

REVIEW



Rhinovirus and the lower respiratory tract[†]

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SUMMARY

Human rhinoviruses (HRVs) are well-recognised causes of common colds and associated upper respiratory tract complications such as sinusitis and otitis media. This article reviews information linking HRV infection to illness in the lower respiratory tract. HRVs are capable of efficient replication *in vitro* at temperatures present in the tracheo-bronchial tree and have been shown to cause productive infection, elaboration of cytokines and chemokines, and up-regulation of cell surface markers in human bronchial epithelial cells. *In situ* hybridisation studies have proven that HRV infection occurs in the tracheobronchial tree following experimental infection. Clinical studies report that HRV infection is the second most frequently recognised agent associated with pneumonia and bronchiolitis in infants and young children and commonly causes exacerbations of pre-existing airways disease in those with asthma, chronic obstructive pulmonary disease or cystic fibrosis. HRV infection is associated with one-third to one-half of asthma exacerbations depending on age and is linked to asthma hospitalisations in both adults and children. Limited information implicates HRV infection as a cause of severe lower respiratory tract illness in older adults and in highly immunocompromised hosts, particularly bone marrow transplant recipients. More information is needed about the pathogenesis of HRV infection with regard to lower respiratory tract complications in these diverse patient groups. Given the large unmet medical need associated with HRV infections, safe and effective antiviral agents are needed for both prevention and treatment of these infections. Copyright © 2004 John Wiley & Sons, Ltd.

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INTRODUCTION

Human rhinoviruses (HRVs) are the most common cause of upper respiratory tract infections in both adults and children. In adults, HRV infections account for approximately 50% of common colds on an annual basis and up to 80% or more during the high prevalence autumn period in the northern hemisphere [1,2]. Large epidemiological studies report a higher incidence of infections in children than adults [3] and almost all children have experienced at least one HRV infection by age 2 years [4]. Reports from the 1960s associated HRV infections

with exacerbations of chronic bronchitis (Table 1) and also described croup, bronchitis and bronchopneumonia in children with HRV infection. One early report suggested that HRVs could cause atypical pneumonia in young adults [5]. More recent evidence increasingly indicates that HRV infections are important causes of exacerbations of multiple types of pre-existing airways disorders and can directly invade the lower respiratory tract to cause disease. This article reviews selected studies that have addressed the pathogenesis, epidemiology and clinical importance of rhinovirus as a lower respiratory tract pathogen.

EVIDENCE FOR REPLICATION IN LOWER RESPIRATORY TRACT

Nearly 40 years ago, studies of experimentally induced HRV infection found that small particle aerosol (0.2–3 µm) delivery of relatively low doses (16–60 50% tissue culture infectious doses [TCID₅₀]) of one HRV strain by nasal inhalation consistently induced infection, as well as rhinitis and cough, in eight seronegative healthy adults [6]. The illness manifested as tracheobronchitis in six (75%) with frequent coughing, substernal chest

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Abbreviations used

BAL, bronchoalveolar lavage; CAP, community-acquired pneumonia; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; HRVs, human rhinoviruses; PEF, peak expiratory flow rates; RANTES, regulated on activation, normal T cells expressed and secreted; RAST, radioallergosorbent test; TCID₅₀, 50% tissue culture infectious doses

Table 1. Representative prospective longitudinal studies of rhinovirus detection in association with lower respiratory tract illness

Study [Ref]	Syndrome/population	No. of patients/episodes	Age (yrs)	Duration of study (mo)	Method of detection	% Rhinovirus (picornavirus) positive
Kellner <i>et al.</i> , 1988 [29]	ARI in hospitalised infants (91%)	519	81% ≤ 1 yr	20	Culture	12% (14.5%)
Horn <i>et al.</i> , 1979 [8]	Wheezy bronchitis in children	22/72	5–15	17	Culture	33%
Andreoletti <i>et al.</i> , 2000 [34]	Bronchiolitis in hospitalised infants	84	0.5–0.8 (mean)	4	RT-PCR	19% (31%)
Papadopoulos <i>et al.</i> , 2002 [33]	Bronchiolitis in hospitalised infants	119	<1	12	RT-PCR	29%
Juven <i>et al.</i> , 2000 [37]	CAP in hospitalised children	254	<1–17	36	Culture, RT-PCR	24%
Jartti <i>et al.</i> , 2002 [36]	Wheezing in hospitalised children	132	<1–16	9	RT-PCR	27% (65%)
Kotaniemi-Syrjanen <i>et al.</i> , 2003 [48]	Wheezing in hospitalised children	81	<2	18	RT-PCR	33% (45%)
Rawlinson <i>et al.</i> , 2003 [27]	Exacerbations in hospitalized asthmatics	179	<1–16	16 (est.)	Culture, RT-PCR	79%
Rakes <i>et al.</i> , 1999 [53]	Exacerbations in asthmatics	22 48	<2 2–16	14	Culture, RT-PCR	41% 71%
Johnston <i>et al.</i> , 1995 [26]	Exacerbations in asthmatics	108/292	7–9	13	Culture, RT-PCR	29% (50%)
Nicholson <i>et al.</i> , 1993 [24]	URI/exacerbations in asthmatics	138/280	19–46	23	Culture, RT-PCR	33%
Teichtahl <i>et al.</i> , 1997 [49]	Exacerbations in hospitalised asthmatics	79	16–66	12	Culture	11%
Eadie <i>et al.</i> , 1966 [7]	ARI/exacerbation in chronic bronchitis	15/75	29–63	30	Culture	16%
McNamara <i>et al.</i> , 1969 [63]	Exacerbations in COPD	29/42	19–75	29	Culture	43%
Smith <i>et al.</i> , 1980 [66]	ARI in COPD	150/798	Adults	96	Culture	6%
Seemungal <i>et al.</i> , 2001 [25]	Exacerbations in COPD	83/168	66 (mean)	12	RT-PCR	23%
Smyth <i>et al.</i> , 1995 [71]	Exacerbations of CF	108/157	2–20	12	RT-PCR	16%
Collinson <i>et al.</i> , 1996 [72]	URI in CF patients	38/119	0.2–18	17	RT-PCR	18% (43%)

