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Human Rhinoviruses

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SUMMARY

Human rhinoviruses (HRVs), first discovered in the 1950s, are responsible for more than one-half of cold-like illnesses and cost billions of dollars annually in medical visits and missed days of work. Advances in molecular methods have enhanced our understanding of the genomic structure of HRV and have led to the characterization of three genetically distinct HRV groups, designated groups A, B, and C, within the genus *Enterovirus* and the family *Picornaviridae*. HRVs are traditionally associated with upper respiratory tract infection, otitis media, and sinusitis. In recent years, the increasing implementation of PCR assays for respiratory virus detection in clinical laboratories has facilitated the recognition of HRV as a lower respiratory tract pathogen, particularly in patients with asthma, infants, elderly patients, and immunocompromised hosts. Cultured isolates of HRV remain important for studies of viral characteristics and disease pathogenesis. Indeed, whether the clinical manifestations of HRV are related directly to viral pathogenicity or secondary to the host immune response is the subject of ongoing research. There are currently no approved antiviral therapies for HRVs, and treatment remains primarily supportive. This review provides a comprehensive, up-to-date assessment of the basic virology, pathogenesis, clinical epidemiology, and laboratory features of and treatment and prevention strategies for HRVs.

INTRODUCTION

Human rhinoviruses (HRVs) were first discovered in the 1950s in an effort to identify the etiology of the common cold. Nearly 60 years later, the search for a "cure" for the common cold virus is still ongoing. Worldwide and nearly year-round, HRV is the most common cause of upper respiratory tract infection (URI), leading to considerable economic burdens in terms of medical visits and school and work absenteeism (1–4). However, while once thought to cause relatively benign upper respiratory tract illness, HRVs are now linked to exacerbations of chronic pulmonary disease, asthma development, and, more recently, severe bronchiolitis in infants and children as well as fatal pneumonia in elderly and immunocompromised adults. Our enhanced understanding of the spectrum of illness of HRVs draws largely from advances in molecular methods that have facilitated the detection and characterization of HRV groups and strains. Indeed, a growing number of clinical laboratories are adopting multiplex PCR-based assays for the detection of respiratory viruses that include HRVs (5).

There are currently no approved antiviral agents for the prevention or treatment of HRV infection. Clinical trials of antiviral therapies have been limited by drug toxicities, drug interactions, and a lack of efficacy when applied to the natural setting. Efforts at vaccine development are hindered by the existence of more than 100 HRV serotypes with high-level sequence variability in the antigenic sites. The treatment of HRV infection remains primarily supportive, including over-the-counter products aimed at symptom relief. Given the frequency of HRV infections and our expanding knowledge of their clinical spectrum, effective control of this virus through treatment and prevention would have significant public health impacts.

In this paper, we provide an up-to-date review of HRVs, including their clinical and molecular epidemiology and disease pathogenesis, laboratory diagnostics, and the status of preventative and therapeutic interventions.

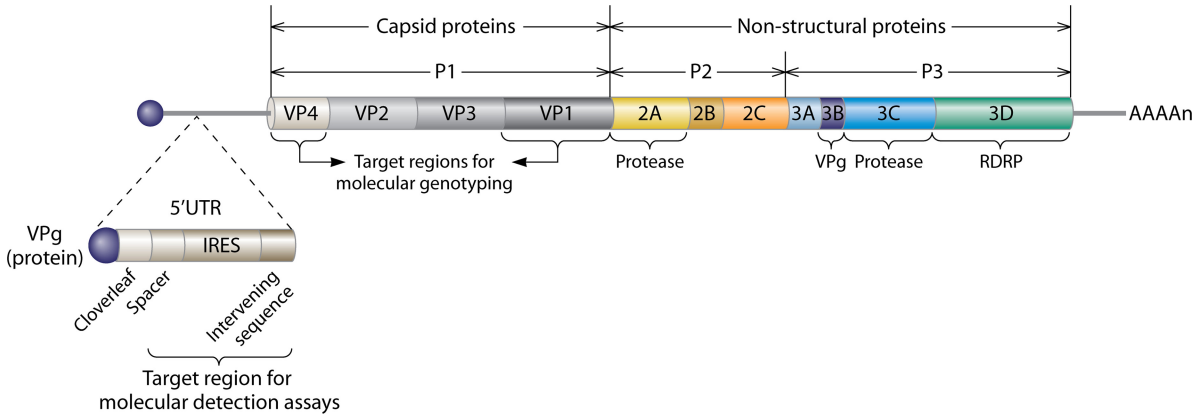

FIG 1 HRV genomic structure. HRV is a 7.2-kb single-stranded, positive-sense RNA virus with a single open reading frame joined to a 5′ untranslated region and a short viral priming protein (VPg). The P1 protein is processed to form the HRV capsid, and P2 and P3 are processed to produce VPg, protease, and RNA-dependent RNA polymerase (RDRP) (6). IRES, internal ribosomal entry subunit.

BASIC VIROLOGY

Virion Structure and Genomic Organization

HRVs, members of the family *Picornaviridae* and the genus *Enterovirus*, are positive-sense, single-stranded-RNA (ssRNA) viruses of approximately 7,200 bp. The viral genome consists of a single gene whose translated protein is cleaved by virally encoded proteases to produce 11 proteins (6) (Fig. 1). Four proteins, VP1, VP2, VP3, and VP4, make up the viral capsid that encases the RNA genome, while the remaining nonstructural proteins are involved in viral genome replication and assembly. The VP1, VP2, and VP3 proteins account for the virus' antigenic diversity, while VP4 anchors the RNA core to the capsid. There are 60 copies each of the four capsid proteins, giving the virion an icosahedral structure, with a canyon in VP1 that serves as the site of attachment to cell surface receptors. More than 90% of known HRV serotypes, the "major group," utilize the cell surface receptor intercellular adhesion molecule 1 (ICAM-1), while the "minor group" attaches to and enters cells via the low-density lipoprotein receptor (LDLR). Some of the major-group HRVs also use heparan sulfate as an additional receptor.

Viral Replication

Depending on the receptor type, virus uptake occurs via clathrin-dependent or -independent endocytosis or via macropinocytosis (Fig. 2). The virions subsequently undergo conformational changes that yield hydrophobic subviral particles. This process is initiated by ICAM-1 and/or the low-pH environment in endosomes. It is thought that the RNA genome crosses the endosome membrane into the cytosol through a pore formed by viral proteins or following membrane rupture. Once inside the cytosol, the host cell ribosome translates the positive-sense, single-stranded RNA into a polyprotein that is eventually processed into its various parts (7).

Serotypes/Genotypes

Until recently, HRVs were classified into two species, HRV-A and -B, based on phylogenetic sequence criteria. Clinical specimens in the 1960s and 1970s yielded approximately 100 different HRV strains (known as the reference or prototype set), which were subsequently serotyped (6). Partial sequencing of viral capsid-encoding regions, noncoding regions, and a limited number of complete genomes led to a division of the original 99 strains into two species: HRV-A (containing 74 serotypes) and HRV-B (containing 25 serotypes). To understand further the molecular and evolutionary biology of the virus and aid in epidemiological investigations, the sequencing of the full genomes of these 99 serotypes was recently completed (8).

The development of highly sensitive molecular techniques for the identification of HRV in clinical specimens led to the identification and designation of a novel species, HRV-C, by the International Committee on Taxonomy of Viruses in 2009 (9). HRV-C strains do not grow in standard cell culture, likely postponing their discovery; therefore, a genetically based classification system was developed. HRV-C strains have a genomic organization similar to that of HRV-A and HRV-B; however, there are several distinct characteristics supporting their classification as a new species. To date, at least 50 different types of HRV-C have been identified by using a threshold of a 13% nucleotide difference in VP1 or at least a 10% nucleotide difference in the VP4/VP2 region if the VP1 sequence is unavailable (10, 11). In 2011, Bochkov et al. were the first to grow HRV-C *in vitro* by utilizing sinus mucosal tissue, and they demonstrated that the species uses a distinct cell attachment mechanism (12). At present, the specific cellular receptor and unique pathogenic mechanisms of HRV-C strains are not known.

Transmission

HRVs are transmitted from person to person via contact (either direct or through a fomite) or aerosol (small or large particle) (13, 14). HRV infection is efficiently initiated by intranasal and conjunctival inoculation but not by the oral route. In studies of natural and experimental HRV infection, the virus is regularly deposited onto the hands and introduced into the environment. HRV is detected in 40% of naturally infected volunteers' hands and 6% of objects in the home (15). In a study of 24 married couples with one experimentally infected partner, the transmission of HRV infection occurred in 9 couples during contact periods ranging from 63

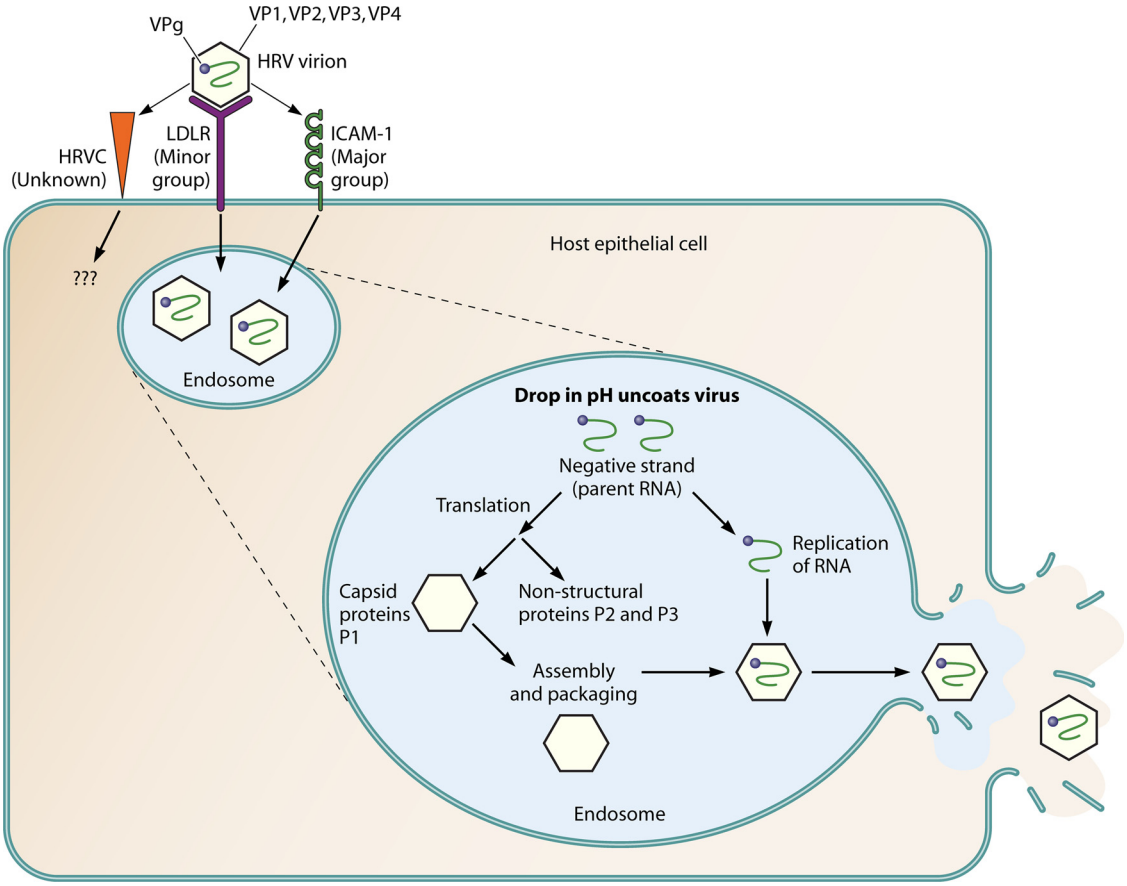

FIG 2 Viral replication in airway epithelial cells. Depending on the receptor type, virus uptake occurs via clathrin-dependent or -independent endocytosis or via macropinocytosis. A drop in the pH leads to viral uncoating. Negative-strand (parental) RNA is replicated as well as translated into structural (capsid) and nonstructural proteins. The virion is then assembled and packaged prior to cellular export via cell lysis (6, 7). LDLR, low-density-lipoprotein receptor; ICAM-1, intercellular adhesion molecule 1.

to 149 h (16). Under experimental conditions, HRV will survive in an indoor environment for hours to days at an ambient temperature and on undisturbed skin for 2 h (17). The frequency and duration of HRV shedding in aerosols are not well understood. In one study, HRV was transmitted via an aerosolized route to 56% of 18 volunteers who played cards for 12 h with experimentally infected subjects (18).

PATHOGENESIS AND HOST RESPONSE

Infection of Nasal Epithelial Cells

To understand how HRV is introduced into the nasal mucosa, Winther et al. delivered 25 μl of a viral inoculum to the right conjunctival sac near the tear duct or to the posterior nasopharyngeal wall of healthy volunteers (19). Cultures for HRV were obtained daily from the inferior turbinates and the nasopharynx via epithelial brush sampling. In general, HRV was initially detected at the nasopharynx and then spread anteriorly to one or both inferior turbinates. One potential mechanism for this pattern of spread is nose blowing, which may propel virus-laden mucus anteriorly (20). The nasopharynx also serves as the endpoint of mucociliary clearance from the nose, paranasal sinuses, and middle ear cavities (20).

