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CHAPTER 58

Respiratory Viral Infections

José Luiz Proença-Módena • Gustavo Olszanski Acrani • Cynthia B. Snider • Eurico Arruda

INTRODUCTION

Acute respiratory infections (ARIs) are prevalent worldwide[1] and rival diarrhea as the leading cause of death in developing countries (***Fig. 58.1***).[1,2] Unlike pathogens restricted to tropical areas, the respiratory viruses are distributed globally, are efficiently transmitted from person to person, and have impact on all age groups. In impoverished urban populations in South America, ARI symptoms may be present on an almost continuous basis, making it difficult to determine symptom-free days and estimate attack rates.[3,4] The most striking disparity between developing and developed countries with regard to ARI epidemiology is the case-fatality rate of lower respiratory infection (LRI), mainly pneumonia, bronchiolitis, and influenza,[5,6] in children under 5 years of age, which may reach 16% in some areas.[1]

Several community-based studies have established the importance of common respiratory viral infections in tropical countries[7] (***Table 58.1***). In impoverished populations LRI may occur simultaneously with measles, diarrhea, and malnutrition, and can potentially become life-threatening.[1,13]

In most health care facility-based LRI studies conducted in tropical countries, human respiratory syncytial virus (HRSV) has been the virus most frequently detected (11–33%), followed by human metapneumovirus (7–43%), parainfluenza viruses (1–13%), human bocavirus (2–19%), adenoviruses (2–34%), and influenza viruses (1–4%) (***Table 58.2***). Except for a few more recent reports, human rhinovirus (HRV) and human coronaviruses (HCoV) have not been tested in prospective studies conducted in tropical countries. A pathogenic role has been well established for most respiratory viruses, but there is considerable overlap of clinical and pathological features between different viral diseases. Therefore, a direct role of certain viruses in the pathogenesis of a specific respiratory illness is not possible to establish on clinical grounds, which is further complicated when multiple respiratory viruses are detected simultaneously in the same specimen.[22] Detecting viral genomes in respiratory secretions does not necessarily correlate with viral replication and may reflect latency or prolonged shedding unrelated to the current symptoms. Therefore, approaches based on the detection of viable viral progeny or markers of active viral replication should be used in studies of pathogenesis of respiratory viruses.

Saffold virus, a cardiovirus in the family *Picornaviridae*, initially detected in stools from a febrile infant,[23] was recently detected in respiratory samples of children with ARI.[24,25] However, since very little is known regarding the pathogenic role of this agent in respiratory disease, it will not be further discussed here.

Few specific interventions have become available to reduce the impact of viral ARI,[2] and their application may be further hampered by epidemiologic conditions in equatorial regions. Poor housing and crowding, lack of clear seasonal outbreaks in some tropical areas, and insufficient resources to provide influenza immunization or antiviral treatment or HRSV immunoprophylaxis prevail in most developing nations.[26,27] In addition, nutritional and educational interventions, such as reinforcing breastfeeding,[28] vitamin A supplementation for measles,[29] and access to oral rehydration therapy,[30] may have significant effect on the morbidity and mortality due to LRI in its interface with diarrhea.

This chapter addresses the most common viral respiratory infections (***Table 58.3***), highlighting features unique to the developing world.

INFLUENZA VIRUSES

In tropical countries influenza activity may occur year-round, as well as in outbreaks more typical of temperate regions. Twice yearly outbreaks are noted in some areas of Southeast Asia. These infections can have high impact on morbidity and mortality, since impoverished populations have limited access to medical care, including vaccination and antiviral treatments.[31] Influenza virus is considered a prototypic emerging virus, because it undergoes antigenic drift and shift. Antigenic drift occurs by the accumulation of point mutations, where host antibodies from previous circulating strains are ineffective. Antigenic shift, usually caused by reassortment of genes from viruses of animal origin, or sometimes by the crossing of species barriers by animal influenza viruses, generates emerging influenza virus strains that may cause localized outbreaks or pandemics, with enormous potential impact for health on a global scale.[32]

Since the 1997 outbreak of avian influenza A/H5N1 and the 2003 epidemic of SARS coronavirus, global surveillance programs have been implemented to identify emerging infections. In April 2009, such programs identified infections in patients from Southern California, Texas, and subsequently Mexico with a novel influenza A(H1N1). This previously unknown virus was rapidly identified to be a reassortant with gene segments from avian, swine and human viruses[33] and quickly circulated throughout the world, affecting more than 213 countries and being responsible for at least 16713 deaths as of March 2010[34] (see ***Fig. 14.1***).

THE AGENT

Influenza viruses of the family *Orthomyxoviridae* are pleomorphic, enveloped with segmented negative-strand RNA genomes, distributed in three genera: *Influenzavirus A*, *B*, and *C*, based on the antigenicity of the nucleoprotein (NP) and matrix protein. The type species influenza A virus is further classified in subtypes based on the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA).[35] Among the 16 HA and 9 NA recognized subtypes, 6 HA (H1, H2, H3, H5, H7, and H9) and 3 NA (N1, N2, and N7) have been detected in humans.[32] However, only three

subtypes of HA (H1, H2, and H3) and two of NA (N1 and N2) have caused pandemics and sustained circulation in human populations.[33] The genomes of influenza viruses consist of eight negative-strand RNA segments in influenza A and B viruses, and seven RNA segments in influenza C.[35]

The viral HA binds to sialic acid-containing cell receptors, and mediates fusion and penetration. Proteolytic cleavage of HA by cellular serine proteases exposes hydrophobic fusion domains that mediate membrane fusion. The NA cleaves terminal sialic acid from glycoconjugates present on respiratory mucins, cells, and progeny virions. This action destroys receptors recognized by HA and allows budding virus to be released from infected cells and to spread within the respiratory tract. Influenza C virus contains a single surface glycoprotein, which binds to receptor, promotes fusion of membranes, and also cleaves sialic acid.[35]

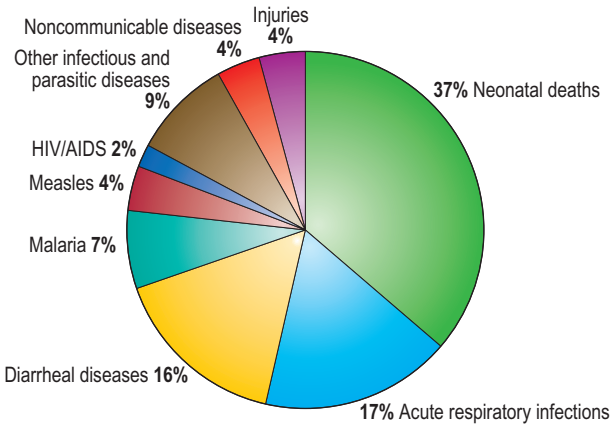

Figure 58.1 Causes of death in children under 5 years of age in the world. (Data from WHO. The global burden of diseases: 2004 update. Geneva: WHO; 2008.)

Virus-receptor binding is followed by internalization into endosomes, acid-dependent fusion of viral and endosomal membranes, and release of genome in the cytoplasm, from where it is transported to the nucleus. In influenza A viruses, the envelope M2 protein serves as an ion channel that facilitates RNA release. Transcription of the negative-strand genomic RNA into positive-strand messenger RNA (mRNA) and complementary RNA (cRNA) is mediated by a viral RNA polymerase complex in the nucleus. cRNA serves as a template for the synthesis of negative-strand RNA genome segments, and mRNA directs viral protein synthesis. Newly assembled nucleocapsids acquire an envelope as they bud through the cell surface and only viruses with a full set of genome segments are infectious.[35]

Influenza A viruses are primarily viruses of aquatic birds, particularly ducks and shore birds, that harbor all of the subtypes recognized to date. Selected subtypes naturally infect a range of terrestrial (swine, horses, humans) and aquatic (seals) mammals; influenza B virus infects humans and uncommonly seals, dogs, cats, and swine; and influenza C virus is primarily a virus of humans. Depending on the virus type and subtype, experimental infection can be induced in mice, ferrets, chickens, swine, and primates, and the viruses can be propagated in primary cultures of

Table 58.1 Detection of Common Respiratory Viruses in Six Representative Community-Based Studies in Tropical Countries

		Respiratory Viruses in Children with Acute Respiratory Infection (%)[a]						
Country	No. of Samples	HRSV	HADV	HPIV	Flu	HRV	HMPV	Reference
Nepal	919	15.1	–	11	11.2	–	4.2	8
Brazil	1052	–	3.6	5.9	2.1	16.7	–	3
Philippines	311	13.0	3.5	5.1	2.2	–	–	9
Thailand	799	4.4	2.0	4.6	1.7	1.7	–	10
Colombia	506	13.2	1.0	4.7	2.0	–	–	11
The Gambia	221	1.8	8.1	6.3	6.3	–	–	12

[a]Viruses were detected by isolation in cell culture or by antigen detection with immunofluorescence or PCR.
HADV, human adenovirus; Flu, influenza virus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; HRV, human rhinovirus; HRSV, respiratory syncytial virus.

Table 58.2 Detection of Common Respiratory Viruses in Eight Representative Hospital-Based Studies in Tropical Countries

		Children with Viral Infection (%)[a]							
Country	No. of Samples	HRSV	HADV	HPIV	Flu	HRV	HMPV	HBoV	Ref
Iran	109	12.9	5.9	22.2	10.9	–	–	–	14
Vietnam	659	23	5	–	15	28	4.5	2	15
Malaysia	180	10.5	1.1	2.2	5.5	4.4	–	–	16
Kenya	822	12.0	1.9	2.3	1.0	6.5	–	–	17
Pakistan	1492	32.9	1.9	0.9	1.3	–	–	–	18
Thailand	596	20.3	6.7	13.2	4.2	–	–	–	19
India	809	20.0	4.0	12.0	4.0	–	–	–	20
Philippines	537	11.2	4.0	5.2	2.8	–	–	–	21

[a]Viruses were detected by isolation in cell culture or by antigen detection with immunofluorescence or PCR. Serology for some viruses was used in addition to viral isolation.
HADV, human adenovirus; Flu, influenza virus; HMPV, human metapneumovirus; HBoV, human bocavirus; HPIV, human parainfluenza virus; HRV, human rhinovirus; HRSV, respiratory syncytial virus.