Some studies did not distinguish between rhinoviruses and enteroviruses or fully characterise isolates/RT-PCR products. Most picornaviruses are likely to be rhinoviruses.

pain, tracheal tenderness, and in two instances, wheezing. In comparison, coryzal illness was common but tracheobronchitis occurred in only two (7%) of 31 volunteers after intranasal inocula-

tion by coarse spray or drops. Other early clinical observations linked rhinovirus infections to exacerbations of chronic bronchitis in adults [7] and acute wheezy bronchitis in children [8]. These studies

also documented recovery of rhinovirus from sputum samples more often than from upper respiratory tract samples. For example, one prospective 30-month study of 15 chronic bronchitis patients found that one-half of colds were preceded or followed by exacerbations [7]. Rhinoviruses were recovered from nasal washings, throat swabs and/or sputum in 23% of 47 illnesses affecting the chest; they were isolated from sputum in five instances, including three for which throat swab samples were negative, and in titres as high as 10^4 TCID₅₀/ml [7]. A 17-month study of 22 children aged 5–15 years who experienced 72 episodes of wheezy bronchitis (acute illness with cough and variable degrees of wheezing, breathlessness and mucus expectoration) found rhinorrhoea in all but one episode and documented rhinovirus infection in 33% of all episodes and 40% of severe ones [8]. Of 24 proven HRV infections, a comparison of initial nose and throat swabs and sputum samples found virus by culture in sputum only in 38%, in upper respiratory samples only in 12%, and in both in 50% of instances. Such differences in virus recovery from lower compared with upper respiratory tract specimens suggested that virus was replicating in the lower airways and not that samples were simply being contaminated by virus from the upper respiratory tract. Subsequently, multiple lines of evidence emerged to indicate that HRVs are capable of replication in the lower respiratory tract.

Lower airway temperatures

Although HRVs are generally temperature-restricted in replication with optimal growth at 33°–35°C, the temperatures observed in the tracheo-bronchial tree are often lower than body core temperatures and so are permissive for HRV replication. Direct monitoring of airstream temperatures has been performed in six healthy adults through the use of multiple thermistors at six points from the trachea to beyond subsegmental bronchi under different conditions [9]. During quiet breathing of room air (26.7°C), the mean inspiratory temperatures gradually increased from 33.2°C at the carina to 35.5°C distally. High levels of ventilation or quiet breathing of very cold air (–18.6°C) were associated with up to approximately 3°–4°C decreases in temperatures at proximal sites. During expiration, heat was continuously transferred back to the bronchial mucosa, so that the mean tempera-

ture decreased from 36.3°C at the periphery to 32.9°C near the glottis. With increased ventilation rate or inspiration of colder (and therefore drier) air, more conditioning of inspired air takes place in the lower airways. During expiration, cooling of the respiratory mucosa occurs to facilitate heat recovery. Such lower airway temperatures would support HRV replication.

Replication in respiratory cells *in vitro*

Many HRV serotypes and clinical strains can replicate efficiently at core body temperatures [10]. One *in vitro* study found that the serial growth of seven of eight HRV serotypes was only modestly less productive (<0.5–1.0 log₁₀ TCID₅₀ differences in infectious titres) in HeLa cell monolayers maintained at 37°C compared with 33°C [10]. Furthermore, four of eight wild-type viruses recovered from nasal lavages replicated as well, and one more efficiently, at the higher temperature compared with 33°C. No evident correlation between temperature-related differences in replication and upper or lower illness were found in this small sample. Another study found slightly lower HRV titres in human bronchial epithelial cells (<1.0 log₁₀ TCID₅₀) at 37°C than 33°C for two serotypes [11].

In vitro studies have established that HRV, as well as other respiratory viruses, are capable of infecting human respiratory epithelial cell lines *in vitro* and inducing pro-inflammatory cytokine and chemokine release. Exposure of the human respiratory epithelial cell line BEAS-2B (derived from bronchial epithelium transformed by an adenovirus 12-SV40 hybrid virus), to rhinovirus 14 resulted in productive viral replication for at least 72 h and increased production of IL-8, IL-6 and GM-CSF without effects on cell viability [12]. IL-8 elaboration was maximal at 24 h, about 3-fold higher than in controls, and correlated with viral titres. Cytokine release required active replication, in that inhibition of viral infection by antibody to ICAM-1 or use of UV light-inactivated virus blocked IL-8 increases. Infection was inefficient, not associated with changes in ICAM-1 expression, enhanced by pre-exposure to TNF-alpha and decreased by exposure to IFN-alpha or gamma. Exposure of A549 cells (a transformed human alveolar cell line) to rhinovirus 9 resulted in non-cytolytic, productive infection and prolonged increases in IL-8 release [13]. Replication was non-sustained with peak titres of <2 log₁₀ TCID₅₀ at

24 h, but IL-8 release continued up to 5 days after infection. Prevention of HRV receptor binding with soluble ICAM-1 completely blocked replication and cytokine release, whereas exposure to UV light-inactivated virus decreased release by about 50%.