Using *in situ* hybridization, HRV replication has been localized to a small proportion of cells in the nasal epithelium and nasopharynx (21), perhaps due to the limited expression of ICAM-1 (22). The majority of HRV-A and -B serotypes (the major receptor group) enter airway epithelial cells via ICAM-1, a member of the immunoglobulin superfamily. In adenoid and nasopharyngeal tissues of healthy children and adults, ICAM-1 is detected in small numbers of single nonciliated lymphoepithelial cells as well as the in basal layer of the ciliated epithelium; however, ICAM-1 is not normally expressed on squamous epithelial cells and on the luminal surface of the ciliated epithelium. However, in normal primary human bronchial epithelial cells (HBECs), HRV upregulates membrane-bound ICAM-1 expression via a NF-κβ-dependent mechanism (23) while downregulating the release of soluble ICAM-1 (24). ICAM-1 upregulation was also observed *in vivo* on nasal epithelial cells following experimental HRV-39 infection of healthy volunteers (25).

While other respiratory viruses, such as influenza virus and respiratory syncytial virus (RSV), cause a destruction of airway epithelial cells, HRV is seldom associated with cytopathology of the upper respiratory tract. Using light and scanning electron microscopy of nasal biopsy specimens from subjects with natural colds, Winther et al. found that epithelial cells were sloughed; however, the epithelial cell lining and borders remained structurally intact (26). A similar preservation of cell morphology and composition was observed for the nasal epithelium during studies of experimental HRV infection, where the amount of viral shedding did not correlate

with the severity of symptoms (19, 27). However, HRV does disrupt epithelial cell barrier function by the dissociation of zona occludens 1 from the tight junction complex (28), thereby facilitating the transmigration of bacteria and exposing basolateral epithelial cell receptors such as Toll-like receptors (TLRs) (29).

Infection of Lower Airway Epithelium

There is mounting evidence from experimental and observational studies to support the role of HRV as a lower respiratory tract pathogen. Although early experiments with HRV-2 suggested that viral replication was optimal at 33°C and markedly reduced at 37°C to 39°C, clinical studies which showed an association between HRV infection and asthma exacerbations prompted researchers to reexamine the temperature sensitivity of HRVs. In 1999, Papadopoulos et al. determined that there were minimal differences in replication capacities at 33°C and 37°C for eight different HRV strains, including when viruses were cultured and titrated at the same temperature (30). Furthermore, virus titers at 37°C for all strains were significantly higher than those required to initiate infection.

In a follow-up study, that same group demonstrated effective HRV replication *in vitro* using primary HBECs and *in vivo* in bronchial biopsy specimens of experimentally infected healthy volunteers via *in situ* hybridization (31). A characteristic cytopathic effect (CPE) was also observed when low-confluence cell cultures of HBECs were exposed to high titers of virus. Moreover, infection of HBECs with rhinovirus type 7 (RV-7) resulted in a significant increase over baseline expression levels for interleukin-6 (IL-6), IL-8, IL-16, and regulated upon activation, normal T cell expressed, and secreted (RANTES).

Gern et al. experimentally infected eight adult allergic volunteers with HRV-16 (32). All subjects developed cold symptoms and had HRV-16 cultured from nasal specimens. In addition, in all subjects, HRV-16 was detected by reverse transcription-PCR (RT-PCR) in lower airway cells obtained via bronchoalveolar lavage (BAL) at 2 to 4 days following infection. Transient abnormal pulmonary function, such as a compliance that is frequency dependent, has also been observed for healthy adults following experimental HRV infection (33).

In the few reported cases of HRV lower respiratory tract infection with human histology, HRV was capable of causing both interstitial and alveolar processes. In those reports, pathological findings included bronchiolitis obliterans with organizing pneumonia (34), interstitial pneumonitis (35; S. E. Jacobs, R. Soave, T. B. Shore, M. J. Satlin, A. N. Schuetz, C. Magro, S. G. Jenkins, and T. J. Walsh, submitted for publication), acute and chronic inflammation with fibrinopurulent alveolar debris (36), and hyperplasia and desquamation of alveolar cells (37). Together, these experiments, as well as clinical observations, confirm that HRV infects the lower airways and induces a proinflammatory response.

Innate and Adaptive Host Response

In addition to a direct effect on respiratory epithelial cells, the innate and adaptive host responses also have a role in the pathogenesis of HRV infection (Fig. 3). Triantafilou et al. conducted a series of *in vitro* experiments with primary HBECs to elucidate the specific recognition of HRV by the innate immune system (38). Those authors determined that the HRV-6 capsid is recognized via TLR2; subsequently, upon HRV-6 ssRNA internalization, the virus genome is recognized by endosomally located TLR7 and TLR8. Once double-stranded RNA (dsRNA) is generated, the type I interferon (IFN) response is mediated by melanoma differentiation-associated gene 5 (MDA-5) and retinoic acid-inducible gene 1 (RIG-1). The engagement of these receptors maximizes HRV-induced IFN-β and IFN-γ and proinflammatory cytokine gene expression, including RANTES, IP-10, IL-6, IL-8, and epithelial cell-derived neutrophil-activating peptide 78 (ENA-78) (38, 39). In particular, IL-8, which has neutrophil chemotactic and activation properties, is an important determinant of the clinical outcome of HRV infection. The HRV-induced stimulation of IL-8 production has been demonstrated for upper and lower airway epithelial cells (40, 41) and is mediated in part by an NF-κβ-dependent transcriptional activation pathway (42). Levels of IL-8 in nasal lavage fluid specimens from experimentally infected subjects correlated with symptom severity (rhinorrhea and nasal obstruction) (40) and peaked at 48 to 72 h after virus inoculation.

Evaluations of promoter polymorphisms of cytokine genes and the associated protein production may provide new insights into symptom expression during HRV infection. IL-6 levels are consistently increased in nasal lavage fluid specimens in controlled studies of experimental and natural HRV infections (42–44). Studies of children and adults with RSV infection found that the C/C genotype of a single-nucleotide polymorphism in the IL-6 promoter at position −174 correlated with illness severity (45, 46). Doyle et al. therefore recently examined the relationship between IL-6 promoter genotypes and the magnitudes of symptoms during HRV infection (47). Following experimental HRV-39 infection, subjects with a phenotype of a low level of production of IL-6 (position −174, C/C genotype) experienced greater symptom severity, although there was no effect on nasal secretion production and mucociliary clearance time. That study also found that the IFN-γ (position +874) phenotype predicted the frequency of seroconversion. There was no relationship between IL-10 or tumor necrosis factor alpha (TNF-α) polymorphisms and seroconversion or symptom outcomes. The observed IL-6 genotype associations are consistent with those of RSV studies; however, they are also unexpected given previous reports that higher IL-6 levels predicted the magnitude of symptoms. Those authors noted that the cytokine phenotypes were assigned based on *in vitro* data, which may not correlate with *in vivo* cytokine production in the more complex nasal mucosa and blood during a viral upper respiratory tract infection. Furthermore, other cytokine polymorphisms that were not measured in the *in vivo* studies may interact with IL-6 genotypes to influence cytokine production. These experiments highlight the complexity in deriving genotype-phenotype associations as well as the challenge of elucidating the complex interactions of cytokines in producing common cold symptoms.

Kinins also play a role in the pathogenesis of symptomatic HRV infections. In both experimental and natural HRV colds, symptomatic subjects show significantly increased levels of kinins, specifically bradykinin and lysylbradykinin, in nasal lavage fluid compared to levels in sham-infected and/or healthy controls (48, 49). Elevated kinin levels are associated with increases in vascular permeability, as indicated by elevated albumin levels and the influx of neutrophils. Of note, histamine levels do not change in symptomatic HRV infection, suggesting that mast cells and basophils do not contribute to the pathogenicity of HRV.

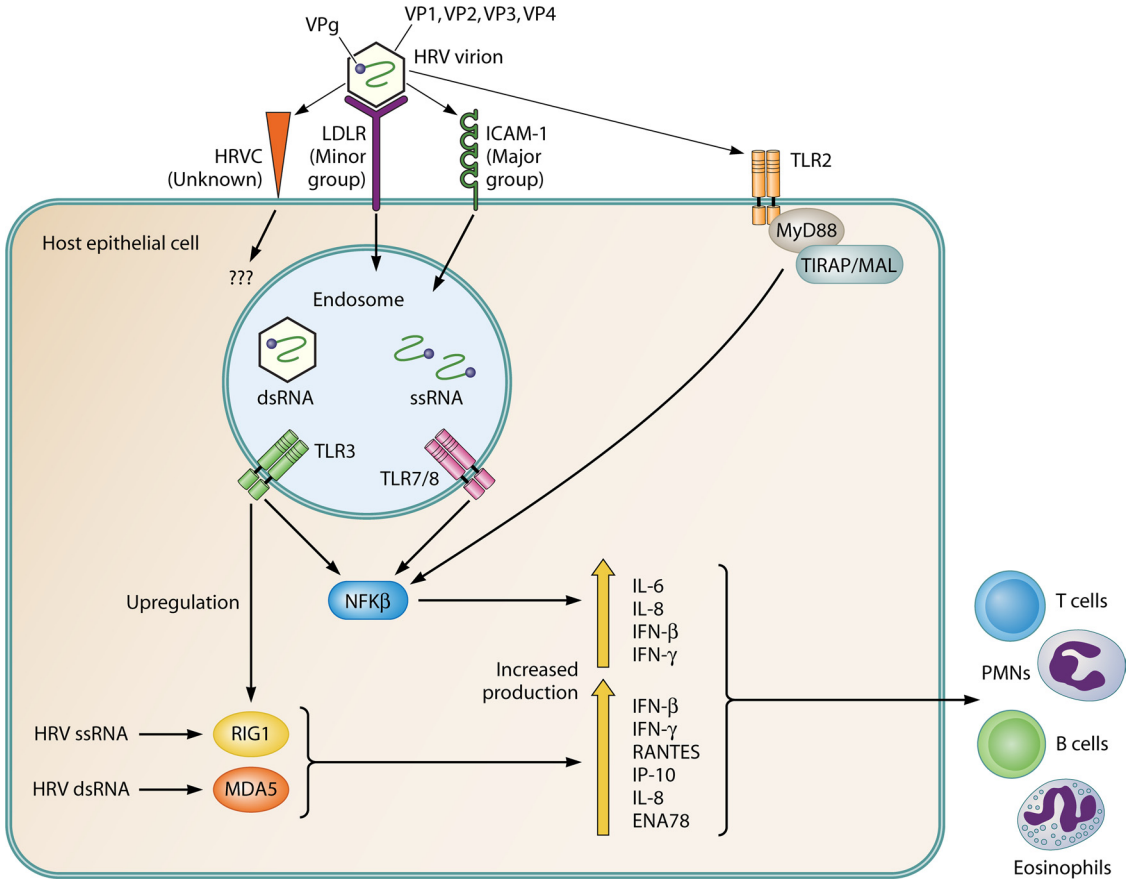

FIG 3 Signal transduction pathways and activation of the innate immune response. In the endosome, viral dsRNA and ssRNA are recognized by TLR3 and TLR7/8, respectively. An interaction with TLR3 triggers the upregulation of the pattern recognition receptors (retinoic acid-inducible gene 1 [RIG-1] and melanoma differentiation-associated protein 5 [MDA-5]) (RNA helicases) in the intracellular compartment. RIG-1 and MDA-5 also recognize newly synthesized viral dsRNA and ssRNA in the cytoplasm. RIG-1 and MDA-5 stimulate HRV-induced IFN gene expression as well as the increased production of T cell and neutrophil cytokines, including regulated, normal T cell expressed, and secreted (RANTES); IFN-γ-induced protein 10 (IP-10); IL-8; and epithelial cell-derived neutrophil-activating peptide 78 (ENA78). An interaction with TLR7/8 triggers IFN-β and IFN-γ production and activates the NF-κβ pathway. HRV also interacts with TLR2 on the cell surface to initiate a proinflammatory cytokine response via a MyD88-dependent pathway (38, 39). LDLR, low-density-lipoprotein receptor; ICAM-1, intercellular adhesion molecule 1; TIRAP, Toll–interleukin-1 receptor (TIR) domain containing adaptor protein; PMNs, polymorphonuclear leukocytes.

Humoral immune responses are important for preventing HRV infection, although the precise mechanism is unknown. HRV infection in antibody-naïve subjects is followed by the development of serotype-specific neutralizing serum antibodies (IgG) as well as secretory antibodies (IgA) in the airways. When seronegative subjects are experimentally infected with HRV-2, specific antibodies are detectable at 1 to 2 weeks, and levels of these antibodies may remain elevated for 1 or more years after infection (50, 51). The persistence of high-titer serotype-specific antibody is associated with protection from infection as well as reduced symptom severity following experimental challenge with the same serotype (52). However, there is little cross-neutralization among serotypes, which presents a challenge to vaccine development, given that there are more than 100 different known HRV serotypes (53). Further support for the role of humoral immunity in the prevention and control of HRV infection was observed in a study of patients with primary hypogammaglobulinemia. These patients experienced more frequent and severe HRV infections than their healthy spouses despite the administration of replacement immunoglobulin therapy (54).