Table 58.3 Common Viral Respiratory Infections

Virus	Types	Principal Syndromes	Virus Detection Method	Specific Therapy	Vaccines
Influenza	A, B, C	Classic "flu," bronchitis, URI, pneumonia, bronchiolitis, croup	Culture, Ag detection, RT-PCR	Oseltamivir, zanamivir (A, B), amantadine/ rimantadine (A)	Inactivated viruses, subunit,[a] cold-adapted, live-attenuated virus, DNA with adjuvant[a]
HRSV	A, B	URI, bronchiolitis, croup, bronchitis, pneumonia	Culture, Ag detection, RT-PCR	Ribavirin, immunoglobulin, palivizumab for prophylaxis, RNAi	(subunit;[a] live attenuated[a])
HPIV	1, 2, 3, 4	URI, croup, bronchiolitis, bronchitis, pneumonia	Culture, Ag detection, RT-PCR	Ribavirin[a]	Live attenuated;[a] recombinant virus[a]
HRV	>100	URI; otitis media; exacerbation of asthma/ COPD	Culture, RT-PCR	Pleconaril;[a] pirodavir[a]	None
HADV	53	URI; PCF; bronchitis; pneumonia	Culture, Ag detection, PCR	Cidofovir[a]	Live oral vaccine (types 4 and 7)
HCoV	OC43, 229E, NL63, HKU1	URI, bronchitis, pneumonia	Culture, RT-PCR	None	None
HMPV	A, B	URI; bronchitis; pneumonia; wheezing	Culture, RT-PCR, ELISA	Ribavirin,[a] immunoglobulin, NMSO3[a]	Live recombinant virus[a]
HBoV	1, 2, 3	URI; bronchitis, pneumonia, wheezing, bronchiolitis, gastroenteritis, tonsillar chronic inflammation	RT-PCR	None	None

[a]Investigational use.

Ag, antigen; COPD, chronic obstructive pulmonary disease; HADV, human adenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; HRV, human rhinovirus; PCF, pharyngoconjunctival fever; HRSV, respiratory syncytial virus; RT-PCR, reverse transcription-polymerase chain reaction; SARS, severe acute respiratory syndrome; SARS-CoV, coronavirus associated with SARS; URI, upper respiratory infection.

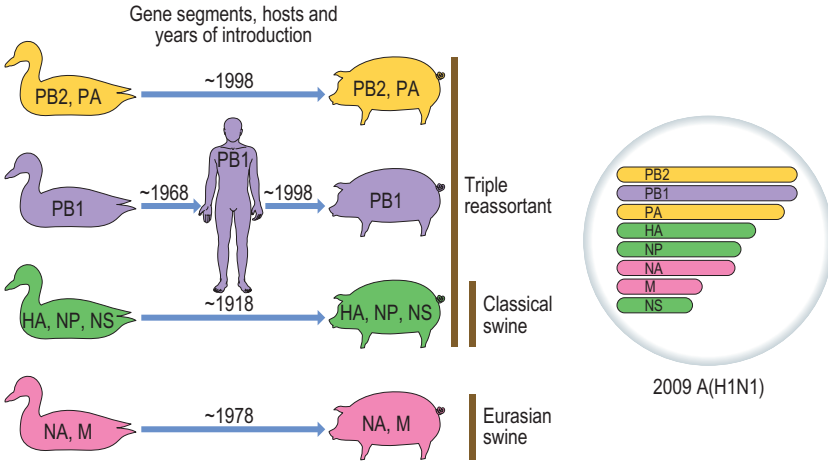

Figure 58.2 Lineage origins for the gene segments of the A/California/04/2009 virus. Phylogenetic analysis shows that six gene segments (PB2, PB1, PA, HA, NP, and NS) were similar to those found in a triple-reassortant swine influenza virus circulating in pigs in North America, with cases previously reported in humans. The NA and M genes entered the Eurasian swine population circa 1979, and that lineage recently combined with the swine triple reassortant. The HA, NP, and NS gene segments are in both the swine triple reassortant and classical swine, which is similar to the human 1918 A(H1N1) lineage. The polymerase PB2 and PA gene segments entered the swine triple reassortant in 1998.[33] The polymerase PB1 gene segment originally entered human population circa 1968 and was seeded into swine from humans in 1998, becoming part of the triple reassortant. (Redrawn from Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science. 2009;325:197.)

kidney cells, continuous cell lines, and also in embryonated hen's eggs.[36] Influenza viruses are inactivated by temperatures above 50°C, and by lipid solvents, acid, formaldehyde, ionizing radiation, and ultraviolet (UV) light.[36]

The 2009 pandemic influenza A(H1N1) virus contains a unique combination of gene segments not previously reported in swine or human influenza viruses, all with an origin traceable to avian viruses (*Fig. 58.2*). Phylogenetic analysis of the genome of A/California/04/2009 showed that its gene segments come from a triple-reassortant swine influenza virus circulating in pigs in North America, combined with a Eurasian swine virus (*Fig. 58.2*). Antigenically this virus is similar to North American swine A(H1N1) viruses, and distinct from seasonal human influenza A(H1N1).[33]

EPIDEMIOLOGY

Influenza virus infections occur throughout the world, causing highly contagious respiratory infections, with high morbidity and excess mortality, particularly in infants and the elderly. In tropical developing countries,

influenza has been associated with an average of 5% of ARIs leading to physician contact.[6,37] These are only the most severe cases, and 30–50% of children under 5 years of age in tropical Africa seroconvert in one outbreak.[37] Previously healthy infants are hospitalized for influenza at rates similar to those for adults at high risk for influenza, and influenza accounts for a great number of outpatient visits and courses of antibiotic therapy in children of all ages.[38] When human influenza virus is introduced into a malnourished population with limited access to health care, high morbidity and mortality rates can occur.[39]

Peaks of influenza activity are associated with excess mortality in tropical and subtropical areas.[40,41] Contrary to the sharp seasonality of influenza A outbreaks in temperate countries, seasonal patterns in tropical countries are variable for reasons that are not clear. In Southern India[20] and Thailand[10] influenza has caused sporadic outbreaks throughout the year, but with consistent outbreaks in June–July and November–January with no apparent association with meteorological factors. In the Philippines, influenza has been more frequent between November and January,[9] while in Senegal, Nigeria, and Taiwan there was a clear association with the rainy season.[27] In southeastern Brazil,[42] Argentina,[43] and South Africa,[44] seasonal outbreaks of influenza A occur from May through August in association with cooler temperatures but not with rainfall. Recent studies show that temperature and humidity play a central role in the timing of influenza circulation in Brazil, where the seasonal wave of influenza activity travels southward from the sparsely populated equatorial region to the densely populated southeast.[45] High temperature and humidity tend to reduce aerosol transmission of influenza virus[46] and thus the reproduction number of influenza outbreaks in tropical regions is reduced as compared to temperate areas.[47]

Influenza B outbreaks occur periodically, yet less frequently than influenza A, both in temperate and tropical regions,[27,36] whereas influenza C is generally nonseasonal.[36]

Ecologists have analyzed migration patterns of influenza viruses throughout the years and have found that there is an East–Southeast Asia seeding of H3N2 spreading to temperate latitudes annually.[48] Interestingly, it has been shown that influenza virus A(H3N2) regularly migrates bidirectionally across hemispheres between seasons.[49] Furthermore, modeling of influenza genealogy suggests there may be North–South transmission where gene flow comes across the Pacific, incorporated into strains in Central America that subsequently migrate to the United States.[50]

Influenza viruses B and C are less prone to antigenic drift. Antigenic shifts occur by the acquisition of genes for novel subtypes of HA or NA, to which humans lack significant immunity.[32] This is typically caused by genetic reassortment in a reservoir host infected simultaneously by human and animal (mostly avian) viruses. Adaptation of such novel influenza A subtypes has led to catastrophic pandemics, including three in the last century. The 1918 H1N1 "Spanish flu" pandemic is estimated to have caused up to 100 million deaths worldwide,[32] while the 1957 H2N2 "Asian flu" and the 1958 "Hong Kong flu" caused an estimated 1–3 million deaths.[32]

Since 1997, clusters of human infections due to highly pathogenic avian influenza viruses H5N1 in Asia have raised concern about new pandemic threats. H5N1 resulted from exposure of humans to infected poultry, but the virus was transmitted inefficiently from person to person.[32,36,51] Other avian viruses, H9N2, H7N7, and H7N3, have also caused mild human disease.[32]

Influenza virus is transmitted from person to person by large droplets and small-particle aerosols, and by fomites with hand contamination and subsequent self-inoculation. Secondary attack rates may be higher than 70% in semi-closed populations, especially among school children and subjects with underlying conditions living in confinement, such as nursing home residents.[36] Children play a major role in influenza outbreaks with respect to propagation of the epidemic virus in families and community.[36]

In March 2009, a previously undescribed influenza virus A(H1N1) quickly spread throughout the community of La Gloria, Veracruz, Mexico with clinical attack rate in children younger than 15 years old twice that in adults, 61% versus 29%.[52] Attack rate of pandemic influenza was estimated at 11%. By July, there were nearly 90 000 laboratory confirmed cases and 382 confirmed deaths globally. The 2009 pandemic A/H1N1 displaced seasonal influenza A(H1N1) and generated fear that its crude mortality rate could surpass that of seasonal influenza in a population lacking neutralizing antibodies. However, total mortality was comparable to that attributed to seasonal influenza, with most deaths associated with predisposing conditions, such as obesity and underlying pulmonary disease, although severe disease was seen with unusually high frequency in younger ages and pregnant women, with a case-fatality rate less than 0.5%.[53]

CLINICAL FEATURES

Classic influenza starts abruptly after an incubation period of 1–4 days, with fever, chills, malaise, headache, myalgia, and prostration, often accompanied by nonproductive cough, sore throat, and mild rhinorrhea. Systemic complaints last 3–5 days, whereas sore throat, hoarseness, and cough, with substernal discomfort, may increase in severity as the systemic symptoms subside. Cough and asthenia often persist for 2 weeks or longer. Respiratory symptoms may be minimal, especially in elderly people or infants. In frail elderly persons, lassitude, lethargy, confusion, low-grade fever, and occasional gastrointestinal complaints may be the primary findings. Influenza B tends to be milder than influenza A, and influenza C typically causes colds or bronchitis.[36] Influenza may also present as unexplained fever, croup, vomiting, diarrhea, and neurologic manifestations in young children.[54] Up to 50% of influenza virus infections in adults are subclinical.[36]

For 2009 H1N1 strain, the incubation period ranged from 1 to 7 days, the most common presenting symptoms were fever, cough, and sore throat, and 25–39% of patients had diarrhea and vomiting, especially children.[55,56] Lymphopenia, and elevated liver transaminases were more common in severely ill patients.[56] Bacterial coinfections were present in 31% of postmortem examinations.[57,58]