Several HRV serotypes have been shown to replicate in primary human bronchial epithelial cells, to cause cytopathic effect under certain conditions, and to induce production of IL-6, IL-8, IL-16, GM-CSF and RANTES (regulated on activation, normal T cells expressed and secreted) [11,14]. For HRV type 16, the magnitude and kinetics of *ex vivo* replication were found to be similar in primary adenoidal and bronchial epithelial cells, although infection occurred much less often in both cell types compared with HeLa cells [15]. Consistent with the finding of scanty foci of replication in nasal mucosal biopsies by *in situ* hybridisation following experimental infection of humans [16], viral infection was documented by infectious center and immunohistochemical assays in less than 10% of exposed cells, despite the use of very high viral inocula. One explanation for this low proportion of infected cells may be the finding of a less efficient eclipse mechanism, that is less uncoating of attached virus, perhaps due to a paucity of ICAM-1 receptors on the surface of bronchial epithelial cells [15]. Inflammatory conditions, including HRV infection itself [17] which up-regulate ICAM-1 expression could theoretically enhance the likelihood of lower respiratory tract replication or perhaps even foster initial infection following inhalation of droplets into the lower respiratory tract.

Detection of HRV in the lower respiratory tract

During naturally occurring colds it is likely that the lower respiratory tract is inoculated with infectious droplets from the upper airway possibly during bouts of coughing or sneezing and during sleep. Studies of experimentally infected volunteers suggest that HRV replicates in the lower airways, usually without visible changes in the bronchial mucosa. The possibility of contamination from the upper airway cannot be excluded in some studies. Following experimental infection by intranasal instillation of virus, HRV was cultured from protected bronchial brush samples of 5 of 13 (38%) subjects who had positive nasal samples [18]. All five had clinical illness including

cough and the bronchoscopy specimen titres were similar to those observed in nasal brush samples; virus was not recovered at bronchoscopy from five infected volunteers without illness. Following inoculation by both intranasal instillation and large particle aerosols, HRV RNA has been detected in lower airway secretions and cells by RT-PCR [19] and directly by *in situ* hybridisation [14]. In eight allergic volunteers inoculated intranasally and by coarse aerosol with HRV 16, viral RNA was detected in nasal lavages and lower airway cells at 2–4 days after infection in all subjects [19]. RNA was detected by the RT-PCR assay in only three of eight acellular BAL samples in comparison to 21 of 26 airway cellular samples, which suggested that HRV16 was largely cell-associated and that the positive results were less likely to be due to contamination from the upper airway. In ten adults experimentally inoculated with HRV by intranasal drops and aerosols, the replicative form of viral RNA was detected in bronchial epithelial biopsies of four subjects [14]. Such findings establish that HRV replication can occur in the lower airways, although the frequency, duration and extent of direct viral involvement and its pathogenetic consequences remain to be defined under natural circumstances. Whether rhinoviruses can replicate in alveolar pneumocytes also remains to be established. However, in 9 experimentally infected healthy volunteers, bronchial biopsy specimens showed 9-fold increases in 5-lipoxygenase positive cells, increased numbers of cyclooxygenase positive cells, and 3–4-fold increases in macrophages and mast cells, as well as doubling of BAL fluid cysteinyl leukotriene levels [20]. These findings indicate that HRV infection can cause bronchial inflammation with enhanced expression of 5-lipoxygenase pathway proteins and cyclooxygenase-2, making them potential targets for therapeutic intervention studies.

CLINICAL EVIDENCE OF LOWER AIRWAYS DISEASE

HRV infections have been associated with lower respiratory tract illness and hospitalisations across the age spectrum. For example, one prospective 4-year study including 1068 children and adults hospitalised with various acute respiratory illnesses found HRV infection by virus isolation from nose and/or throat swabs in 4.3% of children <5 years, 6.0% of those aged 5–35 years and 2.4% of adults

aged >35 years [21]. The most common diagnoses in young children were asthma, bronchiolitis, possible sepsis and pneumonia. Asthma was the principal reason for admission in those aged 5–35 years, whereas pneumonia, congestive heart failure (CHF), chronic obstructive airways disease (COPD) exacerbation and asthma were the admitting diagnoses in the older adults, in whom the average length of hospital stay was 6 days. This and many other studies utilised virus isolation for detection of HRV infections and thus underestimated their contribution. The impact of HRV infections has also been underestimated because many studies were conducted outside high HRV prevalence periods or for short periods of observation. Although HRVs cause infections year-round, the peak prevalence periods are the early fall and late spring months in the northern hemisphere [3]. Consequently, studies focusing on the winter months do not accurately reflect the relative contribution of HRVs to illness on an annual basis. For example, HRV RNA was detected in only 1.9% of 719 adults presenting with influenza-like illness (fever and respiratory plus systemic symptoms) during one influenza season, although it was found in 19% of such illnesses among a subset of 78 persons in whom no other aetiology was established by standard methods [22].

Appreciation of the epidemiological and clinical importance of HRV infections has been enhanced by more sensitive viral diagnostic tests, specifically application of RT-PCR testing. The limitations of diagnosis by RT-PCR are uncertainties about the prevalence of asymptomatic infection and the duration of HRV RNA detectability in the upper respiratory tract of children and adults. For example, older epidemiological studies isolated rhinoviruses in 1.5%–2.1% of respiratory samples collected from well adults and from higher proportions of apparently well children [3]. One study of asymptomatic elderly adults found HRV RNA in 3% of upper respiratory samples [23], whereas another study by the same group of asthmatic adults found no positives in 61 routine samples [24]. A study of 83 stable COPD patients found HRV RNA in 6% at baseline [25]. A study of asthmatic children aged 9–11 years found a positive RT-PCR assay for picornavirus in 12% of samples collected during asymptomatic periods [26]. A recent study of 29 asthmatic children found HRV RNA in 17% of nasopharyngeal aspirates collected

when they were clinically well [27]. Another survey in 107 children aged 1 month to 17 years without respiratory symptoms at the time of admission for surgery detected HRV RNA in 18% and enterovirus RNA in 11% of nasopharyngeal samples [28]. However, 81% of the positive children had recently experienced respiratory symptoms or were in contact with household members with concurrent symptoms, and others developed symptoms after sampling. Overall 5% of apparently healthy children without previous or incipient respiratory symptoms were picornavirus RNA positive.

