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173

Noninfluenza Respiratory Viruses

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KEY CONCEPTS

- Novel strains of adenovirus 11 and 14 have recently been identified as a cause of clinically significant, sometimes severe infection, in otherwise healthy adults and children.
- The novel coronavirus, MERS-CoV, has emerged to cause severe respiratory infections in the Arabian Peninsula.
- A short inhibitory RNA has shown promise in the management of respiratory syncytial virus (RSV) in lung transplant recipients but is not yet approved for the treatment of RSV.
- Human rhinoviruses are the most common cause of the common cold and are also common triggers of exacerbations of asthma and chronic obstructive pulmonary disease (COPD).
- Human metapneumovirus is a significant cause of disease in young children
- Human bocavirus 1 is a newly discovered respiratory pathogen although coinfection with other viruses is often documented.

Adenovirus

Nature

Human adenoviruses (hAdV) are members of the Adenoviridae family, which are enveloped, double-stranded DNA (dsDNA) viruses. hAdV are divided into 57 serotypes and seven species (A, B, C, D, E, F, and G; see Table 173-1) based on serum neutralizing and hemagglutination epitopes, genome sequence and function, oncogenic properties and pathology in humans.[1–3]

Epidemiology

Adenoviruses cause a range of infections from mild, self-limited respiratory viral infections, conjunctivitis and diarrhea to severe disseminated disease.[4] Adenoviruses have a worldwide distribution and infections occur throughout the year without significant seasonal variability. Most infections occur as sporadic events, although local or regional epidemics have been described.[5,6] Infection is more common in children (66.9%), generally less than 5 years of age.[7] About 4.4% of children with diarrhea are infected with adenovirus, typically with AdV 40 and 41.[8] Recently, several, often fatal, outbreaks of AdV 14 occurred starting in 2005 in the USA.[5,6] AdV 55, which is an AdV 11 and 14 recombinant virus, has been recognized to cause significant outbreaks of disease in China. [9,10] Adenovirus is the leading cause of respiratory viral infections among military recruits.[11,12]

Pathogenicity

Adenoviruses infect susceptible hosts by the mouth, nasopharynx or ocular conjunctiva. Key cellular receptors include CAR (coxsackie-adenovirus receptor), CD46, desmoglein 2 and GD1a glycan.[13] In addition, adenovirus hexon-factor X complexes can result in CAR-independent binding involving heparin sulfate glycosaminoglycans or αv integrins.[14]

Replication causes desquamation of epithelial cells, which induces changes in regional lymphatic tissue, including hypertrophy and active, proliferative germinal centers and can result in lymphadenopathy and intussusception, particularly in children.[15,16] The innate and adaptive immune responses are critical for the control of adenovirus replication.[17,18] CD4+ and CD8+ T lymphocytes play a particularly important role in the control and clearance of replicating adenovirus in humans with deficits associated with progressive dissemination in immunosuppressed patients.[4,19–21] Group- and type-specific neutralizing and non-neutralizing antibodies play a significant role, too, in limiting infection.[22]

Prevention

Vaccination and careful attention to infection control practices are the only existing preventative strategies.[11] To date, the vaccine is only available to members of the military in the USA who are 17–50 years old.[11] Contact and droplet precautions are recommended to prevent healthcare-associated and institutional outbreaks of adenovirus infections, including epidemic keratoconjunctivitis.[23] Likewise, careful attention to hand hygiene and standard sterilization of medical equipment that comes in contact with patients with proven or suspected adenovirus infection is essential.[24]

Diagnostic Microbiology

Diagnosis of adenovirus depends on the isolation of the virus from infected tissue and either histopathologic evidence of local replication or clinical symptoms consistent with infection. Confirmation of infection can be confused by latent infection in some tissues, such as the tonsils, and intermittent and sometimes prolonged shedding of virus from throat or in the stool for months to years after primary infection.[25,26] Direct detection of adenovirus from clinical isolates is generally achieved by culture, direct fluorescent antigen detection, or polymerase chain reaction (PCR).[2,27] Adenovirus can readily be grown in HEK and A549 cell lines (see Figure 173-1), with typical cytopathic effect or fluorescent antibodies used to confirm infection. Molecular

TABLE 173-1 Adenovirus Subgroup, Serotype and Major Site of Infection

Subgroup	Serotype	Major Site of Infection
A	12, 18, 31	Respiratory, urinary, gastrointestinal (GI)
B1	3, 7, 16, 21, 50	Respiratory, eye, urinary, GI
B2	11, 14, 34, 35	
C	1, 2, 5, 6, 57	Respiratory, urinary, GI
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56	Eye, GI
E	4	Eye, respiratory
F	40, 41	GI
G	52	GI

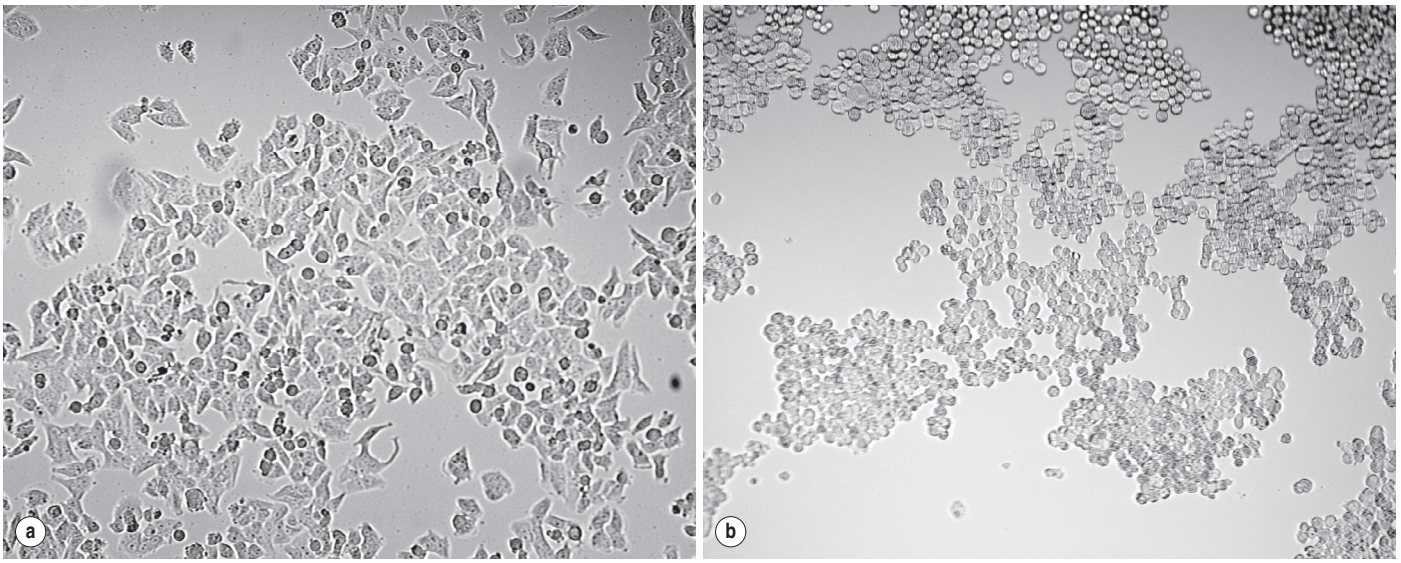

Figure 173-1 Cytopathic effect caused by adenovirus on Hep-2 cell line culture. (a) Uninoculated cell line. (b) Enlarged, refractile, rounded cells forming grape-like clusters.

techniques are used increasingly for the diagnosis of adenovirus and generally have the highest yield of the various diagnostic methods.[27,28] Unfortunately many respiratory virus multiplex panels have low yields for adenovirus in patients with clinical infection; this is especially true if respiratory samples are collected and the respiratory tract is not the primary site of infection.[29] Quantitative PCR assays can be used to predict progression to disseminated disease in pediatric and, to a lesser extent, adult stem cell transplant (HSCT) recipients and to assess response to antiviral therapy.[28,30–33]

Clinical Manifestations

Adenovirus infections are associated with a wide range of clinical manifestations, including respiratory tract, ocular, gastrointestinal tract, genitourinary tract, and central nervous system (CNS) infections, among others. Disseminated infection can also occur, typically in the immunosuppressed host.

RESPIRATORY TRACT INFECTIONS

Pharyngitis, laryngotracheitis, bronchitis and pneumonia have all been described. Typically, symptoms that include nasal congestion, coryza and cough are often accompanied by systemic manifestations, such as generalized malaise, fever, chills, myalgia and headache; abdominal pain, an exudative tonsillitis and cervical adenopathy are often frequently observed.[34,35] If conjunctivitis accompanies the above signs and symptoms, the disease is designated as pharyngoconjunctival fever.[7,36] Rarely a pertussis-like syndrome has been described, typically caused by AdV 5.[37,38] Pneumonia, usually with diffuse bilateral interstitial infiltrates, has been described and may be particularly severe with AdV 3, 7, 14 and 21.[5,39–44] The recent outbreak of severe AdV 14 resulted in significant morbidity and mortality in some patients.[5,44] Most individuals presented with fever and cough and most required hospitalization (76%), supplemental oxygen (61%), and critical care (47%), while a minority received vasopressors (24%) or died (18%).[5] Older age, chronic underlying condition, low absolute lymphocyte counts and elevated creatinine levels were associated with severe illness.

OCULAR INFECTIONS

The two most common manifestations of ocular adenovirus infection are pharyngoconjunctival fever and epidemic keratoconjunctivitis (EKC). Pharyngoconjunctival fever is typically a milder form of acute follicular conjunctivitis that accompanies febrile pharyngitis or cervical adenitis.[7,36] Symptoms typically resolve without sequelae and no treatment is indicated. Epidemic keratoconjunctivitis (EKC) is a more serious disease with a much longer time to recovery. Following an 8–10 day incubation, a follicular conjunctivitis with edema of the eyelids, pain, lacrimation, photophobia, preauricular lymph node hypertrophy and, rarely, self-limited painful corneal opacities develop.[45,46] Epidemic keratoconjunctivitis, typically caused by adenovirus types 8, 19, and 37, was first seen in shipyard workers whose eyes had been slightly injured by chips of rust or paint. Although patients may experience significant pain and blurry vision, EKC typically resolves without permanent corneal damage over 4 weeks.[47,48]

GASTROINTESTINAL TRACT INFECTIONS

Up to 10% of pediatric cases of diarrhea are caused by the group F AdV 40 and 41. Higher rates of adenovirus-induced diarrhea have been described with seasonal outbreaks in low- and middle-income areas of the world.[2] Adenovirus-associated diarrhea is typically prolonged, lasting 8–12 days. Outbreaks in closed communities and clinical settings have been described.[49] Mesenteric adenitis is common and clinically mimics appendicitis or results in intussusception in young children.[16,50] Adenovirus infection may masquerade as rejection in small bowel transplant recipients and should be ruled out on all biopsies from such patients.[4,51]

GENITOURINARY TRACT INFECTIONS

Hemorrhagic cystitis typically presents as microscopic or macroscopic hematuria and pain and cramping of the bladder, and is associated with AdV 11 and 21.[52] Adenovirus tubulointerstitial nephritis occurs mostly commonly in kidney transplant recipients and presents as transient elevation of serum creatinine and hemorrhagic cystitis.[53–55]

OTHER MANIFESTATIONS OF ADENOVIRUS

Other rare manifestations include meningitis, encephalitis, myocarditis and dilated myocardiopathy.[56–58] Additionally, echogenic liver lesions with or without hydrops and neural defects in fetuses have also been described.[59]

ADENOVIRUS DISEASES IN IMMUNOCOMPROMISED PATIENTS

Adenovirus disease in immunocompromised adults and children ranges from asymptomatic shedding to progressive, often fatal disseminated disease.[28,60–63] Risk factors for adenoviral infection in the HSCT population includes allogeneic donor (8.5–30% vs 2–12% for autologous donor), pediatric age groups (20–47% vs 9–13.6% for adults), T cell-depleted grafts (45% vs 11%), use of alemtuzumab, cord blood donor and patients with acute graft-versus-host disease

(GVHD).[4,28,63–65] Although disseminated disease only effects 1–7% of HSCT recipients it is associated with a significant risk of mortality (8–26%).[4,28,63,66] Incidence among solid organ transplant recipient populations is highest in small bowel and liver transplant recipients, pediatric transplant recipients, patients who receive antilymphocyte antibodies, and patients with donor-positive/recipient-negative adenovirus status.[60] Asymptomatic adenovirus DNAemia is common among solid organ transplant recipients and generally is not associated with progression to symptomatic disease.[67,68]

Management

Although no antivirals are specifically approved for the treatment of adenovirus, several have *in vitro* and *in vivo* experimental data to support their use.[4 63,69–73] While no single agent has consistently been effective in all cases, existing data suggest that cidofovir and its lipid ester analogs (CMX001 or brincidofovir) are most effective in managing adenovirus infections.[4,63,74] Adenovirus- and multi-virus-specific T cells are an emerging potential therapy that can safely be used with a low frequency of de novo graft-versus-host disease and a complete or partial response in the adenovirus infection.[75,76] Serial measurement of quantitative viral load is predictive of response to any therapeutic intervention.

Coronavirus

Nature

Coronaviruses are large, lipid-enveloped, positive-sense, single-stranded RNA viruses. Human coronaviruses (e.g. hCoV 229E, OC43, NL63) commonly cause mild upper respiratory tract infections, although occasionally result in more severe disease in immunocompromised individuals.[77] However, two novel human coronaviruses, the severe acute respiratory syndrome-associated coronavirus (SARS-CoV), and a recently identified Middle East respiratory syndrome-associated coronavirus (MERS-CoV) may cause serious viral pneumonitis, leading to hospitalizations and deaths.[78,79,289] Viral genome analyses revealed that SARS-CoV belongs to Group B and MERS-CoV belongs to Group C betacoronavirus, respectively, and both are closely related to coronavirus strains found in bats.[78–80] Intermediate mammalian hosts, such as civet cats, have been implicated for SARS-CoV before its adaptation for human transmission, and emerging evidence (through virus or antibody detection) suggest that the dromedary camels are likely the host for MERS-CoV.[78,79,81,290] The surface spike glycoprotein (S-protein) of coronaviruses is a key virulence factor which attaches the virus to host cells, determining its host range and tissue tropism, and it is a target of the neutralizing antibodies. SARS-CoV uses human angiotensin-converting enzyme 2 (ACE-II) as the primary cellular receptor; the human cellular C-type lectin (DC/L-SIGN) may be the alternative.[82] MERS-CoV has been shown to bind to dipeptidyl peptidase 4, (DPP4; also called CD26), an interspecies-conserved protein found on the surface of several cell types, including the non-ciliated cells in human airways, which can explain its broadened host range and its ability to cause cross-species, zoonotic transmission.[83]

Epidemiology

HCoVs are ubiquitous among humans and are the major cause of respiratory disease, accounting for up to 30% of all common colds. Serologic studies have also suggested that one-half of the infections with coronaviruses are asymptomatic.[84] Two more newly-described coronaviruses, HKU1 and NL63, are also common causes of typically mild, self-limited colds.[85] More severe clinical disease has been described in young children, the elderly and in immunocompromised patients.[86]