T cells contribute to antiviral immunity through the recognition of viral antigens, which trigger both cytotoxic and antibody-mediated immune responses. HRV-infected endothelial cells secrete RANTES and IP-10, which promote T cell chemotaxis (289). IP-10 is a chemokine secreted by bronchial epithelial cells, monocytes, lymphocytes, and neutrophils in response to the cytokines IFN-γ and TNF-α, the levels of which are elevated by HRV infection. Increased numbers of lymphocytes and neutrophils are present in nasal secretions, and bronchial biopsy specimens of HRV-infected subjects with asthma and controls show T cell infiltration of the airway epithelium and submucosa (55) with concomitant lymphopenia. To determine a potential role of circulating lymphocytes in the pathogenesis of HRV infection, Levandowski et al. challenged 15 healthy volunteers with HRV-25 (56). Lymphocyte subsets in peripheral blood leukocytes were classified and quantified by using monoclonal antibodies at baseline and at day 3 and day 7 following HRV inoculation. Among the subsets of T cells, numbers of both $T4^+$ (T helper/inducer) and $T8^+$ (T suppressor/cytotoxic) lymphocytes declined over time, but only the change in the $T4^+$ subset was significant. The duration of virus shedding was

also inversely related to changes in lymphocyte counts. No changes were observed for B cells. In addition, the total leukocyte count increased, likely due to an increased number of neutrophils. A potential explanation for these results is that the lymphocytes migrated to the site of HRV infection, while neutrophils were released from the marginal pools in response to the inflammatory process.

Human T cell clones of HRV-specific T cells are activated by not only serotype-specific but also shared viral epitopes (57). Cross-reactivity among T cells may lead to more potent T cell responses and subsequent cytokine release upon reinfection with different HRV serotypes. In addition, T cell recruitment may facilitate viral clearance via Th1 cytokine production, including IFN-γ and IL-2 (58).

A clinical approach to elucidating the role of the immune system in HRV infection and pathogenesis is to study the effects of pharmacological agents with known mechanisms of action. For example, the cyclooxygenase inhibitor naproxen was evaluated in a randomized, placebo-controlled trial of experimental HRV infection in healthy adults (59). Subjects receiving naproxen had reduced headache, malaise, myalgias, and cough, suggesting that prostaglandins may have a causal role in HRV pathogenesis. Naproxen did not affect the duration of virus shedding or serum-neutralizing antibody responses.

Animal Models

The development of small-animal models is useful to understand further the pathogenesis of HRV infection in both the upper and lower airways; however, there are no known murine rhinoviruses. Experimental animal models using either minor-group receptor HRVs in wild-type mice or major-group receptor HRVs in mice that are transgenic for ICAM-1 have been developed recently (60). Mice were inoculated with 5×10^6 50% tissue culture infective doses of minor-group HRV (HRV-1B) or major-group HRV (HRV-16). Infection with both HRV-1B and HRV-16 resulted in BAL fluid neutrophilia and lymphocytosis; increased Muc5B protein in BAL fluid; increased viral RNA levels in BAL fluid and lung tissue; and the induction of IFNs, chemokines, IL-1β, and virus-specific antibodies. An examination of stained lung sections revealed areas of extensive peribronchial and perivascular cellular infiltration. One limitation to these murine models is that HRV titers exhibit a steep decline within 12 to 24 h, limiting the ability to investigate viral replication and HRV-induced inflammation and airway dysfunction.

A major focus of animal models has been to elucidate the immunology of HRV-induced asthma exacerbations. Using combined mouse models of allergic airway disease and HRV infection, Nagarkar et al. inoculated ovalbumin (OVA)-sensitized and -challenged BALB/c mice with HRV-1B (61). Those authors found that the levels of production of proinflammatory cytokines, including eotaxin-1/CCL11, IL-4, and IL-13, increased following viral infection. Furthermore, levels of bronchoalveolar and lung neutrophils, eosinophils, and macrophages, as well as airway responsiveness, were elevated in the HRV-infected, OVA-treated mice compared to control mice. Activated macrophages played a key role in eosinophilic infiltration and airway responsiveness in HRV-infected OVA-treated versus phosphate-buffered saline (PBS)-treated mice.

Another recently reported strategy is the use of genetically modified mengovirus, a picornavirus that has a phenotype similar to that of poliovirus in its natural murine host (62). The deletion of the poly(C) tract in the distal portion of the 5′ untranslated region (5′UTR) of mengovirus causes an infection in wild-type mice that more closely resembles HRV infection of humans. This murine model of picornaviral infection may be useful for elucidating HRV pathogenesis in humans.

Mechanisms in Chronic Pulmonary Disease

Asthma. Several plausible mechanisms for HRV-induced asthma development have been proposed. Experimental HRV-16 infections in patients with mild atopic asthma and allergic subjects led to a significant reduction in the forced expiratory volume in 1 second (FEV1) in home recordings and potentiated airway inflammation after bronchoprovocation, respectively (63). Potential mechanisms for lower airway dysfunction following HRV infection include the direct infection of the lower airway or the stimulation of inflammatory, immunological, or neurogenic mechanisms. *In vitro* data suggest impaired innate and acquired immune responses in subjects with asthma, including deficient IFN induction and impaired Th1 responses (64–66). Furthermore, HRV can stimulate the synthesis of factors that regulate airway remodeling and alveolar development, including vascular endothelial growth factor, nitric oxide, transforming growth factor β, and fibroblast growth factor (67).

Chronic obstructive pulmonary disease. In order to investigate a causal relationship between HRV and chronic obstructive pulmonary disease (COPD) exacerbations, Mallia et al. experimentally infected 13 patients with COPD and 13 nonobstructed smoker controls (68). Three patients (2 with COPD and 1 control) were excluded from further analysis because HRV was not detected after experimental infection. Of the remaining 11 COPD patients, 10 fulfilled symptom criteria for COPD exacerbation. Pulmonary function tests demonstrated significant reductions in postbronchodilator peak expiratory flow and carbon monoxide diffusion capacity from baseline to HRV infection in COPD patients but no change in controls. Neutrophil elastase and IL-8 levels in sputum supernatants and IL-6 levels in BAL fluid also increased from baseline to HRV infection in COPD patients, whereas the TNF-α levels did not increase significantly in either group. Finally, pulmonary alveolar macrophages from BAL fluid demonstrated deficient IFN-β responses in COPD patients compared to controls, suggesting a potential mechanism for the increased severity of HRV infection in COPD patients. There was a nonsignificant trend toward reduced levels of IFN-α and IFN-γ production ($P = 0.09$ and 0.1, respectively).

Recently, Quint et al. examined serum IP-10 as a potential biomarker of HRV infection during COPD exacerbations (69). At baseline, 136 COPD patients had higher serum IP-10 levels than 70 age-matched controls without COPD. During 2 years of follow-up, serum IP-10 levels increased significantly from baseline during HRV-positive COPD exacerbations and demonstrated no change during HRV-negative exacerbations. Serum IP-10 levels also correlated with sputum HRV viral load. Of note, among 13 COPD patients with HRV positivity at baseline and during exacerbations, serum IP-10 levels and sputum HRV viral loads were significantly higher during exacerbations.

Cystic fibrosis. Mechanisms of HRV-induced pulmonary exacerbations in patients with cystic fibrosis (CF) are not well understood. Two recent studies suggested an impairment in innate immunity, specifically IFN production (70, 71). After infecting CF

and non-CF bronchial epithelial cell lines and primary nasal and bronchial epithelial cells from CF patients and healthy controls, Vareille et al. found that CF cells produced significantly less IFN-β and IFN-γ than did normal cells (70). Deficient IFN production was associated with reduced expression levels of IFN-stimulated genes, including myxovirus resistance A, 2′,5′-oligoadenylate synthetase, viperin, and nitric oxide synthase 2. Secondary viral infections in the setting of chronic bacterial infection are also common and may increase the severity of lung disease in patients with CF. Chattoraj et al. demonstrated *in vitro* that mucoid *Pseudomonas aeruginosa* preinfection impairs HRV clearance by suppressing the antiviral IFN response in primary HBECs from CF patients but not in normal cells (71). Deficient IFN responses occurred via the inhibition of HRV-stimulated Akt phosphorylation and decreased levels of IFN regulatory factor 3 (IRF3) phosphorylation. An exaggerated inflammatory response to viral infection was also postulated to be a mechanism for virus-induced CF exacerbations (72, 73). However, a recent study found no difference in levels of cytokine transcription or production in CF and control nasal and bronchial epithelial cells; indeed, there was a trend toward a reduced cytokine response associated with increased cell death in CF cells (74).

In conclusion, *in vitro* and clinical data indicate that HRVs are a significant upper and lower respiratory tract pathogen. However, the relative roles of the virus itself and the host immune response in HRV pathogenicity and symptomatic illness are still under debate. In the upper airways, HRV has not been shown to cause direct cytopathology; clinical symptoms are likely the result of local and systemic immune responses. In cultured epithelial cells from the lower airways, cytopathology has been observed by using low-confluence monolayers and high viral inocula (31), perhaps suggesting a dose-response relationship to HRV-associated lower respiratory tract infection, to which patients with immune dysregulation (e.g., deficient IFN responses in individuals with asthma and cystic fibrosis) and immunosuppressive conditions are particularly predisposed.

EPIDEMIOLOGY AND CLINICAL SYNDROMES

Seasonal Patterns and Geographic Distribution

HRVs cause respiratory illness throughout the world and throughout the year. Beginning in the 1960s, longitudinal studies of the epidemiology and clinical features of HRV infection in temperate climates reported a peak in incidence in the early fall, with a smaller peak in the spring (75). More recent prospective studies employing molecular detection methods (RT-PCR) have replicated these findings (76–78). In general, HRVs are the most common cause of respiratory viral illness during the spring, summer, and fall months, while influenza virus and RSV predominate in the winter.

Following the identification of a novel HRV genotype, HRV species C (9, 79, 80), studies from diverse geographic regions have attempted to characterize the molecular epidemiology of HRV and identify distinguishing clinical features according to species. All HRV species have been identified in all months, in temperate, tropical, subtropical, and semiarid regions (81–85). HRV-C appears to show seasonality, with peaks in the fall or winter in most temperate or subtropical countries but a possible peak incidence during the rainy season in the tropics. HRV-A and HRV-B may show a similar seasonality (86). Additional longer-duration, large epidemiological studies with symptomatic and asymptomatic subjects are needed to enhance our understanding of the seasonality of HRVs.

Clinical Syndromes

Asymptomatic infections. With the increasing use of molecular methods of viral detection, asymptomatic HRV infection has been noted to be relatively common, particularly in children. The frequent detection of HRV in asymptomatic individuals may also reflect one of several states: prolonged virus shedding after a symptomatic respiratory illness has resolved; mild, unrecognized symptoms; or the incubation period prior to the onset of symptoms. In children less than 4 years old, rates of asymptomatic infection range from 12 to 32% (87–91) and tend to be higher in the youngest age groups (91). A study conducted in Alaska's Yukon Kushkowkim Delta to characterize the etiology of lower respiratory tract-associated hospitalizations in children less than 3 years old selected controls from the community if they had no respiratory symptoms in the previous 2 weeks. Among 425 community control children, 33% tested positive for HRV by real-time PCR of nasopharyngeal swab specimens (87); this rate was not significantly different from that observed for children hospitalized with lower respiratory tract illness. In contrast, Iwane et al. (90) detected HRV in nasal and throat swab specimens from 12.5% of asymptomatic children less than 5 years old recruited at well-child primary care visits in three different regions of the United States (Rochester, NY; Nashville, TN; and Cincinnati, OH). This difference in observed rates of asymptomatic HRV infection may be attributed to the higher prevalence of HRV in the youngest age groups or to environmental factors in the Alaska Native community, including household crowding and a lack of running water, that predispose individuals to respiratory illnesses (92).

Among middle-aged and elderly adults, rates of asymptomatic HRV carriage are less well studied but are considerably lower than those for children. Two studies (91, 93) detected HRV in 0% and 2% of asymptomatic adults, although rates were higher in adult household members of HRV-infected children (94). Of note, the above-mentioned studies were typically conducted over at least a 1-year period, and seasonal differences in rates of asymptomatic infection have not been described.

Upper respiratory infections. (i) Common cold. Studies using both molecular methods and viral culture demonstrated that HRV is the etiology of one-half to two-thirds of common colds (95, 96). The common cold is primarily a self-limited illness in immunocompetent hosts, with an average incubation period of 2 days (97, 98) and a symptom duration period of 7 to 14 days (5, 99). However, in one experimental study, subjects reported nasal irritation and sore or scratchy throat at 2 and 10 h, respectively, after virus inoculation (100). Common symptoms include rhinorrhea, nasal congestion, sore throat, cough, headache, subjective fevers, and malaise. Compared to patients with coronavirus-associated colds, there is no difference in respiratory symptom severity or duration (96). Despite the relatively mild course of illness, the economic burden is considerable. In a survey of 4,051 U.S. households, the economic impact of non-influenza virus respiratory tract infections was estimated to be $40 billion annually in direct and indirect costs (4).