Influenza causes a variety of respiratory complications, including otitis media, sinusitis, tracheobronchitis, and pneumonia. Secondary bacterial infections, especially pneumonia, are common complications and should be suspected in relapses of fever, chest pain, and cough.[36] Other complications include meningococcal infections, exacerbations of asthma, chronic bronchitis, and congestive heart failure. In general, pregnant women, HIV-infected patients and other immunocompromised hosts are at higher risk for severe disease and complications.[36] Reye's syndrome occurs in fewer than 1 per 100 000 cases of influenza in patients under 18 years of age, following the use of salicylates. In contrast to seasonal influenza, which leads to increased hospitalization of elderly and children under the age of 5, almost half the hospitalized patients in the A(H1N1) pandemic were persons under the age of 18 and over one-third were between 18 and 49 years old.[56] Risk factors for severe disease associated with 2009 pandemic A(H1N1) were similar to those for seasonal influenza but also included severe obesity.[56]

PATHOGENESIS AND IMMUNITY

The virus infects the respiratory mucosa causing lysis and desquamation of respiratory epithelium, mononuclear cell infiltrates, and altered mucociliary clearance. Tracheobronchitis is a typical feature, often associated with prolonged abnormalities in small airways pulmonary function and airway hyperreactivity. Primary influenza viral pneumonia results in diffuse alveolar damage, alveolar hemorrhage and exudate, hyaline membranes, and reactive fibrosis. Fatal cases of 2009 pandemic influenza A(H1N1) showed pathological changes of multiorgan dysfunction syndrome, such as brain congestion and swelling, myocardial inflammation, fibrinoid changes in arterioles, thrombosis in branches of pulmonary and splenic arteries, leading to wedged splenic infarcts.[36,58]

Viral replication in the upper respiratory tract generally peaks within 1 or 2 days of symptom onset and, depending on age and prior immunity, continues for about 3–8 days. The severity of illness broadly correlates

with upper respiratory tract viral loads. Constitutional influenza symptoms are due in part to the release of proinflammatory cytokines and chemokines. Levels of interferon (IFN-α and IFN-γ), tumor necrosis factor (TNF-α), interleukins and chemokines (IL-1β, IL-6, IL-8, IL-10, MCP-10, MIP-1α and MIP-1β) are increased in nasal secretions, and IFN, IL-6 and TNF-α are increased in blood in human influenza.[36] The tissue tropism of a strain of influenza virus depends, among other factors, on a combination of susceptibility of its HA to be cleaved by, and tissue availability of, proteases, thus rendering the virus infectious.[59] Extrapulmonary dissemination of virus has been uncommonly documented in humans, but systemic spread is a regular feature of highly pathogenic avian viruses in chickens and sometimes in rodents or other mammalian hosts. Serum and secretory antibodies directed to HA and NA appear 10 days post infection and correlate with durable protection against reinfection by homologous strain. Vaccine-induced protection may last for up to 2–3 years against homotypic virus. Infection also induces cell-mediated immunity detectable 3–6 days later, which seems to be important for recovery.[36] Cytotoxic T-lymphocyte response against internal proteins may provide some degree of heterosubtypic immunity.

Seasonal influenza A(H1N1) replicates mainly in upper airways, whereas 2009 influenza A(H1N1) replicates in both upper and lower respiratory tracts.[60] Postmortem findings in patients with pandemic A(H1N1) were similar to those with A(H5N1) infection, including diffuse alveolar damage, hemophagocytosis, lymphoid atrophy, and elevated levels of inflammatory markers such as IL-6 and MCP-1 in lung parenchyma. Significant correlation was found between disease severity and levels of proinflammatory cytokines, such as IL-6, IL-10, and IL-15.[60]

DIAGNOSIS

During seasonal outbreaks, the diagnosis of influenza is frequently suspected on clinical grounds. A higher index of suspicion, and laboratory diagnostics are needed in sporadic individual cases or outbreaks of febrile respiratory illness. Viral isolation from respiratory specimens can be done in several cell lines (e.g., PRMK, MDCK, LLC-MK2), with confirmation by hemadsorption or immunofluorescence. Rapid detection of conserved influenza antigens (M or NP) in clinical samples can be done by one of several techniques (e.g., IF, EIA), and multiple point-of-care kits are commercially available with turnaround times of 15–30 minutes. The sensitivities of these assays are higher in children (up to 90%) than in adults (generally 50–70%), and depend on the duration of illness and sample type.[36]

Several formats of reverse transcription–polymerase chain reaction (RT-PCR) assays have been used for the detection of influenza A and B RNAs in clinical samples, with the advantage of detecting genomes of noninfectious virus.[36] Real-time RT-PCR has enabled the development of assays that provide rapid quantitative detection of influenza A and B with high sensitivity.[61–63] These assays have great potential to replace other methods, because they are simultaneously rapid, highly sensitive, quantitative, and amenable to being used in multiplex format, which might include probes for several different respiratory pathogens.[62,63] For the 2009 A/H1N1, real-time and conventional RT-PCR assays were the diagnostic assays of choice.[64] Serologic diagnosis of influenza using paired acute and convalescent serum can be done retrospectively by a variety of techniques, but mainly for serologic survey purposes.[36]

TREATMENT AND PROGNOSIS

There are two classes of antiviral drugs currently licensed for human use: M2 ion channel inhibitors known as adamantanes (amantadine and rimantadine) and neuraminidase (NA) inhibitors (oseltamivir and zanamivir). The adamantanes inhibit influenza A virus replication at the uncoating step.[36] In uncomplicated influenza A in adults without underlying diseases, treatment with either drug can reduce the duration of influenza illness by approximately 1–2 days if started early after the onset of symptoms. Amantadine is excreted in an unchanged state in the urine, while rimantadine is extensively metabolized after absorption and less than 10% of the dose is excreted unchanged in the urine. Elderly persons need only half the dose to achieve similar plasma levels. Amantadine or rimantadine may cause gastrointestinal upset and central nervous system (CNS) side effects. CNS intolerance is more common with amantadine and, when severe, can be manifested as agitation, psychosis, seizures, and coma. Mild complaints include insomnia, dizziness, anxiety, dry mouth, anorexia, and nausea and are reversible upon discontinuation. Amantadine and rimantadine are marketed as 100 mg tablets and 10 mg/mL syrup. The recommended dose is 100 mg twice daily for adults <65 years of age (100 mg per day for patients ≥65 years). For children under 10, a rimantadine dose of 5 mg/kg per day (maximum, 150 mg per day) has been suggested.[37] Dose reductions proportional to the creatinine clearance (ClCr) are suggested for patients with renal insufficiency (amantadine for ClCr less than 60–80 mL/min/1.73 m^2; rimantadine for ClCr less than 10–20 mL/min/1.73 m^2). Resistance of seasonal influenza A viruses to adamantanes, due to single nucleotide mutation in the M2 gene, is widespread,[65–67] leading the CDC to recommend the suspension of their use in the United States in 2006.

The neuraminidase inhibitors (NAIs), zanamivir and oseltamivir, inhibit both influenza A and B viruses by blocking the viral neuraminidase active site and inhibiting cleavage of sialic acid and virus release from infected cells.[68] In adults and children older than 5 years, inhaled zanamivir (10 mg twice daily for 5 days) provides 1- to 2.5-day reduction in illness[69] and reduces antibiotic use for lower respiratory complications by 40%. Zanamivir is generally well tolerated, but may rarely induce bronchospasm, particularly in those with influenza and preexisting airways disease.[37] Oral oseltamivir treatment dosage for adults and children older than 12 months and weigh more than 40 kg is 75 mg twice a day for 5 days, but may need an extended period if the patient is immunocompromised. Oseltamivir dosing is reduced to 60 mg twice a day for children weighing 23–40 kg; for 15–23 kg, dosing is 45 mg twice a day; if <15 kg, then 30 mg twice a day. For infants 3–12 months of age, the recommended oseltamivir dose is 3 mg/kg twice a day. For infants <3 months, data are limited in this age group, but a 3 mg/kg dose twice a day would be recommended in the severely ill. The goal of early implementation of antiviral treatment is to reduce illness severity, shorten time to resumption of daily activities by 1–3 days, and reduce complications leading to antibiotic prescription and hospitalizations by 50% in adults. In children 1–12 years of age, oseltamivir reduces the frequency of otitis media and, consequently, antibiotic prescriptions. Side effects include mild nausea or emesis. Dosage of NAIs does not need to be adjusted for the elderly;[36] however, a dosage reduction is required for patients with creatinine clearance <30.

In the first few years after introduction of NAIs (1999–2001) rates of resistance were low, observed in <0.5% of circulating influenza viruses A/H1N1 and A/H3N2. During the 2007–2008 influenza season, unexpectedly high rates of primary resistance to oseltamivir were detected predominantly in Europe, associated with H274Y mutation in the NA gene. Oseltamivir-resistant viruses remain susceptible to zanamivir and adamantanes.[70–73] For 2008–2009 seasonal influenza A(H1N1), treatment recommendations should include zanamivir or combination treatments for those at high risk for sequelae of influenza.[74] In 2009, the CDC recommended that zanamivir, or a combination of oseltamivir and rimantadine, are more appropriate options than oseltamivir or amantadine alone for the treatment of seasonal influenza A(H1N1).[75] The majority of the 2009 A(H1N1) pandemic viruses were susceptible to NAI; but there are sporadic cases of oseltamivir resistance, 80% of them in viruses from patients with prior exposure to oseltamivir therapy or prophylaxis.[34] Recommendations for the clinical management of 2009 H1N1 infections have been proposed by the WHO.[76] In addition to H274Y mutation, a mutation at amino acid 136 of the influenza virus A(H1N1) NA protein has been linked to reduced susceptibility to zanamivir.[77] Rapidly emerging influenza viruses resistant to NAI highlight the need for development of novel antiviral agents for the treatment and prevention of influenza

virus infections. Peramivir, a new NAI for parenteral use, is currently undergoing clinical trials with promising results.[78] Novel therapeutic approaches currently under study are T-705, a selective inhibitor of influenza virus RNA-dependent RNA polymerase,[79] and DAS181, a sialidase fusion protein.[80,81]

Antipyretic-analgesic drugs may be used for influenza-induced fever and aches, but aspirin should be avoided, because of its association with Reye's syndrome.

