Common Cold
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The role of viruses in the etiology and pathogenesis of common cold

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Abstract

Numerous viruses are able to cause respiratory tract infections. With the availability of new molecular techniques, the number of pathogens detected in specimens from the human respiratory tract has increased. Some of these viral infections have the potential to lead to severe systemic disease. Other viruses are limited to playing a role in the pathogenesis of the common cold syndrome. This chapter focuses on the viral pathogens that are linked to common cold. It is not the intention to comprehensively review all the viruses that are able to cause respiratory tract infections – this would go beyond the scope of this book. The list of viruses that are briefly reviewed here includes rhinoviruses, respiratory syncytial virus, parainfluenza virus, adenovirus, metapneumovirus and coronavirus. Bocavirus is discussed as one example of a newly identified pathogen with a less established role in the etiology and pathogenesis of common cold. Influenza virus does not cause what is defined as common cold. However, influenza viruses are associated with respiratory disease and the clinical picture of mild influenza and common cold frequently overlaps. Therefore, influenza virus has been included in this chapter. It is important to note that a number of viruses are frequently co-detected with other viruses in humans with respiratory diseases. Therefore, the viral etiology and the role of viruses in the pathogenesis of common cold is complex, and numerous questions remain to be answered.

Introduction: The role of viruses in the etiology and pathogenesis of common cold

Numerous viruses are able to cause respiratory tract infections. Some of these may also cause severe diseases. Others are limited to a role in the pathogenesis of the common cold syndrome. With the availability of new molecular techniques, the number of pathogens detected in specimens from the human respiratory tract has increased. The association of some of these agents with human respiratory disease is not always clear. It is not the intention of this chapter to comprehensively review the virology of all the viruses that cause or potentially cause respiratory tract infections. The chap-

ter focuses on some pathogens that are linked to common cold. The list of viruses described includes rhinovirus, respiratory syncytial virus, parainfluenza virus, adenovirus, metapneumovirus and coronavirus. Bocavirus is discussed as an example of a newly identified pathogen with a less established role in the etiology and pathogenesis of common cold. Influenza virus does not cause what is defined as common cold, but is associated with respiratory disease and the clinical picture of mild influenza frequently overlaps with that of the common cold. Therefore, influenza viruses are briefly described in this chapter.

For details on the biology of the individual viruses and their role in pathogenesis of respiratory diseases further reading of standard literature and text books of virology is recommended.

Viruses with an established role in common cold are rhinoviruses, adenoviruses, parainfluenza viruses, coronaviruses and the respiratory syncytial virus, and these are reviewed in greater detail here. Their structure and replication, the transmission and epidemiology and the clinical symptoms are described. In addition, some brief comments about current models of pathogenesis and animal models, respectively, complete the respective subchapters.

Table 1 provides an overview of the viruses that cause respiratory tract infections and that do, or may, play a role as a cause or in the pathogenesis of common cold.

A number of viruses are frequently co-detected with other viruses in humans with respiratory diseases. Therefore, the viral etiology and the role of viruses in the pathogenesis of common cold is complex and it is safe to say, not fully understood for each and every virus that is linked to respiratory tract infection.

Recent developments in the field of antivirals are described in the chapter by Tom Jefferson in this book.

Rhinoviruses

Rhinoviruses cause the vast majority of the common colds in humans. Although the infection usually is self limiting and the symptoms of the disease are mild in healthy adults, rhinovirus infections may cause serious illness in children or patients with pre-existing medical problems [1].

Taxonomy, structure and replication

Rhinoviruses (RV) are members of the order *Picornavirales*, family *Picornaviridae*, genus: Enterovirus [2]. (In the virus taxonomy list of the year 2007 of the International Committee on the Taxonomy of Viruses the rhinoviruses still constituted a separate genus: Rhinovirus.)