(ii) Acute otitis media. In both experimental and natural settings, HRV is linked to otitis media (OM), which complicates

approximately one-third of cold-like illnesses in early childhood (101). Among healthy volunteers undergoing intranasal inoculation with HRV, otologic manifestations of HRV infection include Eustachian tube dysfunction, abnormal middle ear pressure, and OM (102, 103). In the Finnish Otitis Media Cohort study of 329 children monitored prospectively from ages of 2 months to 2 years, there were 458 episodes of OM. HRV was detected by real-time PCR in nasopharyngeal aspirate or middle ear fluid (MEF) specimens in 41% of episodes (104). Further support for the role of HRV in upper respiratory tract infections and OM is derived from investigations of adenoid tissue. Using *in situ* hybridization of adenoid tissue removed from children with histories of recurrent OM or adenoid hypertrophy, HRV RNA was detected in 45% of specimens (105). Of note, clinical outcomes of acute OM may not vary according to viral etiology. Among 92 children aged 3 months to 7 years with acute OM, rates of treatment failure or recurrence, defined as recurrent symptoms within 7 to 30 days after initial clinical improvement, were not significantly different between those with HRV detected in MEF specimens (18%), those with RSV or coronavirus detected in MEF specimens (21%), and those with no detectable viral RNA in MEF specimens (27%) (106).

Coinfection with bacterial pathogens is also common during HRV-associated OM episodes. In one study of children with tympanostomy tubes and acute OM onset within the previous 2 days, bacterial-viral coinfection occurred in 66% of patients, with picornaviruses accounting for two-thirds of cases (107). Another prospective study of 121 otitis-prone children tested nasopharyngeal swabs by PCR for respiratory viruses and by culture for bacterial pathogens during three study visits over a 6-month period (108). HRV was detected in 30% and 19% of baseline and follow-up specimens, respectively, and HRV positivity correlated with the culturing of *Moraxella catarrhalis* and *Streptococcus pneumoniae* but not nontypeable *Haemophilus influenzae*. Of note, HRV and bacterial pathogens were found in otitis-prone children even in the absence of clinical symptoms. Further potential mechanisms of HRV-bacterial coinfection are reviewed in "Coinfections with Other Respiratory Pathogens."

(iii) Rhinosinusitis. Sinus abnormalities are frequently detected by computed tomography (CT) (109) and magnetic resonance imaging (MRI) (110) of patients with the common cold. The maxillary and ethmoid sinuses were most commonly involved in healthy young adults challenged with HRV-39 and monitored by serial MRI over several weeks (110). In one study, HRV RNA was detected by RT-PCR of maxillary aspirate specimens and nasal swab specimens in 50% of patients with acute community-acquired sinusitis (111). Furthermore, using *in situ* hybridization, HRV RNA was found in the maxillary sinus epithelium in 7 out of 14 adults with acute sinusitis (112). Nose blowing is one potential mechanism for the spread of nasal fluid carrying viruses and other pathogens to the sinuses in patients with cold symptoms. Gwaltney et al. measured intranasal pressure in adults during nose blowing, coughing, and sneezing (113). In addition, CT scans were performed after the instillation of radiopaque contrast medium into the nasopharynx followed by nose blowing, coughing, and sneezing. Nose blowing, but not coughing or sneezing, generated sufficient pressure to propel nasal fluid into the paranasal sinuses (mean maximum intranasal pressures $\pm$ standard deviations of 66 $\pm$ 14 mm Hg versus 7 $\pm$ 3 mm Hg and 5 $\pm$ 4 mm Hg, respectively).

Lower respiratory infections. (i) Croup. Although most commonly caused by parainfluenza viruses (PIVs), croup has occasionally been reported in children with HRV infection. In a study of U.S. children less than 5 years old hospitalized with acute respiratory infections, 10% and 3% of admission and discharge diagnoses, respectively, were croup among those children with HRV alone isolated from respiratory specimens (77, 90). Croup is a similarly rare manifestation of HRV infection among children and young adults in Japan (81). Another study of children presenting to the emergency department with signs and symptoms of croup detected HRV in 12% of cases, although rates were similar among control children with wheezing illnesses (114). Reflecting patterns of other microbiological surveys for respiratory viruses, HRV was detected more often in samples obtained during the fall (September to November), whereas influenza A virus and RSV were more common in winter (December to February), and PIVs were found most often in winter and spring (December to April).

(ii) Bronchiolitis. Bronchiolitis is the most common clinical manifestation of HRV infection in hospitalized children (115) and accounts for 14% of HRV-associated lower respiratory tract infection admissions to pediatric intensive care units (ICUs) (116). Following RSV, HRV is the second most common cause of bronchiolitis in hospitalized children, as demonstrated in a recent U.S. multicenter prospective study (117). Among 2,207 children less than 2 years old, HRV was detected alone, in combination with RSV, and in combination with non-RSV pathogens in 9%, 15%, and 6% of cases, respectively. Compared to children with RSV infection alone, a hospital length of stay of 3 or more days was less likely for children with HRV infection alone or for children with infection by HRV and non-RSV pathogens but was more likely for those with RSV-HRV coinfection. These findings challenge the notion that the viral etiology of severe bronchiolitis does not affect short-term outcomes and support further research to guide cohorting and therapeutic strategies (117, 118).

Among very-low-birth-weight infants in Argentina, HRV detection (40%) exceeded RSV detection (7%) during bronchiolitis episodes (119). The incidence of HRV infection in this population was 75 per 100 infant years of follow-up. In a multivariable analysis model including bronchopulmonary dysplasia (BPD), weight, breastfeeding status, parental asthma, smoking in the home, and maternal age, BPD was independently associated with a higher risk of HRV-associated bronchiolitis (relative risk, 2.2). The adjusted relative risk of any HRV-associated hospitalization was also increased for infants with BPD and those who were not breastfed.

HRV-associated bronchiolitis in infancy is an independent risk factor for recurrent wheezing at 1 year of age (120) and for the development of asthma. A follow-up study of 81 children conducted 6 years after hospitalization as infants for wheezing found that the risk of childhood asthma was four times higher for children with a history of HRV-associated wheezing than for children with wheezing of a different viral etiology (121). Viral respiratory infections have been shown to induce cell damage as well as alter immune responses. As lung development begins at 4 weeks of gestation and continues through early childhood, HRV infection may have severe direct and indirect effects on lung tissues, leading to chronic lung disease (67).

(iii) Community-acquired pneumonia. Several clinical studies of children hospitalized with community-acquired pneumonia (CAP) have established HRV as a common pathogen in viral CAP,

with rates ranging from 18 to 26%, although it may be difficult to establish a causal role in the presence of bacterial and viral coinfection, which occurs in up to 60% of cases (122–124). Clinical manifestations are potentially severe (125), particularly in children with underlying chronic medical conditions. Indeed, HRV was detected in 49% of children admitted to ICUs with lower respiratory tract infection; in approximately one-half of cases, no other respiratory pathogen was identified (116). Among adults, HRV is identified in only approximately 5% of cases of viral CAP (126, 127). However, in two outbreaks of HRV-associated acute respiratory illness among elderly residents in long-term-care facilities, HRV caused substantial morbidity and one fatality (128, 129).

Infections in Immunocompromised Hosts

With the increasing use of newer molecular platforms for respiratory virus detection, including multiplex real-time PCR assays, HRV is increasingly being recognized as a significant cause of acute respiratory illness in immunocompromised hosts (130, 131). A recent study of patients presenting to the emergency department with influenza-like illness found that the severity of HRV infection in immunocompromised patients, including those with diabetes, human immunodeficiency virus (HIV) infection, malignancy, or organ transplantation, was similar to that of pandemic H1N1 influenza virus. Nearly 40% of patients with HRV-associated respiratory symptoms were admitted to the hospital, and 11% required ICU admission. Two (3%) patients died; however, mortality was due to concurrent illness and was not directly attributable to HRV infection (132).

Lung transplant recipients. Lung transplant recipients provide a unique opportunity to study HRV infection of the lower airways because these patients undergo frequent bronchoscopy and BAL for graft rejection surveillance as well as during episodes of respiratory illness. Kaiser et al. demonstrated active and chronic HRV infection (8 to 15 months) of the lower respiratory tract in three highly immunosuppressed lung transplant patients. HRV was identified by RT-PCR of lower respiratory tract specimens and by using antibodies specifically directed against the infecting HRV strain in interstitial and epithelial cells (133). In a study of 36 adult lung transplant recipients in Italy, HRV was detected in 13% of all BAL fluid specimens obtained from 15 (42%) patients over a 2-year period. All patients were symptomatic at the time of HRV detection, including those with low viral loads of 10^3 to 10^4 RNA copies/ml, and all patients had coinfection with bacterial and/or viral pathogens (134). Compared to adults, HRV is common in pediatric lung transplant recipients, accounting for 22% and 30% of respiratory viral illnesses at 1 year and a median of 22 months, respectively, following transplantation (135, 136). Whether HRV affects transplant outcomes, such as acute rejection and the development of bronchiolitis obliterans syndrome (BOS), is not well understood due to the small numbers of patients under study. In a recent pooled analysis of 34 studies evaluating respiratory viral infection and graft dysfunction, posttransplant BOS occurred in 18% of patients with a history of respiratory viral infection and in 11.6% of patients with no such history (137). In pediatric patients, respiratory viral infection is independently associated with decreased 1-year survival rates (136).

Patients with hematologic malignancy and hematopoietic stem cell transplant recipients. HRV causes both upper and lower respiratory tract infections in hematologic malignancy patients (130, 138–140); however, the relative frequency and severity of HRV infection compared to those of other respiratory viral pathogens remain to be elucidated. Milano et al. obtained weekly surveillance nasopharyngeal swab specimens for HRV and coronavirus testing by real-time RT-PCR and recorded respiratory symptoms in a cohort of allogeneic hematopoietic stem cell transplant (HSCT) recipients. The cumulative incidence of HRV infection was 22% in the first 100 days posttransplantation. In a multivariable regression model, a positive HRV sample within the last week was significantly associated with rhinorrhea, sinus congestion, postnasal drip, sputum production, and cough (139). One-third of HSCT recipients will have clinical and/or radiographic evidence of sinusitis (35). Asymptomatic infection also occurs in a minority of patients (139, 140).

Progression to HRV pneumonia is uncommon but is associated with substantial morbidity and mortality (35, 139, 141). In the majority of reported cases of HRV in the lower airways, coinfection with one or more bacterial, viral, and/or fungal copathogens is present (35, 141). Recently, our group described 63 HSCT recipients with one or more HRV infections since our institution implemented multiplex PCR testing of respiratory viruses in 2008 (Jacobs et al., submitted). We identified 25 HSCT recipients with pneumonia and HRV detected in BAL fluid, of which 10 (40%) patients had no other respiratory pathogen identified via culture or molecular methods. A review of the CT scan findings for these 10 patients revealed a characteristic peribronchiolar patchy ground-glass infiltrate in the majority of cases. For the remaining 15 cases, copathogens were bacterial ($n = 7$), fungal ($n = 5$), and viral ($n = 3$). Compared to patients with respiratory coinfection detected in BAL fluid, patients with HRV alone detected in BAL fluid were not significantly different in terms of age, symptoms, ICU admission, transplant type, conditioning regimen, graft-versus-host disease, or receipt of antibiotics. The mortality rate at 1 year following the first posttransplantation HRV infection was 55% in patients with BAL-confirmed HRV pneumonia, versus 41% in the total cohort of 63 patients. Risk factors for HRV pneumonia may include lymphopenia (absolute lymphocyte count of <500 cells/μl) (142) and hypoalbuminemia (Jacobs et al., submitted); however, further research is needed to elucidate fully the epidemiology and pathogenesis of HRV pneumonia in this patient population.

Exacerbations of Chronic Pulmonary Diseases

Asthma. Wheezing illnesses during infancy are associated with recurrent wheezing and the development of asthma in childhood. In a prospective cohort study of 259 children monitored from birth to 6 years of age, children with outpatient HRV-associated wheezing illnesses from birth to 3 years of age were 10 times more likely to have developed asthma at 6 years of age. Nearly 90% of children who wheezed with HRV in year 3 had asthma by 6 years of age (143). Infants hospitalized with viral bronchiolitis have a 2- to 3-fold-increased risk of asthma later in childhood. This risk appears to be greatest with HRV compared to RSV, PIV, and influenza virus infections (144).

HRV is also associated with exacerbations of asthma. Subjects with asthma do not have more cold illnesses than nonasthmatic individuals, nor are there differences in the severity or duration of HRV-associated upper respiratory tract symptoms; however, they do experience more frequent and severe lower respiratory tract symptoms (144, 145). On average, approximately two-thirds of

respiratory virus-associated asthma exacerbations are due to HRV. In a cross-sectional study of children hospitalized with asthma exacerbations and controls hospitalized with acute respiratory illnesses, HRV was detected in 85% of cases and 33% of controls. Of note, HRV-C was associated with asthma exacerbations, whereas rates of HRV-A and HRV-B detection were similar between groups (146).