SARS-CoV emerged in Southern China (Guangdong province) in late 2002; the first victims were those involved in live animal trade and food handlers at restaurants which serve exotic animal meat.[79] Through international air travel, the disease quickly spread to Hong Kong, Vietnam, Singapore, Taiwan and Canada in a matter of weeks; at the end of the 6-month epidemic, more than 30 countries were affected, resulting in 8096 confirmed infections, and 774 deaths (9.6%).[87] The primary mode of transmission was via respiratory droplets; the basic reproductive number (R0) of SARS was in the range of 2.2–3.7.[79,82,87] However, frequent nosocomial outbreaks and 'super-spreading events' had greatly exacerbated its transmission.[88] Notably, 21% of SARS victims were healthcare workers; and the disease attack rate in hospitals was between 10% and 60%.[78,82] Viral kinetics (peak at the time of clinical deterioration), application of aerosol-generating procedures and devices (e.g. intubation, resuscitation, oxygen or nebulizer therapy, bilevel positive airway pressure (BiPAP)), overcrowdedness and lack of proper isolation facilities in hospitals are some of the explanations.[88–90] A community 'super-spreading event' that occurred in a private housing estate in Hong Kong involved over 300 residents. Drying up of a 'U-shaped' bathroom floor drain and backflow of contaminated sewage (from a SARS patient with diarrhea), coupled with the toilet's exhaust fan, might have created infectious aerosols that rose with warm air along the building's air-shaft; these were then dispersed by wind flow, causing long-range transmission to nearby buildings.[90] This and other evidence suggested that SARS could be 'opportunistically' airborne.[78,89]

The first cases of MERS-CoV infections emerged in June 2012 in Saudi Arabia and Qatar. As of May 2015, a total of 1180 confirmed infections and 483 (41%) deaths had been reported.[78,91] The Middle Eastern countries of Saudi Arabia, Qatar, Jordan and United Arab Emirates were predominantly affected, but imported cases to UK, Netherlands, USA and Asia were reported.[91,291] The largest outbreak outside the Middle East occurred in South Korea in 2015, which involved multiple hospitals and 185 individuals, causing 36 (19.5%) deaths. Although zoonotic transmission is implicated, the majority of infected cases did not report a history of direct animal contact. Epidemiological investigations suggested sporadic transmission of disease with multiple introductions into the at-risk population. It was found that occupational exposure to camels (e.g. shepherds, slaughter-house works) was associated with 15–23 times higher risk of seroconversion (2.3–3.6%) than the general population (<0.2%). Also, a large number of younger (15–44 years) infected individuals, who develop no or mild symptoms might exist and serve as the source of infection for those without direct animal contact.[78,91,92,292] Secondary transmission in household is not uncommon, which occur in over 20% of case clusters. Transmission is typically highly efficient in the hospital settings via infectious droplets/aerosols, leading to frequent occurrence of nosocomial outbreaks, as in the case of SARS.[78,93–96,293,294]

Pathogenicity

The cellular receptor for HCoV-229E is aminopeptidase N (APN) or CD13; the cellular receptor for HCoV-OC43 is 9-*O*-acetylated sialic acid on the cell surface. Like SARS-CoV, HCoV-NL63 binds to the ACE2.[85]

Humans have no pre-existing immunity to SARS-CoV or MERS-CoV. These novel viruses have the ability to evade innate host defenses (e.g. type I interferon responses and related mechanisms), and replicate efficiently in host tissues (respiratory and intestinal tract cells; kidney cells also for MERS-CoV).[78,80,82,83] In addition to lytic cell damage, uncontrolled replication of SARS-CoV leads to unabated inflammatory cytokine activation (commonly known as 'cytokine storms'), which is implicated in the development of progressive pneumonitis, diffuse alveolar damage and acute respiratory distress syndrome (ARDS), and hemophagocytic syndrome.[79,97] High viral load and slow viral clearance due to inefficient host responses are associated with progressive disease and fatal outcomes.[82,98,99] Pathogenesis data for MERS-CoV are limited; a macaque model has shown active viral replication in lung tissues causing localized-to-widespread lesions and clinical illness, which only abates after 1 week of illness. Infected marmosets can develop severe interstitial pneumonia similar to humans cases. Neutrophil and macrophage infiltration and alveolar oedema are noted.[78,100,101]

Prevention

There is no vaccine or chemoprophylaxis available for coronaviruses at present.[102] Droplet and contact precautions, including the use of face masks, are advisable to prevent transmission. As transmission could be opportunistically airborne, appropriate isolation precautions (in negative-pressure facilities if available) should be implemented in all hospitalized patients confirmed with novel coronavirus infections, particularly when respiratory procedures and devices are applied.[88–91]

Diagnostic Microbiology

A high index of suspicion, together with detailed clinical and epidemiological assessments (e.g. travel history to affected areas, case clustering), is required for the diagnosis of novel coronavirus infections.[88,103] RT-PCR is the diagnostic test of choice. A combination of upper respiratory (nasal, pharyngeal, nasopharyngeal), lower respiratory (higher yield due to higher viral levels, e.g. sputum, tracheal aspirate, bronchoalveolar lavage (BAL) whenever available), blood and fecal samples, and repeated sampling should be considered to maximize the chance of virus detection.[103–105] A single negative test from an upper respiratory sample may be insufficient to rule out the diagnosis. For SARS-CoV, plasma RT-PCR can detect viremia as early as day 2–3 after symptom onset, and its level may have prognostic value.[88,99] Virus culture (e.g. using Vero cells) is confirmatory, but the delay to a result prevents its use for clinical management; also biosafety level-3 facilities are required. Serological diagnosis is retrospective and largely used for epidemiological surveillance purposes.[103] Clinicians should refer to their local reference laboratories for coronavirus testing (e.g. pan-coronavirus RT-PCR, specific SARS-CoV and MERS-CoV RT-PCR).[87,91]

Clinical Manifestations

The incubation period of SARS is about 4–6 days (range 2–16 days). Patients initially develop fever, chills and rigor, which partially subside in a few days. These are then followed by resurgence of high fever, cough, shortness of breath and the development of pneumonia.[78,88] Chest radiographs first reveal patches of consolidation and ground-glass changes, which rapidly progress in the next few days to involve multiple lobes. CT scan of thorax may show features resembling bronchiolitis obliterans or organizing pneumonia, such as peripheral air-space consolidation.[78,79,88] Laboratory features include lymphopenia, thrombocytopenia, elevated transaminases, creatinine kinase and lactate dehydrogenase.[88] Around day 10–14, 15–25% of patients further deteriorate and develop refractory respiratory failure and ARDS.[106] About 20% of patients develop profuse diarrhea which contains highly infectious virus particles; renal failure is rare.[107] Other complications include ventilator-associated pneumonia (e.g. MRSA), pneumothorax and pneumomediastinum.[106] The overall death rate of SARS was 6–16%. The age-stratified case fatality rate was: <25 years: <1%; 25–44 years: 6%; 45–64 years: 15%; and >65 years: >50%.[78,87,106] Young children (e.g. <5 years) typically have mild disease; fatality is rare.

Available data indicate that the clinical features of MERS-CoV are similar to those of SARS. Patients develop high fever and chills, cough, shortness of breath and progressive pneumonia in about 1 week after symptom onset. Imaging findings include multiple, patchy consolidations and ground-glass changes. In addition to lymphopenia, thrombocytopenia and elevated liver enzymes, acute renal failure seems to be a common feature.[80,92,95,104,108] The majority of adult patients with severe infection are older (mean age 60 years), and had underlying conditions (e.g. diabetes mellitus, renal impairment) and required ICU care because of respiratory failure and ARDS; the associated fatality rate in such patients rate can be as high as 60–76% despite maximal medical support.[92,108,109] Notably, affected children usually have mild or no symptoms, as in the case of SARS.[78,79,110]

Management

At present, there is no established therapy for coronavirus infection. During the SARS outbreak in 2003, a range of agents had been used but their efficacies are questionable.[78,102,111] Ribavirin, though shown to be active *in vitro*, did not seem to provide any clinical benefit. An HIV protease-inhibitor (lopinavir-ritonavir) with *in vitro* activity against SARS-CoV was reported to cause viral load reduction and fewer ARDS and deaths in 41 patients; however, the study was uncontrolled.[112] Another study reported that 19 patients who received 'convalescent-plasma' from recovering individuals, which contained neutralizing antibodies, had better clinical outcomes (survival 100% vs 66%, discharge rates 78% vs 23%).[113] Subsequent *in vitro* and animal (ferrets, hamsters, macaques) studies have shown that monoclonal antibodies targeting the S-protein may provide neutralizing activity against SARS-CoV, resulting in viral load reduction and resolution of lung lesions.[114,115] *In vitro* and animal (mice, macaques) studies show that type I interferons, if given prophylactically or shortly after exposure, may protect against SARS.[116] In a small clinical study (n=9), interferon-alpha given within 5 days was associated with lower rates of intubation (11% vs 23%) and death (0% vs 8%).[117] Against MERS-CoV, available data have shown that type I and type III interferons are active *in vitro*; a combination of IFN-α 2b and ribavirin given hours after infection appears to reduce lung injury in a macaque model.[118] Clinical data are limited; among the few patients who received such treatment, no consistent result was observed. In one retrospective study, survival was 30% versus 17% at 28 days, but it did not reach statistical significance.[80,92,95,104,108–110,295] Animal models have suggested potential benefits of other antiviral agents or antibody-based therapies, but no clinical data have been published to date.[119,296,297]

Systemic corticosteroid treatment is highly controversial. While favorable clinical and radiological responses have been reported, controlled data are lacking.[102,106,111] In the only randomized, placebo-controlled study performed during the SARS outbreak, early corticosteroid treatment within the first few days delayed viral clearance.[98] A later systematic review has ded that corticosteroid is not associated with definite benefit, but is likely to be harmful (e.g. metabolic side effects, bacterial and fungal superinfections, avascular osteonecrosis, acute psychosis).[111] Currently, corticosteroid therapy is not recommended in SARS-CoV, MERS-CoV and avian influenza infections, perhaps except in cases with refractory septic shock and adrenal insufficiency, and should only be given at a low dose (e.g. hydrocortisone 50 mg Q8H).[78,91]

Respiratory Syncytial Virus

Nature and Pathogenicity

Respiratory syncytial virus (RSV) is an enveloped, single-stranded RNA paramyxovirus that includes two major groups, A and B, each of which consists of 5 to 6 genotypes. The RSV genome encodes two nonstructural (NS1 and NS2) and nine structural proteins, including the F (fusion) and G (attachment) glycoproteins on the viral envelope. Antibodies against the F and G proteins are neutralizing, and have been shown to confer protection against RSV infection in animal models. Immunity after primary infection (which generally occurs by 2 years of age) is partial and short-lived; thus, reinfections can occur throughout life.[120] Low serum neutralizing antibody levels in adults predicts infection risk and disease severity.[121] Immunologic mechanisms (e.g. cytokine responses) have been implicated in the pathogenesis of severe RSV diseases; however, emerging evidence suggests that uncontrolled viral replication, as indicated by high respiratory tract viral load, drives disease manifestations and is associated with severe clinical outcomes.[120,122–125] Such findings provide an important rationale for the approach to antiviral drug development against RSV.[122]

Epidemiology

RSV is known to be an important cause of lower respiratory tract infection in infants and young children (e.g. acute bronchiolitis, wheezy attacks), resulting in hospitalizations and deaths.[126] In adults, it has been estimated that RSV infects 3–10% of the population

annually. Although most infections are mild, severe lower respiratory tract infections can occur, especially among older adults (e.g. >65 years) and those with underlying conditions (e.g. chronic lung diseases, chronic cardiovascular diseases).[120,127] RSV has been shown to account for 5–15% of community-acquired pneumonia, 9–10% of hospital admissions for acute cardiorespiratory diseases and excessive deaths among adults during seasonal peaks.[120,128,129] Outbreaks among nursing home residents are common, but under-recognized. The disease burden of RSV has been shown to approach that of seasonal influenza.[129–131] Patients who are profoundly immunosuppressed, such as hematopoietic stem cell transplant (HSCT) recipients, are at particularly high risk for severe RSV infection (2–17%), which can be rapidly fatal.[132]

Diagnostic Microbiology

RSV infection is clinically indistinguishable from other viral respiratory infections and diagnosis requires laboratory testing. Upper respiratory tract specimens (e.g. nasal, throat, nasopharyngeal) are commonly used, but lower respiratory samples (e.g. tracheal aspirate, BAL) should be considered whenever available. The gold standard for diagnosis is by RT-PCR; other tests such as antigen assays (e.g. enzyme immunoassays) and culture have much lower sensitivities (see Figure 173-2), especially among adults because of their lower viral loads.[120,131] A negative antigen assay result cannot be used to rule out RSV infection. Serology to detect RSV-specific IgG antibodies may also assist with the diagnosis and, if available, can be used in combination with RT-PCR to maximize the yield.[120]

Clinical Manifestations

RSV infections in infants and young children can lead to severe lower respiratory tract illnesses such as pneumonia and bronchiolitis.[126] The clinical manifestations of RSV infection in adults are diverse and often determined by the underlying conditions (e.g. chronic lung diseases) and degree of immunosuppression. In healthy young adults, RSV may cause self-limiting upper respiratory illnesses; in the profoundly immunosuppressed, progressive pneumonitis can occur (17–84%), resulting in high mortality (7–83%).[120,132] Older adults hospitalized for RSV infection may present with fever, cough, sputum production, wheezing and dyspnea. Although wheezing and dyspnea may be more common with RSV, and the magnitude of fever sometimes lower, such findings could not reliably differentiate it from influenza.[120,131] Radiographically, about 50–60% of cases show active pneumonic changes such as consolidation and ground-glass opacities, which are typically small, patchy and unilateral.[97,120,131] The majority (>70%) of adults hospitalized with RSV develop severe lower respiratory complications, including pneumonia, acute bronchitis and exacerbations of COPD/asthma resulting in respiratory failure and hypoxemia, and 10–15% develop cardiovascular complications such as congestive heart failure or acute coronary syndrome.[127–129,131] Bacterial superinfection occurs in at least 12–17%.[120,127,131] Published data suggest that around 10–18% of patients required ventilatory support because of respiratory failure and the overall mortality was approximately 8–10%; the outcomes are generally comparable to that of seasonal influenza.[128,131]

Treatment and Prevention

At present, there is no established antiviral therapy or vaccine available for RSV. Ribavirin has *in vitro* activity against RSV and in animal models, palivizumab (an RSV-specific monoclonal antibody directed against the F glycoprotein) has been shown to reduce viral titers and replication in pulmonary tissues.[120,133] In randomized clinical trials, palivizumab given prophylactically to very young high-risk children was shown to reduce hospitalizations related to RSV infections.[134] In immunocompromised adults, ribavirin (aerosolized or systemic administration) and palivizumab have been used to treat RSV infection with the aim of reducing progression to lower respiratory disease and death, with variable results.[120,132,133,135] There is lack of controlled data, and it is not known whether these approaches can be applied to older, non-immunocompromised adults. New antiviral agents (e.g. fusion protein and polymerase inhibitors, siRNA) and newer generation antibody therapies are under active research.[120,136,137,298] Systemic corticosteroids are commonly used to treat wheezing and exacerbations of COPD/asthma in adults, including those triggered by viral infections. Randomized controlled trials of corticosteroid therapy in young children with RSV infections have revealed a lack of clinical benefit and inconsistent control of inflammatory cytokine responses.[138,139] A recent study of corticosteroids in adults reported that virus control seemed to be unaffected but humoral immunity against RSV was diminished.[140] It is suggested that the decision to treat RSV patients with corticosteroids should be weighed against the potential risks (e.g. bacterial superinfections) and be limited to a short course if used.[131,140] Because of the high rates of secondary infections, it is prudent to test and treat bacterial pathogens according to local resistance profiles. In addition to *Streptococcus pneumoniae* and *Haemophilus influenzae*, *Pseudomonas aeruginosa* and other gram-negative bacilli