PREVENTION AND CONTROL

Immunization with formalin-inactivated or live-attenuated multivalent vaccines and chemoprophylaxis are methods available for preventing influenza. Inactivated influenza vaccine is used prior to the influenza season and currently includes one strain of influenza B, and two strains of influenza A viruses, subtypes H3N2 and H1N1, chosen by the WHO among the viruses most likely to circulate in the next influenza season.[82] It has 70–90% efficacy in preventing illness in healthy children and adults, and reduces hospitalizations and mortality in elderly and high-risk patients.[82] The CDC recommendations applied to the United States have been expanded to include not only elderly and high-risk patients but also all children, and now basically recommend universal immunization. During the influenza season when patients often seek medical care, another important group to immunize is health care workers. There is an increasing movement towards mandatory influenza vaccination amongst health care workers, or at least prioritizing those with the most patient contacts in cases of vaccine shortages.[36,82] The inactivated vaccine, administered as a single intramuscular (IM) dose shortly before influenza season (two doses in previously unimmunized children <9 years), is safe during pregnancy but should be avoided in persons with a history of anaphylactic reactions to eggs.[82] Vaccine has a favorable safety profile with 77–91% efficacy in 1- to 15-year-old children. Inactivated vaccine is not currently recommended for children younger than 6 months since the vaccine efficacy is lower in infants; however, vaccination of household contacts and caregivers reduces the risk of these high-risk children contracting influenza. Healthy people aged 2–49 years who are not contacts of immunosuppressed patients can receive either inactivated or intranasal live-attenuated vaccines.[82]

The composition of the inactivated influenza vaccine given in tropical countries is based on viruses that circulate in the southern hemisphere and is given prior to the influenza peak season, which for most countries in the South is between May and July.[83] In South America, annual vaccination of the elderly has reduced hospitalizations and mortality for respiratory diseases.[84]

Live-attenuated, cold-adapted vaccines administered intranasally are well tolerated, genetically stable, rarely transmissible, and have the advantage of inducing local secretory IgA responses.[36] This vaccine was licensed in the United States in 2003, as an option for healthy persons aged 5–49 years, including those in close contact with groups at high risk and those wanting to avoid influenza.[82] This vaccine is not recommended for persons with asthma and other chronic disorders of the pulmonary or cardiovascular systems; persons with underlying medical conditions, including diabetes, renal dysfunction, and hemoglobinopathies; or persons with known or suspected immunodeficiencies or who are on immunosuppressive therapies; children or adolescents receiving aspirin or other salicylates; persons with a history of Guillain–Barré syndrome; pregnant women; and persons with a history of hypersensitivity to eggs.[82] The inactivated vaccine has been more efficient than the live attenuated vaccine in preventing influenza in healthy adults, but appears less effective in young children.[85]

A promising live attenuated influenza vaccine lacking the NS1 protein was shown to be effective in animal models, and induced significant levels of lineage-specific and cross-reactive neutralizing antibodies in healthy volunteers.[86] Other investigational approaches have been explored in influenza vaccine development, including recombinant HA produced in insect cells, virosomes incorporating surface glycoproteins, M2 protein conjugated with hepatitis B virus core, and naked DNA encoding influenza virus nucleoprotein or HA.[36] Cell culture-based vaccines (MDCK, Vero) have been approved in Europe and may offer an alternative to the limitations of the current egg-grown vaccines.

Chemoprophylaxis with antivirals should be considered for non-vaccinated elderly people, immunodeficient patients, patients in chronic care institutions during influenza outbreaks, people with contraindications for vaccination, and those who received a vaccine strain different from the one causing the outbreak. Approved prophylaxis dose is once daily (i.e., one half of treatment dose) for oseltamivir or zanamivir which should be started as early as possible and continued for 7–10 days for post-exposure prophylaxis.[76] However, reports of high rates of influenza resistance to antiviral drugs should prompt more rigorous judgment in the decision to use antiviral prophylaxis. Antiviral agents, especially the neuraminidase inhibitors, could significantly help in the control of a future pandemic of influenza, by reducing lower respiratory complications, hospitalizations, and person-to-person transmission. Availability of antivirals also pose a limitation in resource-constraint settings.[87] Therefore, policies to ensure a stockpile of these drugs, as well as directions to optimize their use, are important issues to be considered.[32]

More emphasis should also be placed upon non-pharmaceutical interventions such as handwashing and use of facemask to curtail the spread of influenza. These measures appear to reduce household transmission of influenza virus as long as they are implemented within 36 hours of the onset of symptoms in the index case.[88]

HUMAN RESPIRATORY SYNCYTIAL VIRUS

Human respiratory syncytial virus (HRSV) is the single most important viral etiology of LRI and a major cause of morbidity and mortality in children worldwide. HRSV is the leading cause of hospitalization in young children[77] and has been the most frequently detected virus in hospital-based ARI studies of children in tropical areas.[7]

THE AGENT

HRSV, the only known human pathogen of the genus *Pneumovirus* in the family *Paramyxoviridae*, is a negative-strand RNA virus with helical nucleocapsid and a lipid-containing envelope. The 15.2 kb HRSV genome encodes 11 distinct viral proteins, and the antigenic differences in the G, F, and SH envelope proteins permit classification of HRSV into groups A and B, each with subgroups.[89] The G glycoprotein interacts with host cell receptors and mediates adsorption to the cell surface,[90] resulting in fusion of virus and host cell membranes mediated by the F glycoprotein.[89] The expression of F protein on the cell surface results in fusions of adjacent cells forming syncytia, the hallmark of paramyxovirus cytopathic effect.[89]

HRSV is sensitive to ether, chloroform, detergents, and pH less than 5, is inactivated at 55°C, survives poorly on porous surfaces, and loses infectivity by slow freezing and storage at temperatures above 4°C.[89]

EPIDEMIOLOGY

HRSV occurs worldwide, causing annual outbreaks in temperate climates in the winter and early spring, with sporadic cases throughout the year.[90] In tropical regions, where temperature fluctuates less, HRSV outbreaks occur in the rainy seasons, as in Malaysia, Hong Kong, India, Papua New Guinea, Colombia, Kenya, and The Gambia.[27] In southeast Brazil, HRSV occurs from February through July, after the rainy season, when temperatures tend to be cooler.[91] In regions with colder winter temperatures, such as Sao Paulo city and the southernmost parts of Brazil, as well as in Argentina and Chile, HRSV peak activity tends to occur in July–August.[92–96] The duration of HRSV season is longer in overcrowded areas with larger families.[97]

HRSV from both groups A and B may co-circulate during a seasonal peak.[98] A recent long-term study in Brazil[99] showed that several amino

acid residues in immunodominant epitopes of HRSV G protein undergo back and forth positive selection between seasons, generating a "flip-flop" variation pattern. Changes in herd immunity may select mutants within a limited repertoire of functionally viable HRSV variants. A new variant of HRSV B with a conspicuous 30 amino acid duplication in the G protein has become the dominant genotype in several regions, including Argentina, Brazil, and India.[99–101] HRSV co-circulates with other respiratory viruses and sensitive molecular methods show that coinfections of HRSV with other viruses are common,[102] without evidence of increased severity of the HRSV disease.[103]

Most children develop anti-HRSV antibody by age 2, although reinfections are common throughout life.[104] HRSV transmission occurs by large-particle aerosols or by contamination of hands and subsequent inoculation into the eyes or nose. Transmission to siblings and adults occurs efficiently in household settings. It is estimated that 30% of all infants will have HRSV infection requiring medical attention and up to 2% of them will be hospitalized.[77] An estimated 10% of all children will have bronchiolitis in their first year of life, and 60–90% of those are caused by HRSV.[105]

In southeast Brazil HRSV is responsible for up to 85% of child hospitalizations for LRI during HRSV peak months.[96]

CLINICAL FEATURES

After an incubation period of 3–7 days,[106] HRSV symptoms start, usually with mild upper respiratory infection (URI),[107] but may progress to severe LRI, including pneumonia, bronchiolitis, tracheobronchitis, and croup. Most commonly bronchiolitis follows URI symptoms, with tachypnea, dyspnea, cough, expiratory wheezing, air trapping and, in more severe cases, intercostal muscle retractions and cyanosis. Half of the patients have fever, and chest X-ray may show lung hyperaeration and segmented atelectasis.[107] Blood counts usually show lymphocytosis and increased neutrophils suggest bacterial superinfection, the most common being acute otitis media.[108] HRSV RNA can be detected in up to 75% of middle ear effusions in children with HRSV infection and acute otitis media.[108] While serious bacterial infections are rare in previously healthy infants with HRSV in developed regions,[109] such infections are more likely to occur in tropical areas among children previously debilitated by other diseases and malnutrition.

Those at risk for severe and fatal HRSV infections include: premature infants, infants with congenital heart disease or underlying pulmonary conditions such as cystic fibrosis or bronchopulmonary dysplasia, as well as immunocompromised hosts of any age. HIV-infected children with HRSV infections have a higher rate of pneumonia, prolonged illness and virus shedding, but no increase in severity of the HRSV disease.[107] Differential diagnosis of acute bronchiolitis includes asthma, pneumonia, congenital heart and lung diseases, and cystic fibrosis. The most frequent HRSV illness in children over 3 years of age and adults is characterized by symptoms of URI often with low-grade fever. Exacerbations of chronic pulmonary diseases and wheezing episodes can also be seen in adults.[110] Neurological complications including seizures and encephalopathy occur rarely in severe HRSV infections requiring intensive care.[111] HRSV, as well as influenza and parainfluenza, also contributes to wheezing and asthma exacerbations in infants.[112–115] HRSV has also been increasingly recognized in LRI in the elderly, mainly interstitial pneumonia, with prolonged cough and dyspnea in patients with chronic pulmonary conditions.[116]

PATHOGENESIS AND IMMUNITY

HRSV replicates in respiratory epithelium, reaching titers of 10^6 $TCID_{50}$ (tissue culture infectious dose for 50% of the test units per milliliter) per mL in nasal secretions of infected babies, with virus shedding as prolonged as 3 weeks after symptoms disappear.[89] HRSV spreading from cell to cell may involve the entire respiratory tree, reaching bronchioles 1–3 days after the onset of rhinorrhea. Replication in the bronchiolar epithelium causes necrosis of ciliated cells, syncytia formation, peribronchiolar inflammation with abundant lymphocytes and macrophages, impairment of secretion clearance, small airway obstruction and lung hyperaeration.[89] HRSV nonstructural proteins NS1 and NS2 counteract production of type I interferon (IFN), contributing to pathogenesis.[117] HRSV may also modulate surfactant expression in human pulmonary epithelial cells, which can contribute for disease severity.[118] Pneumonia frequently coexists with bronchiolitis, evidenced by interstitial mononuclear infiltrate, eosinophilic cytoplasmic inclusions in epithelial cells, and multinucleated giant cells. The innate immune response to HRSV is triggered by recognition via Toll-like receptor (TLR)4, TLR3, TLR2 and RIG-I, resulting in expression of proinflammatory cytokines and chemokines.[119] HRSV infects dendritic cells (DCs), causing them to lose their ability to stimulate HRSV-specific T cells.[120] B-lymphocyte-stimulating factors derived from infected DC and epithelial cells are determinants of the mucosal antibody response and disease progression.[121]

Immunity to HRSV is incomplete and short-lived, but reinfections tend to be less severe. Local secretory IgA correlates better with protection than does serum antibody level and age. Cell-mediated immune response is important for recovery from HRSV infection, and patients with suppressed cell-mediated immune response are at risk of severe pulmonary disease and fatal outcome.[89,107]

The type of immune response to the virus is probably a major factor in the development of wheezing. A bias towards a Th2 cytokine profile seems to be associated with more severe disease, whereas a Th1 profile leads to effective viral clearance and milder illness. It has been suggested that HRSV bronchiolitis at early age may predispose to wheezing or asthma later in life.[122]

DIAGNOSIS

HRSV isolation from respiratory samples is usually done in cultures of HEp-2 cells, in which HRSV induces syncytia in 3–5 days. Various assays for HRSV antigen detection directly in respiratory secretion are available, some requiring equipment such as immunofluorescence microscope or EIA readers, and others requiring no equipment, such as the membrane-based immunochromatographic assays ideal for field studies.[123] Such rapid tests may have very low sensitivity for HRSV detection in samples from adult patients.