Chronic obstructive pulmonary disease. Several longitudinal studies have demonstrated a significant association between respiratory viral infections and exacerbations of COPD in both inpatient and outpatient settings. In a study of 62 patients with COPD monitored for a mean of 26 months, 58% of office visits and 6% of emergency room visits were associated with respiratory viral infections (147). Among patients hospitalized with acute exacerbations of COPD (AECOPD), respiratory viruses were detected in 37% of AECOPD patients, compared to 12% of control subjects with stable COPD and 12% of nonobstructed smokers. HRV was the most common respiratory viral infection, isolated in 24% of AECOPD patients. HRV was also detected in 4% of stable COPD patients and zero nonobstructed smokers. Therefore, compared to stable COPD patients, those with COPD exacerbations were 4.4 times more likely to have a respiratory viral infection (148). Two additional prospective studies of patients hospitalized with AECOPD found that picornaviruses were the most common viral infection detected by RT-PCR in nasal lavage fluid, nasopharyngeal swab, or induced sputum specimens, occurring in 36 to 50% of cases (149, 150). In the outpatient setting, approximately two-thirds of AECOPD cases are associated with common cold symptoms (increased nasal congestion and/or increased rhinorrhea) in the 18 days preceding the onset of the exacerbation, and HRV remains the most common cause of viral infection (151). The presence of common cold symptoms appears to be associated with prolonged recovery in patients with a moderate to severe obstruction (152).

Compared to patients with nonviral causes of AECOPD, fever is more frequent in those with documented viral infection (148, 149). Two studies also found increased lengths of stay for patients hospitalized with AECOPD and respiratory viral infection (151, 153). Another study found no difference in lengths of stay among AECOPD patients with and without respiratory viral infection; however, rates of bacterial infection were higher in the nonviral infection group (150). Respiratory viral infections do not occur more commonly according to baseline COPD severity (0.38 and 0.52 respiratory viral infections per year in patients with mild obstruction and moderate to severe obstruction, respectively) (147).

Cystic fibrosis. Several studies have examined the effects of respiratory viruses on respiratory exacerbations in CF patients. Among patients with a proven viral etiology, HRV is the most commonly detected pathogen when molecular detection methods are employed; in one study, HRV was detected during 16% of all respiratory exacerbations (154, 155). Unlike other respiratory viruses, including influenza virus, PIV, and RSV, HRV was not associated with decrements in pulmonary function (154). However, the effects of HRV on the lower airways may be species or strain dependent. In a study of 103 Brazilian children with CF tested for respiratory viruses during routine visits or respiratory exacerbations over a 1-year period, HRV-C but not HRV-A or HRV-B was significantly associated with respiratory exacerbations (156).

Health Care-Associated Infections

Data are limited on the frequency and risk factors for the health care-associated transmission of HRV infection, including health care worker (HCW)-to-patient and patient-to-patient transmission, perhaps due to limited methods for HRV detection at facilities that do not use molecular detection methods. Hospital HRV outbreaks have been reported primarily in neonatal ICUs (157–159). In one study, 7 out of 11 infected infants acquired HRV infection during their hospital stay (159). The most common symptoms were respiratory distress, apnea, rhinorrhea, and hypothermia; all infants required respiratory support. Chest radiographs revealed perihilar streakiness, atelectasis, focal consolidation, and hyperinflation.

HRV outbreaks have also been described in long-term-care facilities, where they have been linked to pneumonia, hospitalization, and, rarely, death (128, 129, 160). One of the first studies to report an HRV outbreak described an investigation in a nursing home in rural Wisconsin in 1993. In this elderly population, 66% of residents had lower respiratory signs and symptoms, including productive cough, dyspnea, hoarseness, and abnormalities upon lung auscultation (wheezing, rhonchi, and rales) (161). More recently, using data from an active surveillance network in Ontario, Canada, Longtin et al. identified 297 respiratory disease outbreaks in long-term-care facilities reported to the Ontario Public Health Laboratory from 1 July to 31 December 2009 (160). Among the 234 (79%) outbreaks for which a pathogen was identified, 174 (59%) pathogens were determined to be HRV by using multiplex PCR. Deaths were potentially associated with the HRV outbreak in four facilities. Among the 13 patients who died, 7 had clinical data available; 6 of these 7 patients died from pneumonia/respiratory illness (162). In all of the above-described outbreaks, different strains of HRV were identified.

Although HCWs have a higher risk of acquisition and transmission of respiratory viruses, few studies have thoroughly assessed their role in health care-associated HRV outbreaks. In a 2-year prospective study in Sao Paulo, Brazil, 203 HCWs presenting to the Health Care Worker Medical Assistance Service with acute respiratory illness of a possible viral etiology were tested for a panel of respiratory viruses, including influenza virus (163). HRV testing was performed by using RT-PCR on influenza virus-negative samples. Overall, HRV was the most frequently detected virus (38%). Sixteen of the HRV-infected HCWs, representing 77% of HRV cases, worked with high-risk patients, including immunocompromised and obstetric patients and those with cardiovascular and pulmonary diseases. Another study involving an outbreak of HRV in an intensive care nursery found that 42% and 31% of clinical staff reported upper respiratory symptoms in the previous 4 weeks and 1 week, respectively, preceding the onset of symptoms in the infants (158). Of 29 nasal washes performed in 29 clinical staff, HRV was isolated from one nurse via cell culture; cross-neutralization testing could not confirm if the strain was the same as that isolated from the infected infants.

The U.S. Centers for Disease Control and Prevention Healthcare Infection Control Practices Advisory Committee 2007 guidelines for hospital isolation recommend droplet precautions for patients with HRV infection, with the addition of contact precautions if "copious moist secretions" are present or if close contact is likely to occur (164).

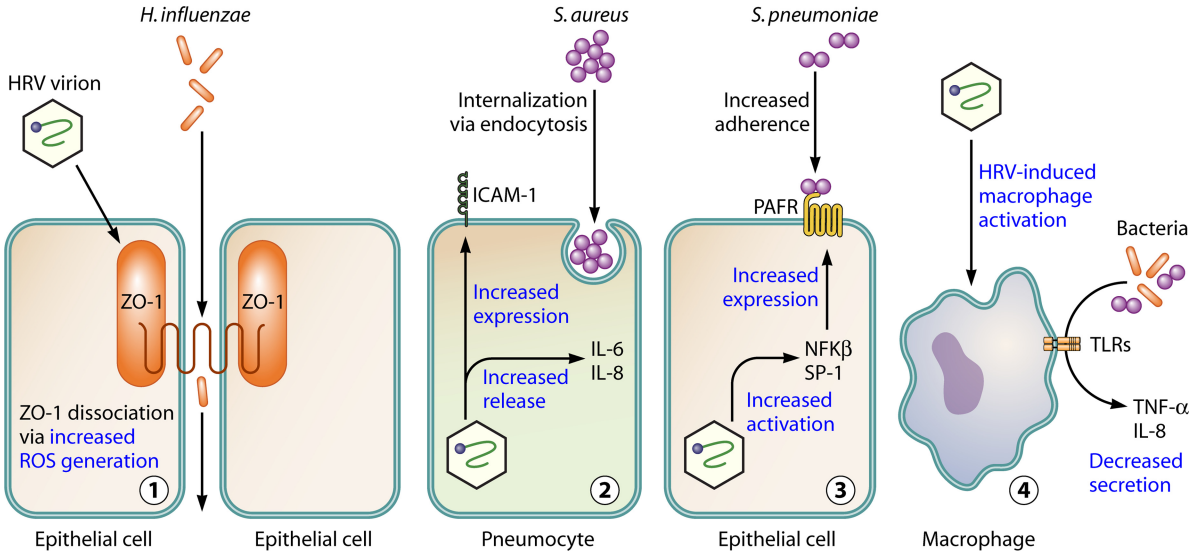

FIG 4 Mechanisms by which HRV increases susceptibility to bacterial infection. (1) HRVs disrupt epithelial cell barrier function by the dissociation of zona occludens 1 (ZO-1) from the tight junction complex via the increased generation of reactive oxygen species (ROS), thereby facilitating the transmigration of bacteria (28). (2) HRVs promote *Staphylococcus aureus* internalization into non-fully permissive cultured pneumocytes via the increased release of IL-6 and IL-8 and expression of intercellular adhesion molecule 1 (ICAM-1) on neighboring uninfected cells (175). (3) HRVs stimulate *Streptococcus pneumoniae* adhesion to human tracheal epithelial cells by inducing the surface expression of platelet-activating factor receptor (PAFR) via NF-κβ expression (173) and to nasal epithelial cells via increased gene and protein expression levels of fibronectin, PAFR, and carcinoembryonic antigen-related cell adhesion molecule (174). (4) Compared to non-HRV-activated macrophages, HRV-activated macrophages demonstrate reduced levels of secretion of TNF-α and IL-8 when exposed to bacterial Toll-like receptors (TLRs) (lipopolysaccharide and lipoteichoic acid) (176). SP-1, promoter-specific transcription factor 1.

Clinical Microbiology Correlations

HRV species. Several early reports suggested that HRV-C causes more severe respiratory illness in adults and children as well as more asthma hospitalizations than HRV-A or -B (84, 165, 166). In addition, two recent case reports described systemic HRV-C infection involving BAL and pericardial fluid, plasma, urine, and feces (125, 167). Also of note, when serum specimens from hospitalized children with HRV-associated severe respiratory illness were tested for HRV viremia, 12% of specimens were positive for HRV. Among children with HRV-C infection, 31% were viremic, compared to 3% and 0% of children with HRV-A and HRV-B infections, respectively (168). More recently, however, well-designed studies found that the clinical manifestations and prevalences of lower respiratory tract infection among children with HRV-A and HRV-C infections appear to be similar (90, 122, 169). Whether illness severity is virus or host (e.g., immune status) dependent requires further study.

HRV viral load. The quantification of the HRV viral load may predict disease severity at higher levels. Piralla et al. found that a viral load higher than 10^7 RNA copies/ml in nasopharyngeal aspirate specimens and patient age less than 5 years were independently associated with lower respiratory tract infection among children and adults hospitalized for acute respiratory illness (170). The viral load also correlated with illness severity scores for children more than 11 months old with lower respiratory tract illnesses (171). In other patient populations and clinical settings, however, lower viral loads of 10^3 to 10^4 RNA copies/ml have been variably associated with symptomatic infection (134), and serial measurements are not useful for monitoring illness resolution (172). Furthermore, results from individual studies are difficult to interpret due to differences in real-time PCR methods and sampling techniques, and therefore, they may not be generalizable to other patient populations and clinical settings. For further discussion of the utility of quantitative real-time RT-PCR as a clinical and research tool, please see "Quantitative real-time RT-PCR" below.

Coinfection with Other Respiratory Pathogens

Bacterial pathogens. Several potential mechanisms through which HRV increases susceptibility to bacterial infection have been demonstrated *in vitro* in epithelial cells of the upper and lower airways (Fig. 4). HRVs stimulate *Streptococcus pneumoniae* adhesion to human tracheal epithelial cells via increases in levels of platelet-activating factor receptors (PAFRs) (173) and to nasal epithelial cells via increased gene and protein expression levels of fibronectin, PAFR, and carcinoembryonic antigen-related cell adhesion molecule (174). HRVs also promote *Staphylococcus aureus* internalization into non-fully permissive cultured pneumocytes (175) and disrupt epithelial cell barrier function by the dissociation of zona occludens 1 from the tight junction complex, thereby facilitating the transmigration of bacteria (28). HRV also impairs immune responses to bacterial products in human alveolar macrophages (176). Compared to non-HRV-activated macrophages, HRV-activated macrophages demonstrate reduced levels of secretion of TNF-α and IL-8 when exposed to bacterial TLRs.

A large ecological study in Finland identified a temporal association between HRV infection in the community and the incidence of invasive pneumococcal disease (IPD) in children (177). Using data from national registries and large epidemiological cohort studies, the authors of that study determined that the mean IPD rates in children less than 5 years old were 2.9 cases per week (95% confidence interval [CI], 2.5 to 3.3) during periods of high-level HRV activity (September to November) and 1.4 (95% CI, 1.2 to 1.6) during periods of low-level HRV activity (April and May)

($P < 0.001$). In comparison, the mean IPD rate was only moderately increased during periods of high- versus low-level RSV activity (2.1 versus 1.7 cases per week; $P = 0.008$) and was unchanged during periods of high- and low-level influenza virus activity.

Viral pathogens. HRV-virus coinfection is common in prospective observational studies; several studies have attempted to elucidate the significance and directionality of this association. Greer et al. (178) showed a statistically significant reduction in HRV-virus codetection compared to other respiratory viruses. Among 1,247 clinical respiratory specimens from all seasons of 2003 tested for 17 respiratory viruses using PCR-based analyses, 131 (11%) contained two or more viruses. HRV was the most commonly detected virus, and 24% of HRV-positive specimens demonstrated virus coinfection. HRV detection was associated with a reduced probability of detection of human adenoviruses, coronaviruses, bocavirus, metapneumovirus, RSV, PIV, influenza A virus, and the KI and WU polyomaviruses. Those authors suggested that the HRV mediation of IFN-stimulating genes may induce a protective antiviral state.