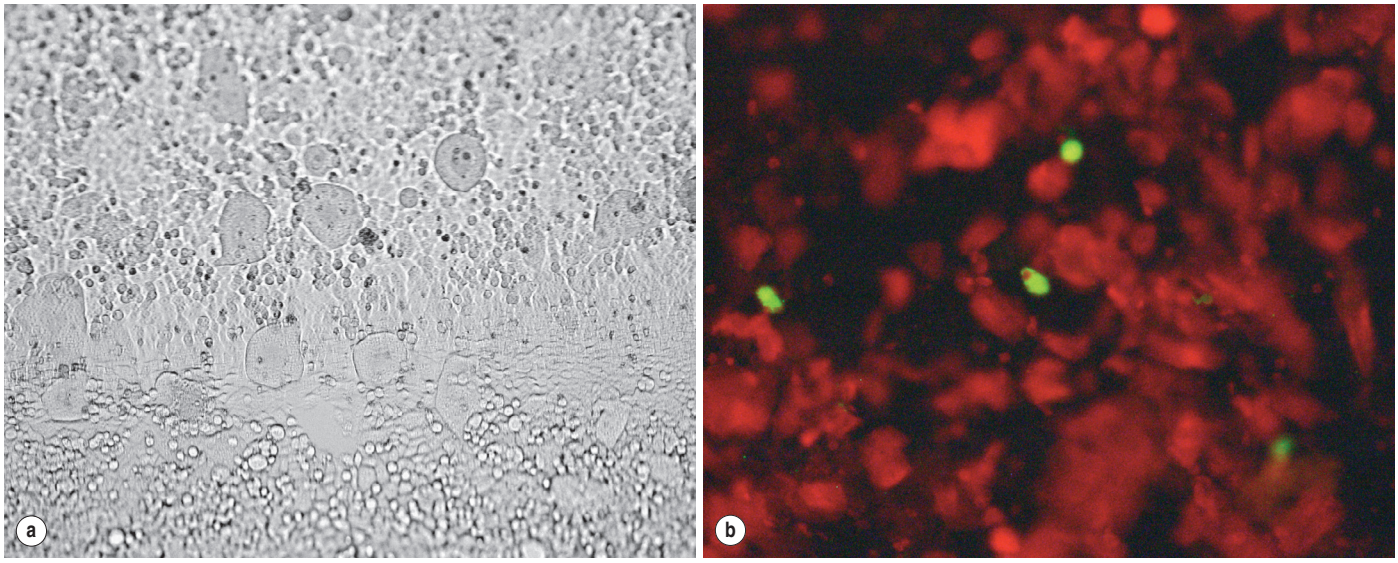

Figure 173-2 Cytopathic effect of RSV on Hep-2 cell line culture and identification of RSV antigen by means of IFA. (a) Syncytia formation in cell line culture. (b) Positive cells coloring green under IF microscope.

may need to be considered in patients with underlying chronic lung diseases.[127,131]

Rhinovirus

Nature

Human rhinoviruses (HRVs) are members of the family Picornaviridae and the genus *Enterovirus*. The HRVs are positive-sense, single-stranded-RNA viruses that encode a single protein that is cleaved by the virally mediated protease. The VP1-4 proteins make up the viral capsid.[141] There are well over 100 serotypes that are generally phylogenetically organized into three different species: HRV-A (74 serotypes), HRV-B (23 serotypes) and a more recently identified HRV-C (>50 serotypes have been identified to date).[142,143]

Epidemiology

HRV infections occur worldwide with peak incidence in the early fall and spring in temperate climates, although infection can occur year round. HRV-C has its peak incidence in the fall and winter in temperate regions and during the rainy season in tropical regions.[144,145] Infections occur in all age groups. HRVs are responsible for over half of all common colds and have been increasingly associated with more severe upper and lower respiratory tract infections in children, the elderly and the immunocompromised.[141] Young children are typically responsible for introducing the virus in household settings with a secondary attack rate of about 50%. With challenge studies, most (95%) develop infection while about 75% demonstrate clinical illness.[146]

Pathogenicity

HRVs are transmitted from person to person via contact (either direct or through a fomite) or aerosol (small or large particle).[147] HRVs can survive from a few hours to as long as 4 days on nonporous surfaces and for over 2 hours on human skin.[148] The majority of HRVs bind to intercellular adhesion molecule 1 (ICAM-1) whereas a minor group of HRVs adhere to the low-density lipoprotein receptor (LDLr).[141] Heparan sulfate has also been demonstrated as an additional receptor for some HRVs. Following infection of the respiratory epithelium, significant inflammatory cytokines, including IFN-β and IFN-γ and RANTES, IP-10, IL-6, IL-8, epithelial cell-derived neutrophil-activating peptide 78 (ENA-78), bradykinins, prostaglandins and histamine, are released locally.[141,149,150] This, in turn, results in vasodilation of nasal blood vessels, transudation of plasma and increased glandular secretions.[151] Together, these result in local congestion and trigger sneeze and cough reflexes. Immunity, which is type-specific and short-lived, results in eventual clearance of virus locally.

While the majority of replication was initially felt to be limited to the upper airway, a growing body of evidence suggests that HRVs can and frequently may replicate in the lower airways. This is likely to be more common among individuals with lower airway signs and symptoms and may contribute to pneumonia, which may occur rarely in immunocompromised adults and children. Further, this lower airway involvement may contribute to asthma and COPD exacerbations.[141,152]

Prevention

There are currently no available vaccines against HRV. The cornerstone against transmission is diligent hand hygiene. Symptomatic adults should be advised to wash hands frequently and use disposable tissues. Currently, droplet precautions are recommended to prevent nosocomial transmissions of rhinoviral infections.

Diagnostic Microbiology

While HRV can be grown in cell culture, molecular diagnostics are more sensitive and are currently widely available.[141,153] Typical cytopathic effects can be seen when HRV are grown on human embryonic kidney and human fibroblast cell lines, including WI-38, human foreskin fibroblasts (HFF), MRC-5 and HeLa (see Figure 173-3). HRV are acid labile which allows differentiation from other enteroviruses; they preferentially grow at pH 7.0 and not at 3.0.[148] Most molecular assays target the 5'UTR, a region highly conserved among all HRVs and enteroviruses; as a result, most molecular assays detect but do not differentiate between HRV and enteroviruses, although newer diagnostic platforms are circumventing this problem.[141,153]

Clinical Manifestations

The most common manifestations of HRV infections include the common cold and exacerbations of asthma and COPD, although more serious infections, including pneumonia, have been described, particularly in immunocompromised adults and children. Following a typically 2-day incubation period, patients with colds then develop watery rhinorrhea, nasal congestion, sneezing, cough, sore throat, headaches and sometimes fever that last 7–14 days.[154] Rhinoviral colds cannot be differentiated from those caused by other pathogens. Primary viral

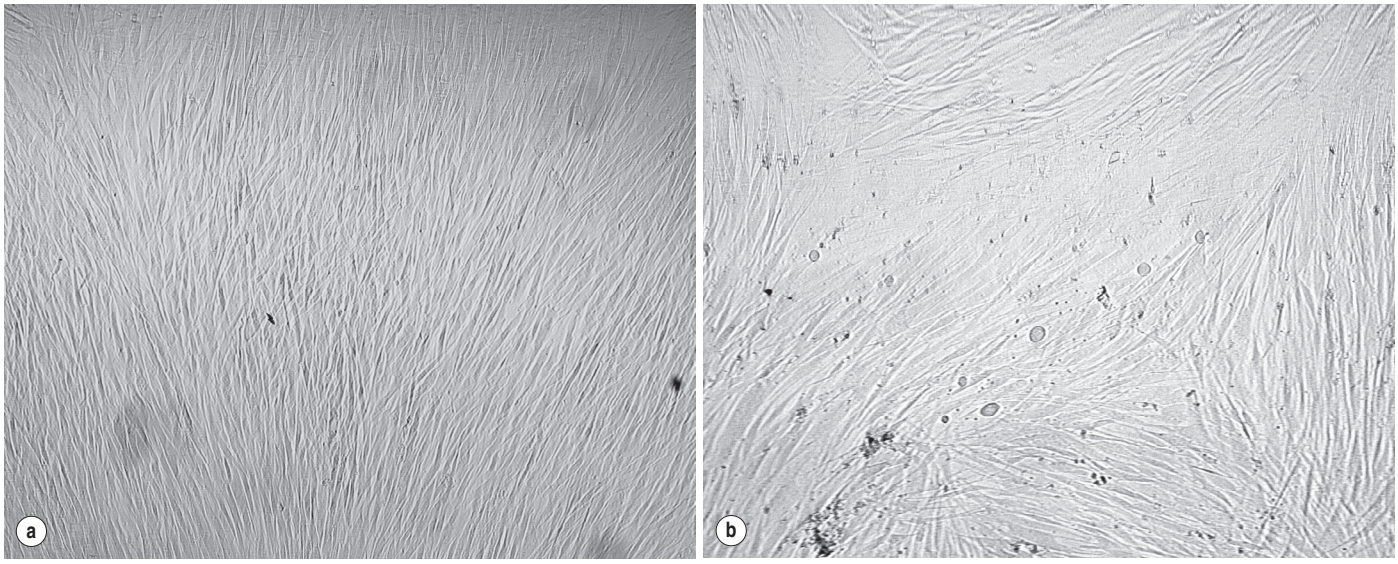

Figure 173-3 Cytopathic effect caused by rhinovirus on human foreskin fibroblasts (HFF) cell line culture. (a) Uninoculated cell line. (b) Formation of small teardrop- to oval-shaped highly refractile cells indicative of adenovirus-induced cytopathic effect.

otitis or rhinosinusitis may also occur with HRV and typically occurs earlier than bacterial superinfections that occur later in the course of colds. Lower airway infections, including croup, bronchiolitis and pneumonia have been described.[148,155,156] HRV are common triggers for exacerbation of asthma and COPD and may be caused by the direct infection of the lower airway or the stimulation of inflammatory, immunological, or neurogenic mechanisms.[157]

Management

While a number of small molecule antivirals (capsid-binding agents: pleconaril, vapendavir and pirodavir; 3C protease inhibitors: rupintrivir; soluble ICAM-1: tremacamra) and complementary medical interventions (echinacea, zinc) have been studied for the treatment of rhinoviral colds, none has been approved for use. Interferons have been shown to prevent HRV colds, but they have not been documented to have positive therapeutic effects. As a result, the mainstay of treatment of rhinoviral colds remains symptomatic treatment with analgesic agents, decongestants, antihistamines and antitussives.[141]

Parainfluenza Virus

Nature

Parainfluenza viruses (PIVs) are single-stranded, enveloped RNA viruses belonging to the genus *Paramyxovirus* in the Paramyxoviridae family.[158,159] The single strand of negative-sense RNA encodes at least six viral proteins: the nucleocapsid protein (NP), the phosphoprotein (P), the matrix protein (M), the fusion glycoprotein (F), the hemagglutinin-neuraminidase glycoprotein (HN), and the RNA polymerase (L).[160] There are four major serotypes of human PIV (PIV-1, 2, 3, 4) that are defined by complement fixation and hemagglutinating antigens.[161–163] PIV-1 and 3 are members of the genus *Respirovirus*, whereas PIV-2 and 4 are members of the genus *Rubulavirus*.

Epidemiology

Initial infection with parainfluenza typically occurs early in childhood, most commonly in children less than 5 years old. Serologic studies have demonstrated that PIV-3 affects up to 50% of children within the first year of life with PIV-1 and 2 causing initial infections later, generally between 3 and 5 years old.[164,165] Most adults have antibodies to PIV. PIV is responsible for 20–40% of lower respiratory tract infections and is the second most common viral cause of hospitalization in children.[166] With contemporary molecular diagnostics, PIV has also been demonstrated to be the second or third most commonly isolated virus in hospitalized adults, aged 16–64 years old.[167–169] In both adults and children, re-infection is common despite the presence of antibodies formed during prior infection.[170]

In tropical and subtropical regions, parainfluenza viruses do not show seasonal variations, while in temperate regions, such as the USA, PIV-1 and PIV-2 cause seasonal outbreaks in the fall (September–December) and PIV-3 causes epidemics during the spring (April–June).[171,172] In years when there is no PIV-1 circulating (typically even-numbered years), PIV-3 activity is generally greater.[172] PIV-4, which generally causes a milder degree of illness, is more common in the autumn and winter months.[173]

Parainfluenza is one of the more common causes of respiratory viral infections in patients with hematologic malignancies, hematopoietic stem cell transplantation, or solid organ transplantation and is associated with a high rate of progressive disease involving the lower airway and an increased mortality rate.[174,175]

Pathogenicity

Parainfluenza viruses are transmitted by direct person-to-person contact through large respiratory droplets and contact with fomites contaminated with respiratory secretions.[176] Clinical symptoms typically develop after a 2–6 day incubation period. After initial infection of upper airway ciliated epithelial cells,[177] infection spreads to the large and small airways.[178] Peak symptoms correlate with peak viral replication. Infection of the larynx and upper trachea is associated with croup whereas bronchiolitis and pneumonia are associated with infection of the distal airways.[179]

The host immune response appears to play a more important role in the pathogenesis of PIV infection than direct viral replication.[179,180] Specifically, the key host immune responses which contribute to viral clearance, including the innate immune responses, CD8+ and CD4+ T-cell responses, interferon production and local and system IgA, IgE and IgG responses, are also the key drivers of the clinical signs and symptoms of infection.[179] While both humoral and cellular immune responses are critical for clearance of infection, defects in cytotoxic T-cell response are associated with progressive disease, as is demonstrated by the increased risk of progressive and fatal disease in HSCT recipients.[174]

Prevention

There is currently no licensed vaccine against PIV, although several candidates that appear to produce neutralizing antibodies to the HN and F proteins are being investigated.[163] Most of the contemporary vaccine candidates have focused on reverse genetic technology applied to live, attenuated intranasal vaccines.[159] Further, some of the candidate vaccines afford protection against both PIV and RSV.[181] Nosocomial outbreaks of PIV infection have been documented, highlighting the importance of standard and contact precautions and use of private rooms whenever possible.[24] Respiratory precautions are not necessary, because the droplets are large and do not aerosolize.

Diagnostic Microbiology

PIV can be diagnosed by culture, antigen detection, or nucleic acid testing. PIV is stable in viral transport medium at 4°C for up to 5 days. Freezing to −20°C does decrease infectivity of the virus, but long-term storage can be achieved easily by adding sucrose or glycerol to the holding media and freezing to less than −70°C.[162] LLC-MK2 rhesus monkey kidney, Vero African green monkey kidney, and NCI-H292 human lung carcinoma cell lines using standard and shell vial techniques can be used to culture PIV;[163] fixed-mixed cell lines, such as R-Mix (Diagnostic Hybrids, Athens, OH), have a sensitivity that approaches that of standard cell culture lines for the detection of PIV.[162,182–184] Trypsin is required for the growth of PIV-1 and PIV-4 but not PIV-2 and PIV-3.[163] Hemadsorption-inhibition, hemagglutination inhibition or immunofluorescence is used to identify the PIV in cultures (see Figures 173-4 and 173-5).[162] There are currently no approved rapid antigen kits available for the detection of PIV although monoclonal antibodies can be used to detect the PIV serotypes in primary patient samples and from cell cultures.[162,185]

Polymerase chain reaction (PCR)-based tests, typically directed toward the hemagglutinin-neuraminidase (HN) gene, are now considered the gold standard for the diagnosis of PIV. Such assays typically have higher yield over cultures for the diagnosis of PIV.[186–189] Despite the advantages of highly multiplexed PCR-based systems in detecting a wide range of viruses, the diagnostic yield for PIV is not consistent for all available systems.[190–192]

Clinical Manifestations

Parainfluenza viruses cause a variety of upper and lower respiratory tract illnesses, ranging from mild cold-like syndromes to life-threatening pneumonias.