Detection of HRSV RNA by conventional or real-time RT-PCR is becoming widely available, with the added convenience of being quantitative and amenable for simultaneous detection and subtyping of HRSV directly in clinical specimens.[124] Recently, an ultrasound-based RNA extraction method combined with a multicomponent nucleic acid enzyme amplification procedure was developed to detect HRSV in difficult respiratory samples, as noted in elderly respiratory samples which often bear low viral loads.[125] HRSV serology has limited value for case management, but may be useful in epidemiologic surveys.[107]

TREATMENT AND PROGNOSIS

URI caused by HRSV requires no specific treatment and the use of antibiotics is recommended only in the presence of bacterial otitis media or sinusitis.[107] The supportive treatment of infants with HRSV bronchiolitis consists in preventing hypoxemia and electrolyte imbalance, in addition to aerosolized bronchodilators. Chest X-ray should be recommended only for severely ill or deteriorating infants.[105] To prevent hypoxemia, requirements may vary from simple removal of respiratory secretions and proper positioning of the infant, to mechanical respiratory assistance and even extracorporeal membrane oxygenation (ECMO). Pulse oximetry has been advocated to assess oxygen needs, but in tropical developing areas oximeters may not be available and serial clinical assessment is essential to monitor disease progression. For this purpose, crackles and cyanosis seem to correlate better with hypoxemia than tachypnea and intercostal retraction.[105] Correction of hypoxemia can be accomplished with 40% or lower oxygen concentrations.[107] Oxygen should be humidified with saline and delivered by mask if head boxes or tents are

unavailable. The role of corticosteroids remains unclear, with a variety of evidence showing they are not beneficial.[105]

Ribavirin, delivered by small-particle aerosol via a mist tent, mask, oxygen hood, or ventilator, is recommended only for infants and young children with an underlying condition, such as congenital heart disease, cystic fibrosis, or immunosuppression. Premature infants, infants younger than 6 weeks of age, and those severely ill may also be considered for therapy.[126] This treatment requires a specific nebulization device and generates potential exposure of health care workers to a teratogenic agent. Humanized monoclonal antibodies to HRSV, which are beneficial for prophylaxis, have shown no benefit in treatment of HRSV infections.[107] Recently, the use of small interfering RNAs (siRNAs) has become a promising strategy to treat HRSV infections. One such siRNA to the nucleocapsid protein had anti-HRSV activity both *in vitro* and *in vivo*[127] and an inhaled preparation is undergoing clinical testing.[128]

PREVENTION AND CONTROL

Disease enhancement caused by a formalin-inactivated HRSV vaccine in the 1960s and results of more recent unsuccessful trials of live attenuated vaccines have significantly slowed progress toward an HRSV vaccine.[2] However, there is recent evidence that the lack of protection of the formalin-inactivated vaccine of the 1960s was due to reduced antibody affinity resulting from poor stimulation of TLR. This suggests that the efficacy and safety of inactivated HRSV vaccine may be improved by inclusion of TLR agonists in the formulation.[129] An HRSV-A subunit vaccine containing F, G, and M proteins was tested in elderly patients and elicited potentially protective neutralizing antibody.[130]

Passive immunization of high-risk infants with monthly doses of HRSV humanized monoclonal antibodies during the HRSV season reduces incidence and severity of infections in high-risk children.[131] Although the commercially available preparations palivizumab and motavizumab are much too expensive to be routinely used in underprivileged tropical regions, their judicious use for high-risk premature babies, children with congenital heart disease, and children less than 2 years of age with bronchopulmonary dysplasia, may result in net cost savings for some health care systems.[132]

Hospitalized infants with HRSV should be isolated or grouped together to prevent cross-infection. Handwashing, use of eye–nose goggles, gowns, and gloves, and decontamination of surfaces and fomites are additional nosocomial infection control measures.[107]

HUMAN PARAINFLUENZA VIRUSES

Human parainfluenza viruses (HPIVs) are leading causes of croup in infants and children worldwide, and are among the most frequent causes of LRI in infants.[133,134] HPIVs can be detected in up to 13% of children in hospital-based LRI studies in developing countries.[37,135]

THE AGENT

The HPIVs are distributed in two genera of the family *Paramyxoviridae*, sharing structural and biological characteristics of HRSV. HPIVs are classified antigenically into types 1 to 4, with HPIV-4 subdivided into subtypes A and B. HPIV types 1 and 3 are classified in the genus *Respirovirus*, while HPIV types 2 and 4 are in the genus *Rubulavirus*. HPIV-1 and -3 are the types most frequently associated with LRI in children, the immunocompromised, the chronically ill and the elderly, whereas HPIV-4 causes mostly URI both in children and adults.[133]

Binding of HPIV to sialic acid in the cell membrane is mediated by the viral glycoprotein HN, which contains both hemagglutinin and neuraminidase activities. Fusion of viral and cell membranes is mediated by the viral F protein, which is cleaved by cellular proteolytic enzymes.[136] Once inside the cell, the replication cycle is similar to that of HRSV. HPIV budding is finalized when the neuraminidase function of the HN protein cleaves sialic acid, permitting the release of virions from the cell.[137]

EPIDEMIOLOGY

Primary HPIV infection occurs early in childhood, and by age 5 virtually all children are seropositive for one or more HPIVs.[133] Up to one-third of all viral LRIs in children in the United States are caused by HPIV-1 and HPIV-3.[133,138] In tropical areas HPIVs may account for up to 15% of hospital admissions of children due to LRI.[37] In temperate regions HPIV-1 and HPIV-2 cause epidemics in the fall of alternate years, either in co-circulation or alternating with one another. The biennial pattern of HPIV-1 circulation has been found in both hemispheres.[133] HPIV-1 causes most croup outbreaks, whereas HPIV-2 more frequently causes mild illness, although it also causes croup.[133] HPIV-3 occurs endemically throughout the year, with sporadic spring outbreaks mainly among infants, and HPIV-4 occurs sporadically throughout the year in children and adults.[133] In an emergency room study in Fortaleza, northeast Brazil, HPIVs were detected in 4% of children with ARI and HPIV-3 was the most frequently detected and occurred seasonally, with most cases observed from September to November, in inverse relationship to the rainy season.[139] However, community-based ARI studies in children under 5 years of age have shown higher HPIV activity during rainy seasons in tropical countries.[3,10]

HPIVs spread mainly within families and closed communities, such as nurseries, daycare centers and pediatric wards, with high secondary attack rates. In a longitudinal ARI study conducted in children less than 2 years of age in daycare for low-income families in northeast Brazil, HPIVs represented 11% of the viruses detected.[140]

HPIVs are transmitted mainly by large droplets and fomites[107] and virus shedding lasts 3–10 days, but HPIV shedding for months has been reported in very young children and immunosuppressed hosts.[141]

CLINICAL FEATURES

Primary HPIV infection may cause rhinitis, pharyngitis, laryngotracheobronchitis (croup), bronchiolitis, and pneumonia.[133] Approximately two-thirds of HPIV infections in children result in febrile URI, frequently associated with otitis media, and the remaining one-third are cases of croup, bronchiolitis, and pneumonia.[133] HPIVs, principally types 1 and 2, cause up to 74% of all cases of croup,[133] mainly between 6 and 36 months of age.[142] Croup is manifested by inspiratory stridor, barking cough, and hoarseness caused by subglottic edema, preceded by rhinorrhea, mild cough, and low-grade fever.[142] Most children recover in 2–5 days, but some may develop bronchiolitis and pneumonia.[142]

Immunity to HPIV is incomplete and infections occur throughout life, but adults present only nonspecific URI, commonly with hoarseness.[107] HPIVs are detected in 5.8% of cases of influenza-like illness (ILI) in the United States[143] and in 3.2% in Peru.[144]

HPIVs can cause severe disease in immunocompromised hosts, especially children with severe combined immunodeficiency and recipients of bone marrow transplants, reaching mortality rates of 10–20%.[107,145]

PATHOGENESIS AND IMMUNITY

HPIVs cause epithelial cytolysis, spreading from the upper respiratory tract down the respiratory tree. Similar to influenza, the extent of HPIV infection depends on viral F protein cleavage by tissue proteases.[107] Larynx and trachea are mostly involved in the croup syndrome, and extensive involvement of the lower respiratory tree may be present in tracheobronchitis, bronchopneumonia, and bronchiolitis.[133,141,142]

Host immunity is largely directed to the two surface proteins HN and F, and secretory antibody to the HN glycoprotein is the best marker of protection,[141] but such protection is limited and repeated infections occur. T-cell immune response is involved in both the

clearance of virus and inflammation, with edema and excess mucus secretion.[133]

In the lungs HPIVs cause mononuclear interstitial infiltrate, epithelial necrosis, alveolar exudate, and hyaline membrane formation.[107]

DIAGNOSIS

HPIV can be recovered from respiratory secretions up to 8 days from the onset of symptoms in several continuous cell lines and virus isolation can be confirmed by immunofluorescence.[133,141] Immunofluorescence done directly on exfoliated respiratory epithelial cells has produced disappointing results due to low sensitivity.[133] Detection of HPIV RNA by real-time RT-PCR is quite sensitive and has become a standard diagnostic method.[146,147]

TREATMENT

At present, only supportive and symptomatic treatment is available for HPIV infections. Management of croup includes supplemental oxygen and racemic epinephrine nebulization in hospitalized patients. Mist therapy, although traditional, has no proven value.[142] Short-term, high-dose systemic corticosteroids may reduce the need for intubation, and nebulized budesonide has a rapid effect and is as safe and efficacious as nebulized epinephrine in moderately severe croup.[142] There is no approved antiviral treatment for HPIV infections, but compounds designed to bind and inhibit the functions of HN protein have been effective *in vitro* and in an experimental animal model.[148,149]

