surfaces. Persistently infected cells do not fuse with each other because of the decreased level of cell surface neuraminic acid, fusing only with uninfected cells containing neuraminic acid [31]. The study authors speculated that the persistence of infection is achieved through a high MOI inoculum of the virus, reflecting the fact that high levels of neuraminidase induce the rapid destruction of cell surface sialic acid and prevent viral spread through cell-to-cell fusion. Moreover, Ah-Tye et al. demonstrated that this phenomenon also depends on the virus serotype. These authors showed that only infections with a high MOI of hPIV-3 block cell fusion, which was not the case for hPIV-1 and hPIV-2 infections [32]. This finding would explain why infection control programmes focusing on disease symptoms are ineffective against PIV.

Parainfluenza viruses and chronic airway diseases

Viral infections caused by the *Paramyxoviridae* family (e.g. RSV) have been implicated in the induction of not only transient asthmatic symptoms in children but also persistent asthmatic phenotypes later in life. Patients hospitalized for RSV and rhinovirus bronchiolitis during first 2 years of life had an increased risk of asthma at 15–18 years of age compared with control populations [33]. Infections with hPIV in childhood have been postulated as inducing similar effects because this virus may have unique features that trigger immune reprogramming, resulting in a chronic asthma phenotype [34•].

Respiratory viral infections are considered the most frequent cause of asthma and COPD exacerbations, with rhinoviruses the most commonly involved. However, both epidemiological and experimental data strongly suggest a role for parainfluenza viruses in the exacerbation of chronic respiratory disorders. A summary of these findings is presented in Table 4. hPIV has been considered an important causative agent of virus-induced asthma exacerbations [35, 36]. In a longitudinal study of 138 asthma subjects, Nicholson et al. [37] revealed that hPIV infections occurred in 3.6% of asthma exacerbations and, after rhinoviruses and coronaviruses, ranked third among non-bacterial causes associated with asthma symptoms. In children 9–11 years old with bronchial hyperreactivity, Johnson et al. [38] detected hPIV in 7.2% of the respiratory episode cases. In a small group of adult asthmatics with asthma exacerbation, 60% of the cases were infected with hPIV-3, and among those individuals infected during mild cold-induced asthma exacerbation, a significant release of cysteinyl leukotrienes into the induced sputum was observed [39]. Frost et al. [10] in a large-scale retrospective analysis of 11,533 specimens collected from children with respiratory symptoms revealed that 752 samples (6.5%) were positive for hPIV1–4. Of the 316 samples that were positive for only hPIV, 41 (13%) were collected from children with asthma; however, no significant differences were observed in the prevalence of the four HPIV serotypes in these subjects. In another study of 309 nasopharyngeal samples collected from children with lower respiratory tract infections, 19 (6.1%) samples were positive for parainfluenza virus. A more detailed analysis revealed that hPIV was present in 7.7% of the bronchitis patient samples, 7.1% of the asthma patient samples, 6.8% of the pneumonia patient samples and 4.4% of the bronchiolitis patient samples [40]. Lee et al. [41] summarized historical data to estimate the age-specific rates of acute respiratory illness (ARI), LRI and hospitalization resulting from hPIV-3 infections among US children <5 years of age. These authors showed that age-specific rates might vary fivefold in different studies conducted in different populations using different clinical and laboratory definitions. The annual medical burden for hPIV-3 in children younger than 5 years was estimated as 3.24 million cases of medically attended ARI, 1.08 million cases of LRI and 29,000 cases of hospitalization. Marx et al. [42] demonstrated that during their respective epidemic seasons, hPIV-1 and hPIV-3 were among the most commonly identified infections in adult patients hospitalized for lower respiratory tract infections, accounting for 2.5 and 3.1% of the episodes, respectively. Azevedo et al. [36] showed that the viral identification rate (with a 2.8% hPIV detection frequency) was higher in asthmatic children, suggesting potential susceptibility to viral infections in this subgroup. Zhu et al. [43] suggested that virus detection in Chinese children with acute respiratory infection should include respiratory viruses considered relatively uncommon, particularly when severe ARI-related clinical illnesses are manifested. In that study, hPIV was detected in 4.8% of specimens and was less common than respiratory syncytial virus, adenovirus, human rhinovirus, metapneumovirus, human bocavirus and coronaviruses. Glezen et al. [3] examined 1029 patients