PEDIATRIC DISEASE

Most infections in children are limited to the upper respiratory tract with only about 15% involving the lower respiratory tract.[193] Upper tract disease may involve the entire airway, including the middle ear and sinuses.[193–195] PIV-1 and -2 are associated with croup or laryngotracheobronchitis in children, which is characterized by fever, rhinorrhea and pharyngitis and is typically followed by a barking cough.[161,196]

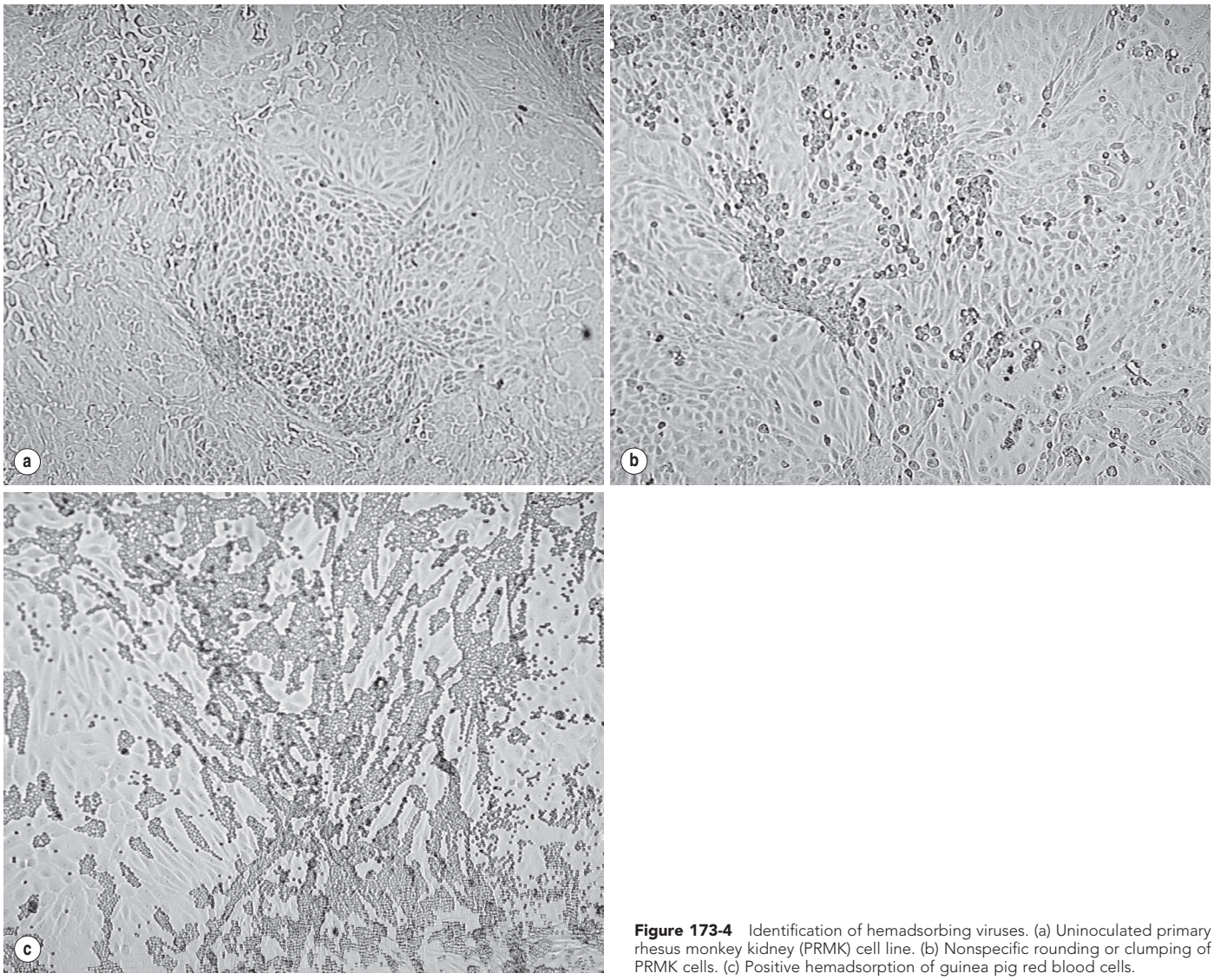

Figure 173-4 Identification of hemadsorbing viruses. (a) Uninoculated primary rhesus monkey kidney (PRMK) cell line. (b) Nonspecific rounding or clumping of PRMK cells. (c) Positive hemadsorption of guinea pig red blood cells.

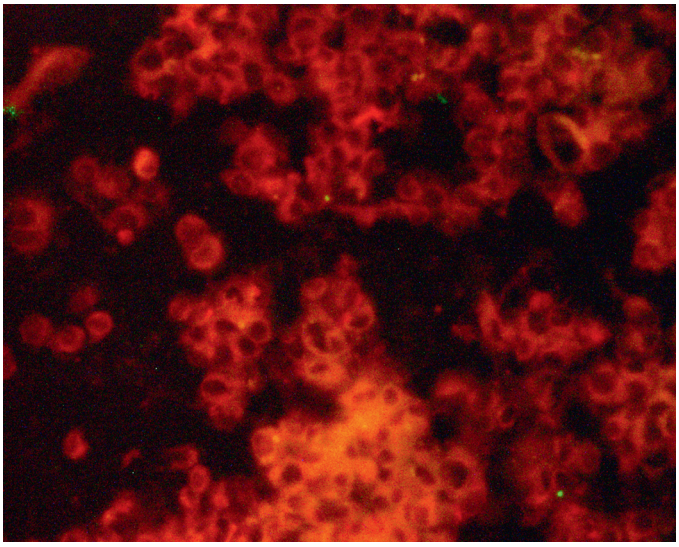

Figure 173-5 Differentiation of hemadsorbing viruses by means of IFA. Negative control.

PIV-3 is associated with more distal lung involvement, particularly in the first 6 months of life.[161,197] PIV-4 is generally associated with mild upper respiratory infections or asymptomatic infection, although more severe disease has been described in children with underlying cardiopulmonary disease or immune compromise.[161,198] Nonrespiratory complications of PIV include meningitis, myocarditis, pericarditis, and Guillain–Barré Syndrome.[199–202]

ADULT DISEASE

PIV is responsible for 1–15% of acute, typically mild respiratory illnesses in adults with higher rates and more significant morbidity in older adults.[203,204] Higher rates of pneumonia (11–14%) have been described in the elderly.[205,206] Wider use of more sensitive molecular assays have demonstrated that PIV is a significant cause of community-acquired pneumonia and exacerbations of asthma and chronic bronchitis.[207–213]

PARAINFLUENZA VIRUS IN IMMUNOCOMPROMISED PATIENTS

Parainfluenza virus causes significant direct and indirect morbidity and mortality among immunocompromised adults and children.[174] PIV can cause asymptomatic shedding, particularly among HSCT

recipients, which may contribute to nosocomial spread.[214] Progression to the lower respiratory tract complicates 13–43% of cases and is associated with enhanced mortality.[175,214–219] Rarely, lower airway involvement will be characterized by small peribronchial nodules on CT radiography.[220] Steroids, in a dose dependent manner, are associated with increased risk of progression from upper to lower tract disease and mortality.[217,221,222] Other risk factors for progressive disease include onset early post-transplant, allogeneic (matched unrelated and matched related) donor, presence of lymphocytopenia, active graft-versus-host disease and pediatric age group.[174,175,221,223] Reduced intensity conditioning appears to be a risk factor for late onset (≥ 30 days) PIV infection.[223] PIV infection is associated with a 17.9 greater odds of developing severe airflow declines following infection.[224]

Parainfluenza has also been demonstrated to cause severe disease in patients undergoing chemotherapy, with enhanced risk of progression to severe lower respiratory infection in patients with severe lymphocytopenia.[225,226]

Parainfluenza virus infections among lung transplant recipients have been associated with significant short- and long-term pulmonary dysfunction.[71,227] In addition to local acute, direct virologic effects on the lung of transplant recipients, PIV, particularly with lower tract infection, is associated with development or progression of bronchiolitis obliterans or bronchiolitis obliterans syndrome (BOS).[71,227–230]

Management

There are currently no antivirals with proven efficacy approved for the treatment of PIV infections. For children with croup, glucocorticoids and nebulized epinephrine have been associated with improved clinical outcomes.[231,232] When approved antivirals such as ribavirin have been studied, typically in immunocompromised patients, treatment was not associated with reduction in viral shedding or mortality.[175,202,216–219,223,227,233–240] One investigational agent, DAS181, which acts by cleaving the sialic acid receptors from the surface of human respiratory epithelial cells, has been shown to improve symptoms, oxygenation, pulmonary function and nasopharyngeal viral loads in treated subjects with PIV infection.[241–244]

Human Metapneumovirus

Nature

Human metapneumovirus (hMPV) is a nonsegmented negative-sense RNA virus that is a member of the Pneumovirinae subfamily of the Paramyxoviridae. There are two subgroups each with two clades of hMPV (A1, A2, B1 and B2); although all four subtypes typically co-circulate, each season often has one subtype that predominates.[245,246] hMPV codes for similar genes to RSV except that the order is slightly different (3'-N-P-M-F-M2-SH-G-L-5') and there are no NS1 or NS2 genes.[247]

Epidemiology

hMPV results in typically self-limited upper and lower respiratory tract infections in all age groups with a global distribution.[248] Symptomatic disease appears to be more common among young children and the elderly.[249] Risk factors for hospitalization from hMPV infections in children include age <6 months and the presence of three or more children in the home, whereas female gender, prematurity and genotype B infection were risk factors for severe disease.[250] About 4–9% of children require hospitalization for severe respiratory symptoms or pneumonia.[248,250] hMPV is identified in 8.5% of adults hospitalized with acute cardiorespiratory illness.[251] Risk factors for hospitalization and severe disease in the elderly include a diagnosis of COPD, asthma, cancer or lung transplantation. The average rate of hospitalization for hMPV (9.8 per 10 000 residents) is similar to the rate for influenza (11.8 per 10 000 residents) in adults ≥ 50 years old.[252] Severe, sometimes fatal infections, including pneumonia, have been described more frequently among immunocompromised patients and the elderly.[248,251,253]

Most children are infected by 5 years of age.[254] hMPV is the second most frequently isolated viral pathogen, after RSV, in children presenting with bronchiolitis.[248] Infections typically occur in the late winter and spring in temperate climates and in the spring and summer in subtropical regions.[254,255] Epidemic peaks typically occur 1–2 months later than RSV epidemics annually.[248]

Pathogenicity

hMPV is likely to be transmitted by direct or close contact with contaminated secretions; large particle aerosols, droplets and fomites may also result in transmission. There is typically a 5-day interval between onset of symptoms in an index case and the onset of symptoms in household exposed contacts.[256] hMPV G protein binds to the integrin α-V-β-1 receptors on respiratory epithelial cells.[248,257] Local replication in the airway is associated with upper respiratory tract signs and significant airway inflammation.[258] Viral replication also induces mucus hyperproduction and hyperplasia of the respiratory epithelium which may result in airway obstruction and hyperresponsiveness to methacholine challenge.[259] Replication results in an increase in IL-8, IL-12, tumor necrosis factor, IL-6, and IL-1β, although levels are lower than those observed with RSV infection.[260]

Prevention

There is currently no licensed vaccine against hMPV, although several are currently under investigation. Most of the candidate vaccines utilize chimeric viruses, live-attenuated viruses and subunits of the virus.[248] Nosocomial outbreaks have been described which highlight the importance of droplet and contact precautions to prevent transmission.

Diagnostic Microbiology

Although hMPV may be grown in tertiary monkey kidney cells, Vero cells, LLC-MK2-cells, BEAS-2B cells, A549 cells and HepG2 cells, yields are often low and require prolonged incubation.[248] Cytopathic effects typically can be observed after 10–21 days and range from syncytia formation to rounding of the cells.[254] Direct immunofluorescence assays (DFA) with virus-specific antibodies are available and allow detection of virus in direct patient specimens and cell culture. DFA is less sensitive than PCR but is often more widely available.[248] Molecular diagnostics, as either a singleplex or multiplex assay, are commercially available and have the highest yield in detecting hMPV. Most PCR assays detect all hMPV genotypes and rely on conserved and essential regions within the N, F or L gene.[248,261] The sensitivity varies by assay and is lower for most multiplex assays as compared to singleplex assays. Serologic diagnosis can be made by ELISA methods but is not widely available.[248]

Clinical Manifestations

hMPV is associated with respiratory tract infections in all age groups. Among children, the most common symptoms are rhinorrhea, cough or fever, although conjunctivitis, vomiting, diarrhea and rash have been reported infrequently.[262] Encephalitis is a rare manifestation of hMPV infection.[263,264] Although hMPV and RSV are generally indistinguishable, fever is more frequent in children with hMPV, while rhinorrhea is more commonly observed in RSV-infected individuals.[248,265] Children appear to be at higher risk of developing bronchiolitis, pneumonia, croup and possibly asthma exacerbation, particularly when younger than 2 years of age. Most hospitalizations are the result of bronchiolitis and pneumonia in children. Coinfection with hMPV and RSV results in more severe disease and an increased risk for admission to the ICU and a 10-fold increase in the need for mechanical ventilation.[266] The typical duration of symptoms is around a week with viral shedding typically for 1–2 weeks.[254,255] Repeat infection has clearly been documented although subsequent infections are typically limited to

the upper airway. Severe, sometimes fatal infections, often involving the lower airway, have been described in adults and children with malignancy, hematopoietic stem cell and solid organ transplantation. The severity of illness is likely related to reduced cellular immune responses. Likewise, elderly adults, adults with severe cardiopulmonary disease, and residents of long-term care facilities have a higher incidence of hMPV infection with an increased risk of lower airway involvement, complications including bacterial superinfections, and death.[248] Although asymptomatic infection is common for otherwise healthy adults, such individuals may present with cold- and influenza-like illnesses.[267] Adults commonly present with cough, nasal congestion and rhinorrhea, but rarely have fever.[130] The role of hMPV in exacerbations of COPD is unclear as one large study demonstrated an association whereas another failed to identify hMPV in patients with COPD exacerbations.[268,269]

Management

Generally, patients can be safely managed with supportive care, including supplemental oxygen and intravenous hydration when indicated. Bronchodilators and corticosteroids are often used empirically although there are no controlled trials of these medications for hMPV.[248] Ribavirin shows equivalent activity against hMPV (mean EC50 74μM) and RSV (mean EC50 88 μM).[270] Likewise, standard IgIV has neutralizing antibodies against hMPV (10 $\log_2$/0.05mL) and RSV (11 $\log_2$/0.05mL), whereas RSV-specific monoclonal antibodies have no activity against hMPV.[270] Neither agent has been studied prospectively for the treatment of hMPV, although there are a number of case series demonstrating clinical response in some cases.[135,271–273]

Human Bocavirus

Nature

Human bocaviruses were first identified through molecular screening in 2005.[274] Bocaviruses are non-enveloped, linear, single-stranded DNA viruses that are members of the Parvoviridae family.[275] There are currently four species of human bocaviruses (HBoV1-4); HBoV2 can be further divided into two strains (A and B).[276] The viral genome encodes two forms of the nonstructural protein (NS1), nuclear phosphoprotein (NP1) and two major structural proteins (VP1 and VP2).[275,277]