Paediatric pneumonia and bronchiolitis

Bronchiolitis and pneumonia are the most common syndromes in children aged <5 years who are admitted to hospital with HRV infection (Table 1) [21]. A prospective 20-month study of infants, approximately 80% of whom were aged 12 months or less and 91% of whom were hospitalised, found that nasopharyngeal aspirates were culture positive for rhinovirus in 11.8% of 519 illnesses, second only to RSV [29]. HRV was especially important in infants aged 6–12 months, in whom it was associated with 14% of lower respiratory illnesses and with pneumonia as often as RSV. A 5-year review of 40 infants \leq 30 days of age hospitalized with viral pneumonia found that HRVs were cultured from 15% and enteroviruses from 15%, compared to 55% for RSV [30]. A retrospective, 6-year hospital-based study of 93 HRV culture-positive paediatric patients, 72% of whom were 12 months of age or younger, found that 95% had acute lower respiratory illness and 14% had fever with suspected sepsis [31]. Fifteen of the 64 with chest radiographs had interstitial or lobar pneumonias, and five children required ventilatory support. Overall, 31% were considered to be otherwise healthy, but the majority had one or more underlying conditions including prematurity, reactive airways and congenital cardiac disease. Another 2-year study of infants and young children with bronchopulmonary dysplasia identified eight cases of severe, culture proven HRV lower respiratory illness, three of which were nosocomially acquired, among 40 patients [32]. HRV was second to RSV in frequency and less often associated with the need for mechanical ventilation.

One prospective study, which examined 118 otherwise healthy children aged 18 months and

younger hospitalised with a clinical diagnosis of bronchiolitis, found viral infection in 74% of patients by RT-PCR [33]. Among those with documented viral infection, RSV was detected in 72%, HRV in 29%, adenovirus in 8.5%, and influenza, parainfluenza and coronavirus each in 2.6%. Compared with infants with RSV infection alone, those with HRV infection alone were hospitalised earlier in their illness course (1.8 versus 3.1 days) and were somewhat older (5.2 versus 3.2 months). HRV infection was associated with higher clinical severity score on admission. A study of 84 infants hospitalised with bronchiolitis found picornavirus RNA in 31% of nasal aspirates, compared with 54% of samples positive for RSV [34]. Picornavirus was the only virus identified in 18% of illnesses. Another 1-month study during a period of RSV and influenza circulation found evidence for picornavirus infection by RT-PCR of nasopharyngeal secretions, predominately HRV, in 22% of 50 infants admitted with bronchiolitis compared with 54% with RSV [35]. A recent 9-month study of 132 children aged 4 months–13.5 years hospitalised for acute expiratory wheezing (25 with bronchiolitis, 59 with wheezy bronchitis, 48 with asthma) utilised RT-PCR and detected HRV in 27%, enterovirus in 22% and non-typable picornavirus in 16%, so that nearly two-thirds of these episodes had an associated picornavirus infection [36].

The frequencies of primary viral pneumonia and secondary bacterial infection associated with HRV infection in infants and young children are not well characterised. One prospective study cultured HRV from 11.9% of 126 children with pneumonia, almost all of whom were aged 1 year or less [29]. A 3-year prospective Finnish study of 254 children (43% aged 2 years or less, 33% aged 2–5 years) hospitalised with radiographically documented community-acquired pneumonia (CAP) utilised antigen and RT-PCR assays of nasopharyngeal aspirates, as well as viral cultures and paired serologic studies to examine the role of 17 pathogens [37]. A potential causative agent was detected in 85% of patients with viral infection in 62%, bacterial infection in 53% and both in 37%. The three most commonly detected agents were *S. pneumoniae* in 37%, RSV in 29% and HRV in 24%. About one-half of those with viral infection had evidence for concomitant bacterial infection. Of 58 HRV infections, 52% had evidence of concomitant bacterial infection by serologic testing,

compared with 44% of 73 RSV infections. One retrospective study of 93 hospitalised children with culture-proven HRV infection found concurrent infection with other respiratory viruses in four and bacterial infection in four others [31].

Direct evidence for HRV invasion of the pulmonary parenchyma has been found in individual paediatric patients with pneumonia by virus isolation from bronchoalveolar lavage (BAL) [38] and by immunohistochemistry of lung tissue in one case [39]. The latter case of fatal disease in a 2-month-old infant found histologic evidence of hyperplasia and desquamation of the alveolar lining cells and localisation of HRV antigen in alveolar epithelial cells and macrophages [39]. Another case report described recovery of HRV 47 from the postmortem lung and blood of an 11-month-old asthmatic child who died suddenly and was found to have necropsy findings of eosinophilic bronchial inflammation and plugging, interstitial pneumonia with hyaline membranes and severe tracheobronchitis [40]. In aggregate, the available evidence suggests that HRV is the second most commonly recognised viral cause of bronchiolitis and pneumonia in infants and young children after RSV and uncommonly may be associated with severe viral pneumonitis, as well as secondary bacterial infection. It remains to be determined whether such illnesses predispose to subsequent risk of wheezing and asthma.