Table 4   Associations of hPIV infections with asthma and COPD (studies listed by the year of publication)

Study group	Number of subjects/ collected material	Number of hPIV infections (%)	Other viruses with higher frequency	Reference
Young adults (19–46 years of age) with asthma enrolled from the general population (community (43%), general practice (32%), and hospital (25%))	138 (48 men, 90 women)/229 paired (acute and convalescent) serum samples	Total: 5/229 (2.2%) During exacerbations: 3/84 (3.6%)	Rhinovirus, coronavirus OC43 and 229E	[37]
Children 9–11 years old with bronchial hyperreactivity	108 (58 boys, 50 girls)—42 with asthma diagnosed/292 reported respiratory episodes	21/292 (7.2%)	Rhinovirus/enterovirus, coronavirus	[38]
Adult patients hospitalized due to acute lower respiratory tract infection	Serum samples tested for hPIV-specific IgG antibodies during the respective epidemic seasons of hPIV1-3	18/721 (2.5%) positive for hPIV-1 IgG 2/1057 (0.2%) positive for hPIV-2 IgG 22/705 (3.1%) positive for hPIV-3 IgG		[42]
Patients hospitalized due to acute respiratory tract conditions (pneumonia, tracheobronchitis, croup, asthma exacerbations, COPD)	1029 patients	78/1029 (7.6%) positive for hPIV-1-3	RSV, influenza	[3]
104 children (2–4 years old) included in four groups: I: asthmatics with acute attack and URTI; II: asthmatics without URTI; III: non-asthmatics with URTI; IV: non-asthmatic, asymptomatic children	123 nasal mucosa cells collected and cultured for virus detection	1/36 (2.7%)	Respiratory syncytial virus, rhinovirus, adenovirus	[36]
Summary of different cohort studies to estimate age-specific rates of acute respiratory illness (ARI), lower respiratory illness (LRI) and hospitalization resulting from hPIV-3 infections among US children <5 years of age		The annual medical burden for hPIV-3 in children <5 years old: 3.24 million cases of medically attended ARI, 1.08 million cases of LRI and 29 thousand cases of hospitalization		[6]
Adult asthmatics with mild asthma exacerbations	19 (including 15 cold-induced asthma patients)	9/15 (60%)	–	[39]
<5 year-old children with respiratory symptoms	11,533 nasopharyngeal washes, tracheal aspirates and bronchoalveolar lavages tested using respiratory virus PCR 316 samples (without bacterial/viral coinfections) included in further analysis	752/11533 (6.5%) positive for hPIV-1-4 41/316 (13%) from asthmatic donors	Rhinovirus, respiratory syncytial virus	[10]
Children with lower respiratory tract infections (pneumonia, bronchitis, bronchiolitis or asthma)	309 nasopharyngeal aspirates	19/309 (6.1%): pneumonia; 11/163 (6.8%) bronchitis; 2/26 (7.7%) bronchiolitis; 4/92 (4.4%) asthma: 2/28 (7.1%)	Adenovirus, human metapneumovirus, respiratory syncytial virus, rhinovirus, Influenza virus	[40]
Children with acute respiratory infection	270 patients	13/270 (4.8%) samples positive for hPIV-3	Respiratory syncytial virus, adenovirus, human rhinovirus, metapneumovirus, human bocavirus and coronaviruses	[43]
Review of 19 studies of COPD patients	1728 patients	3.35% (pooled prevalence)	Coronavirus, influenza, rhinovirus, respiratory syncytial virus	[44]
Patients with COPD exacerbations	63 pharyngeal swabs	Parainfluenza 4 (5.4%), parainfluenza 3 (2.7%)	Rhinovirus, respiratory syncytial virus, human metapneumovirus, influenza A	[45]

hospitalized for pneumonia, tracheobronchitis, bronchiolitis, croup, and asthma and COPD exacerbations, showing that hPIVs ranked as the third most common (7.6%) causes of serious acute respiratory conditions, after RSV and influenza virus.