Epidemiology

Human bocaviruses have been detected worldwide from respiratory and stool specimens. HBoV1 is predominantly a respiratory pathogen whereas HBoV2-4 have been mostly found in stool and are less clearly associated with respiratory tract infections. Although HBoV1 is most commonly detected in children 6–24 months old, it has been detected less frequently in other age groups.[275] Infection appears to occur year round with peaks of detection in the winter and spring. Although HBoV2-4 are felt to be predominantly gastrointestinal viruses, HBoV2 has been detected in nasopharyngeal specimens.[278,279] Most children have antibodies to HBoV1 by age 6 and most adults have detectable HBoV1 antibodies.[275]

Pathogenicity

A key challenge to understanding the pathogenesis and clinical implications of HBoV is the fact that up to 83% of HBoV DNA-positive respiratory samples have evidence of coinfection with other respiratory viruses. Further, the virus is often shed for at least 6 months after initial infection in immunocompetent individuals. In studies of wheezing children, 64% had serologic evidence of primary infection. Further, several studies that include asymptomatic control patients have documented an association between presence of HBoV and clinical symptoms. Additionally, these studies have documented a positive correlation between high copy numbers of HBoV1 DNA and HBoV1 monoinfection and respiratory symptoms.[275]

Although the exact route of transmission of human bocaviruses is unknown, transmission by inhalation or contact with infectious sputum, feces or urine is probable. The mechanism of cell entry and host range are not known. Although most HBoV1 is detected in the upper airway, clinical symptoms and positive BAL specimens suggest that HBoV1 is capable of infecting the lower airways down to at least the bronchioles.[275] HBoV1 DNA is detectable in the serum suggesting dissemination beyond the airway. Likewise, HBoV1 can rarely be detected in stool with and without gastrointestinal symptoms suggesting passive spread from the respiratory to the gastrointestinal tract.[275]

In vitro, HBoV1 induces IL-13, IFN-γ and IL-10 in CD4+ T cells. Children with HBoV1-associated bronchiolitis have increased levels of IFN-γ, IL-2 and IL-4 in nasopharyngeal aspirates. [280–282]

Prevention

There is no vaccine available for HBoV.[275] Specialist advice should be sought regarding appropriate isolation practice for patients with proven HBoV infections.

Diagnostic Microbiology

Diagnosis of HBoV depends on molecular and serologic methods. While HBoV can be cultured on differentiated human airway epithelial cells, these are not widely available in the clinical microbiology laboratory.[283] PCR is not an optimal diagnostic method because of prolonged shedding and the potential for persistent infection in some tissues. Presence of HBoV1 DNA in serum may provide enhanced specificity of active infection as it correlates with HBoV1-specific IgM 61% of the time.[284,285] Quantitative virology may also help improve the probability that HBoV detection is associated with the clinical presentation, as high viral loads ($>2 \times 10^8$ genomes/mL) in nasopharyngeal aspirates appear to correlate with illness severity and fewer coinfections.[275,286] Serology, particularly IgG avidity EIA, may also increase the probability that detected HBoV is truly associated with clinical disease.[275] Given the limitations of diagnostic strategies, most would recommend using two diagnostic modalities to diagnose HBoV infection.

Clinical Manifestations

Because of the limitations outlined above, there remains controversy in the causal link between HBoV1 and respiratory disease. A number of studies have correlated HBoV1 detection and the common cold, asthma, acute wheezing, bronchiolitis, pneumonia, acute otitis media and plastic bronchitis. The strongest link in the available literature is that HBoV1 may be the cause of wheezing and pneumonia. One study found a link between HBoV1 DNA in serum, cerebrospinal fluid (CSF) or stool and Kawasaki disease,[287] although this study was not confirmed by two other groups.[278,288]

Management

No comparative studies of available antivirals have been conducted in patients with HBoV infection. Supportive measures are the only currently available therapy.[275]

References available online at expertconsult.com.

KEY REFERENCES

Advisory Committee on Healthcare Infection Control Practices, Centers for Disease Control and Prevention: Guidelines for preventing health-care-associated pneumonia, 2003 recommendations of the CDC and the Healthcare Infection Control Practices Advisory Committee. *Respir Care* 2004; 49:926-939.

Centers for Disease Control and Prevention: Acute respiratory disease associated with adenovirus serotype 14 – four states, 2006–2007. *MMWR Morb Mortal Wkly Rep* 2007; 56:1181-1184.

Chan J.F., Lau S.K., To K.K., et al.: Middle East respiratory syndrome coronavirus: another zoonotic

betacoronavirus causing SARS-like disease. *Clin Microbiol Rev* 2015; 28(2):465-522.

Chan J.F., Yao Y., Yeung M.L., et al.: Treatment With Lopinavir/Ritonavir or Interferon-β1b Improves Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. *J Infect Dis* 2015; 212(12):1904-1913.

Corti D., Zhao J., Pedotti M., et al.: Prophylactic and post-exposure efficacy of a potent human monoclonal antibody against MERS coronavirus. *Proc Natl Acad Sci USA* 2015; 112(33):10473-10478.

DeVincenzo J., Lambkin-Williams R., Wilkinson T., et al.: A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc Natl Acad Sci USA* 2010; 107: 8800-8805.

DeVincenzo J.P., Whitley R.J., Mackman R.L., et al.: Oral GS-5806 activity in a respiratory syncytial virus challenge study. *N Engl J Med* 2014; 371(8):711-722.

Drosten C., Meyer B., Müller M.A., et al.: Transmission of MERS-coronavirus in household contacts. *N Engl J Med* 2014; 371(9):828-835.

Garbino J., Gerbase M.W., Wunderli W., et al.: Respiratory viruses and severe lower respiratory tract complications in hospitalized patients. *Chest* 2004; 125: 1033-1039.

Gern J.E.: Rhinovirus and the initiation of asthma. *Curr Opin Allergy Clin Immunol* 2009; 9:73-78.

Hall C.B.: Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001; 344:1917-1928.

Hui D.S., Memish Z.A., Zumla A.: Severe acute respiratory syndrome vs. the Middle East respiratory syndrome. *Curr Opin Pulm Med* 2014; 20:233-241.

Jacobs S.E., Lamson D.M., St George K., et al.: Human rhinoviruses. *Clin Microbiol Rev* 2013; 26:135-162.

Khalafalla A.I., Lu X., Al-Mubarak A.I., et al.: MERS-CoV in Upper Respiratory Tract and Lungs of Dromedary Camels, Saudi Arabia, 2013-2014. *Emerg Infect Dis* 2015; 21(7):1153-1158.

Korea Centers for Disease Control and Prevention: Middle East Respiratory Syndrome Coronavirus Outbreak in the Republic of Korea, 2015. *Osong Public Health Res Perspect.* 2015; 6(4):269-278.

Louie J.K., Kajon A.E., Holodniy M., et al.: Severe pneumonia due to adenovirus serotype 14: a new respiratory threat? *Clin Infect Dis* 2008; 46:421-425.

Müller M.A., Meyer B., Corman V.M., et al.: Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. *Lancet Infect Dis* 2015; 15(5):559-564.

Oboho I.K., Tomczyk S.M., Al-Asmari A.M., et al.: 2014 MERS-CoV outbreak in Jeddah – a link to health care facilities. *N Engl J Med* 2015; 372(9):846-854.

Omrani A.S., Saad M.M., Baig K., et al.: Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. *Lancet Infect Dis* 2014; 14(11):1090-1095.

Peiris J.S., Yuen K.Y., Osterhaus A.D., et al.: The severe acute respiratory syndrome. *N Engl J Med* 2003; 349: 2431-2441.

Raza K., Ismailjee S.B., Crespo M., et al.: Successful outcome of human metapneumovirus (hMPV) pneumonia in a lung transplant recipient treated with intravenous ribavirin. *J Heart Lung Transplant* 2007; 26:862-864.

Schildgen V., van den Hoogen B., Fouchier R., et al. Human Metapneumovirus: lessons learned over the first decade. *Clin Microbiol Rev* 2011;24:734-754.

Walsh E.E.: Respiratory syncytial virus infection in adults. *Semin Respir Crit Care Med* 2011; 32:423-432.

REFERENCES

1. Berk J.: Adenoviridae. In: Knipe D.M., Howley P.M., eds. *Fields Virology*, 6th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2013:1704-1731.
2. Wold W.S.M., Ison M.G.: Adenoviruses. In: Knipe D.M., Howley P.M., eds. *Fields Virology*, 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013:1732-1767.
3. Kajon A.E., Erdman D.D.: Assessment of genetic variability among subspecies b1 human adenoviruses for molecular epidemiology studies. *Methods Mol Med* 2007; 131:335-355.
4. Ison M.G.: Adenovirus infections in transplant recipients. *ClinInfect Dis* 2006; 43:331-339.
5. Lewis P.F., Schmidt M.A., Lu X., et al.: A community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. *J Infect Dis* 2009; 199:1427-1434.
6. Centers for Disease Control and Prevention: Acute respiratory disease associated with adenovirus serotype 14 – four states, 2006–2007. *MMWR Morb Mortal Wkly Rep* 2007; 56:1181-1184.
7. Cooper R.J., Hallett R., Tullo A.B., et al.: The epidemiology of adenovirus infections in Greater Manchester, UK 1982–96. *Epidemiol Infect* 2000; 125:333-345.
8. Li L., Phan T.G., Nguyen T.A., et al.: Molecular epidemiology of adenovirus infection among pediatric population with diarrhea in Asia. *Microbiol Immunol* 2005; 49:121-128.
9. Liu J., Nian Q.G., Zhang Y., et al.: In vitro characterization of human adenovirus type 55 in comparison with its parental adenoviruses, types 11 and 14. *PLoS ONE* 2014; 9:e100665.
10. Lu Q.B., Tong Y.G., Wo Y., et al.: Epidemiology of human adenovirus and molecular characterization of human adenovirus 55 in China, 2009–2012. *Influenza Other Respir Viruses* 2014; 8:302-308.
11. Hoke C.H. Jr, Snyder C.E. Jr: History of the restoration of adenovirus type 4 and type 7 vaccine, live oral (Adenovirus Vaccine) in the context of the Department of Defense acquisition system. *Vaccine* 2013; 31:1623-1632.
12. Kajon A.E., Moseley J.M., Metzgar D., et al.: Molecular epidemiology of adenovirus type 4 infections in US military recruits in the postvaccination era (1997–2003). *J Infect Dis* 2007; 196:67-75.
13. Wang H., Li Z.Y., Liu Y., et al.: Desmoglein 2 is a receptor for adenovirus serotypes 3, 7, 11 and 14. *Nat Med* 2011; 17:96-104.
14. Waddington S.N., McVey J.H., Bhella D., et al.: Adenovirus serotype 5 hexon mediates liver gene transfer. *Cell* 2008; 132:397-409.
15. Duncan S.J., Gordon F.C., Gregory D.W., et al.: Infection of mouse liver by human adenovirus type 5. *J Gen Virol* 1978; 40:45-61.
16. Arbizu R.A., Aljomah G., Kozielski R., et al.: Intussusception Associated with Adenovirus. *J Pediatr Gastroenterol Nutr* 2014; 59(5):e41.
17. Liu Q., Muruve D.A.: Molecular basis of the inflammatory response to adenovirus vectors. *Gene Ther* 2003; 10:935-940.
18. Nazir S.A., Metcalf J.P.: Innate immune response to adenovirus. *J Investig Med* 2005; 53:292-304.
19. Walls T., Shankar A.G., Shingadia D.: Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. *Lancet Infect Dis* 2003; 3:79-86.
20. Leen A.M., Bollard C.M., Myers G.D., et al.: Adenoviral infections in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; 12:243-251.
21. Kojaoghlanian T., Flomenberg P., Horwitz M.S.: The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 2003; 13:155-171.
22. Kuschner R.A., Russell K.L., Abuja M., et al.: A phase 3, randomized, double-blind, placebo-controlled study of the safety and efficacy of the live, oral adenovirus type 4 and type 7 vaccine, in U.S. military recruits. *Vaccine* 2013; 31:2963-2971.
23. Advisory Committee on Healthcare Infection Control Practices, Centers for Disease Control and Prevention: Guidelines for preventing health-care-associated pneumonia, 2003 recommendations of the CDC and the Healthcare Infection Control Practices Advisory Committee. *Respir Care* 2004; 49:926-939.
24. Siegel J.D., Rhinehart E., Jackson M., et al.: 2007 Guideline for isolation precautions: preventing transmission of infectious agents in health care settings. *Am J Infect Control* 2007; 35:S65-S164.
25. Fox J.P., Brandt C.D., Wassermann F.E., et al.: The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VI. Observations of adenovirus infections: virus excretion patterns, antibody response, efficiency of surveillance, patterns of infections, and relation to illness. *Am J Epidemiol* 1969; 89:25-50.
26. Hillis W.D., Cooper M.R., Bang F.B.: Adenovirus infections in West Bengal. I. Persistence of viruses in infants and young children. *Indian J Med Res* 1973; 61:980-988.
27. Ison M.G., Hayden R.T.: Adneovirus. In: Hayden R.T., Carroll K.C., Tang Y.W., et al., eds. *Diagnostic microbiology of the immunocompromised host*. Washington, DC: ASM Press; 2008:129-140.
28. Breuer S., Rauch M., Matthes-Martin S., et al.: Molecular diagnosis and management of viral infections in hematopoietic stem cell transplant recipients. *Mol Diagn Ther* 2012; 16:63-77.
29. Wessels E., Rusman L.G., van Bussel M.J., et al.: Added value of multiplex Luminex Gastrointestinal Pathogen Panel (xTAG GPP) testing in the diagnosis of infectious gastroenteritis. *Clin Microbiol Infect* 2014; 20(3):O182-O187.
30. Bruno B., Gooley T., Hackman R.C., et al.: Adenovirus infection in hematopoietic stem cell transplantation: effect of ganciclovir and impact on survival. *Biol Blood Marrow Transplant* 2003; 9:341-352.
31. Chakrabarti S., Mautner V., Osman H., et al.: Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 2002; 100:1619-1627.
32. Erard V., Huang M.L., Ferrenberg J., et al.: Quantitative real-time polymerase chain reaction for detection of adenovirus after T cell-replete hematopoietic cell transplantation: viral load as a marker for invasive disease. *Clin Infect Dis* 2007; 45:958-965.
33. Leruez-Ville M., Minard V., Lacaille F., et al.: Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. *Clin Infect Dis* 2004; 38:45-52.
34. Edwards K.M., Thompson J., Paolini J., et al.: Adenovirus infections in young children. *Pediatrics* 1985; 76:420-424.
35. Pacini D.L., Collier A.M., Henderson F.W.: Adenovirus infections and respiratory illnesses in children in group day care. *J Infect Dis* 1987; 156:920-927.
36. Xie L., Yu X.F., Sun Z., et al.: Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. *J Clin Microbiol* 2012; 50:1879-1888.
37. Nelson K.E., Gavitt F., Batt M.D., et al.: The role of adenoviruses in the pertussis syndrome. *J Pediatr* 1975; 86:335-341.
38. Sturdy P.M., Court S.D., Gardner P.S.: Viruses and whooping-cough. *Lancet* 1971; 2:978-979.
39. Carballal G., Videla C., Misirlian A., et al.: Adenovirus type 7 associated with severe and fatal acute lower respiratory infections in Argentine children. *BMC Pediatr* 2002; 2:6.
40. Chuang Y., Chiu C.H., Wong K.S., et al.: Severe adenovirus infection in children. *J Microbiol Immunol Infect* 2003; 36:37-40.
41. Kajon A.E., Murtagh P., Garcia Franco S., et al.: A new genome type of adenovirus 3 associated with severe lower acute respiratory infection in children. *J Med Virol* 1990; 30:73-76.
42. Lang W.R., Howden C.W., Laws J., et al.: Bronchopneumonia with serious sequelae in children with evidence of adenovirus type 21 infection. *BMJ* 1969; 1:73-79.
43. Levy Y., Nitzan M., Beharab A., et al.: Adenovirus type 3 infection with systemic manifestation in apparently normal children. *Isr J Med Sci* 1986; 22:774-778.
44. Louie J.K., Kajon A.E., Holodniy M., et al.: Severe pneumonia due to adenovirus serotype 14: a new respiratory threat? *Clin Infect Dis* 2008; 46:421-425.
45. Kaufman H.E.: Treatment of viral diseases of the cornea and external eye. *Prog Retin Eye Res* 2000; 19:69-85.
46. Dawson C.R., O'Day D., Vastine D.: Letter: Adenovirus 19, a cause of epidemic keratoconjunctivitis, not acute hemorrhagic conjunctivitis. *N Engl J Med* 1975; 293:45-46.
47. Ersoy Y., Otlu B., Turkcuoglu P., et al.: Outbreak of adenovirus serotype 8 conjunctivitis in preterm infants in a neonatal intensive care unit. *J Hosp Infect* 2012; 80:144-149.
48. Centers for Disease Control and Prevention: Adenovirus-associated epidemic keratoconjunctivitis outbreaks – four states, 2008–2010. *MMWR Morb Mortal Wkly Rep* 2013; 62:637-641.
49. Van R., Wun C.C., O'Ryan M.L., et al.: Outbreaks of human enteric adenovirus types 40 and 41 in Houston day care centers. *J Pediatr* 1992; 120:516-521.
50. Bines J.E., Liem N.T., Justice F.A., et al.: Risk factors for intussusception in infants in Vietnam and Australia: adenovirus implicated, but not rotavirus. *J Pediatr* 2006; 149:452-460.
51. Ison M.G., Green M., Practice A.S.T.I.D.: Community of Practice. Adenovirus in solid organ transplant recipients. *Am J Transplant* 2009; 9(Suppl. 4):S161-S165.
52. Mufson M.A., Belshe R.B.: A review of adenoviruses in the etiology of acute hemorrhagic cystitis. *J Urol* 1976; 115:191-194.
53. Ito M., Hirabayashi N., Uno Y., et al.: Necrotizing tubulointerstitial nephritis associated with adenovirus infection. *Human Pathol* 1991; 22:1225-1231.
54. Akiyama H., Kurosu T., Sakashita C., et al.: Adenovirus is a key pathogen in hemorrhagic cystitis associated with bone marrow transplantation. *Clin Infect Dis* 2001; 32:1325-1330.
55. Hofland C.A., Eron L.J., Washecka R.M.: Hemorrhagic adenovirus cystitis after renal transplantation. *Transplantation Proc* 2004; 36:3025-3027.
56. Andreoletti L., Leveque N., Boulagnon C., et al.: Viral causes of human myocarditis. *Arch Cardiovasc Dis* 2009; 102:559-568.
57. Bowles N.E., Ni J., Kearney D.L., et al.: Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol* 2003; 42:466-472.
58. Frange P., Peffault de Latour R., Arnaud C., et al.: Adenoviral infection presenting as an isolated central nervous system disease without detectable viremia in two children after stem cell transplantation. *J Clin Microbiol* 2011; 49:2361-2364.
59. Baschat A.A., Towbin J., Bowles N.E., et al.: Is adenovirus a fetal pathogen? *Am J Obstet Gynecol* 2003; 189:758-763.
60. Florescu D.F., Hoffman J.A., Practice ASTIDCo: Adenovirus in solid organ transplantation. *Am J Transplant* 2013; 13(Suppl. 4):206-211.
61. Florescu D.F., Kwon J.Y., Dumitru I.: Adenovirus infections in heart transplantation. *Cardiol Rev* 2013; 21:203-206.
62. Florescu M.C., Miles C.D., Florescu D.F.: What do we know about adenovirus in renal transplantation? *Nephrol Dial Transplant* 2013; 28:2003-2010.
63. Matthes-Martin S., Feuchtinger T., Shaw P.J., et al.: European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transpl Infect Dis* 2012; 14:555-563.
64. Myers G.D., Krance R.A., Weiss H., et al.: Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant* 2005; 36:1001-1008.
65. Robin M., Marque-Juillet S., Scieux C., et al.: Disseminated adenovirus infections after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcome. *Haematologica* 2007; 92:1254-1257.
66. Suparno C., Milligan D.W., Moss P.A., et al.: Adenovirus infections in stem cell transplant recipients: recent developments in understanding of pathogenesis, diagnosis and management. *Leukemia Lymphoma* 2004; 45:873-885.