Dual viral infections

HRV infection is one of the most frequent pathogens implicated in dual respiratory viral infections. It has also been hypothesised that dual viral infections, particularly those related to picornaviruses or adenoviruses, could enhance illness severity, although this remains to be definitively established. In one bronchiolitis study [33], a combination of viruses was detected in 19.5%, most often dual infection with RSV and HRV (69% of instances). Nearly one-half of those with HRV infection had more than one virus detected, but there was not a significantly greater disease severity on admission in this group. Patients with dual infections were admitted later in their disease course than those with single pathogens, a finding which suggested sequential infection. Another study of bronchiolitis in hospitalised children found dual viral infections in 21% of children, including 42% of those with picornavirus

infections, but no evidence for greater severity of illness compared with single infection [34]. It is unclear from such hospital-based studies whether dual infections increase the severity of illness and subsequent likelihood of hospitalisation. An extensive analysis of respiratory viral studies identified dual infections in 5% of 1341 illnesses across the age spectrum; the diagnostic method was a major variable, such that the proportion of dual infections increased to 11.6% in studies employing RT-PCR [41]. Influenza A and HRV were the most commonly implicated viruses, and the likelihood was higher in infants. Patients with dual infections were more likely to be hospitalised than those with infection due to a single virus.

Adult bronchitis and pneumonia

Ambulatory elderly persons with HRV infection experience a high frequency of prolonged illness and lower respiratory tract symptoms, particularly those with underlying airways disease. In an HRV outbreak among elderly residents of a long-term care facility, the 35 proven HRV infections were associated with lower respiratory symptoms in 66%, systemic symptoms in 71%, auscultatory changes in 52%, and requirement for bronchodilator therapy in 29% [42]. One developed radiographically documented pneumonia, and another died from respiratory failure. Those with underlying lung disease were more likely to develop dyspnoea, bronchospasm and protracted cough. One prospective study of 533 ambulatory adults aged 60–90 years detected HRV RNA in 24% of 497 respiratory illnesses during two winter seasons [23]. Among those with first or sole HRV infection, the median duration of illness was 16 days, and 63% had lower respiratory tract symptoms (productive cough, wheeze, chest pain), 26% were unable to perform routine activities, 19% were confined to bed, 43% consulted their physicians, and one died from exacerbation of COPD. The presence of chronic medical conditions or smoking increased the likelihood of lower respiratory illness by approximately 40% in elderly adults with HRV infection. These authors estimated that HRV infections caused the greatest overall disease burden, exceeding that of influenza and other respiratory viruses [43].

A single winter season study (November–April) of elderly adults (aged ≥ 65 years) hospitalised with a clinical diagnosis of acute respiratory illness

including pneumonia, COPD exacerbation, bronchitis or CHF found evidence of HRV infection by RT-PCR in five of 100 randomly selected nasopharyngeal samples from patients who were negative for influenza or RSV infection [44]. Two of the five had radiographically proven pneumonia, but all recovered and were discharged after an average 8-day hospitalisation. All patients had significant underlying medical problems, principally severe COPD and CHF. However, a prospective 4-year study of 417 adults aged ≥ 45 years who were hospitalised with acute respiratory illness in association with various underlying conditions found culture-positive HRV infection in only 2.2% [45].

Asthma

Many studies of both children and adults have linked HRV infection to exacerbations of asthma (Table 1), although the frequency of HRV detection has depended heavily on the sensitivity of the laboratory methods [46]. One longitudinal 13-month study of 108 children aged 9–11 years examining the relationship between exacerbations of asthma and viral infection found 292 reported respiratory illness episodes (mean, 2.5 per child-year), in which picornavirus infection was detected in 147 (50%) [26]. Most were detected by RT-PCR and were proven or likely to be HRVs. Overall a virus infection was found in about 80% of reported illnesses with lower respiratory manifestations (cough, wheezing, shortness of breath) or falls in peak expiratory flow rates. The median delay between onset of respiratory symptoms and decreases in peak expiratory flow rates was only 1 day; the delay was 2 or more days in 46% of those presenting with upper respiratory symptoms. Such findings indicate that the time window for therapeutic intervention to prevent exacerbations after the onset of respiratory symptoms is narrow. A follow-up analysis documented strong correlations between the seasonal patterns of viral respiratory infections and asthma hospitalisations for both children and adults [47]. Upper respiratory illnesses and asthma admissions were both more common during school attendance than during breaks; peaks were associated with new school terms and HRV infections were associated with all of the four peaks in admissions identified during this 1-year study. A recent study of 179 asthmatic children hospitalized with exacerbations

during two winter and two spring/summer seasons detected HRV RNA in nasopharyngeal aspirates in 79%, a significantly greater proportion than the observed frequencies of 17% in 29 asymptomatic asthmatic children and 52% in 50 non-asthmatic ambulatory children with upper respiratory tract illness [27]. Another study of 81 children less than 2 years of age who were hospitalised with wheezing found HRV RNA in 33% and enterovirus RNA in 12%; HRV was the sole pathogen in 81% of 27 positive patients, and it was most common in those aged 12–17 months (65% of children) [48]. HRV infection was associated with atopic dermatitis in infancy, and follow-up at approximately 6 years found a possible association between HRV infection at the initial admission and subsequent diagnosis of early school-age asthma. Further study is needed to determine whether HRV-associated wheezing in infancy may be associated the development of asthma.