PREVENTION AND CONTROL

No interventions are licensed for the prevention of HPIV infections. Recently, a live attenuated cold-adapted HPIV-3 vaccine was found to be safe and immunogenic in children, with a seroconversion rate of 79%.[150] More recently, a Sendai virus vaccine, with extensive homology with HPIV-1, was found to be naturally attenuated in humans and induced high-titer specific neutralizing antibodies.[151]

HUMAN METAPNEUMOVIRUS

Human metapneumovirus was initially detected in children with ARI in the Netherlands in 2001. The agent is a paramyxovirus of the subfamily *Pneumovirinae*, closely related to avian pneumovirus of the genus *Metapneumovirus*, and is now recognized by serological studies as having circulated for at least five decades.[140,141]

THE AGENT

HMPV is enveloped, pleomorphic, with negative-sense single-stranded RNA contained in a helicoidal nucleocapsid, surrounded by an envelope with glycoproteins that mediate attachment (G), and fusion (F).[152,153] HMPV glycoprotein (F) binds to $\alpha v\beta 1$ integrins on the cell surface to mediate cell entry.[154]

HMPV isolates cluster into two main subgroups, named A and B, and based on the sequence of the F and G genes the two subgroups can be subdivided into two genetic sub-lineages named A1, A2 and B1, B2;[153,155] the A2 sublineage is further divided into two sub-clusters, A2a and A2b.[156]

EPIDEMIOLOGY

HMPV is globally distributed and a frequent cause of ARI in all continents. At the age of 5 virtually all children have become seropositive for the agent.[157–159] HMPV infections are more frequent in the colder months in temperate regions and different strains of both subgroups A and B co-circulate during the same year.[160] However, little is known of HMPV seasonality in tropical countries. In South Africa, HMPV peak activity occurs in the winter season.[161]

Rates of HMPV detection in respiratory samples from ARI patients in tropical countries are quite variable. Hospital-based ARI studies in children have recorded frequencies of HMPV of 7.4% in South Africa,[162] 5.4% in Thailand,[163] 7% in Yemen,[164] and 15.7% in South Korea.[165] In northeast Brazil, HMPV was detected alone or simultaneously with HRSV in 24% of children younger than 3 years of age, in April and May 2002,[157] whereas in the following year it was not detected by the same method.[166] In contrast, in southeast Brazil, HMPV was detected in 5.6% of infants hospitalized for ARI.[167] A 4-year study also done in southeast Brazil found an average frequency of 11.4% in children with ARI, with the two HMPV subgroups co-circulating, with peak frequency in winter or spring.[168]

CLINICAL FEATURES

Clinically, HMPV infections resemble those caused by HRSV, ranging from mild upper ARI to severe bronchiolitis and pneumonia. The median age of children hospitalized for HMPV is higher than that of those with HRSV. The most frequent symptoms are fever, dyspnea, cough, wheezing/stridor, rhinitis, and sore throat.[160,169] All infected children in one study had pneumonia or bronchiolitis, frequently accompanied by otitis media.[160,170,171] HMPV may cause more serious infections in immunocompromised patients, those with previous conditions, the very young and the elderly.[160] HMPV infection in adults may present as influenza-like illness, acute bronchitis, or URI.[160]

HMPV has been increasingly recognized as cause of acute wheezing in children. A study from Finland found HMPV in 8% of wheezing children; in addition, a significantly higher level of IL-8 was found in nasal secretions of those cases.[172] In Brazil, 47% of children with HMPV had wheezing and 31% had chest indrawing.[157] Previous history of asthma has been more frequently associated with HMPV than with HRSV infection; HMPV-infected patients are more often treated with bronchodilators and corticosteroids than are HRVS-infected patients.[169] Remarkably, HMPV was detected in 8.3% of children younger than 5 years with community-acquired alveolar pneumonia in Israel.[173]

PATHOGENESIS AND IMMUNITY

HMPV infection occurs at young age, but reinfections remain common at later ages.[152] Knowledge about HMPV pathogenesis is still limited, but it is clear that it infects both upper and lower respiratory tracts[160] and both subgroups A and B are equally pathogenic.[174] Experimental infections in animal models indicate that peak viral shedding occurs from 2 to 8 days following infection.[153] In humans, HMPV infections are associated with increased levels of IL-8, and reduced levels of inflammatory cytokines IL-12, TNF-α, IL-6 and IL-1β in the upper respiratory tract, as compared with HRSV infections.[175,176]

Interestingly, in experimentally infected animals HMPV replicates in respiratory epithelial cells, and migrates to neuronal processes in the lungs, where it may persist when infection of epithelial cells is no longer detectable. Whether this happens in humans and may contribute to later wheezing is not known.[177]

A common finding in epidemiological studies is the coinfection with HMPV and HRSV and some studies reported that this correlates with increased severity of HRSV disease.[178,179]

DIAGNOSIS

HMPV can be isolated from respiratory samples in LLC-MK2 cells, with late cytopathic effect characterized by syncytia formation or cell

rounding and detachment.[160] Sensitive RT-PCR assays for HMPV have rapidly become standard for diagnosis.[180] A real-time RT-PCR assay for HMPV was more sensitive than conventional RT-PCR[180] and other non-molecular methods, such as direct fluorescence assay.[181] A rapid immunochromatography assay for HMPV antigen has been developed which can be completed in approximately 15 minutes and does not require equipment.[182]

TREATMENT, PREVENTION, AND CONTROL

Other than supportive measures, such as oxygen therapy, bronchodilators, corticosteroids, and mechanical ventilation, there is no specific antiviral treatment for this agent.[169] Ribavirin is inhibitory for HMPV *in vitro*[183] and there have been anecdotal reports of its successful use in association with immunoglobulin for HMPV pneumonia in immunocompromised patients.[184] Although an HMPV vaccine is not available, promising preclinical studies have been done with a live recombinant human parainfluenza virus expressing HMPV F protein,[185] and with a chimeric bovine/human parainfluenza virus 3 expressing HMPV F protein.[186] Other approaches successfully tested in animal models were soluble recombinant F protein, DNA vaccine, and live attenuated vaccines.[187] Humanized neutralizing monoclonal antibody to F protein is active in experimentally infected animals.[188,189]

HUMAN RHINOVIRUSES

Human rhinoviruses (HRVs) are the most frequent respiratory pathogens of humans.[190] They were the most frequently isolated viruses in children under 5 years old with ARI in an urban slum in tropical northeast Brazil.[3]

THE AGENT

HRVs are small, nonenveloped, positive-strand RNA viruses in the genus *Enterovirus*, family *Picornaviridae*,[191] with over 100 identified serotypes.[187] HRVs comprise three different species named *Human rhinovirus A* (with 75 serotypes), *B* (25 serotypes), and the recently described group *C*.[192,193]

HRV serotypes are also classified by receptor-specificity into two groups: the major group includes 90% of the serotypes, whose receptor is intercellular adhesion molecule-1 (ICAM-1), and the minor group contains the remaining serotypes, whose receptor is the low-density lipoprotein receptor (LDLR). HRVs are acid-labile, which distinguishes them from other enteroviruses.[190] Genomic analysis of HRVs indicates that a large number of genotypes co-circulate in a short period, causing a high frequency of reinfections. Recombinations between serotypes may be a driving force in rhinovirus evolution.[194]

The HRV genome is a positive single-stranded RNA packed in an icosahedral capsid. Receptor binding destabilizes the capsid, triggering internalization and exposure of the viral genome to the cytoplasm, where the whole replicative cycle takes place, leading to production of mature virions released by cell lysis.[190]

HRVs are stable for days on environmental surfaces, are resistant to ethanol, ether, chloroform, and nonionic detergents, but are sensitive to UV light, pH <5, halogens such as chlorine, bromine, and iodine, and phenolic disinfectants.[190]

EPIDEMIOLOGY

HRV infections occur in people from all continents, including remotely located populations, such as Bushmen from the Kalahari Desert, native Alaskans, and isolated Amazon Indian tribes.[195] HRV causes up to 80% of all colds in adults in temperate climates[196] but very few community-based studies of ARI have included HRV detection methods in the tropics.[3] HRV is frequently associated with ARI in children in tropical Brazil, where it was isolated in 46% of samples from children under 5 with ARI in Fortaleza, and detected by RT-PCR in 52% of toddlers with ARI in a daycare center for the underprivileged in Salvador.[3,140] In southeast Brazil, HRV represented 36% of the respiratory viruses in adults with ARI in Sao Paulo, with seasonality similar to that of influenza virus.[197] Health care facility-based studies of young children with ARI have detected HRV in 30% in Thailand,[198] and 35.4% in Hong Kong,[199] highlighting its importance as a frequent cause of ARI worldwide.

HRV transmission requires close exposure, occurs mainly by hand contact with self-inoculation into the eyes or nostrils, but also by droplet spread, and children play an important role spreading HRV in households.[200] Once HRV reaches the nasal cavity, infection occurs in virtually all exposed susceptible subjects, with 75–80% of the infected developing illness after a 1–4-day incubation.[106]

CLINICAL FEATURES

HRV is responsible for the majority of common colds, which are clinically indistinguishable from colds of other viral causes, consisting of nasal discharge, nasal obstruction, sneezing, sore or scratchy throat, hoarseness, cough, headache, and uncommonly feverishness and malaise. The symptoms last for approximately 7 days, but may persist for up to 2 weeks in 25% of cases. Infants and toddlers may display only nasal discharge, being otherwise asymptomatic.[190] The use of the RT-PCR method has revealed that approximately 20% of HRV infections are asymptomatic.[201]

Most patients with HRV have obstruction and mucosal abnormalities of the sinus cavities, eustachian tubes, and middle ear, which predispose to secondary bacterial sinusitis and otitis media, complications found in approximately 2% of all colds.[202] HRV RNA may be detected in maxillary sinus brushings in 40% of adults presenting with acute sinusitis[203] and in 25% of middle ear fluid samples from children with acute otitis media.[204,205]

HRV infections frequently trigger exacerbations of chronic obstructive pulmonary disease and asthma.[114,206] In adults, HRV is associated with 60–70% of asthma exacerbations.[207] Furthermore, it was recently shown that the HRV species C is more closely related to lower respiratory tract infections, causing febrile wheezing and asthmatic exacerbation in children.[192,193]

PATHOGENESIS AND IMMUNITY

HRV infects only higher primates, causing illness only in humans. It replicates mainly in ciliated cells of the nose and nonciliated cells of the nasopharynx.[208] HRV infection induces little tissue damage, although it appears that viral replication and the associated host pro-inflammatory and neurogenic responses drive the illness.[209] The localized viral replication triggers release of cytokines, chemokines, and inflammatory mediators which, together with stimulation of the local parasympathetic nerve endings, results in cold symptoms. Kinins, prostaglandins, and proinflammatory cytokines and chemokines contribute to vasodilation, increased vascular permeability, influx of leukocytes, exocrine gland secretion, and nerve ending stimulation, resulting in nasal obstruction, rhinorrhea, sneezing, cough, and sore throat.[190]

In addition to the upper airways, HRV can cause LRI, inducing local inflammatory response.[210] Lower respiratory symptoms may also result from the inflammatory response to rhinovirus infections of the upper airways.[211] In this regard, individuals with asthma develop T-cell infiltration of the airway epithelium and submucosa upon experimental HRV infection,[212] suggesting a role for these cells in the pathogenesis of asthma exacerbations caused by HRV.