67. Humar A., Kumar D., Mazzulli T., et al.: A surveillance study of adenovirus infection in adult solid organ transplant recipients. *Am J Transplant* 2005; 5:2555-2559.
68. Humar A., Doucette K., Kumar D., et al.: Assessment of adenovirus infection in adult lung transplant recipients using molecular surveillance. *J Heart Lung Transplant* 2006; 25:1441-1446.
69. Renaud C., Englund J.A.: Antiviral therapy of respiratory viruses in haematopoietic stem cell transplant recipients. *Antivir Ther* 2012; 17:175-191.
70. Skevaki C.L., Galani I.E., Pararas M.V., et al.: Treatment of viral conjunctivitis with antiviral drugs. *Drugs* 2011; 71:331-347.
71. Vu D.L., Bridevaux P.O., Aubert J.D., et al.: Respiratory viruses in lung transplant recipients: a critical review and pooled analysis of clinical studies. *Am J Transplant* 2011; 11:1071-1078.
72. Morfin F., Dupuis-Girod S., Frobert E., et al.: Differential susceptibility of adenovirus clinical isolates to cidofovir and ribavirin is not related to species alone. *Antivir Ther* 2009; 14:55-61.
73. Morfin F., Dupuis-Girod S., Mundweiler S., et al.: In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther* 2005; 10:225-229.
74. Florescu D.F., Pergam S.A., Neely M.N., et al.: Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012; 18:731-738.
75. Leen A.M., Bollard C.M., Mendizabal A.M., et al.: Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood* 2013; 121:5113-5123.
76. Sukdolak C., Tischer S., Dieks D., et al.: CMV-, EBV- and ADV-specific T cell immunity: screening and monitoring of potential third-party donors to improve post-transplantation outcome. *Biol Blood Marrow Transplant* 2013; 19:1480-1492.
77. El-Sahly H.M., Atmar R.L., Glezen W.P., et al.: Spectrum of clinical illness in hospitalized patients with 'common cold' virus infections. *Clin Infect Dis* 2000; 31:96-100.
78. Zumla A., Hui D.S., Perlman S.: Middle East respiratory syndrome. *Lancet* 2015; 386(9997):995-1007.
79. Peiris J.S., Yuen K.Y., Osterhaus A.D., et al.: The severe acute respiratory syndrome. *N Engl J Med* 2003; 349:2431-2441.
80. Zaki A.M., van Boheemen S., Bestebroer T.M., et al.: Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012; 367:1814-1820.
81. Azhar E.I., El-Kafrawy S.A., Farraj S.A., et al.: Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med* 2014; 370:2499-2505.
82. Peiris J.S., Guan Y., Yuen K.Y.: Severe acute respiratory syndrome. *Nat Med* 2004; 10:S88-S97.
83. Raj V.S., Mou H., Smits S.L., et al.: Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013; 495:251-254.
84. Monto A.S., Lim S.K.: The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974; 129:271-276.
85. Pyrc K., Berkhout B., van der Hoek L.: The novel human coronaviruses NL63 and HKU1. *J Virol* 2007; 81:3051-3057.
86. van Elden L.J., van Loon A.M., van Alphen F., et al.: Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J Infect Dis* 2004; 189:652-657.
87. Severe acute respiratory syndrome (SARS). Available: http://www.who.int/csr/sars/en/.
88. Lee N., Hui D., Wu A., et al.: A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003; 348:1986-1994.
89. Hui D.S., Chow B.K., Chu L.C., et al.: Exhaled air and aerosolized droplet dispersion during application of a jet nebulizer. *Chest* 2009; 135:648-654.
90. Yu I.T., Xie Z.H., Tsoi K.K., et al.: Why did outbreaks of severe acute respiratory syndrome occur in some hospital wards but not in others? *Clin Infect Dis* 2007; 44:1017-1025.
91. Middle East respiratory syndrome coronavirus (MERS-CoV). Available: http://www.who.int/csr/disease/coronavirus_infections/en/.
92. Al-Tawfiq J.A., Hinedi K., Ghandour J., et al.: Middle East respiratory syndrome coronavirus: a case-control study of hospitalized patients. *Clin Infect Dis* 2014; 59:160-165.
93. Al-Abdallat M.M., Payne D.C., Alqasrawi S., et al.: Hospital-associated outbreak of Middle East respiratory syndrome coronavirus: a serologic, epidemiologic, and clinical description. *Clin Infect Dis* 2014; 59(9):1225-1233.
94. Assiri A., McGeer A., Perl T.M., et al.: Hospital outbreak of Middle East respiratory syndrome coronavirus. *N Engl J Med* 2013; 369:407-416.
95. Guery B., Poissy J., el Mansouf L., et al.: Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. *Lancet* 2013; 381:2265-2272.
96. Memish Z.A., Zumla A.I., Al-Hakeem R.F., et al.: Family cluster of Middle East respiratory syndrome coronavirus infections. *N Engl J Med* 2013; 368:2487-2494.
97. Wong C.K., Lam C.W., Wu A.K., et al.: Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol* 2004; 136:95-103.
98. Lee N., Allen Chan K.C., Hui D.S., et al.: Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. *J Clin Virol* 2004; 31:304-309.
99. Ng E.K., Hui D.S., Chan K.C., et al.: Quantitative analysis and prognostic implication of SARS coronavirus RNA in the plasma and serum of patients with severe acute respiratory syndrome. *Clin Chem* 2003; 49:1976-1980.
100. de Wit E., Rasmussen A.L., Falzarano D., et al.: Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc Natl Acad Sci USA* 2013; 110:16598-16603.
101. Munster V.J., de Wit E., Feldmann H.: Pneumonia from human coronavirus in a macaque model. *N Engl J Med* 2013; 368:1560-1562.
102. Groneberg D.A., Poutanen S.M., Low D.E., et al.: Treatment and vaccines for severe acute respiratory syndrome. *Lancet Infect Dis* 2005; 5:147-155.
103. Chan P.K., To W.K., Ng K.C., et al.: Laboratory diagnosis of SARS. *Emerg Infect Dis* 2004; 10: 825-831.
104. Drosten C., Seilmaier M., Corman V.M., et al.: Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. *Lancet Infect Dis* 2013; 13:745-751.
105. Memish Z.A., Al-Tawfiq J.A., Makhdoom H.Q., et al.: Respiratory tract samples, viral load, and genome fraction yield in patients with Middle East respiratory syndrome. *J Infect Dis* 2014; 210(10):1590-1594.
106. Sung J.J., Wu A., Joynt G.M., et al.: Severe acute respiratory syndrome: report of treatment and outcome after a major outbreak. *Thorax* 2004; 59:414-420.
107. Leung W.K., To K.F., Chan P.K., et al.: Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* 2003; 125: 1011-1017.
108. Assiri A., Al-Tawfiq J.A., Al-Rabeeah A.A., et al.: Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013; 13:752-761.
109. Arabi Y.M., Arifi A.A., Balkhy H.H., et al.: Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. *Ann Inter Med* 2014; 160:389-397.
110. Memish Z.A., Al-Tawfiq J.A., Assiri A., et al.: Middle East respiratory syndrome coronavirus disease in children. *Pediatr Infect Dis J* 2014; 33(9):904-906.
111. Stockman L.J., Bellamy R., Garner P.: SARS: systematic review of treatment effects. *PLoS Med* 2006; 3:e343.
112. Chu C.M., Cheng V.C., Hung I.F., et al.: Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 2004; 59:252-256.
113. Soo Y.O., Cheng Y., Wong R., et al.: Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. *Clin Microbiol Infect* 2004; 10:676-678.
114. Sui J., Li W., Murakami A., et al.: Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. *Proc Natl Acad Sci USA* 2004; 101: 2536-2541.
115. Zhu Z., Chakraborti S., He Y., et al.: Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. *Proc Natl Acad Sci USA* 2007; 104:12123-12128.
116. Haagmans B.L., Kuiken T., Martina B.E., et al.: Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat Med* 2004; 10:290-293.
117. Loutfy M.R., Blatt L.M., Siminovitch K.A., et al.: Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. *JAMA* 2003; 290:3222-3228.
118. Falzarano D., de Wit E., Rasmussen A.L., et al.: Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. *Nat Med* 2013; 19:1313-1317.
119. Tang X.C., Agnihothram S.S., Jiao Y., et al.: Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. *Proc Natl Acad Sci USA* 2014; 111:E2018-E2026.
120. Walsh E.E.: Respiratory syncytial virus infection in adults. *Semin Respir Crit Care Med* 2011; 32:423-432.
121. Walsh E.E., Peterson D.R., Falsey A.R.: Risk factors for severe respiratory syncytial virus infection in elderly persons. *J Infect Dis* 2004; 189:233-238.
122. DeVincenzo J.P., Wilkinson T., Vaishnaw A., et al.: Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010; 182:1305-1314.
123. Duncan C.B., Walsh E.E., Peterson D.R., et al.: Risk factors for respiratory failure associated with respiratory syncytial virus infection in adults. *J Infect Dis* 2009; 200:1242-1246.
124. Walsh E.E., Peterson D.R., Kalkanoglu A.E., et al.: Viral shedding and immune responses to respiratory syncytial virus infection in older adults. *J Infect Dis* 2013; 207:1424-1432.
125. Miyairi I., DeVincenzo J.P.: Human genetic factors and respiratory syncytial virus disease severity. *Clin Microbiol Rev* 2008; 21:686-703.
126. Nair H., Nokes D.J., Gessner B.D., et al.: Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; 375:1545-1555.
127. Walsh E.E., Peterson D.R., Falsey A.R.: Is clinical recognition of respiratory syncytial virus infection in hospitalized elderly and high-risk adults possible? *J Infect Dis* 2007; 195:1046-1051.
128. Falsey A.R., Hennessey P.A., Formica M.A., et al.: Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005; 352:1749-1759.
129. Jansen A.G., Sanders E.A., Hoes A.W., et al.: Influenza- and respiratory syncytial virus-associated mortality and hospitalisations. *Eur Respir J* 2007; 30:1158-1166.
130. Falsey A.R., Erdman D., Anderson L.J., et al.: Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 2003; 187:785-790.
131. Lee N., Lui G.C., Wong K.T., et al.: High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. *Clin Infect Dis* 2013; 57:1069-1077.
132. Shah J.N., Chemaly R.F.: Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011; 117:2755-2763.
133. Hynicka L.M., Ensor C.R.: Prophylaxis and treatment of respiratory syncytial virus in adult immunocompromised patients. *Ann Pharmacother* 2012; 46:558-566.
134. Committee On Infectious D, Bronchiolitis Guidelines C, Committee On Infectious D, et al.: Updated guidance for palivizumab prophylaxis among infants and