The above-described findings are in contrast to those of Tanner et al., who demonstrated a significant positive association between HRV and PIV, HRV and RSV, and HRV and adenovirus during the 2009 and 2010 winter seasons (179). A negative association was found only between HRV and influenza A virus, which has been observed in other settings (180). These differences may be due to geographical location, seasonality, or the host response and warrant further study. The clinical significance of virus codetection is also unknown. In one study, hospitalized children with bronchiolitis with HRV and RSV coinfection had longer hospital stays than those with HRV infection alone or HRV coinfection with non-RSV pathogens (117).

Fungal pathogens. With the exception of immunocompromised hosts, HRV-fungus coinfection has not been described. *Aspergillus* species are the most common fungal pathogens identified in patients with hematological malignancy and HSCT recipients (35, 141) but has not been associated with HRV infection.

LABORATORY DIAGNOSTICS

Specimen Collection and Processing

Specimens should be collected for laboratory diagnosis as soon as possible after the onset of symptoms. HRV titers are highest in the respiratory tract during the first 2 days of presentation, although the virus may be isolated from 1 day before to 6 days after the onset of symptoms (181). For the investigation of upper respiratory tract infections, nasopharyngeal swabs or aspirates rather than oropharyngeal swabs are preferred, with flocked swabs generally yielding higher virus recovery rates than wrapped swabs, especially for adult patients (182). Nasal wash specimens are also a good source of virus for HRV detection (183, 184), although they are suboptimal for some other respiratory viruses and therefore are not recommended unless no other pathogens are being investigated. Swabs should be placed into viral transport medium.

For lower respiratory tract infections, samples may include tracheal or bronchial aspirate, BAL fluid, or, less frequently, lung biopsy specimens. In a recent study, Harvala et al. reported the detection of HRV in approximately 10% of stool samples from hospitalized patients with enteric disease, predominantly from children under the age of 2 years or adults over 65 years of age (185). In contrast, that study detected HRV in only 1 of 1,500 cerebrospinal fluid (CSF) samples collected from patients with central nervous system disease. However, in general, HRV is not usually suspected of causing infections at other anatomical sites. If respiratory specimens are being transported to an off-site facility, they should be refrigerated and shipped on cold packs, or if delays of more than 2 days are anticipated, they should be frozen at −70°C and shipped on dry ice.

Antigen Detection and Serology

There is no common antigen among HRVs, and an increasingly large number of serotypes have been described; therefore, antigen detection assays are not used for routine detection. Antibodies are measured in both serum and nasal secretions by neutralization, plaque reduction, complement fixation, and enzyme-linked immunosorbent assays (ELISAs) in research settings (104, 186). As with antigen assays, however, the lack of a common antigen across all strains of HRV makes the detection of antibody responses impractical for diagnostic purposes. Furthermore, antibodies are not detectable for 1 to 3 weeks postinfection. Therefore, while antibody measurement is useful for epidemiological studies (187), it is not useful for diagnosing acute infections.

Virus Culture

Conventional virus culture. While well-designed molecular methods are clearly more sensitive for overall detection, HRVs occasionally grow in culture that were otherwise missed due to oligonucleotide mismatches or technical errors. Additionally, cultured isolates are important for studies of virus characteristics and disease pathogenesis (12). HRVs were originally isolated from primary monkey kidney cells, although these cells will support the growth of only some strains (181). Human fetal embryonic lung fibroblast cell lines, certain HeLa cell clones, and human embryonic kidney cell lines are most commonly used for HRV culture in clinical laboratories. In a recent study, HRV-infected susceptible HeLa cells were shown to be a good model compared to HBECs for the study of viral RNA synthesis, translation, protein processing, intracellular protein localization, and disruption of host cell functions (188). In a study of HRV recovery from nasal wash specimens, WI-38 and an HRV-susceptible HeLa clone (with high levels of ICAM-1 expression) were found to be the most sensitive cell types for HRV culture (183). As noted by other investigators (189), those authors suggested a combination of different cell lines as the best approach for the optimal recovery of HRV.

Inoculated cultures are maintained at a neutral pH, since HRVs are acid sensitive (181). Early studies showed enhanced growth with incubation at 33°C rather than at 36°C or 37°C (190, 191). However, a later study demonstrated that for the majority of several serotypes, replication was enhanced only slightly by incubation at the lower temperature and in some instances was equal or better at 37°C (30). Continuous rotation has been demonstrated to provide clear improvements in the detection and virus yields of HRV for many years (192–194).

CPE is usually visible in most cell lines, although CPE-negative strains have been reported (195). Morphological changes are easiest to observe in fibroblast lines and include foci of small and large rounded, refractile cells with pyknotic nuclei and cellular debris (181).

Conventional cultures should be incubated for up to 14 days, and presumptive identification is usually made on the basis of CPE

in appropriate cell lines, although the appearance is the same or very similar to that of enteroviruses (EVs). The viruses are distinguished by acid stability testing: EVs are relatively resistant to low pHs compared to HRV, and a 2- to 3-log reduction in titers after exposure to pH 3.0 will indicate that the virus is HRV (181). A more rapid confirmation method was recently reported, utilizing a combination of staining with a pan-enterovirus reagent which cross-reacts with EV- and HRV-positive cultures and non-cross-reactive EV antibodies (196). Serotyping with specific antisera can be performed with immunofluorescence-labeled antisera but is increasingly being replaced by molecular genotyping (described below).

Rapid culture methods. Rapid culture methods for the detection of infectious HRV have been described. These methods include multichamber slides with HRV-susceptible HeLa cells combined with virus antigen detection using immunofluorescence at 48 h postinfection (197). However, despite reports of a reasonable detection sensitivity compared to that of conventional culture, rapid methods are not commonly used in routine clinical practice. While respiratory screening panels have been developed with pools of labeled antibodies for the simultaneous immunofluorescence detection of many other cultured respiratory agents, HRV is usually not included on the list of detectable viruses (198). Again, the absence of a common antigen makes the development of reliable and specific immunofluorescence reagents difficult. Centrifugation-enhanced cultures can be stained prior to the development of CPE with some EV detection reagents that cross-react with HRV; however, laboratories are left without a specific diagnosis.

A large prospective study reported the use of a centrifugation-enhanced culture method with HuH7 cells in 48-well microplates followed by RT-PCR for HRV on cultures that were CPE positive within 4 days (199). This method had been previously validated for a very large number of HRV genotypes as well as many other respiratory viruses. Over three winter seasons, 4,032 nasal aspirate specimens that had tested negative for respiratory viruses by a direct fluorescence assay were tested for HRV and other respiratory viruses by the rapid culture method, and 272 specimens tested positive for HRV. However, the authors of that study did not report the detection sensitivity compared to that of the direct application of the HRV RT-PCR assay to specimens or how it compared to conventional culture with other cell lines.

Organ culture. Historically, organ cultures of fetal nasal or tracheal epithelia were used to isolate HRVs (200, 201), but these techniques are not used in clinical laboratories. Nasal and tracheal mucosa organ cultures are still used in research settings for pathogenesis research (202), and sinus organ culture has been used to grow an isolate of HRV-C (12).

Molecular Methods

Conventional and real-time RT-PCR. HRVs are somewhat fastidious *in vitro*, and due to the specific conditions required for optimal culture, isolation rates have been generally low except in large reference virology laboratories. Furthermore, since the viruses were believed to cause only "common colds," most laboratories did not consider the detection and identification of HRV infections worth their time and effort. In the late 1980s, methods for PCR-based assays capable of detecting HRV in primary respiratory samples were reported (203–206). The use of these early molecular tests resulted in an increased HRV detection rate. Although identification had to be confirmed with dideoxy sequencing due to cross-reactivity with human DNA, these tests shortened the time to diagnosis from up to 2 weeks to a few days. However, HRV testing continued to be performed primarily in specialized laboratories under limited clinical circumstances or, for specific studies, for many years.

Multiple RT-PCR and real-time RT-PCR techniques have been developed for the detection of HRV since the late 1980s. In general, most of these assays target the 5′UTR, a region highly conserved among all HRVs and EVs, causing cross-reactivity between assays for the two viruses and making their differentiation difficult. RT-PCR tests that can differentiate HRV from EV by amplicon size (207, 208), restriction fragment length polymorphism (RFLP) (206), hybridization with HRV-specific probes (205), RT-PCR followed by sequencing (209), and real-time RT-PCR (210) have also been reported. Since the International Committee on Taxonomy of Viruses reclassified all HRVs into the EV genus, however, the reporting of diagnostic test results as HRV or EV without further testing has become acceptable.

Quantitative real-time RT-PCR assays. While some reports have indicated a correlation between higher viral loads and symptomatic disease (172, 211), which makes quantitative testing attractive for verifying the clinical relevance of positive results, there are currently no commercially available quantitative molecular HRV tests. Additionally, due to the intertypic variability of HRV, there is no standard for the quantification of all HRV types, although some studies have used a chimeric HRV internal standard to limit assay variability between types (212). Other challenges that arise when attempting to provide an accurate and relevant quantification include factors such as the sample type and collection procedure, test methodology, age of the patient, and immune status. All these factors affect viral load and the potential standardization of assays and collectively impact the clinical interpretation of quantitative HRV data such that generally applicable guidelines are not feasible with any reasonable level of confidence.

Additional amplification techniques. Isothermal and other nucleic acid amplification chemistries, in addition to RT-PCR, have also been employed for the molecular detection of HRV. In clinical laboratories, the most commonly used of the alternative chemistries is nucleic acid sequence-based amplification (NASBA). First developed in the late 1990s (213), NASBA assays for HRV were improved several years later by the generation of additional sequence data and appropriate changes to primers (214). A clinical evaluation showed a performance comparable to that of RT-PCR and a sensitivity superior to that of culture, with sensitivity and specificity values of 85.1% and 98.3%, respectively, for the NASBA assay and 82.9% and 93.4%, respectively, for RT-PCR (215). Recently, a quantitative real-time NASBA assay using molecular beacon probes was reported (216), which provides a simplified format and the additional benefit of viral load assessment. However, reports on its clinical evaluation have not yet been published.

Respiratory virus detection panels. With the expanding number of highly multiplexed respiratory virus detection arrays available in recent years and their increasing simplicity of operation, respiratory virus detection has entered a new era (217). Numerous agents that previously could be tested at only a few sophisticated molecular laboratories can now be detected rapidly in most microbiology facilities. As data accumulated on the involvement of HRV in more serious clinical disease, the importance of the inclu-

sion of assays for the virus in these new panels became clear. In turn, as these new assays have been introduced into clinical practice, more data have emerged on the high incidence of HRV infection, resulting in the further awareness of the widespread and sometimes serious disease manifestations. Additionally, some HRVs, such as the group C viruses, are uncultivable by routine culture techniques. Many studies have now demonstrated that HRV infection can lead to an influenza-like illness, lower respiratory tract infections, chronic infections, and secondary bacterial infections, especially in immunocompromised patients (132), children with asthma (218–221), and adults with COPD (152).

Genotyping. HRVs were originally characterized by their growth patterns in human embryonic and monkey kidney cells (222, 223). However, this system demonstrated limited use, since growth characteristics changed with time *in vitro* (224). A subsequent classification system, based on cell receptors, divided HRVs into a major group that utilizes ICAM and a minor group that utilizes LDL (225). An additional system grouped 100 HRV types into 2 groups, designated groups A and B, based on the activities of 15 antiviral compounds (226, 227). Serotyping was also attempted but, due to the large amount of cross-reactivity, did not produce a clear delineation between types.

Since the discovery of PCR in the 1980s, multiple investigators have employed PCR to amplify different genes and nontranslated regions, to further characterize HRV. Three groups of HRVs are now genetically distinguished: group A, group B, and the more recently discovered group C (9). This characterization is best demonstrated by the amplification and sequence analysis of either the VP1 (91) or VP4 (208) gene of the virus. While VP4 sequence data are generally more easily obtained and readily distinguish different HRV groups, the VP1 sequence usually provides more powerful data for HRV strain comparisons of viruses within a group. Sequence analysis can be performed on other regions in the HRV genome, such as the RNA-dependent RNA polymerase (3D) gene and the 5′UTR. However, while analyses of these regions can differentiate groups A, B, and C, they do not have the power to distinguish related strains within the same genetic group.

The motivations for genetic characterizations of HRV include outbreak source determinations and investigations of relatedness or the emergence of a previously uncharacterized strain. In laboratories that perform phylogenetic analyses, RT-PCR amplification and sequence analysis of either the VP1 or VP4 gene are commonly used for this purpose.

Whole-genome sequencing. In 2009, the full-length genomic sequences of all known human HRV serotypes, including the group C viruses, were published (8). A recently updated phylogenetic tree, generated from full-genome HRV sequences, is shown in Fig. 5 (228). This work has harmonized genetic characterizations and provided a reference for the comparison of multiple gene sequences and the determination of type for all subsequently detected HRVs. The importance of full-length genomic sequencing has become increasingly evident with recent studies that demonstrated HRV recombination (229, 230). In such cases, the HRV type determined based on one specific gene would be misleading.

With the increasing availability of techniques for whole-genome sequencing (WGS), it is feasible that more laboratories could perform these methods on clinical isolates of HRV. The increased volume of WGS data will help identify additional genomic regions for analysis. Furthermore, it could provide a better understanding of HRV evolutionary changes, including recombination (229, 230), and help us understand what makes strains of HRV more virulent, prone to causing chronic infections, exhibit tropism for certain tissues or cell types, or target patients with particular underlying conditions or genetic predispositions for more severe outcomes.