Table 1. Viruses that cause illness of the respiratory tract (for references see text)

Agents	Taxonomy	Disease/symptoms	Epidemiology	Transmission	Treatment	Prophylaxis
Rhinovirus (RV)	**Order**: Picornavirales **Family**: Picornaviridae **Genus**: Enterovirus **Species**: Human rhinovirus A / B	Rhinitis, pharyngitis, cough, fever, otitis media, sinusitis, acute disease or exacerbation of chronic pulmonary diseases	~60–80% of the patients with a common cold syndrome, between August and November	Direct contact human-to-human, aerosols	Symptomatic	Hygiene, disinfection, chemoprophylaxis
Respiratory Syncytial Virus (RSV)	**Order**: Mononegavirales **Family**: Paramyxoviridae **Subfamily**: Pneumovirinae **Genus**: Pneumovirus **Species**: Human respiratory syncytial virus	Rhinitis, pharyngitis, cough, bronchiolitis, pneumonia, complications in patients with immunodeficiency or underlying conditions like cystic fibrosis, chronic heart disease etc.	WHO: RSV causes 64 million infections and 160000 deaths annually, seasonal: winter/ early spring	Epidemic, mostly saliva/respiratory droplets, human-human, hand-to-mouth or hand-to-eye, contaminated surfaces	Immunoglobulins, antibodies, ribavirin; symptomatic	Vaccines in development
Parainfluenza virus (PIV) 1 / 3	**Order**: Mononegavirales **Family**: Paramyxoviridae **Subfamily**: Paramyxovirinae **Genus**: Respirovirus **Species**: Human parainfluenza virus1/ 3	Rhinitis, pharyngitis, cough, hoarseness, fever, croup, bronchiolitits, pneumonia	Children, infants, most children infected by 5 years of age, PIV 1: epidemics in fall PIV 3: epidemics in early spring	Human-to-human, aerosols	Symptomatic	Hygiene, disinfection, no effective vaccines available

Table 1 (continued)

Agents	Taxonomy	Disease/symptoms	Epidemiology	Transmission	Treatment	Prophylaxis
Parainfluenza virus (PIV)	**Order**: Mononegavirales **Family**: Paramyxoviridae **Subfamily**: Paramyxovirinae **Genus**: Rubulavirus **Species**: Human parainfluenza virus 2/ 4	Rhinitis, pharyngitis, cough PIV 2: croup, bronchiolitits, pneumonia PIV 4: mild upper respiratory tract disease	PIV 2 epidemics mainly in autumn	Human-to-human, aerosols	Symptomatic	Hygiene, disinfection, no effective vaccines available
Adenovirus (AV)	**Order**: not assigned **Family**: Adenoviridae **Genus**: Mastadenovirus Species: Human adenovirus C (B) (A-F)	URTI, rhinitis, conjunctivitis, tonsillitis, (gastroenteritis)	5% of URTI in children, institutional infections, AV account for 10% of pneumonia in children; respir. infections seasonal (mainly late winter to early summer)	Aerosols, human-human	Symptomatic, cidofovir in immunosuppressed patients	Hygiene
Metapneumovirus (hMPV)	**Order**: Mononegavirales **Family**: Paramyxoviridae **Subfamily**: Pneumovirinae **Genus**: Metapneumovirus **Species**: Human metapneumovirus virus	Cough, wheezing, coryza, fever, diarrhea, vomiting bronchiolitis, pneumonia, complications in patients with immunodeficiency or underlying conditions such as asthma, COPD	Seasonal distribution in temperate regions (i.e., late winter, spring) third leading cause of ARTI in humans	Epidemic, mostly saliva/respiratory droplets (not aerosols), human-human, hand-to-mouth or hand-to-eye, contaminated surfaces	Symptomatic, ribavirin	Hygiene

Boca Virus (HBoV)	**Order**: not assigned **Family**: Parvoviridae **Subfamily**: Parvovirinae **Genus**: Bocavirus **Species**: Human Bocavirus (not listed in the 2008 ICTV database)	Rhinitis, pharyngitis, cough, LRTI	Role to be established, frequent co-infections			
Coronavirus (hCoV)	**Order**: Nidovirales **Family**: Coronaviridae **Genus**: Coronavirus **Species**: Human coronavirus (several species: 229E, HKU1, NL 63, OC43)	Rhinitis, pharyngitis, cough, otitis media	Up to 25% of common cold cases are estimated to be caused by HCoV	Aerosols, human-human, winter season	Symptomatic	Hygiene
Influenza A/B Virus (IAV, IBV)	**Order**: not assigned **Family**: Orthomyxoviridae **Genus**: Influenza virus **Species**: Influenza virus A, B	Influenza, URTI, manifestations possible in the central nervous system, muscle (myositis/rhabdomyositis), heart (myocarditis), toxic/septic shock	- WHO: 5–15% of the population are affected with URTI/year - 3–5 million cases of severe illness - 250 000–500 000 deaths/year worldwide - most deaths >65 years of age - seasonal distribution in temperate regions (i.e., winter)	Epidemic, pandemic (not IB), aerosols, human-human, seabirds, poultry, animals	Symptomatic, neuraminidase-inhibitors	Vaccination, hygiene