young children at increased risk of hospitalization for respiratory syncytial virus infection. *Pediatrics* 2014; 134:415-420.
135. Park S.Y., Baek S., Lee S.O., et al.: Efficacy of oral ribavirin in hematologic disease patients with paramyxovirus infection: analytic strategy using propensity scores. *Antimicrob Agents Chemother* 2013; 57:983-989.
136. Bonavia A., Franti M., Pusateri Keaney E., et al.: Identification of broad-spectrum antiviral compounds and assessment of the druggability of their target for efficacy against respiratory syncytial virus (RSV). *Proc Natl Acad Sci USA* 2011; 108:6739-6744.
137. DeVincenzo J., Lambkin-Williams R., Wilkinson T., et al.: A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc Natl Acad Sci USA* 2010; 107:8800-8805.
138. Fernandes R.M., Bialy L.M., Vandermeer B., et al.: Glucocorticoids for acute viral bronchiolitis in infants and young children. *Cochrane Database Syst Rev* 2010; (10):CD004878.
139. van Woensel J.B., Vyas H., Group S.T.: Dexamethasone in children mechanically ventilated for lower respiratory tract infection caused by respiratory syncytial virus: a randomized controlled trial. *Crit Care Med* 2011; 39:1779-1783.
140. Lee F.E., Walsh E.E., Falsey A.R.: The effect of steroid use in hospitalized adults with respiratory syncytial virus-related illness. *Chest* 2011; 140:1155-1161.
141. Jacobs S.E., Lamson D.M., St George K., et al.: Human rhinoviruses. *Clin Microbiol Rev* 2013; 26:135-162.
142. Bochkov Y.A., Gern J.E.: Clinical and molecular features of human rhinovirus C. *Microbes Infect* 2012; 14:485-494.
143. Palmenberg A.C., Spiro D., Kuzmickas R., et al.: Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science* 2009; 324:55-59.
144. Briese T., Renwick N., Venter M., et al.: Global distribution of novel rhinovirus genotype. *Emerg Infect Dis* 2008; 14:944-947.
145. Gwaltney J.M. Jr, Hendley J.O., Simon G., et al.: Rhinovirus infections in an industrial population. I. The occurrence of illness. *N Engl J Med* 1966; 275:1261-1268.
146. Jackson G.G., Dowling H.F., Spiesman I.G., et al.: Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *AMA Arch Intern Med* 1958; 101:267-278.
147. Hendley J.O., Gwaltney J.M. Jr: Mechanisms of transmission of rhinovirus infections. *Epidemiol Rev* 1988; 10:243-258.
148. Jacobs S.E., Soave R., Shore T.B., et al.: Human rhinovirus infections of the lower respiratory tract in hematopoietic stem cell transplant recipients. *Transpl Infect Dis* 2013; 15:474-486.
149. Noah T.L., Henderson F.W., Wortman I.A., et al.: Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 1995; 171:584-592.
150. Proud D., Naclerio R.M., Gwaltney J.M., et al.: Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990; 161:120-123.
151. Turner R.B., Weingand K.W., Yeh C.H., et al.: Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. *Clin Infect Dis* 1998; 26:840-846.
152. Blair H.T., Greenberg S.B., Stevens P.M., et al.: Effects of rhinovirus infection of pulmonary function of healthy human volunteers. *Am Rev Respir Dis* 1976; 114:95-102.
153. Gama R.E., Horsnell P.R., Hughes P.J., et al.: Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. *J Med Virol* 1989; 28:73-77.
154. Pappas D.E., Hendley J.O., Hayden F.G., et al.: Symptom profile of common colds in school-aged children. *Pediatr Infect Dis J* 2008; 27:8-11.
155. Ison M.G., Hayden F.G., Kaiser L., et al.: Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. *Clin Infect Dis* 2003; 36:1139-1143.
156. Costa C., Bergallo M., Astegiano S., et al.: Detection of human rhinoviruses in the lower respiratory tract of lung transplant recipients. *Arch Virol* 2011; 156:1439-1443.
157. Gern J.E.: Rhinovirus and the initiation of asthma. *Curr Opin Allergy Clin Immunol* 2009; 9:73-78.
158. Lambert D.M., Barney S., Lambert A.L., et al.: Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proc Natl Acad Sci USA* 1996; 93:2186-2191.
159. Schmidt A.C., Schaap-Nutt A., Bartlett E.J., et al.: Progress in the development of human parainfluenza virus vaccines. *Expert Rev Respir Med* 2011; 5:515-526.
160. Heilman C.A.: From the National Institute of Allergy and Infectious Diseases and the World Health Organization. Respiratory syncytial and parainfluenza viruses. *J Infect Dis* 1990; 161:402-406.
161. Hall C.B.: Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001; 344:1917-1928.
162. Piedra P.A., Englund J., Glezen W.P.: Respiratory syncytial virus and parainfluenza viruses. In: Richman D.D., Whitley R.J., Hayden F.G., eds. *Clinical virology*. Washington, DC: ASM Press; 2002:763-790.
163. Karron R.A., Collins P.L.: Parainfluenz viruses. In: Knipe D.M., Howley P.M., eds. *Fields Virology*. Philadelphia: Williams and Wilkins; 2007:1497-1526.
164. Parrott R.H., Vargosko A.J., Kimhw, et al.: Acute respiratory diseases of viral etiology. III. parainfluenza. Myxoviruses. *Am J Public Health Nations Health* 1962; 52:907-917.
165. Weinberg G.A., Hall C.B., Iwane M.K., et al.: Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr* 2009; 154:694-699.
166. Chanock R.M., Parrott R.H.: Acute Respiratory Disease in Infancy and Childhood: Present Understanding and Prospects for Prevention. *Pediatrics* 1965; 36:21-39.
167. Gaunt E.R., Harvala H., McIntyre C., et al.: Disease burden of the most commonly detected respiratory viruses in hospitalized patients calculated using the disability adjusted life year (DALY) model. *J Clin Virol* 2011; 52:215-221.
168. Nisii C., Meschi S., Selleri M., et al.: Frequency of Detection of Upper Respiratory Tract Viruses in Patients Tested for Pandemic H1N1/09 Viral Infection. *J Clin Microbiol* 2010; 48:3383-3385.
169. Walker E., Ison M.G.: Respiratory viral infections among hospitalized adults: experience of a single tertiary healthcare hospital. *Influenza Other Respir Viruses* 2014; 8:282-292.
170. Glezen W.P., Frank A.L., Taber L.H., et al.: Parainfluenza virus type 3: seasonality and risk of infection and reinfection in young children. *J Infect Dis* 1984; 150:851-857.
171. Chew F.T., Doraisingham S., Ling A.E., et al.: Seasonal trends of viral respiratory tract infections in the tropics. *Epidemiol Infect* 1998; 121:121-128.
172. Fry A.M., Curns A.T., Harbour K., et al.: Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. *Clin Infect Dis* 2006; 43:1016-1022.
173. Vachon M.L., Dionne N., Leblanc E., et al.: Human parainfluenza type 4 infections, Canada. *Emerg Infect Dis* 2006; 12:1755-1758.
174. Ison M.G.: Respiratory viral infections in transplant recipients. *Antivir Ther* 2007; 12:627-638.
175. Shah D.P., Ghantoji S.S., Mulanovich V.E., et al.: Management of respiratory viral infections in hematopoietic cell transplant recipients. *Am J Blood Res* 2012; 2:203-218.
176. Ansari S.A., Springthorpe V.S., Sattar S.A., et al.: Potential role of hands in the spread of respiratory viral infections: studies with human parainfluenza virus 3 and rhinovirus 14. *J Clin Microbiol* 1991; 29:2115-2119.
177. Massion P.P., Funari C.C., Ueki I., et al.: Parainfluenza (Sendai) virus infects ciliated cells and secretory cells but not basal cells of rat tracheal epithelium. *Am J Respir Cell Mol Biol* 1993; 9:361-370.
178. Castleman W.L., Brundage-Anguish L.J., Kreitzer L., et al.: Pathogenesis of bronchiolitis and pneumonia induced in neonatal and weanling rats by parainfluenza (Sendai) virus. *Am J Pathol* 1987; 129:277-286.
179. Porter D.D., Prince G.A., Hemming V.G., et al.: Pathogenesis of human parainfluenza virus 3 infection in two species of cotton rats: *Sigmodon hispidus* develops bronchiolitis, while *Sigmodon fulviventer* develops interstitial pneumonia. *J Virol* 1991; 65:103-111.
180. Prince G.A., Porter D.D.: Treatment of parainfluenza virus type 3 bronchiolitis and pneumonia in a cotton rat model using topical antibody and glucocorticosteroid. *J Infect Dis* 1996; 173:598-608.
181. Belshe R.B., Newman F.K., Anderson E.L., et al.: Evaluation of combined live, attenuated respiratory syncytial virus and parainfluenza 3 virus vaccines in infants and young children. *J Infect Dis* 2004; 190:2096-2103.
182. Weinberg A., Brewster L., Clark J., et al.: consortium A. Evaluation of R-Mix shell vials for the diagnosis of viral respiratory tract infections. *J Clin Virol* 2004; 30:100-105.
183. LaSala P.R., Bufton K.K., Ismail N., et al.: Prospective comparison of R-mix shell vial system with direct antigen tests and conventional cell culture for respiratory virus detection. *J Clin Virol* 2007; 38:210-216.
184. Kim J.S., Kim S.H., Bae S.Y., et al.: Enhanced detection of respiratory viruses using cryopreserved R-Mix ReadyCells. *J Clin Virol* 2008; 42:264-267.
185. Ray C.G., Minnich L.L.: Efficiency of immunofluorescence for rapid detection of common respiratory viruses. *J Clin Microbiol* 1987; 25:355-357.
186. Kuypers J., Wright N., Ferrenberg J., et al.: Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. *J Clin Microbiol* 2006; 44:2382-2388.
187. Kuypers J., Campbell A.P., Cent A., et al.: Comparison of conventional and molecular detection of respiratory viruses in hematopoietic cell transplant recipients. *Transpl Infect Dis* 2009; 11:298-303.
188. Weinberg G.A., Erdman D.D., Edwards K.M., et al.: Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. *J Infect Dis* 2004; 189:706-710.
189. Krunic N., Merante F., Yaghoubian S., et al.: Advances in the diagnosis of respiratory tract infections: role of the Luminex xTAG respiratory viral panel. *Ann N Y Acad Sci* 2011; 1222:6-13.
190. Pabbaraju K., Wong S., Tokaryk K.L., et al.: Comparison of the Luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol* 2011; 49:1738-1744.
191. Pabbaraju K., Wong S., Lee B., et al.: Comparison of a singleplex real-time RT-PCR assay and multiplex respiratory viral panel assay for detection of influenza 'A' in respiratory specimens. *Influenza Other Respir Viruses* 2011; 5:99-103.
192. Endimiani A., Hujer K.M., Hujer A.M., et al.: Are we ready for novel detection methods to treat respiratory pathogens in hospital-acquired pneumonia? *Clin Infect Dis* 2011; 52(Suppl. 4):S373-S383.
193. Reed G., Jewett P.H., Thompson J., et al.: Epidemiology and clinical impact of parainfluenza virus infections in otherwise healthy infants and young children <5 years old. *J Infect Dis* 1997; 175:807-813.
194. Vesa S., Kleemola M., Blomqvist S., et al.: Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. *Pediatr Infect Dis J* 2001; 20:574-581.
195. Gwaltney J.M. Jr, Scheld W.M., Sande M.A., et al.: The microbial etiology and antimicrobial therapy of adults with acute community-acquired sinusitis: a fifteen-year experience at the University of Virginia and review of other selected studies. *J Allergy Clin Immunol* 1992; 90:457-461, discussion 62.
196. Denny F.W., Clyde W.A.J.: Acute lower respiratory tract infections in nonhospitalized children. *J Pediatr* 1986; 108:635.
197. Denny F.W. Jr: The clinical impact of human respiratory virus infections. *Am J Respir Crit Care Med* 1995; 152:S4-S12.
198. Slavin K.A., Passaro D.J., Hacker J.K., et al.: Parainfluenza virus type 4: case report and review of the literature. *Pediatr Infect Dis J* 2000; 19:893-896.
199. Arisoy E.S., Demmler G.J., Thakar S., et al.: Meningitis due to parainfluenza virus type 3: report of two cases and review. *Clin Infect Dis* 1993; 17:995-997.
200. Roman G., Phillips C.A., Poser C.M.: Parainfluenza virus type 3: isolation from CSF of a patient with Guillain-Barre syndrome. *JAMA* 1978; 240:1613-1615.