Viral infections have also been associated with COPD exacerbations, considering that respiratory viruses are frequently detected in both upper and lower respiratory tract samples. A systematic review of 19 studies with 1728 patients revealed that parainfluenza virus infections were associated with 3.35% of COPD exacerbations, and although hPIV was the fifth most frequent infection, the prevalence of this disease was higher than that of adenovirus (2.07%), hMPV (2.78%) or bocaviruses (0.56%) [44]. In a recent prospective study of COPD patients, viral infection was detected in 25.7% of exacerbations. Although rhinovirus is the most frequently isolated virus (51.4%), parainfluenza 4 and 3 were the fourth most prevalent viruses, accounting for 81% of COPD exacerbations [45]. Thus, parainfluenza viruses should be included among potential triggers of COPD exacerbations (Table 4).

Epidemiological data suggesting a causative role for hPIV in exacerbations of chronic airway diseases (asthma or COPD) have been further supported by experimental data from animal models of airway inflammation. In an animal model of allergen-induced pulmonary disease, hPIV-3 infection resulted in inflammatory cell recruitment and a concomitant increase in airway responsiveness to histamine [46]. Following hPIV-3 infection, the allergen-induced airway obstruction in ovalbumin-sensitized animals shifted from the two typical transient phases to a single sustained response lasting up to 12 h. Interactions between viral- and allergy-induced pulmonary inflammation may alter bronchial smooth muscle responses such that allergy-induced pulmonary obstructions last longer and are more resistant to glucocorticoid treatment, suggesting that hPIV-3 infection in asthmatic patients, similar to rhinovirus infection, may decrease the effectiveness of glucocorticoid treatment.

Parainfluenza viruses are a major cause of post-infectious olfactory dysfunction. A comparison of the monthly frequency of this disorder with the incidence of the isolation of various viruses suggested that parainfluenza virus type 3, rather than influenza virus, respiratory syncytial virus, or herpes virus, was the most likely causative agent [47]. In another study, all patients with postviral olfactory disorder had increased serum antibody titres for parainfluenza virus type 3 [48]. Furthermore, parainfluenza virus 3 was detected in the turbinate epithelial cells of 87% of the postviral olfactory dysfunction patients compared with 9% of the control patients [49]. These studies suggest a potential role for hPIV infection in the pathogenesis of chronic rhinosinusitis, which is frequently associated with the most severe forms of asthma, including aspirin-exacerbated respiratory disease [50] (Table 4).

Prospects for antiviral treatment

Because lower respiratory tract infections caused by different viruses, including hPIVs, lead to the exacerbation of chronic respiratory diseases, early antiviral treatment could potentially prevent such exacerbations. Although no effective antiviral treatment for hPIV is available for clinical practice, new strategies that may be applicable to hPIV respiratory infections are being developed. The most direct approach is based on interference with viral entry through the removal of the sialic acid receptors used for binding by the parainfluenza HN protein, thereby preventing the first step in infection. DAS18, a recombinant sialidase protein, has been suggested to effectively inhibit HPIV infection. DAS18-mediated desialylation and anti-HPIV activity have been described in HAE and in the cotton rat hPIV-3 infection model. These data suggest that this anti-hPIV strategy warrants further studies to assess its potential clinical utility for hPIV infection and suggests the likelihood of the development of an antiviral agent against hPIV [51].

Another therapeutic concept based on peptide insertion into the cell membrane may enable the use of fusion-inhibitory peptides for viruses that fuse in the cell interior. Porotto et al. [52] reported that adding a cholesterol moiety to a paramyxovirus HRC-derived peptide significantly increased its antiviral potency. These authors suggested that this enhanced activity reflects the targeting of the peptide to the plasma membrane, where fusion occurs. The cholesterol-tagged peptides on the cell surface create a protective antiviral shield, directly target the F protein at its site of action, and expand the potential utility of inhibitory peptides for paramyxoviruses [52]. This antiviral potency was shown to increase further through cholesterol tagging combined with the dimerization of the HR-derived sequence, as observed for human parainfluenza virus, Nipah virus, and HIV-1 [53].