A 23-month longitudinal study of 138 asthmatic adults (89% allergy history, 38% prior hospitalisation for asthma) identified 315 self-diagnosed acute respiratory illness episodes, during which 27% had objective evidence of an asthma exacerbation (≥ 50 L/min decrease in peak expiratory flow rate; PEFr) [24]. Colds were reported in 80% of episodes with lower respiratory symptoms and 89% of colds were associated with asthma symptoms. Of 229 episodes sampled, HRV infection was detected by culture in 8% and by RT-PCR in 33%. Lower respiratory symptoms were present in about 70% of proven viral infections and PEFr decreases ≥ 50 L/min lasting at least 7 days after symptom onset in 24%. Another longitudinal study in adult asthmatics encompassing 137 acute respiratory illnesses, 63% of which had an associated exacerbation, found respiratory viral infection in 44% of exacerbations, most commonly picornavirus followed by parainfluenza, influenza and coronavirus [46]. A related emergency room-based study enrolling adults with asthma exacerbations found that 76% of patients had upper respiratory tract symptoms and that picornaviral RNA was detectable in 36% of 148 episodes [46]. Most of the picornaviruses in both studies were characterised as rhinoviruses. A 1-year study detected HRV by culture from 11% of adults hospitalised with severe asthma exacerbations [49]. A recent prospective study collected nasal aspirates every 2 weeks irrespective of symptoms from 76

cohabiting couples, one partner of which had asthma [50]. HRV RNA was detected at similar frequencies, 10% and 8.5% of samples from asthmatic and healthy partners, and the severity of associated upper respiratory tract illnesses (URTI) did not differ between the groups. However, lower respiratory tract illness occurred more often in asthmatics, 43% versus 17% following the first HRV infection, and they had greater average decrements in PEFrs. Such findings indicate that asthmatic adults are not more susceptible to HRV infection but are more likely to develop lower respiratory illness.

The likelihood of wheezing following HRV infection appears to depend on host factors, including age and presence of allergy, as well as severity of the acute respiratory illness. Not all acute respiratory illnesses are associated with airway obstruction. One study of asthmatic children found that 15 (74%) of 21 HRV infections were associated with wheezing and that the severity of HRV-associated illness correlated with the occurrence of asthma attacks [51]. An emergency room-based study of wheezing children found that HRV infection was detected by culture somewhat less often in those less than 2 years old (15% of episodes) than in those older than 2 years (23% of episodes) [52]. In those aged 2 years and older, the presence of IgE antibody to inhalant allergens was an important risk factor, particularly in conjunction with viral infection. A follow-up study also determined that HRV infection was significantly associated with episodes in children aged 2 years and older, in whom viral RNA was detected in 71% compared with 36% in controls [53]. In this older group of children, the odds ratio for wheezing was much higher in those with a combination of RT-PCR positivity and presence of IgE to aeroallergens by radioallergosorbent test (RAST), nasal eosinophilia or elevated nasal eosinophilic cationic protein. In adults experimentally infected with HRV 16, significantly greater changes in airway responsiveness to inhaled histamine have been observed in volunteers with allergies compared with healthy control subjects, who in general do not show changes in reactivity [54]. Such observations are consistent with the concept that HRV infection potentiates pre-existing airway inflammation in asthmatics.

The mechanisms responsible for the association between HRV infection and asthma exacerbations

are incompletely defined but in part relate to the wide variety of inflammatory mediators produced by airway epithelial cells in response to viral infection (reviewed in [54–56]). One of the histologic hallmarks of asthma is eosinophilic infiltration. During experimental HRV infection of both asthmatic and otherwise healthy persons, eosinophilic infiltration was found in the bronchial mucosa at 4 days after infection [57], although another study did not find increased eosinophils in nonatopics [20]. In contrast to healthy subjects, those with asthma continued to show eosinophilic infiltration on repeat biopsies 6–8 weeks after infection. The chemokine IL-8 may play a critical role in both eosinophil and neutrophil recruitment and activation in HRV-associated asthma exacerbations. In asthmatic children with virus-induced exacerbations predominantly due to HRV infection, increased nasal lavage levels of IL-8 and neutrophil myeloperoxidase are correlated with each other and with the severity of upper respiratory symptoms [58]. Similarly, increased nasal lavage levels of IL-8 were correlated with bronchial hyper-reactivity in experimental HRV infections of asthmatic adults [59]. Such observations suggest that IL-8 might be a target for therapeutic intervention.

Viral infections also potentiate the inflammatory and bronchoconstrictive responses to inhaled allergens. Allergic subjects experimentally infected with HRV develop both immediate and late allergic responses with increased BAL histamine concentrations after segmental allergen challenge, in contrast to uninfected subjects or those without allergy [60]. Bronchoconstriction can also follow altered neural control including enhanced parasympathetic responses and other mechanisms [55]. Viral infections may cause reduced activity of the enzyme-neutral endopeptidase that inactivates tachykinins, a group of peptide transmitters produced in sensory nerves that result in bronchoconstriction, increased vascular permeability, glandular secretion and leukocyte influx [56]. Airway parasympathetic neurons also produce eotaxin which attracts eosinophils. Infections can augment vagally mediated bronchoconstriction through multiple mechanisms including inhibition of the expression and action of the M2 muscarinic receptors inhibitory for M3 receptor mediated constriction. Major basic protein from eosinophils also blocks the function of M2 receptors. With respect to treatment, corticosteroids can increase M2

receptor activity, and topical anticholinergics, specifically ipratropium bromide, can provide therapeutic benefit in acute exacerbations [56]. However, one placebo-controlled study in 104 school-aged asthmatic or atopic children found no prophylactic benefit of inhaled corticosteroids in ameliorating episodes of reduced expiratory flow or of lower respiratory tract illness during 6 months of dosing [61]. Viral aetiology studies were not determined in this study. Another placebo-controlled study of inhaled corticosteroid prophylaxis in a small number of atopic adults found no significant effects on the number of inflammatory cells in bronchial biopsy specimens following experimental HRV infection [62]. More information on the clinical and virologic effects of inhaled and oral corticosteroids for HRV-associated exacerbations of asthma is needed.