ARTI: acute respiratory tract infection

More than 100 serotypes, strains and isolates of RV have been isolated from humans. Two human RV species have been described: Human rhinovirus (HRV) A and B. Eighteen serotypes and 2 subtypes (HRV 1A and 1B) belong to HRV A. Five serotypes are assigned to HRV B and 82 serotypes are not yet assigned to a species including bovine rhinoviruses (BRV) 1–3.

RV are non-enveloped viruses with an icosahedral symmetry. The virus is small and has a diameter of approximately 30 nm. Four capsid proteins, VP1–4 have been described. A protomer is composed of one copy of VP1, VP3 and VP0 (a precursor where VP4 and VP2 are covalently linked). Cleavage of VP0 is the final step of the assembly process [3]. One or two copies of VP0 will remain uncleaved; no role for this uncleaved VP0 has been established yet. Five protomers are arranged symmetrically about a fivefold axis, forming a pentamer that represents a corner of the icosahedron. The capsid is formed by 12 pentamers. RV have, similar to human enteroviruses, the same comparatively uneven surface with its characteristic canyon around the fivefold axis [4]. The canyon serves as an attachment site for the cell receptor [5]. In CsCl, RV have a buoyant density of 1.38–1.42 g/cm^3. Virions are unstable at a pH below 5–6, a feature, which distinguishes RV from other enteroviruses. However, as they are non-enveloped viruses, RV are stable against detergents and most organic solvents. On the other hand, alcohol and phenol are effective virucidal agents.

The RV genome is organized as a single-stranded positive-sense RNA of approximately 7100–7200 nucleotides in length with its 5' terminus covalently linked to a small protein, VPg. The 5'-untranslated region (UTR) of approximately 0.65 kb is shorter than that of other enteroviruses, owing to a deletion of approximately 100 nucleotides between the internal ribosomal entry site (IRES) and the translation start site. One open reading frame (ORF) of about 2150 codons, a 3'-UTR of approximately 40 nucleotides and a 3' poly(A) tail complete the structure of the genome. RV have a characteristic nucleotide composition with a preponderance of A and U, particularly in the third position of the codons. The genome is fully sequenced and has the accession number [K02121]; [K02021] [2].

Replication is initiated through attachment to the cell receptor. For most RV serotypes this is intercellular adhesion molecule-1 (ICAM-1) [6, 7]. It is hypothesized that the viral canyon structure releases a lipid moiety upon ICAM-1 binding, which, in turn, leads to a change in conformation, destabilization and the release of the viral RNA into the cytoplasm [8]. RV shut off host cell protein synthesis by inactivating the cap binding complex. Their IRES allows them to replicate despite this inactivation.

The RNA serves as a messenger RNA encoding a single polyprotein which is cleaved post-translationally by virus-encoded proteases. Once the first round of translation and subsequent processing is complete a 3D^{pol} RNA-dependent RNA polymerase produces negative-sense RNA from the genomic template, which in turn serve as template for the production

of positive-sense genomic RNA. The synthesis of a single virus polyprotein requires post-translational processing to facilitate subsequent steps in viral replication. At least two proteolytic activities are encoded by the virus: $2A^{pro}$ performs the first cleavage releasing capsid precursors and $3C^{pro}$ catalyzes most other cleavage reactions. The protease has a trypsin-like structure but the active site of the enzyme is a cysteine sulfydryl [9].