Vaccines

Despite multiple attempts over the past decades to develop and clinically test live-attenuated hPIV-3 vaccines, currently no licenced vaccine is available for the prevention or treatment of human parainfluenza infections. In the classic Jennerian approach, related viruses from heterologous species have been examined as potential vaccine candidates. Thereby, bovine PIV-3 (bPIV-3) was described as a promising candidate, because this virus generated a marked response in seronegative children and adults, despite not producing significant immune responses in seropositive subjects [54–57]. A live attenuated bPIV-3 vaccine was evaluated in a phase 2 clinical trial wherein it was administered intranasally to infants at 2, 4 and 6 months of age [41]. This trial demonstrated that bPIV-3 was safe and well tolerated in infants; moreover, the immunization elicited a humoral antibody response to bPIV-3, which cross-reacted with hPIV-3. Because

the bPIV-3 vaccine used in this phase 2 trial was prepared using foetal rhesus monkey lung-2 (FRhL-2) cells, other attempts were necessary to identify suitable cell substrates for the commercial production of bPIV-3 given that the routine vaccine manufacture using FRhL-2 cells is not feasible. Promising results were obtained for antigenically and genetically stable attenuated strains of hPIV-3, derived using a classic attenuation approach of successive passage at lower temperatures (cold adaptation) and which appeared to be even more immunogenic than bPIV-3 [54, 58, 59].

More recently, the cDNA-derived recombinant vaccine rHPIV3cp45 was developed, containing 15 known attenuating mutations present in this biologically derived virus [60]. Such genetic modification enables the use of a virus with a short and well-characterized passage history [61]. The rHPIV3cp45 vaccine was evaluated in a phase I trial in children 6–12 months old, who received two doses of the vaccine 4–10 weeks apart [62]. The first dose of rHPIV3cp45 was safe and infective, similar to the biologically derived virus; however, the second dose failed to boost serum antibody responses [62]. Englund et al. [63] confirmed the safety, acceptable tolerance and good rates of the infectivity and antibody responses following a single dose of the vaccine. Moreover, these authors demonstrated that after two doses, administered 6 months apart, rHPIV3cp45 infects children under 3 years of age who were not infected with a single dose of vaccine. Senchi et al. [64] examined the effectiveness of oligomannose-coated liposomes (OMLs) as an antigen-delivery system in combination with a synthetic double-stranded RNA analogue for the induction of mucosal and systematic immunity against hPIV-3. These authors showed that the intranasal administration of haemagglutinin-neuraminidase protein encapsulated in OMLs with the synthetic double-stranded RNA adjuvant, polyriboinosinic-polyribocytidylic acid [poly (I:C)] generated significant viral-specific systemic and mucosal immune responses, evidenced by the prominent induction of serum IgG and nasal wash IgA, respectively. Most recently, a live-attenuated hPIV-1 vaccine, designated rHPIV-1/84/del 170/942A, was evaluated in a phase I clinical trial in adults, hPIV-1–seropositive children, and hPIV-1–seronegative children. An effective hPIV-1 vaccine could prevent a substantial amount of respiratory tract illness in young children but was overattenuated in hPIV-1–seronegative children [65]. Thus, despite decades of research and several vaccines tested under experimental conditions, no vaccines are currently licenced for the prevention of hPIV infection.

Conclusions

Considering the biology the virus and wide range of symptoms observed in both children and adults after hPIV infection, the role of parainfluenza viruses in respiratory tract infections must not be neglected. Moreover, hPIV infections are important causes of viral exacerbations of chronic airway diseases, asthma and COPD. A potential causative role for hPIV in chronic rhinosinusitis has also been suggested. Further studies on the biology of this virus and its host-virus interactions are required to allow for development of specific therapies, which may counteract exacerbations of chronic respiratory diseases.

Compliance with Ethical Standards

Conflict of Interest   Drs. Pawełczyk and Kowalski declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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• Of importance
•• Of major importance

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