Chronic obstructive pulmonary disease

Approximately one-half or more of COPD exacerbations are associated with colds or pharyngitis. Wide disparities exist in HRV detection rates among studies examining acute respiratory illness and exacerbations of COPD; the differences relate in part to differences in virologic methods and the populations studied. Older prospective studies of COPD patients, which employed virus culture as the means for HRV detection, found that approximately 14%–43% of exacerbations were associated with HRV infection [63]. One 10-month trial [64] collected nasal washings and throat swabs monthly and at the time of respiratory illness from 34 chronic bronchitis patients and from 19 control subjects. HRV infection was found in 14.3% and 19.0% of samples during acute respiratory illnesses, respectively, in each group and in 2.1% and 1.1% of samples during periods of quiescence, respectively. Exacerbations occurred in about 90% of the bronchitics with respiratory illness, and 68% of 56 exacerbations were associated with upper respiratory symptoms. Such results indicate that patients with chronic bronchitis are not more susceptible to HRV infection but that such infections are more likely to be associated with lower respiratory tract symptoms, a conclusion supported by comparable findings in COPD [65] and asthma patients (discussed above). A similar study of patients with moderate to severe obstructive airway disease employed prospective cultures of throat samples during clinic visits and

found that HRVs were recovered in 43% of exacerbations and that 78% of HRV isolations were associated with an exacerbation [63]. Another 3-year prospective study in patients with COPD cultured rhinoviruses from 6.1% of illnesses studied, making it the most common virus identified, and found that 88% of HRV isolations were associated with illness [66]. HRV infections were associated with cough (71% of episodes) but, unlike influenza, not with deterioration in forced expiratory volume (FEV₁) measurements [67]. A more recent study in adults with COPD utilised serology and culture for virus over an average 26 month follow-up period and found that respiratory illnesses were more common in those with COPD but that such illnesses were less often associated with proven viral infections (19%) than in controls with ARI (39%) [65]. Picornaviruses accounted for 23% and 31% of the documented viral infections in the COPD and controls, respectively. Only one hospitalisation was associated with HRV infection.

One 2.5 year prospective study of 101 moderate-severe COPD patients found that 35% of 504 exacerbations were associated with common colds and 12% with sore throat at onset [69]. The presence of cold symptoms at the onset of exacerbations was associated with both significant decreases in PEFr and more protracted recovery. Only 75% of all exacerbations had returned to baseline lung function by 5 weeks follow-up. Corticosteroids but not antibiotic therapy appeared to benefit the rate of PEFr recovery; neither significantly affected symptom recovery. A follow-up study utilising RT-PCR for HRV detection found that 64% of exacerbations were associated with colds occurring up to 18 days beforehand and that HRV was detected in 23% of 168 exacerbations and in 58% of identified viral infections [25]. Viral infections were associated with slower recovery time (median, 13 versus 6 days) and higher plasma fibrinogen and IL-6 levels than episodes without virus detected. Because colds have been associated with more protracted recovery, corticosteroids have been proposed as early treatment of COPD exacerbations associated with colds [69]. However, corticosteroids have also been shown to enhance HRV replication in the upper respiratory tract during colds [70,71], so that it will be important to assess whether their use affects HRV replication in the lower airways during exacerbations of pre-existing airways disease.

Cystic fibrosis

Studies relying on cell culture isolation have detected low frequencies of viral infection in cystic fibrosis patients, possibly related to technical difficulties in recovering virus from tenacious mucoid secretions. In contrast, a 6-month prospective study of 108 cystic fibrosis patients (median age, 8 years) detected HRV in nasopharyngeal aspirates by RT-PCR in association with 16% of all 157 exacerbations studied and in 57% of the 44 with a proven viral aetiology [72]. Dual infection with another respiratory virus was found in five instances. HRV infections were associated with significantly greater duration of intravenous antibiotic therapy but not with differences in pulmonary function testing compared with those without detectable viral infection. Another 17-month prospective study of colds in 38 children with cystic fibrosis (median age, 7.5 years) found that picornavirus RNA was detected in 43% of 119 nasal and pharyngeal swab samples during colds; approximately 40% of these or 18% of sampled episodes were shown to be due to HRV infection [73]. Decrements in spirometric values were comparable in picornavirus- and non-picornavirus-associated episodes. Children with more frequent colds had evidence of more rapid disease progression, and most new bacterial infections were associated with colds.

Immunocompromised hosts

Increasing evidence also indicates that HRV infections are associated with lower respiratory tract involvement and severe disease in iatrogenically immunocompromised hosts, particularly transplant recipients. Our retrospective analysis of 431 virus-positive BAL or bronchial biopsy cultures over a 10-year period at an academic medical centre identified 28 (7%) that were HRV-positive; HRV represented the third most common virus detected after CMV (64% of positive samples) and HSV (21%) [74]. These positives occurred in 20 hospitalised patients aged 2.5–86 years, all of whom had acute respiratory illness, abnormal chest radiographs, and the presence of an immunosuppressing condition (including solid organ transplantation in 35%, malignancy in 20% and AIDS in 10%) and/or immunosuppressive therapy (90%). Leukopenia was present in 30%. One lung transplant recipient had intermittent recovery of HRV from respiratory samples over a 15-week period, although attempts to detect HRV RNA in

bronchial biopsies were unsuccessful [74]. Other pathogens were detected in 70% of patients including fungi in 45%, bacteria in 40%, and other viruses in 25%. Histopathologic abnormalities were found in seven of 13 biopsies, and two biopsies from patients without other recognised infections showed acute inflammation with fibropurulent debris in alveoli. Detection of HRV was associated with a poor prognosis; 60% of the patients were cared for in an intensive care unit and 25% died during that hospitalisation. The findings suggested that HRV infection could be associated with severe lower respiratory tract disease in some highly immunocompromised patients.