Pathogenesis, immunology and clinical symptoms

RV are transmitted mainly by direct contact and less frequently through aerosols (for details see the chapter by Diane Pappas and Owen Hendley). Virus can frequently be isolated from the hands of an infected individual and is transmitted to other individuals or to objects in the environment. During RV infection virus titers in nasal secretions are as high as 10^2–10^3 $TCID_{50}$/ml of nasal lavage fluid [10, 11].

Infection of humans is very effective and less RV may be needed for infection of seronegative volunteers by nasal drops than needed for infection of a human embryonic fibroblast tissue culture [12]. In contrast, when the same virus was used for inhalation of aerosols a 20-fold disparity in infectious dose has been described [13], suggesting that the lower respiratory tract is less susceptible for infection than the nasopharynx.

After a short incubation period of 1–4 days virus is shed, peaks after another 2–3 days, and declines thereafter [13, 14]. The primary site of viral replication is ciliated epithelial cells as detected by *in situ* hybridization [15].

The histopathology of RV infection is not as yet very detailed. Nasal mucosa biopsies reveal only few or no histopathological abnormalities despite active virus shedding. Explant cultures inoculated with rhinovirus failed to develop cytopathic effects (CPE) [16]. Biopsies showed marked edema of connective tissue, sparse infiltration of inflammatory cells, hyperemia and exudation of seromucous fluids [17–19]. Infection of bovine tracheal organ cultures with BRV leads to shedding of ciliated cells.

It has been suggested that the immune response of the host contributes to the symptom complex. Increased concentrations of the pro-inflammatory cytokines IL-8, IL-1 and IL-6 [20–22] have been found in nasal secretions of subjects with symptomatic RV infection. A direct correlation between concentration in nasal fluids and symptom severity has been described for IL-6 [22].

RV infection is usually accompanied by the typical common cold symptoms: nasal discharge and obstruction, sneezing, coughing, sore throat and, less frequently, fever. Gastrointestinal symptoms are sometimes observed in children. The infection is usually limited to the upper respiratory tract. It is commonly believed that RV infection increases the risk for subsequent or secondary bacterial infections. In patients predisposed with existing under-

lying diseases, like cystic fibrosis or chronic bronchitis, and in immunocompromised patients, elderly and infants, RV can cause serious infections of the lower respiratory tract. RV infection of the lower respiratory tract (LRTI) was demonstrated by Papadopoulos and co-workers [23] using *in situ* hybridization techniques. These authors demonstrated RV infection not only in epithelial cells but also in underlying submucosal cells. Exacerbation of chronic bronchitis or asthma may be a consequence in these patients [24, 25]. Indeed, approximately 80% of asthma exacerbations in children [26] and about 70% in adults [27] are associated with respiratory virus infections, and the vast majority of these are RV infections [28]. Examination of the early innate immune responses to RV infection in asthmatic bronchial epithelia revealed profound impairment of virus-induced interferon (IFN)-β expression leading to impaired apoptotic responses and enhanced RV replication [29].

RV infections lead to the production of type-specific IgA, IgG and IgM antibodies. However, frequencies of response to natural infection have been reported to vary between 37% and 92% [30]. Infected patients usually develop neutralizing antibodies to the infecting virus within 1–3 weeks after infection. IgA is the dominant immunoglobulin in nasal secretions, has a protective role and may prevent re-infections with homotypic viruses or reduce the symptoms upon reinfection. The involvement in the viral clearance process is less clear and other mechanisms like the induction of an innate immune response are being discussed. Both serum and secretory antibodies persist for several years after infection.

Epidemiology, diagnosis and treatment

In the absence of effective antiviral treatments, the diagnosis of RV infection for guiding an anti-RV therapy is not useful. The diagnosis of RV infection largely relies on the clinical symptoms. Approximately 60–80% of the patients with a common cold syndrome of afebrile prominent nasal symptoms but minimal systemic disease that occurs between August and early November have RV infection [31]. However, the general method for identifying the etiological agent is the isolation and propagation in cell culture. In addition, polymerase chain reaction (PCR) can be used for rapid identification of RV in specimens. Point-of-care diagnostics are under development.

Experimental models

A major obstacle to understanding disease pathogenesis has been the lack of a small-animal model for RV infection. RV have shown a high degree of species specificity, limiting the use of experimental animal systems.