Serotype-specific mucosal IgA can be detected by day 3 post infection, followed by IgM, and finally by IgG, 7–8 days later.[213] Protection from infection is correlated with both mucosal and serum antibody. Although detectable, the importance of cell-mediated immunity for recovery from

infection is unclear, except perhaps for cases of severe lung disease in transplant patients.

DIAGNOSIS

HRV shedding in nasal secretions peaks around 48 hours post infection, declining rapidly thereafter, but remaining at low levels for up to 3 weeks.[190] HRV can be isolated from respiratory secretions in cells kept at 33–35°C, with cytopathic effect developing in 10–14 days. RT-PCR, either by conventional or real-time techniques, is more sensitive and less tedious than HRV isolation,[214,215] and revealed that coinfections of HRV with other respiratory viruses can be documented in 50% of all HRV-positive samples.[22,216]

Novel multitarget molecular methods for HRV detection include multiplex PCR with tagged primers associated with tag-specific colored microspheres and flow cytometry, which allows for the rapid detection of 17 different respiratory viruses in clinical specimens.[217] MassTag is another technique that can identify viral PCR products with the advantage of detecting novel HRV sequences.[218]

TREATMENT

There are no licensed antiviral treatments for HRV infections. Several capsid-binding antiviral compounds inhibit *in vitro* replication of most rhinoviruses and enteroviruses,[219] and one such compound, pleconaril, when used orally reduced the duration and severity of natural colds in adults.[220]

Symptomatic relief from cold symptoms can be obtained with a broad variety of nonprescription medications. Systemic sympathomimetic decongestants, such as pseudoephedrine, may reduce nasal obstruction, first-generation antihistamines may reduce sneezing and rhinorrhea, and nonsteroidal anti-inflammatory drugs, such as naproxen or ibuprofen, may reduce headache, cough, and systemic symptoms.[190] However, these treatments have side effects, such as sedation with the antihistamines and CNS toxicities with the sympathomimetics, which are contraindicated in infants and young children.

PREVENTION AND CONTROL

The large number of HRV serotypes with minimal cross-antigenicity hampers the development of an HRV vaccine. It is possible to reduce HRV exposure by handwashing or virucidal hand treatment after contact with a cold sufferer or objects contaminated with respiratory secretions.[221] Short-term postexposure prophylaxis with intranasal IFN-α significantly reduced the incidence of HRV colds in household contacts of an index case,[222] but is investigational and causes mucosal irritation with sustained use.

HUMAN CORONAVIRUSES

Four species of human coronaviruses (HCoV) cause ARI: 229E, OC43, and the more recently discovered NL63 and HKU1.[223]The known HCoVs are distributed in two of the three known genera of coronaviruses: HCoV-229E and NL63 belong to the *Alphacoronavirus* genus, while HCoV-OC43 and HKU1 are members of the *Betacoronavirus* genus. The other known human coronavirus, discovered in 2003 as the agent of severe acute respiratory syndrome (SARS) is discussed in Chapter 59.

THE AGENT

HCoVs are enveloped viruses with long and widely spaced peplomers on the surface, resembling a crown (*corona*) and a 27–32 kb positive-strand RNA genome, which is the largest known viral RNA genome.[224] The envelope S glycoprotein contains neutralizing antibody and T-cell epitopes and attaches to the cell surface receptor, which for HCoV-229E is the aminopeptidase N and for HCoV-NL63 is the angiotensin-converting enzyme 2 (ACE2).[225] The hemagglutinin (HE) of group 2 HCoVs binds to the receptor 9-*O*-acetylated sialic acid.[226] Coronavirus replication occurs in the cytoplasm and new virions assemble by budding through intracellular membranes, being released through vesicles of the secretory pathway.[224]

EPIDEMIOLOGY

HCoVs are second only to rhinoviruses as causative agents of common colds and may cause up to 35% of mild URI in temperate climate regions,[227] but rates may vary significantly from year to year. In temperate areas HCoV infections occur mainly in the winter and spring months, but summer activity has also been documented.[227,228] However, their impact in tropical countries has not been well studied. HCoV-NL63 has been found in 2–4% of patients with ARI and pneumonia in Hong Kong and Australia[229,230] and HKU1 in 0.3–4.4% of patients with ARI in Hong Kong.[231,232]

In Brazil, the role of HCoV-229E in causing nonhospitalized respiratory infections in children in the community was first documented by serology in the early 1970s, with seropositivity of 26% in adults.[233] A recent study found that 5.7% of adult patients with ARI tested positive for 229E or OC43 in nasal secretions by RT-PCR.[197] A hospital-based study in Thailand detected HCoV-229E and -OC43 in respectively 3.5% and 1% of young children with LRI.[234] Hospital-based studies of children with ARI have detected HCoV-NL63 in 1.3% in Taiwan[235] and 2.4% in South Africa.[236]

CLINICAL FEATURES

Children seroconvert to HCoV-OC43 and -229E in the first 5 years of life, but symptomatic reinfections occur.[224] Clinical manifestations of HCoV infections are typical of common colds, with average incubation period 1 day longer than for HRV, and duration of 6–7 days. Low-grade fever may be present in up to 20% of patients, and cough and sore throat occur frequently. More serious infections of the lower respiratory tract have been documented.[227] In addition, HCoVs have been detected in 8% of influenza-like illnesses in frail elderly people in the United States.[237]

HCoV has been associated with exacerbations of asthma, chronic bronchitis, and recurrent wheezing in children in several parts of the world, including tropical countries such as Brazil, West Indies, and Trinidad.[112,227,238] HCoV infections frequently complicate with otitis media and maxillary sinusitis in children and adults. HCoV were detected by RT-PCR in the middle ear and/or nasopharyngeal aspirate from 16 out of 92 (17%) children presenting with acute otitis media in Finland,[205] and in nasal swabs from 3 out of 20 adults presenting with acute maxillary sinusitis.[203]

HCoV infections cause mostly symptoms of URI, but HCoV-NL63 has been implicated in LRI, including bronchitis and pneumonia,[239] and was detected in 15% of cases of croup in children in Taiwan.[235] HCoV-HKU1 has also been associated with LRI in elderly patients and children with underlying disease.[232]

PATHOGENESIS AND IMMUNITY

There are no convenient animal models to study HCoV pathogenesis, and humans naturally or experimentally infected have been the only source of information. HCoVs are transmitted by the respiratory route and virus shedding begins 48 hours post infection, coincident with onset of symptoms, and in adults lasts approximately 5 days.[227] Both entry and release of HCoV-229E happen on the apical surface of epithelial

cells[240] and the virus causes cell damage and loss of cilia on day 3 post infection.[241]

DIAGNOSIS

Except for HCoV-NL63, which can be cultured in LLC-MK2 and Vero cells, isolation of HCoVs in cell culture is tedious.[226] RT-PCR-based assays for HCoVs have become the best alternative for clinical studies.[223] Serology by EIA is sensitive and specific, and is useful in epidemiologic surveys.[224]

TREATMENT AND PREVENTION

Studies with RNAi[242] and broad-spectrum protease inhibitors[243] have been reported, but no specific antiviral therapy is available for HCoV. No vaccines are currently available for HCoV.

RESPIRATORY ADENOVIRUSES

Human adenoviruses (HAdV), the first respiratory viruses isolated, were obtained from cultured adenoid tissue, in which they may remain quiescent for a long time. Respiratory illnesses are among the most frequent consequences of adenoviral infections, particularly in children younger than 5 years.[243] Adenovirus-caused disease may present a wide spectrum of clinical manifestations, including respiratory symptoms, gastroenteritis, and conjunctivitis. HAdV have been frequently detected in ARI studies in tropical countries.[135]

THE AGENT

HAdV are nonenveloped, icosahedral DNA viruses of the genus *Mastadenovirus*, family *Adenoviridae*,[244] grouped into seven species (A–G), based on biological properties and phylogenetic relationships.[245] HAdV are distinguished antigenically into 53 types, which can be further classified into genomic subtypes.[244] HAdV types 7, 3, 4, and 21 are most frequently associated with severe disease.[246]

Adenovirus capsids are formed by three morphologically, antigenically, and functionally distinct types of capsomers: hexons, penton bases, and fibers that project from the penton bases. The hexon and penton bases contain complement-fixing, group-specific antigens common to all human adenoviruses, and the fibers, which are the virus-receptor binding domain, contain the neutralizing and hemagglutinating, type-specific antigens.[244] The adenovirus genome consists of linear double-stranded DNA of almost 36 kb, encoding approximately 40 genes.[244] A small single-stranded DNA parvovirus named adeno-associated virus is commonly detected concurrently with adenovirus, but does not seem to cause disease.[247] The virus fiber binds to a cell surface protein named CAR (Coxsackie and adenovirus receptor), but class I MHC and heparan sulfate serve as receptors for HAdV 5 and 1, respectively.[244,248] Receptor binding facilitates interaction of penton bases with cell surface integrins, triggering entry.[249] Upon endocytosis, double-stranded genomic DNA is transported to the nucleus, where "early" and "late" sets of viral genes are transcribed into mRNAs coding for structural and nonstructural proteins. Early genes encode mostly nonstructural proteins that orchestrate gene expression to maximize progeny production, while late genes encode structural proteins.[250]

Virus assembly takes place in the nucleus, releasing up to 1 million virions by cell lysis.[244] HAdV replicates well in continuous epithelial cell lines, such as HEp-2, HeLa, and A549, and can be adapted to grow in human embryonic lung fibroblasts.[244] HAdV are stable over a wide pH range (5–9), resistant to alcohol, ether and chloroform, stable for weeks at room temperature, and can be lyophilized. They are inactivated by sodium hypochlorite and by 2 minutes of heating at 60°C.[251]

EPIDEMIOLOGY

Respiratory transmission of HAdV occurs at all ages worldwide. Outbreaks happen in military recruits and sometimes in other semi-closed populations such as boarding schools and chronic care facilities. HAdV ocular transmission is associated with swimming pools or clinics where instrument sterilization or handwashing have been inadequate. Asymptomatic HAdV infection and prolonged shedding are common.[244]

HAdV species C, serotypes 1, 2, 3, and 5 are more frequent in children younger than 5 years, accounting for 5–20% of URI cases and approximately 5% of LRI cases in children.[244] In adults, HAdV occurs sporadically and causes mostly URI. Infections by HAdV types 4 and 7 are usually epidemic, with attack rates of 6–16% per week in newly assembled confined groups, such as military recruits, whose HAdV carriage rate may be as high as 18%.[244] In this group adenoviral syndromes vary from mild colds to severe LRI, with attack rates of up to 80%, with 20–40% of individuals requiring hospitalization.[244]

In temperate climates adenoviral infections are more frequent in late winter, spring and early summer, while in tropical northeast Brazil they seem to occur year-round,[135] being detected in 11% of ARI in children younger than 2 years in daycare.[140] In tropical areas the incidence of HAdV infections in military recruits is lower, and different serotypes may be involved.[244]

Adenovirus type 14 had not been associated with severe disease until 2006, when a cluster of severe disease was reported in military recruits, likely due to lack of preexisting immunity.[252] This agent is now known to occur in the general population.