ANTIVIRAL AGENTS

Table 1 summarizes the outcomes of clinical trials of agents for HRV/enterovirus prevention and treatment.

Capsid-Binding Agents

The viral capsid was one of the first viral proteins targeted for the development of inhibitors of viral replication. These compounds bind to the hydrophobic pocket of the viral capsid, inducing a conformational change that increases the stability of the virion and interferes with its ability to interact with the cellular receptor (231). Selection for drug resistance is of concern among this class of agents due to the error-prone nature of the viral polymerase and variable conservation of the viral structural proteins (231). To reduce the risk of resistance, the combined use of antiviral therapies with different mechanisms of action may be a useful strategy (232).

Pleconaril. Pleconaril has 70% bioavailability, a long half-life, and good central nervous system penetration, making it an attractive therapy for EV infections (233). A case series of 38 patients treated under a compassionate plea with pleconaril showed that 78% had favorable clinical responses. Clinical syndromes included chronic enterovirus meningoencephalitis and agammaglobulinemia or hypogammaglobulinemia, neonatal enterovirus sepsis, myocarditis, vaccine-associated or wild-type poliovirus infection, postpolio muscular atrophy syndrome, or enterovirus encephalitis as well as bone marrow transplant patients infected with EV (234).

The most rigorous studies of pleconaril were conducted with patients with respiratory illnesses. Two phase 3 multicenter studies in the United States and Canada randomized 2,096 healthy subjects with self-diagnosed colds into groups receiving pleconaril at 400 mg orally twice daily or matching placebo for 5 days. The primary endpoint was the duration of illness. In the primary-efficacy population, which included 1,363 subjects with picornavirus RNA detected in nasal mucus, pleconaril-treated subjects experienced a 1-day reduction in the duration of illness (7.3 days versus 6.3 days; $P < 0.001$) compared to the placebo group (235). In subsequent analyses of subjects with cultivable picornavirus at baseline, pleconaril treatment was associated with a more rapid loss of cultivable virus. In addition, pleconaril-treated subjects infected with more highly susceptible viruses experienced a greater reduction in the duration of symptoms than subjects with reduced viral susceptibility (236). Despite these results, the Food and Drug Administration declined to license pleconaril due to concerns about resistance and safety, including interactions with hormonal contraception and drugs used to treat HIV (233, 237). Pleconaril was subsequently developed into a nasal spray. A phase 2 study evaluating the efficacy of pleconaril nasal spray in preventing asthma exacerbations and common cold symptoms was completed in 2007; results have not yet been disclosed (ClinicalTrials.gov identifier NCT00394914 [http://www.clinicaltrials.gov/ {accessed 20 September 2012}]).

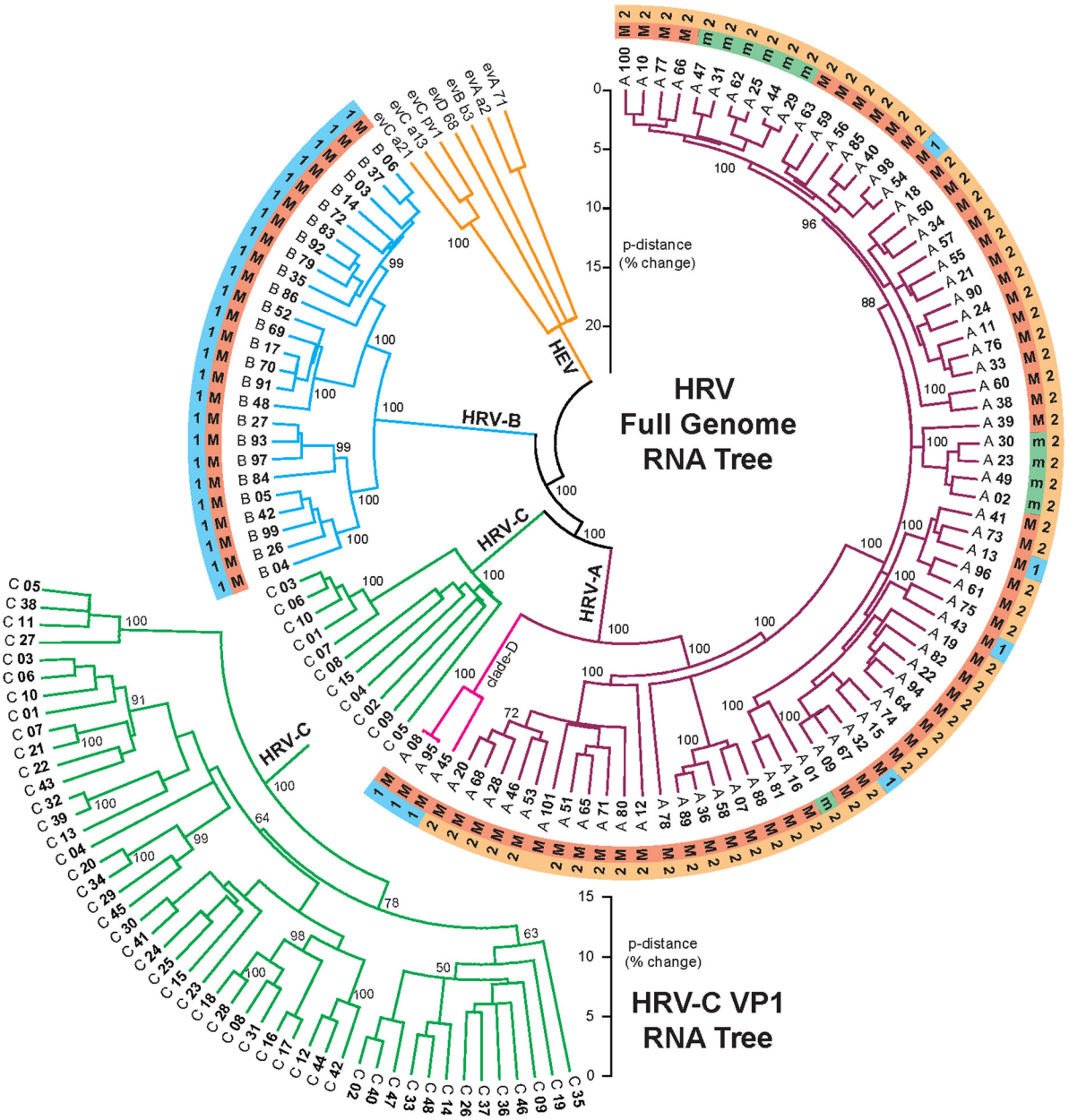

FIG 5 Phylogenetic tree. Shown are circle phylogram relationships for known genotypes of HRV-A, HRV-B, and HRV-C. The tree was calculated with neighbor-joining methods from aligned, full-genome RNA sequences and rooted with data for human enterovirus species A, B, and C. The outer ring ("1" or "2") indicates anticapsid drug group types, if known. The inner ring shows members of the major ("M") (ICAM-1) and minor ("m") (LDLR) receptor groups. The HRV-C receptor is unknown. Since few HRV-C strains are fully sequenced, the determination of relationships among these genotypes relies on partial VP1 RNA data (bottom left). Bootstrap values (percentages of 2,000 replicates) are indicated at key nodes. (Reprinted from reference 228 with permission of Wolters Kluwer Health/Lippincott Williams & Wilkins.)

Vapendavir. Vapendavir is a novel orally administered investigational agent that binds to the HRV VP1 capsid protein, thus preventing the release of viral RNA into the target cell. Vapendavir exhibits antiviral activity against known HRV-A and HRV-B serotypes as well as selected EVs; activity against HRV-C has not yet been established. In a phase 2a clinical trial with healthy volunteers inoculated with HRV-39 2 days after randomization into vapendavir treatment or placebo groups, vapendavir was well tolerated and reduced the incidence of HRV and the peak viral load (238). A phase 2b treatment study with asthmatic adults to assess the effect of vapendavir on URI symptom severity and asthma exacerbations was recently completed; results have not yet been disclosed (ClinicalTrials.gov identifier NCT01175226 [http://www.clinicaltrials.gov/ {accessed 20 September 2012}]).

Pirodavir. Pirodavir is an intranasal capsid-binding agent that reached phase 3 clinical trials for HRV prevention and treatment in the 1990s. Although the compound decreased viral replication and shedding, it failed to show a significant reduction in the duration or severity of symptoms (239, 240). The development of pirodavir has been discontinued (241).

TABLE 1 Agents for HRV/enterovirus prevention and treatment[b]

Drug or therapy	Mechanism of action	Formulation	Study objective(s)[a]	Status	Result(s)	Comments	Source or reference(s)
Vapendavir	Capsid binder	Oral	Prevention of asthma exacerbations and reduction in URI symptom severity and duration	Phase 2b completed	Results not yet disclosed		ClinicalTrials.gov
Pleconaril	Capsid binder	Oral	Treatment of natural HRV colds	Phase 3 completed	1-day reduction in illness duration compared to placebo	U.S. FDA licensing withheld due to safety concerns	235, 236
Pirodavir	Capsid binder	Intranasal spray	Prevention of exptl HRV colds; treatment of exptl and natural HRV colds	Phase 3 completed	For prevention, reduced HRV infection rates when administered 6 times daily but not when given 3 times daily; for treatment, no difference in symptom severity or duration	Compound development discontinued	239, 240
Ruprintrivir	3C protease inhibitor	Intranasal spray	Prevention and treatment of exptl HRV infection	Phase 2 completed	For prevention, reduced frequency of positive viral cultures but not of colds; for treatment, reduced symptom severity by 33%	No effect on viral load or symptom severity in natural HRV infection (data not reported)	242
IFN-α2, IFN-β	Immunomodulatory and possible mediation of viral replication	Intranasal spray or drops	Prevention and treatment of exptl and natural HRV colds	Studies completed	For prevention, reduced HRV colds using IFN-α2, no benefit using IFN-β; for treatment, no difference in symptom severity or duration	Limited use as prophylaxis due to nasal irritation and nasal mucosal friability and bleeding	246, 274–278
Echinacea	Immunomodulatory	Oral	Prevention and treatment of exptl and natural HRV colds in adults and children	Phase 3 completed	For prevention, no reduction in HRV infection rates; for treatment, majority of studies showed no reduction in symptom severity or duration using various *Echinacea* extracts	EMA approved *E. purpurea* for treatment of respiratory tract infections in 2010	248–254
Vitamin C	Antioxidant	Oral	Prevention and treatment of natural colds in adults and children	Phase 3 completed	For prevention, no reduction in HRV colds; for treatment, modest reduction in symptom severity and duration	Treatment benefit may be limited to higher doses of vitamin C and to subjects undergoing brief physical stress	281
Zinc	Unknown[c]	Oral	Prevention and treatment of natural colds in adults and children	Phase 3 completed	For prevention, reduced cold incidence, school absenteeism, and antibiotic prescriptions in children; for treatment, reduced symptom severity and duration when taken within 24 h of symptom onset	Optimal dosage, formulation, and duration are unknown; side effects of zinc lozenges include bad taste and nausea	261
Antihistamines	H_1 receptor inverse agonist	Oral	Treatment of exptl and natural HRV colds	Phase 3 completed	Reduced sneezing and rhinorrhea with no change in total symptom severity scores	Use limited by side effects of dry eyes, nose, and mouth; generally not recommended for treatment of HRV colds	263–265
Tremacamra	Recombinant sICAM-1	Intranasal spray or inhaled powder	Treatment of exptl HRV infection	Study completed	Reduced symptom severity and nasal mucus wt	Potential concerns of 6-times-daily administration affecting adherence and antibody response to sICAM-1; no further development currently reported	284
Enviroxime	Unknown; targets protein 3A	Intranasal spray or oral	Prevention of exptl HRV infection; treatment of exptl and natural HRV colds	Phase 2 completed	For prevention, no reduction in HRV infection; for treatment, no difference in symptom severity or wt of nasal secretions	Oral formulation with gastrointestinal side effects; lack of response to intranasal spray may be due to inconsistent drug levels	285–288

[a] All agents were evaluated in adults only unless otherwise specified.
[b] HRV, human rhinovirus; FDA, Food and Drug Administration; ICAM-1, intercellular adhesion molecule 1; sICAM-1, soluble ICAM-1; IFN, interferon; EMA, European Medicines Agency.
[c] See the text.

Proteolytic Enzyme Inhibitors

Rupintrivir. A virally encoded enzyme, 3C protease, cleaves viral proteins from precursor polyproteins and is essential for viral replication and the assembly of the virion. Rupintrivir was one of the most potent compounds to inhibit 3C protease *in vitro* and was active against a broad panel of HRVs and EVs. In an HRV challenge trial, rupintrivir was well tolerated and reduced viral loads and respiratory symptoms (242). However, in trials of natural infection, rupintrivir did not significantly affect viral loads and symptom severity (243).