Therefore, aspects of pathogenesis have been studied in experimentally induced colds in human volunteers.

Infection of rabbits, guinea pigs or weanling mice by parenteral routes was not successful with certain strains of the virus [32–34], but infection of mice was possible using a tissue-culture adapted HRV-2 [35]. Chimpanzees or gibbons has been experimentally infected using specific strains of HRV [36, 37].

Approximately 90% of the RV use human ICAM-1 as their cell receptor and do not bind mouse ICAM-1; the remaining 10% use a member of the low-density lipoprotein receptor family and can bind the mouse counterpart. Recently, three novel mouse models of RV infection: minor-group RV infection of BALB/c mice, major-group RV infection of transgenic BALB/c mice expressing a mouse-human ICAM-1 chimera and RV-induced exacerbation of allergic airway inflammation were described by Bartlett et al. [38]. These models have features similar to those observed in RV infection in humans, including augmentation of allergic airway inflammation, and may be useful in the development of future therapies for colds and asthma exacerbations. Association between common cold symptoms and inflammatory mediators is an important aspect of understanding common cold and RV infection. Although this association seems obvious, the exact mechanisms are less clear and one might expect a better understanding of the detailed mechanism(s) once inhibitors of viral replication are available for studies in humans. In addition, the new animal models developed by Bartlett et al. [38] can be expected to support efforts to study pathogenesis of RV infection *in vivo* in greater detail.

Respiratory syncytial virus

Human respiratory syncytial virus (RSV) was first isolated from a laboratory chimpanzee with upper respiratory tract infection (URTI) in 1956 [39]. RSV is today recognized as the leading viral agent in upper respiratory tract disease in infancy and childhood. The spectrum of RSV-caused diseases includes rhinitis, otitis media, pneumonia and bronchiolitis. The latter two diseases can be associated with a substantial morbidity and mortality. In addition, there is growing recognition for its importance as a causative agent for diseases in elderly and immunocompromised patients [40]. The World Health Organization estimates that RSV causes 64 million infections and 160 000 deaths annually [41]. A bovine RSV (BRSV) has been described causing economically important respiratory diseases in cattle [42]. Another animal RSV is the pneumonia virus of mice (PVM) [43], suggesting that there was an interspecies spread in the evolution of these viruses. However, an animal reservoir for human RSV has not been described so far [44]. Despite the importance of RSV as a leading cause for respiratory diseases, the pathogenesis of RSV infection is not fully understood and efficacious vaccines are not available.

Taxonomy, structure and replication

RSV is a member of the order *Mononegavirales*, which includes several non-segmented negative-strand RNA viruses. RSV is a member of the family of *Paramyxoviridae*, subfamily *Pneumovirinae* and represents the type species for the genus Pneumovirus [43].

RSV virions consist of an envelope and a nucleocapsid. The viral gene expression and nucleic acid replication occur in the cytoplasm. The envelope is acquired by cell budding. Virions are spherical to pleomorphic; filamentous and other forms are common. They measure 150–300 nm in diameter and up to 1000–10 000 nm in length [45]. The surface of the virion is covered by projections (spikes) formed by fusion (F) glycoproteins. The spikes are 11–20 nm long and are spaced 6–10 nm apart; they mediate attachment and penetration. The helical nucleocapsid is filamentous with a length of 600–800(1000) nm and a width of 12–15 nm [40]. The nucleocapsid does not enter the cells by surface fusion typical for paramyxoviruses but rather by membrane fusion which may involve clathrin-mediated endocytosis [46].

The unsegmented genome contains a single molecule of linear negative-sense, single-stranded RNA. Virions occasionally contain a positive-sense single-stranded copy of the genome (partial self-annealing of extracted RNA may occur). The complete genome is approximately 15 300 nucleotides long and fully sequenced. The genome has the accession number(s) [D00386] – [D00397] [43]. The RNA genome has a 3'-extragenic leader region, followed by the ten viral genes and a 5' trailer region. Each gene is transcribed into a separate mRNA encoding for a single viral protein with the exception of the M2 mRNA. This contains two overlapping ORF, expressed by a ribosomal stop-restart mechanism into two proteins, M2-1 and M2-2 [47]. Although gene expression is consistent with that of other members of the order of *Mononegavirales* M2-1 and M2-2 have some regulatory features unique to RSV [44].