201. Wilks D., Burns S.M.: Myopericarditis associated with parainfluenza virus type 3 infection. *Eur J Clin Microbiol Infect Dis* 1998; 17:363-365.
202. Kalimuddin S., Sessions O.M., Hou Y., et al.: Successful clearance of human parainfluenza virus type 2 viraemia with intravenous ribavirin and immunoglobulin in a patient with acute myocarditis. *J Clin Virol* 2013; 56:37-40.
203. Walker T.A., Khurana S., Tilden S.J.: Viral respiratory infections. *Pediatr Clin North Am* 1994; 41:1365-1381.
204. Falsey A.R., Walsh E.E.: Viral pneumonia in older adults. *Clin Infect Dis* 2006; 42:518-524.
205. Fransen H., Heigl Z., Wolontis S., et al.: Infections with viruses in patients hospitalized with acute respiratory illness, Stockholm 1963–1967. *Scand J Infect Dis* 1969; 1:127-136.
206. Public Health Laboratory Service Communicable Disease Surveillance Center: Parainfluenza infections in the elderly 1976-82. *Br Med J* 1983; 287:1619.
207. Choi S.H., Hong S.B., Ko G.B., et al.: Viral infection in patients with severe pneumonia requiring intensive care unit admission. *Am J Respir Crit Care Med* 2012; 186:325-332.
208. Diederen B.M., Van Der Eerden M.M., Vlaspolder F., et al.: Detection of respiratory viruses and *Legionella* spp. by real-time polymerase chain reaction in patients with community acquired pneumonia. *Scand J Infect Dis* 2009; 41:45-50.
209. Johnstone J., Majumdar S.R., Fox J.D., et al.: Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. *Chest* 2008; 134:1141-1148.
210. Johnston N.W.: The similarities and differences of epidemic cycles of chronic obstructive pulmonary disease and asthma exacerbations. *Proc Am Thorac Soc* 2007; 4:591-596.
211. Hansbro N.G., Horvat J.C., Wark P.A., et al.: Understanding the mechanisms of viral induced asthma: new therapeutic directions. *Pharmacol Ther* 2008; 117:313-353.
212. Matsuse H., Kondo Y., Saeki S., et al.: Naturally occurring parainfluenza virus 3 infection in adults induces mild exacerbation of asthma associated with increased sputum concentrations of cysteinyl leukotrienes. *Int Arch Allergy Immunol* 2005; 138:267-272.
213. Dimopoulos G., Lerikou M., Tsiodras S., et al.: Viral epidemiology of acute exacerbations of chronic obstructive pulmonary disease. *Pulm Pharmacol Ther* 2012; 25:12-18.
214. Peck A.J., Englund J.A., Kuypers J., et al.: Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. *Blood* 2007; 110:1681-1688.
215. Kim Y.J., Boeckh M., Englund J.A.: Community respiratory virus infections in immunocompromised patients: hematopoietic stem cell and solid organ transplant recipients, and individuals with human immunodeficiency virus infection. *Semin Respir Crit Care Med* 2007; 28:222-242.
216. Dignan F., Alvares C., Riley U., et al.: Parainfluenza type 3 infection post stem cell transplant: high prevalence but low mortality. *J Hosp Infect* 2006; 63:452-458.
217. Nichols W.G., Corey L., Gooley T., et al.: Parainfluenza virus infections after hematopoietic stem cell transplantation: risk factors, response to antiviral therapy, and effect on transplant outcome. *Blood* 2001; 98:573-578.
218. Nichols W.G., Gooley T., Boeckh M.: Community-acquired respiratory syncytial virus and parainfluenza virus infections after hematopoietic stem cell transplantation: the Fred Hutchinson Cancer Research Center experience. *Biol Blood Marrow Transplant* 2001; (Suppl. 7):11S-15S.
219. Whimbey E., Bodey G.P.: Viral pneumonia in the immunocompromised adult with neoplastic disease: the role of common community respiratory viruses. *Semin Respir Infect* 1992; 7:122-131.
220. Ferguson P.E., Sorrell T.C., Bradstock K.F., et al.: Parainfluenza virus type 3 pneumonia in bone marrow transplant recipients: multiple small nodules in high-resolution lung computed tomography scans provide a radiological clue to diagnosis. *Clin Infect Dis* 2009; 48(7):905-909.
221. Srinivasan A., Wang C., Yang J., et al.: Symptomatic parainfluenza virus infections in children undergoing hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011; 17:1520-1527.
222. Chemaly R.F., Hanmod S.S., Rathod D.B., et al.: The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. *Blood* 2012; 119:2738-2745, quiz 969.
223. Ustun C., Slaby J., Shanley R.M., et al.: Human parainfluenza virus infection after hematopoietic stem cell transplantation: risk factors, management, mortality, and changes over time. *Biol Blood Marrow Transplant* 2012; 18:1580-1588.
224. Erard V., Chien J.W., Kim H.W., et al.: Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. *J Infect Dis* 2006; 193:1619-1625.
225. Christensen M.S., Nielsen L.P., Hasle H.: Few but severe viral infections in children with cancer: a prospective RT-PCR and PCR-based 12-month study. *Pediatr Blood Cancer* 2005; 45:945-951.
226. Srinivasan A., Wang C., Yang J., et al.: Parainfluenza virus infections in children with hematologic malignancies. *Pediatr Infect Dis J* 2011; 30:855-859.
227. Vilchez R.A., Dauber J., McCurry K., et al.: Parainfluenza virus infection in adult lung transplant recipients: an emergent clinical syndrome with implications on allograft function. *Am J Transplant* 2003; 3:116-120.
228. Billings J.L., Hertz M.I., Savik K., et al.: Respiratory viruses and chronic rejection in lung transplant recipients. *J Heart Lung Transplant* 2002; 21:559-566.
229. Garbino J., Gerbase M.W., Wunderli W., et al.: Respiratory viruses and severe lower respiratory tract complications in hospitalized patients. *Chest* 2004; 125:1033-1039.
230. Vilchez R., McCurry K., Dauber J., et al.: Influenza and parainfluenza respiratory viral infection requiring admission in adult lung transplant recipients. *Transplantation* 2002; 73:1075-1078.
231. Bjornson C., Russell K.F., Vandermeer B., et al.: Nebulized epinephrine for croup in children. *Cochrane Database Syst Rev* 2011; (10):CD006619.
232. Russell K.F., Liang Y., O'Gorman K., et al.: Glucocorticoids for croup. *Cochrane Database Syst Rev* 2011; (1):CD001955.
233. Chakrabarti S., Collingham K.E., Holder K., et al.: Parainfluenza virus type 3 infections in hematopoetic stem cell transplant recipients: response to ribavirin therapy. *Clin Infect Dis* 2000; 31:1516-1518.
234. Cobian L., Houston S., Greene J., et al.: Parainfluenza virus respiratory infection after heart transplantation: successful treatment with ribavirin. *Clin Infect Dis* 1995; 21:1040-1041.
235. Elizaga J., Olavarria E., Apperley J., et al.: Parainfluenza virus 3 infection after stem cell transplant: relevance to outcome of rapid diagnosis and ribavirin treatment. *Clin Infect Dis* 2001; 32:413-418.
236. Milstone A.P., Brumble L.M., Barnes J., et al.: A single-season prospective study of respiratory viral infections in lung transplant recipients. *Eur Respir J* 2006; 28:131-137.
237. Sparrelid E., Ljungman P., Ekelof-Andstrom E., et al.: Ribavirin therapy in bone marrow transplant recipients with viral respiratory tract infections. *Bone Marrow Transplant* 1997; 19:905-908.
238. Stankova J., Carret A.S., Moore D., et al.: Long-term therapy with aerosolized ribavirin for parainfluenza 3 virus respiratory tract infection in an infant with severe combined immunodeficiency. *Pediatr Transplant* 2007; 11:209-213.
239. Wendt C.H., Weisdorf D.J., Jordan M.C., et al.: Parainfluenza virus respiratory infection after bone marrow transplantation. *N Engl J Med* 1992; 326:921-926.
240. Wright J.J., O'Driscoll G.: Treatment of parainfluenza virus 3 pneumonia in a cardiac transplant recipient with intravenous ribavirin and methylprednisolone. *J Heart Lung Transplant* 2005; 24:343-346.
241. Chen Y.B., Driscoll J.P., McAfee S.L., et al.: Treatment of parainfluenza 3 infection with DAS181 in a patient after allogeneic stem cell transplantation. *Clin Infect Dis* 2011; 53:e77-e80.
242. Guzman-Suarez B.B., Buckley M.W., Gilmore E.T., et al.: Clinical potential of DAS181 for treatment of parainfluenza-3 infections in transplant recipients. *Transpl Infect Dis* 2012; 14:427-433.
243. Drozd D.R., Limaye A.P., Moss R.B., et al.: DAS181 treatment of severe parainfluenza type 3 pneumonia in a lung transplant recipient. *Transpl Infect Dis* 2013; 15:E28-E32.
244. Chalkias S., Mackenzie M.R., Gay C., et al.: DAS181 treatment of hematopoietic stem cell transplant patients with parainfluenza virus lung disease requiring mechanical ventilation. *Transpl Infect Dis* 2014; 16:141-144.
245. Boivin G., Mackay I., Sloots T.P., et al.: Global genetic diversity of human metapneumovirus fusion gene. *Emerg Infect Dis* 2004; 10:1154-1157.
246. Mackay I.M., Bialasiewicz S., Jacob K.C., et al.: Genetic diversity of human metapneumovirus over 4 consecutive years in Australia. *J Infect Dis* 2006; 193:1630-1633.
247. van den Hoogen B.G., Bestebroer T.M., Osterhaus A.D., et al.: Analysis of the genomic sequence of a human metapneumovirus. *Virology* 2002; 295:119-132.
248. Schildgen V., van den Hoogen B., Fouchier R., et al.: Human Metapneumovirus: lessons learned over the first decade. *Clin Microbiol Rev* 2011; 24:734-754.
249. Boivin G., Abed Y., Pelletier G., et al.: Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. *J Infect Dis* 2002; 186:1330-1334.
250. Papenburg J., Hamelin M.E., Ouhoummane N., et al.: Comparison of risk factors for human metapneumovirus and respiratory syncytial virus disease severity in young children. *J Infect Dis* 2012; 206:178-189.
251. Walsh E.E., Peterson D.R., Falsey A.R.: Human metapneumovirus infections in adults: another piece of the puzzle. *Arch Intern Med* 2008; 168:2489-2496.
252. Widmer K., Zhu Y., Williams J.V., et al.: Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012; 206:56-62.
253. Egli A., Bucher C., Dumoulin A., et al.: Human metapneumovirus infection after allogeneic hematopoietic stem cell transplantation. *Infection* 2012; 40:677-684.
254. van den Hoogen B.G., de Jong J.C., Groen J., et al.: A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001; 7:719-724.
255. Williams J.V., Harris P.A., Tollefson S.J., et al.: Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004; 350:443-450.
256. Matsuzaki Y., Itagaki T., Ikeda T., et al.: Human metapneumovirus infection among family members. *Epidemiol Infect* 2013; 141:827-832.
257. Cseke G., Maginnis M.S., Cox R.G., et al.: Integrin alphavbeta1 promotes infection by human metapneumovirus. *Proc Natl Acad Sci USA* 2009; 106:1566-1571.
258. Kuiken T., van den Hoogen B.G., van Riel D.A., et al.: Experimental human metapneumovirus infection of cynomolgus macaques (*Macaca fascicularis*) results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. *Am J Pathol* 2004; 164:1893-1900.
259. Hamelin M.E., Prince G.A., Gomez A.M., et al.: Human metapneumovirus infection induces long-term pulmonary inflammation associated with airway obstruction and hyperresponsiveness in mice. *J Infect Dis* 2006; 193:1634-1642.
260. Laham F.R., Israele V., Casellas J.M., et al.: Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. *J Infect Dis* 2004; 189:2047-2056.
261. Mackay I.M., Jacob K.C., Woolhouse D., et al.: Molecular assays for detection of human metapneumovirus. *J Clin Microbiol* 2003; 41:100-105.
262. van den Hoogen B.G., Osterhaus D.M., Fouchier R.A.: Clinical impact and diagnosis of human metapneumovirus infection. *Pediatr Infect Dis J* 2004; 23:S25-S32.
263. Schildgen O., Glatzel T., Geikowski T., et al.: Human metapneumovirus RNA in encephalitis patient. *Emerg Infect Dis* 2005; 11:467-470.
264. Sanchez Fernandez I., Rebollo Polo M., Munoz-Almagro C., et al.: Human Metapneumovirus in the

Cerebrospinal Fluid of a Patient With Acute Encephalitis. *Arch Neurol* 2012; 69:649-652.
265. Beneri C., Ginocchio C.C., Manji R., et al.: Comparison of clinical features of pediatric respiratory syncytial virus and human metapneumovirus infections. *Infect Control Hosp Epidemiol* 2009; 30:1240-1241.
266. Semple M.G., Cowell A., Dove W., et al.: Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 2005; 191:382-386.
267. Hamelin M.E., Abed Y., Boivin G.: Human metapneumovirus: a new player among respiratory viruses. *Clin Infect Dis* 2004; 38:983-990.
268. Vicente D., Montes M., Cilla G., et al.: Human metapneumovirus and chronic obstructive pulmonary disease. *Emerg Infect Dis* 2004; 10:1338-1339.
269. Beckham J.D., Cadena A., Lin J., et al.: Respiratory viral infections in patients with chronic, obstructive pulmonary disease. *J Infect* 2005; 50:322-330.
270. Wyde P.R., Chetty S.N., Jewell A.M., et al.: Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. *Antiviral Res* 2003; 60:51-59.
271. Kitanovski L., Kopriva S., Pokorn M., et al.: Treatment of severe human metapneumovirus (hMPV) pneumonia in an immunocompromised child with oral ribavirin and IVIG. *J Pediatr Hematol Oncol* 2013; 35:e311-e313.
272. Raza K., Ismailjee S.B., Crespo M., et al.: Successful outcome of human metapneumovirus (hMPV) pneumonia in a lung transplant recipient treated with intravenous ribavirin. *J Heart Lung Transplant* 2007; 26:862-864.
273. Shachor-Meyouhas Y., Ben-Barak A., Kassis I.: Treatment with oral ribavirin and IVIG of severe human metapneumovirus pneumonia (HMPV) in immune compromised child. *Pediatr Blood Cancer* 2011; 57:350-351.
274. Allander T., Tammi M.T., Eriksson M., et al.: Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; 102:12891-12896.
275. Jartti T., Hedman K., Jartti L., et al.: Human bocavirus-the first 5 years. *Rev Med Virol* 2012; 22:46-64.
276. Kapoor A., Simmonds P., Slikas E., et al.: Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J Infect Dis* 2010; 201:1633-1643.
277. Chen A.Y., Cheng F., Lou S., et al.: Characterization of the gene expression profile of human bocavirus. *Virology* 2010; 403:145-154.
278. Han T.H., Chung J.Y., Hwang E.S.: Human bocavirus 2 in children, South Korea. *Emerg Infect Dis* 2009; 15:1698-1700.
279. Song J.R., Jin Y., Xie Z.P., et al.: Novel human bocavirus in children with acute respiratory tract infection. *Emerg Infect Dis* 2010; 16:324-327.
280. Chung J.Y., Han T.H., Kim J.S., et al.: Th1 and Th2 cytokine levels in nasopharyngeal aspirates from children with human bocavirus bronchiolitis. *J Clin Virol* 2008; 43:223-225.
281. Kumar A., Filippone C., Lahtinen A., et al.: Comparison of Th-cell immunity against human bocavirus and parvovirus B19: proliferation and cytokine responses are similar in magnitude but more closely interrelated with human bocavirus. *Scand J Immunol* 2011; 73:135-140.
282. Lindner J., Zehentmeier S., Franssila R., et al.: CD4+ T helper cell responses against human bocavirus viral protein 2 viruslike particles in healthy adults. *J Infect Dis* 2008; 198:1677-1684.
283. Dijkman R., Koekkoek S.M., Molenkamp R., et al.: Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* 2009; 83:7739-7748.
284. Karalar L., Lindner J., Schimanski S., et al.: Prevalence and clinical aspects of human bocavirus infection in children. *Clin Microbiol Infect* 2010; 16:633-639.
285. Soderlund-Venermo M., Lahtinen A., Jartti T., et al.: Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis* 2009; 15:1423-1430.
286. Christensen A., Nordbo S.A., Krokstad S., et al.: Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J Clin Virol* 2010; 49:158-162.
287. Catalano-Pons C., Giraud C., Rozenberg F., et al.: Detection of human bocavirus in children with Kawasaki disease. *Clin Microbiol Infect* 2007; 13:1220-1222.
288. Lehmann C., Klar R., Lindner J., et al.: Kawasaki disease lacks association with human coronavirus NL63 and human bocavirus. *Pediatr Infect Dis J* 2009; 28:553-554.
289. Chan J.F., Lau S.K., To K.K., et al.: Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin Microbiol Rev* 2015; 28(2):465-522.
290. Khalafalla A.I., Lu X., Al-Mubarak A.I., et al.: MERS-CoV in Upper Respiratory Tract and Lungs of Dromedary Camels, Saudi Arabia, 2013-2014. *Emerg Infect Dis* 2015; 21(7):1153-1158.
291. Korea Centers for Disease Control and Prevention: Middle East Respiratory Syndrome Coronavirus Outbreak in the Republic of Korea, 2015. *Osong Public Health Res Perspect.* 2015; 6(4):269-278.
292. Müller M.A., Meyer B., Corman V.M., et al.: Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. *Lancet Infect Dis* 2015; 15(5):559-564.
293. Oboho I.K., Tomczyk S.M., Al-Asmari A.M., et al.: 2014 MERS-CoV outbreak in Jeddah–a link to health care facilities. *N Engl J Med* 2015; 372(9):846-854.
294. Drosten C., Meyer B., Müller M.A., et al.: Transmission of MERS-coronavirus in household contacts. *N Engl J Med* 2014; 371(9):828-835.
295. Omrani A.S., Saad M.M., Baig K., et al.: Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. *Lancet Infect Dis* 2014; 14(11): 1090-1095.
296. Corti D., Zhao J., Pedotti M., et al.: Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus. *Proc Natl Acad Sci USA* 2015; 112(33):10473-10478.
297. Chan J.F., Yao Y., Yeung M.L., et al.: Treatment With Lopinavir/Ritonavir or Interferon-β1b Improves Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. *J Infect Dis* 2015; 212(12):1904-1913.
298. DeVincenzo J.P., Whitley R.J., Mackman R.L., et al.: Oral GS-5806 activity in a respiratory syncytial virus challenge study. *N Engl J Med* 2014; 371(8):711-722.