Pharyngoconjunctival fever, commonly caused by HAdV types 3 and 7, may be epidemic or endemic among children in the summer in temperate climates, commonly associated with inadequate chlorination or filtration of swimming pools.[253] The incubation period of HAdV infections averages 10 days.[244]

CLINICAL FEATURES

HAdV respiratory diseases may involve all parts of the respiratory tract, but up to 50% of nonepidemic HAdV infections are asymptomatic. In fact, adenoviruses were discovered because of their propensity for latency in adenoids.[244]

Most HAdV illnesses are febrile colds, but children may have prolonged high fever. Pharyngitis is common and may be associated with fever, pharyngeal exudate, granular appearance of the mucosa, and anterior cervical adenopathy, similar to streptococcal pharyngitis.[244] HAdV can be detected in up to 20% of small children with pharyngitis. In addition to pharyngitis, pharyngoconjunctival fever caused by HAdV types 3 and 7 includes conjunctivitis, which may last 1–2 weeks, with preauricular adenopathy, cough, rhinitis, malaise, and fever.[253] The most frequent complication of HAdV ARI is acute otitis media, which occurs in up to 30% of cases.[254]

Adenovirus LRIs are mainly bronchitis and pneumonia, and may account for more than 10% of childhood LRIs in temperate areas.[255] HAdV may cause permanent lung parenchymal damage, especially when concurrent with measles.[255] Epidemic HAdV infections in military recruits have a spectrum of clinical manifestation including severe pneumonia. Typically, however, the manifestations are fever, pharyngeal symptoms, cough, chest pain, headache, and malaise.[244] More severe clinical manifestations have more recently been associated with infections of adults by an emerging mutant of HAdV type 14.[246]

Overwhelming pneumonitis may be part of disseminated HAdV infections in newborn infants and patients with immunodeficiencies including AIDS. However, frequent coinfections with other respiratory pathogens in patients with AIDS make an association of disease severity with HAdV uncertain.[256]

HAdV is also an important cause of epidemic keratoconjunctivitis.[244] HAdV 7 and 19 were the predominant serotypes associated with conjunctivitis in Brazil.[257]

PATHOGENESIS AND IMMUNITY

Respiratory disease caused by HAdV results from necrosis of cells of the airway epithelia, with viremia that may lead to disseminated infection in immunocompromised persons. Bronchiolitis, interstitial pneumonia, and mononuclear cell infiltrates are part of the inflammatory process in the lungs. The severity of HAdV diseases in children correlates with detection of higher levels of IL-6, IL-8 and TNF-α in the serum.[258] It remains unclear why certain HAdV strains are more virulent than others. For example, the genomic variant B7h was associated with fatal lower respiratory disease in a study in South America.[259] Adenoviruses may cause persistent infection in epithelial and lymphoid cells and this helps to keep viral circulation in the population.[244] HAdV glycoprotein E3 interferes with expression of class I MHC molecules, reducing display of viral epitopes on cell surface and contributing to persistence.[260] In addition, HAdV protein E1A inhibits interferon response and reduces HAdV-induced apoptosis of infected cells.[261]

Protection from HAdV infection and disease is mainly due to type-specific neutralizing antibodies, but reinfections, mostly asymptomatic, may occur. T-cell-mediated immunity is important for recovery from HAdV infection and immunocompromised patients are at higher risk for severe disease.[261]

DIAGNOSIS

HAdV can be detected in several kinds of clinical samples, but clinical correlation is required, since asymptomatic shedding is common. HAdV is amenable to isolation in human cell lines, but the direct detection of viral antigens by IF, or viral DNA by PCR, are attractive alternatives.[244] Rapid antigen detection is around 95% sensitive and easy to use in point-of-care diagnosis. However, conventional or real-time PCR are more sensitive than other methods.[262] HAdV serology has limited clinical utility.[245]

TREATMENT

At present, there is no licensed antiviral treatment for adenovirus infections.[263] Cidofovir has shown some efficacy in the rabbit model of ocular HAdV infection, while iododeoxyuridine and adenine arabinoside have been unsuccessful in treatment of keratoconjunctivitis.[244]

PREVENTION AND CONTROL

A live vaccine consisting of wild-type adenovirus packaged in capsules for enteric release induces immunity without infection of the respiratory tree. This approach has been successful in military recruits immunized with HAdV types 4 and 7.[244] Proper sterilization, handwashing, and chlorination can avoid adenovirus spread through tonometers, hands, and swimming pools.

HUMAN BOCAVIRUS

Human bocavirus (HBoV) was first detected in 2005 by an elegant viral metagenomic survey in nasopharyngeal aspirates from Swedish children with ARI.[264] Since then HBoV has been detected by PCR in children and adults with ARI essentially worldwide.[214,265]

THE AGENT

HBoV is a small, nonenveloped, icosahedral single-strand DNA virus of the family *Parvoviridae*, genus *Bocavirus*,[264] with a 5.3 kb genome encoding four proteins: non-structural proteins NS1 and NP-1, and capsid proteins VP1 and VP2.[264] HBoV capsid proteins bind to unknown cell receptors and the replication of HBoV occurs in the nucleus, where viral DNA replication is mediated by the host cell DNA polymerase.[265] After assembly the viral progeny may be released by exocytosis or by cell lysis.[265] Since DNA synthesis occurs in the S phase of the cell cycle, parvoviruses replicate more efficiently in actively dividing tissues, such as the bone marrow and respiratory and digestive epithelia.[266] Like other parvoviruses, HBoV is resistant to porous environmental surfaces, slow freezing, storage at temperatures above 4°C, or treatment with alcohol.[267]

EPIDEMIOLOGY

Three species of HBoV are recognized: HBoV1, the main species, was detected in most studies prior to 2009; HBoV2 and 3, which differ from HBoV1 by 23% at the nucleotide level, were initially detected in 2009 in stools from children with acute gastroenteritis in Pakistan and Australia, and later also in China and South Korea.[268,269]

HBoV has been detected mostly in children with respiratory and/or gastrointestinal symptoms, with frequencies ranging from 2% to 30%, and often found as a co-pathogen.[270] Its transmission may happen by respiratory and oral routes[270] and the virus can be detected in river water, suggesting potential waterborne transmission.[271]

In temperate climates HBoV is more frequent in winter and spring,[270] but in tropical areas it occurs year round.[217] Serological surveys suggest that most people are infected by HBoV early in life and in Japan 94% of people have been infected by age 6.[272]

CLINICAL FEATURES

HBoV is often detected in samples from patients with ARI[273] most often associated with wheezing, cough, rhinorrhea, and fever.[270] The most frequent clinical diagnosis of patients with HBoV infections are bronchiolitis, pneumonia, common cold, bronchitis, exacerbation of asthma, and croup.[274,275] Symptoms usually last 1–2 weeks, but occasionally may be prolonged.[276] Importantly, HBoV has been detected in association with symptoms of ILI and pneumonia. In a study conducted in Thailand, HBoV was detected in 4% of 512 patients with ILI and in 4.5% of 1168 patients with community-acquired pneumonia.[275] In approximately 90% of the patients, HBoV was present in coinfection wtih other respiratory viruses. When only children younger than 5 years were considered, HBoV was detected in 12% of those with pneumonia, highlighting its importance as a cause of LRI in that age group.[275]

HBoV has also been detected in feces of children with diarrhea.[277] Although not yet firmly established, a causative role of HBoV in diarrhea has been suggested by its detection as a single agent in studies of diarrhea, including one in Brazil.[278] Diarrhea with HBoV is more common in young children, with watery stools, nausea and vomiting.[279]

PATHOGENESIS AND IMMUNITY

Case-control studies consistently find HBoV more frequently in ARI patients than in asymptomatic controls.[274,275] In addition, HBoV viral loads are significantly higher in symptomatic patients than in asymptomatic ones.[280] Little is known about HBoV pathogenesis, including routes of infection, portal of entry, replication sites, mechanisms of tissue injury, duration of shedding, and protective efficacy of immune response. Pathogenesis studies have been hampered by the lack of growth of the virus in routine cell cultures and of experimental animal models of infection, hence Koch's postulates have not been fulfilled. Furthermore, detection of HBoV in asymptomatic individuals or in association with other respiratory viruses with established pathogenic potential complicates the diagnosis.[217,281] However, similar to other parvoviruses, HBoV may cause persistent infection with prolonged shedding.[282] HBoV causes viremia, which is uncommon in other respiratory viral infections.[274] HBoV DNA has been found in 32% of tonsils and adenoids removed from children.[283] HBoV DNA has been detected as single agent in feces from 0.8–7.8% of children with gastroenteritis

without ARI symptoms.[277,278,284,285] The role of immune response in pathogenesis and protection against infection remains unclear; but both Th1 and Th2 cytokines are increased in children with bronchiolitis and HBoV.[286]

DIAGNOSIS

HBoV replicates in primary cultures of respiratory cells,[287] but cannot be propagated in common cell lines, and rapid antigen detection methods have not become available. Real-time PCR assays have become the standard for HBoV diagnosis, but with limited availability in most developing countries.[288]

TREATMENT, PREVENTION, AND CONTROL

The actual clinical impact of HBoV respiratory and digestive infections is still uncertain, and no specific therapeutic or prophylactic approaches are available for this agent.

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