The five nucleocapsid-associated proteins are the N (nucleocapsid) protein, the phosphoprotein P (co-factor for RNA synthesis), the L protein (large, a 2165-amino acid subunit of viral polymerase), the M2-1 (transcription processivity factor) and the M2-2 protein (which possesses regulatory functions) [44, 48]. The N protein binds the genomic and the antigenomic (positive-sense intermediate) RNA and protects is against degradation. In addition, it reduces the detection and responses by the host's immune system (for instance Toll-like receptors, TLRs) and intracellular RNA recognition helicases, which initiate innate immune responses [44, 49, 50].

The viral envelope is formed by four RSV proteins that associate with the lipid bilayer: a matrix (M) protein that is located at the inner surface and is important for the assembly of the virion [51], a glycosylated (G) protein, a fusion (F) protein and a small hydrophobic (SH) protein. Two other RSV proteins, the NS1 and NS2 proteins are a minor part of the virion [44]. NS1

and NS2 are thought to modulate the host response to RSV infection. The G glycoprotein (~90 kDa) has a peptide backbone with 24–25 side chains and is important for viral attachment to the host cell [52]. A second secretory form of the G protein exists that arises from a second initiation codon in the G ORF. Proteolytic trimming removes additional amino acids, and the final protein lacks the 65 N-terminal residues, including the membrane anchor [44, 52]. The ectodomain of the G protein has a mucin-like structure that differs from attachment proteins of other paramyxovirus. Its function is not clear but it is thought to contribute to virus spread or to prevent trapping by mucus [44].

The F protein has two distinct functions: penetration into the host cell by membrane fusion and a syncytia-forming property. The F protein matures by activation through a furin-like intracellular protease that cleaves the precursor, F_0 into three fragments, F_1, F_2 and p27. F_1 and F_2 are linked by a disulfide bond and represent the active form of F [53]. The hydrophobic N terminus of F_1 is conserved within the RSV and it is thought that this domain inserts into the host cell membrane when fusion occurs [44].

NS1 and NS2 are thought to modulate the host's immune response to RSV [44]. Lack of M2-1 results in reduced expression of NS1 and NS2, and it is thought that this down-regulation of the host-defense antagonists may help to facilitate persistent RSV infection [44].

Pathogenesis, immunology and clinical symptoms

Infection occurs through direct contact, through large respiratory droplets and, to a lesser extent, through small droplets. The site of the first replication is the nasopharynx. After an incubation period of 4–5 days the virus spreads to the lower respiratory tract [54, 55]. The clinical signs include cough, rhinitis, fever and signs of bronchiolitis like air trapping, wheezing and increased airway resistance. The most prominent clinical symptoms are cough and rhinorrhea, which occur in approximately 90% of primary RSV infections in infants and to some lesser extent in reinfected adults [56]. Fever occurs in 30–40% of both infected infants and adults and otitis media is reported in approximately 20% of infected infants. Ear and sinus pain is reported in 20–30% of infected adults. Symptoms of LRTI, including bronchiolitis, pneumonia, croup, wheeze and tracheobronchitis, are observed in 30–40% of infected infants and to some lesser extent in adults, with wheeze and tracheobronchitis being the most prominent disease symptoms. Hospitalization is necessary in approximately 3% of infected children and below 0.1% of infected adults. RSV is the single most important agent in children younger than 3 years of age. Importantly, children with a mild RSV disease have also been reported to have recurrent wheezing for up to 10 years after the primary acute disease [57].

Extrapulmonary dissemination may occur in immunocompromised patients [58]. The virus may spread to kidneys, liver, the central nervous system and the heart. Virus can be isolated from the nasopharynx of children for up to 14 days. In immunocompromised patients, virus recovery is possible for up to 1 month or even longer. In immunocompetent individuals the viral infection is usually restricted to the superficial cells of the epithelia and viral spread outside the respiratory tract is uncommon [54, 59]. An exception, however, is the middle ear: the virus frequently causes otitis media [60].