The frequency of HRV infections and risk factors for lower respiratory tract disease in transplant recipients have not been well characterised. An 8-year prospective study of marrow and stem cell transplant patients identified 31 patients with HRV infection, representing one-quarter of community respiratory viral infections [75]. BAL was performed routinely for radiographic evidence of pneumonia. In 28 of 29 instances (97%) the isolate was obtained from the upper respiratory tract, and only one BAL was positive for HRV. These findings suggested that HRV infections rarely caused lower respiratory tract disease, although the timing of these infections in relation to transplantation was not delineated [75]. A 7-year study of lung transplant recipients which obtained routine BAL specimens after transplantation found that six of seven patients with HRV detection were symptom free, although three had evidence for rejection or organising pneumonia [68]. In contrast, another prospective 5-year study in adult blood and marrow transplant recipients who were undergoing conditioning or were myelosuppressed before engraftment identified 22 HRV infections among approximately 2000 persons (~1%) undergoing transplantation [76]. HRV infections accounted for about 8% of persons with acute respiratory illness. All presented with manifestations of upper respiratory illness within 10 days after transplantation, and 64% of infections were nosocomially acquired following 7–22 days of hospitalisation. These illnesses occurred sporadically throughout the year without seasonal predominance. In 15 patients the illness remained uncomplicated, although associated with a high frequency of fever (47%), and lasted an average of 14 days. In seven (34%) patients the infection was complicated by

pneumonia and death due to progressive respiratory failure. Fever (86%), wheezing (57%), and dyspnoea (71%) were common in these patients. The mean time from onset of respiratory symptoms until ventilatory support was 12 days (range, 3–21 days), and HRV was cultured from antemortem BAL or tracheal aspirate in six of seven patients. In five autopsies, four showed findings consistent with viral pneumonia, including interstitial pneumonitis and/or acute respiratory distress syndrome with diffuse alveolar damage, congestion and hyaline membranes. One autopsy revealed invasive aspergillosis but the other four found no other pathogens in the lung, although three of four had other serious infections antemortem. An important issue in these patients was the extent to which chemotherapy- or radiation-induced pulmonary damage and/or myelosuppression might have predisposed to the development of pneumonia [76], although the findings suggested that infection early post-transplant had increased risk of an unfavourable outcome. A recent retrospective analysis of 77 marrow transplant recipients with pneumonia during one respiratory season identified eight BAL samples (6% of 122 tested) in six patients (8%) that were positive for HRV RNA by RT-PCR [77]. The samples were collected an average of 151 days post-transplant, indicating that almost all were community acquired infections. In two patients repeat BAL samples were positive at intervals of 31 and 44 days after the initial positive one, which raised the possibility of prolonged lower respiratory tract replication. The fatality rate in HRV-infected patients was 83% but all of them had significant co-infections. The mean time from respiratory illness onset to death was 54 days; no autopsy data were obtained. The available evidence indicates that HRV may be associated with lower respiratory tract disease in highly immunocompromised marrow transplant recipients, frequent co-infections and often poor prognosis. However, the contribution of HRV to overall pathogenesis remains uncertain with regard to causing direct viral damage or predisposition to secondary invaders. The timing of infection in relation to transplantation and type of transplant may be an important variable with regard to severity of disease, and more data are needed from both HSCT and solid organ, particularly lung transplant recipients.

Implications for antiviral use

The considerable burden of HRV infections with respect to both upper and lower respiratory tract disorders highlights the medical need for effective interventions. Unfortunately, no safe and effective HRV-specific antiviral agents are currently available for treatment or prevention of HRV illness. An important limitation of the available data regarding HRV infection in most target populations is the lack of information regarding viral dynamics, specifically quantitative infectious viral levels at different sites (upper versus lower respiratory tract) and time points in illness. Consequently, the importance of ongoing viral replication in disease pathogenesis has not been defined for most of the lower respiratory syndromes associated with HRV. In this regard, controlled studies with selective antiviral agents would be helpful in assessing the contribution of HRV infections to lower respiratory tract disease in many target groups.

Prevention of HRV infections by antiviral chemoprophylaxis or other control measures (e.g. handwashing) would be expected to reduce the risk of lower respiratory tract illness, particularly in those with underlying conditions. In contrast, given the short time between onset of colds and exacerbation of pre-existing airways diseases, it is unclear whether early treatment could prevent such exacerbations. Whether antiviral treatment might modify an established exacerbation of asthma or COPD would depend heavily on the extent of HRV replication and its role in promoting deleterious host responses. Combinations of antiviral and interventions directed at such host responses would seem to offer the highest likelihood of therapeutic success. Treatment studies of anti-HRV agents in infants and young children and in immunocompromised hosts could provide important data regarding the role of HRV infection in causing viral pneumonia, as well as potentially providing clinical benefit.

Among investigational antivirals studied in the past (reviewed in reference [78]), only intranasal interferons have been proven to have prophylactic efficacy against natural HRV infections. However, intranasal interferons are associated with mucosal intolerance after prolonged use and are not therapeutically effective in established HRV colds. While nasal application of antivirals is protective in immunocompetent adults, it remains to be

determined whether delivery to the lower respiratory tract is important for prophylactic efficacy in those with pre-existing airways disorders. Currently two investigational anti-HRV agents, the orally administered capsid-binder pleconaril and the topically applied 3C protease inhibitor rupintrivir, are candidates for use [79]. Pleconaril is the first antiviral to be proven effective in treating HRV colds in otherwise healthy adults [80]. In addition, pleconaril has been used in treating several severe enterovirus infections and one HRV infection in marrow transplant patients with reported clinical improvement [81] and is available on a compassionate use basis.

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