Alpha-2 Interferon

Interferons have antiviral, antiproliferative, and immunological effects that impact host cell susceptibility to infection. All of these effects are mediated through cell receptor signal transduction pathways (244). Although intranasal IFN-α2 has shown some benefit for the prevention of HRV colds, studies focusing on treatment have yielded equivocal results. Hayden and Gwaltney randomized healthy adult volunteers to receive recombinant IFN-α2 or placebo via intranasal spray or drops 28 h after experimental HRV infection. Both treatment modalities were associated with significant reductions in the quantity and duration of viral shedding; however, only nasal drops were associated with a significant albeit modest reduction in nasal symptom scores on days 2 and 3 only after viral challenge (245). Subsequently, a placebo-controlled trial using two different doses of recombinant IFN-α2b nasal spray was performed with subjects with naturally occurring colds to determine the effect of interferon after symptoms are established as well as household transmission rates. The treatment arms showed no benefit for symptom severity or duration. In fact, subjects receiving the higher dose of interferon nasal spray experienced a longer duration of symptoms and more severe sore throat and nasal congestion, likely a toxicity of the treatment (246). Given the limited clinical benefit and the side effects, including nasal mucosal bleeding, intranasal interferon has not been adopted as a therapy for HRV infections.

Echinacea

Echinacea preparations are among the most widely used herbal medicines. Preparations include mainly the leaves and roots of dried or fresh *Echinacea purpurea*, *Echinacea angustifolia*, and *Echinacea pallida* and are manufactured by using a range of extraction methods. Chemical constituents that may be important in *Echinacea* health effects include alkylamides, polysaccharides, and caffeic acid derivatives. *Echinacea* is best known for its immune effects, including the stimulation of macrophages, other monocytes, and natural killer cells. Despite the *in vitro* demonstration of immunomodulatory properties, human clinical trials of *Echinacea* therapy for experimental and natural clinical colds yielded conflicting results (247). When healthy volunteers were challenged with HRV-39, three different *E. angustifolia* extracts did not affect rates of infection or symptom severity compared to placebo (248). Among 282 healthy adults with common cold symptoms randomized in a double-blind fashion to treatment with *E. purpurea* extract or placebo, total daily symptom scores were 23% lower in subjects treated with *Echinacea* than in subjects treated with placebo (249). However, in two similarly well-designed studies of children and college students using a dried extract of *E. purpurea* juice of the herb and a dried mixture of *E. angustifolia* root, respectively, *E. purpurea* root and *E. purpurea* herb failed to show a reduction in URI symptom severity or duration (250, 251). More recently, a trial of *E. purpurea* and *E. angustifolia* root extract for the treatment of common cold symptoms employed a two-way factorial design, randomizing subjects into groups with various degrees of clinical interactions and with no pills, blinded placebo, blinded *Echinacea*, or unblinded open-label *Echinacea*. That study found a nonstatistically significant trend toward reduced symptom severity and duration in subjects assigned to the *Echinacea* treatment group (252).

Explanations for the conflicting study results include variations in extracts, medication regimens, and study design, including the timing of administration relative to the onset of symptoms. A Cochrane review concluded that despite some studies that showed a benefit, there is no conclusive evidence that *Echinacea* products effectively treat or prevent the common cold (253). That review cited concerns about publication bias, poor study quality, and the variability of study results. The European Medicines Agency Committee on Herbal Medicinal Products recently assessed *in vitro* and *in vivo* pharmacological and clinical data for *E. purpurea* root and herb, *E. pallida* root, and *E. angustifolia* root for the prevention and treatment of respiratory infections. The committee concluded that herbal drug preparations of *E. purpurea* can be considered safe and effective for the treatment of respiratory tract infections. However, there are insufficient pharmacological and/or clinical data to support the efficacy and clinical use of *E. purpurea*, *E. pallida*, and *E. angustifolia* root (254–256). The U.S. Food and Drug Administration has not evaluated *Echinacea* for safety and efficacy.

Zinc

Zinc has activity against HRVs, although its exact mechanism of action is unknown. Zinc has been shown to inhibit viral replication *in vitro* (257), block HRV binding to ICAM-1 molecules (258), alter the configuration of viral capsid proteins to prevent their role in proteolysis and virus assembly (259), and decrease histamine release (260). A recent Cochrane review included 966 subjects from 13 randomized controlled trials in which all subjects began zinc therapy within 3 days of the onset of symptoms and continued treatment for at least 5 days (261). Formulations included zinc sulfate tablets, zinc sulfate syrup, or zinc gluconate or zinc acetate lozenges. The authors of that study found that zinc supplementation within 24 h of the onset of a cold was associated with significantly reduced symptom severity and duration. Another systematic review and meta-analysis similarly showed a dose-dependent reduction in the duration of symptoms among subjects exposed to zinc (262). However, the benefits were limited to adults, and zinc did not impact symptom severity. Significant trial heterogeneity and a lack of adequate blinding may contribute to the different conclusions of the reviews. When utilized for prevention, zinc supplementation for at least 5 months was associated with reduced cold incidences, school absenteeism, and antibiotic prescriptions in children (261).

Antihistamines

First-generation (i.e., nonselective) antihistamines, including clemastine fumarate and brompheniramine maleate, were evaluated in the 1990s for the treatment of experimental HRV colds. In two separate randomized, placebo-controlled trials of adult subjects with experimental HRV infection, clemastine and brompheniramine reduced sneeze scores and rhinorrhea scores by up to

50% and 62%, respectively, compared to placebo when administered daily for 4 days (263, 264). However, neither the severity of other cold symptoms, including cough, nasal obstruction, and sore throat, nor the total symptom severity scores were significantly different between groups. A Cochrane review of 32 trials confirmed a mild effect of first-generation antihistamines on rhinorrhea and sneezing, but this benefit was not replicated with nonsedating antihistamines (265). Furthermore, in clinical practice, their use is limited by side effects such as dry eyes, nose, and mouth.

Other Agents

Tremacamra, targeting recombinant soluble ICAM-1, and enviroxime, with an unknown mechanism, are two compounds evaluated for HRV prevention and treatment that failed to show a benefit in clinical trials. More information on these agents is available in Table 1.

PREVENTION

Potential modes of person-to-person HRV transmission include small-particle aerosols, large-particle aerosols (18), and contact spread either directly or through a fomite (13, 15). Behavioral strategies to reduce respiratory viral transmission include social distancing, the use of respiratory masks, and hand hygiene. Efforts to develop prophylactic medications and vaccinations specifically for HRV prevention have been unsuccessful.

Social Distancing and Respiratory Masks

Behavioral strategies such as social distancing and respiratory mask application have been evaluated primarily in the context of pandemic influenza A virus and influenza-like illness prevention. Social distancing includes school closures and the avoidance of public gatherings. An assessment of the effectiveness of social distancing in real-world settings is challenging due to the lack of randomization, inadequate reporting, and changing interventions over time (266, 267). Broderick et al. evaluated febrile respiratory illness (FRI) transmission rates in a military training setting where some units were open to potentially infectious convalescents and some units were closed to the entry of potentially infected individuals (268). They found that there was no significant difference in FRI rates between open and closed units; however, any effect of social distancing may have been mitigated by the finding that there was also considerable environmental pathogen contamination in the housing units. Such studies highlight the complexity of systematic evaluations of population-based interventions in natural settings, rather than disprove their efficacy. Indeed, computer simulations have demonstrated that social distancing can be an effective public health prevention measure, reducing epidemic attack rates by as much as 90%, depending on the infectivity of the pathogen (269, 270). The use of masks, particularly among health care workers, is an established effective intervention to reduce respiratory virus transmission (266). It remains uncertain whether N95 respirators confer additional protection over surgical masks, and the degree of difference may vary by pathogen. A well-designed randomized controlled trial designed to show noninferiority found that surgical masks offer protection similar to that of N95 respirators among nurses at the highest risk for exposure to influenza virus (271).

Hand Hygiene

Hand-to-hand HRV transmission appears to be highly efficient, and individuals may also self-inoculate if a contaminated hand contacts nasal secretions. Therefore, the interruption of direct contact in viral transmission presents a potential target for intervention. In a series of experiments with healthy volunteers challenged with HRV-39 on the fingertips, Turner et al. demonstrated that ethanol hand sanitizer was more effective than soap and water in removing detectable virus from the hand (the efficacy rate for the ethanol group of 80%, versus 31% for the group using soap and water) and that the addition of organic acids (2% malic acid and 2% citric acid in a 70% solution of ethanol) provided additional virucidal activity that persisted for at least 4 h (272).

In order to evaluate the efficacy of hand disinfection on HRV prevention in the natural setting, Turner et al. (273) conducted an unblinded, randomized trial with 212 healthy young-adult volunteers. The hand treatment contained 2% citric acid and 2% malic acid in 62% ethanol and was applied every 3 h for 9 weeks during the fall of 2009. In both the intention-to-treat and per-protocol analyses, there was no difference between treatment groups in the primary endpoint, HRV-associated illness, or the secondary endpoint, the incidence of HRV infection. Despite evidence supporting virucidal hand treatments for HRV prevention in the experimental setting, those authors provided several potential explanations for the discrepancy in the results: a lack of control in the natural setting for variables such as compliance and modes of transmission, potential protective effects of nasal secretions, and the potential for routes of virus transmission other than direct-contact self-inoculation. Nonetheless, a systematic review of physical interventions to reduce the transmission of respiratory viruses found that hand washing with or without antiseptic was effective (266). The results were most robust for children, who are least capable of performing hygienic behaviors by themselves.

Alpha-2 and Beta Interferons

Several studies in the 1980s demonstrated that intranasal IFN-α2 reduced respiratory illness when administered either continuously during a respiratory virus season or intermittently as post-exposure prophylaxis in the family setting (274–276). However, local adverse reactions, including nasal irritation, mucosal friability, and bleeding, have limited its use (275, 276). In one study, IFN-β serine appeared to be better tolerated (277); however, a follow-up prophylaxis study in the natural setting failed to show a benefit of IFN-β serine compared to placebo (278).

Echinacea

Several double-blinded, placebo-controlled studies have found that different preparations of *Echinacea* are ineffective for the prevention of HRV infection or the development of HRV colds (248, 279, 280). A 2009 Cochrane review of *Echinacea* for prophylaxis and treatment including three prevention trials in the natural setting confirmed these negative findings (253).

Vitamin C

Vitamin C has been studied for the prevention and treatment of the common cold since 1942 and has been marketed as such since the 1970s. As an antioxidant, vitamin C may protect against the generation of oxidative stress during infections; in animal studies, vitamin C reduces the incidence and severity of bacterial and viral infections. The belief that these benefits extend to human subjects

has existed for many years. In 2010, the Cochrane Library conducted a systematic review and meta-analysis of vitamin C for the prevention and treatment of the common cold (281). Only placebo-controlled trials using doses of 0.2 mg/day vitamin C were included. Twenty-nine trials including more than 11,000 subjects found no difference in the incidences of colds between subjects treated with vitamin C and those given placebo; however, the duration and severity of colds were reduced albeit modestly. Of note, the benefit was most pronounced in subjects undergoing brief periods of high physical stress, e.g., marathon runners. Seven therapeutic trials showed no difference in the severity or duration of symptoms, with the exception of one trial showing that high-dose vitamin C (8 g/day) was associated with more "short" colds (symptom duration of <1 day) than lower-dose vitamin C (4 g/day).

Vaccination

To date, there have been no HRV vaccines evaluated in clinical trials. Challenges to vaccine development include the presence of more than 100 different HRV serotypes, the lack of epidemiological data to identify the most commonly circulating HRV strains, the incomplete understanding of antigenic differences between the recently discovered HRV-C species and known serotypes, and limited animal models of HRV infection to understand viral pathogenesis (282). Effective vaccine development calls for the elucidation of antigenic epitopes common to most known HRV serotypes to induce the production of cross-reactive antibodies (53). Recent work has focused on deriving antigenic peptides from one of the viral capsid proteins, VP1, which plays a central role in receptor binding and subsequent epithelial cell infection and is recognized by HRV-neutralizing antibodies (283). However, no studies have moved beyond the *in vitro* phase; due to the challenges noted above, we are still far from clinical vaccine development.

CONCLUSIONS

Substantial advances in the field of HRV research have occurred in the last decade, due primarily to improvements in molecular diagnostics. HRV is not just a cause of benign upper respiratory illness; rather, it is a significant lower respiratory tract pathogen in patients with chronic pulmonary disease, children, and immunocompromised hosts. Our understanding of HRV pathogenesis, drawn largely from *in vitro* data and *in vivo* studies of experimental infection of healthy adults, implicates both direct viral effects and tissue damage due to the host immune response. The recently published full-length genomic sequences of all known HRV serotypes, including the group C viruses, will facilitate characterizations of HRV strains detected in the future. Additionally, whole-genome sequencing may provide insight into the observed differences in clinical symptoms and outcomes according to the HRV strain. There is also a need to identify other modifiable risk factors for the acquisition and severity of HRV infection. A better understanding of the mechanisms leading to manifestations of HRV infection and the role of the host immune response is needed to guide future efforts at HRV prevention and treatment.

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Daryl M. Lamson has a patent application pending for the discovery of HRV-C sequences and their uses (application number PCT/US 2009/0275636).

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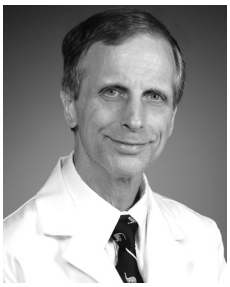

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