The typical pathological findings in RSV-infected tissue include epithelial necrosis and infiltrates of monocytes, T cells and neutrophils [61]. Airways appear obstructed due to sloughed cells, mucus secretion, proliferation of bronchoalveolar epithelium or cellular infiltration. Formation of syncytia in the bronchoalveolar epithelium is sometimes observed [61]. However, giant cell pneumonia or syncytia formation are related to severe T cell-deficient patients [44].

Specific host factors that may influence the clinical signs and outcome of RSV infection including the general health status, the nutritional status [56, 62, 63], gender, ethnic group, levels of maternal antibodies [64], age of first RSV infection [65] and underlying cardiac or pulmonary diseases [66]. In addition, there are several environmental factors (e.g., tobacco use in household, stress, day care) that may influence the course of the disease or severity of symptoms. The role of inflammation and a bias of the host's immune response towards a humoral Th2 response (the typical cytokines are IL-4, IL-5, IL-10 and IL-13) are discussed controversially in the literature. A strong inflammatory response does not seem to be determinative for the severity of clinical symptoms [67], a finding supported by the fact that in many clinical studies patients receiving anti-inflammatory therapy did not significantly benefit from that treatment [68]. However, strong inflammatory responses also have been suggested to enhance the severity of clinical symptoms in RSV disease. The role of inflammatory chemokines in RSV pathogenesis has been supported by many preclinical and clinical studies (reviewed in [44]). For example, genetic polymorphisms that increase IL-8, a major chemoattractant for neutrophils, and CCR5 expression have been associated with increased RSV disease [69]. A link between an increased ratio of Th2/Th1 immune response (a bias toward the humoral vs the classical cytotoxic response) has been suggested by several authors. This discussion is based on evidence for elevated Th2/Th1 response ratios in clinical studies, the role of key Th2 cytokines in the pathogenesis of asthma and the experience with a formalin-inactivated RSV vaccine in the 1960s [70–72]. This vaccine was poorly protective and vaccinated children and infants developed dramatically enhanced disease compared to naive patients upon natural RSV reinfection [48]. Subsequent preclinical studies confirmed a bias toward a Th2-specific $CD4^+$ T cell response in animals treated with formalin-inactivated RSV vaccine vs naturally infected animals [73]. IL-4

and IL-13 support isotype switching to IgE, which is bound to mast cells and eosinophils and, upon antigen contact, induces release of inflammatory mediators like histamine or leukotrienes by these cells. These mediators contribute to the development of the typical clinical symptoms associated with RSV disease.

Several viral proteins play an important role in the pathogenesis of RSV disease (reviewed in [44]). The soluble G protein has been suggested to modulate the innate immune response by down-regulating inflammatory mediators such as IL-6 or IL-8 in epithelial cells as a response to RSV infection [74]. G protein also modulates inflammatory responses of monocytes by acting as a general antagonist for TLR activity [75]. The G-mediated suppression of TLR-4 signaling appears to be counteracted by the F protein, which has been described to induce signaling through this TLR [76], although the significance of this activity is unclear.

Importantly, RSV infection can block the maturation of dendritic cells (DC), which serve as major antigen-presenting cells (reviewed in [44]). This alteration of DC biology may support the shift of the Th2/Th1 balance towards Th2, reduce antiviral interferon activity and limit the mobility of mature antigen-presenting cells, thus qualitatively altering the immune response to RSV infection (reviewed in [44]).

In summary, there are several host factors or viral factors that play roles in the pathogenesis of RSV infection. The picture, however, is highly complex and relative contributions of the various factors to RSV pathogenesis are not entirely understood.

Epidemiology, diagnosis and treatment

As mentioned above RSV is a leading cause of respiratory diseases in children and has increasing importance as a causative agent for respiratory diseases in elderly. In a prospective study of infants and children in the Unites States, RSV was detected in 43% of pediatric hospitalizations for bronchiolitis, 25% for pneumonia, 11% for bronchitis and 10% for croup [54]. Approximately 90% of infants have been infected at least once by 2 years of age [44, 77]. Although RSV is represented by one serotype, a protective immunity against RSV is generally weak and reinfection occurs. Virus-neutralizing antibodies, including secretory IgA found in the respiratory tract, contribute to viral clearance and may play a role in protection against reinfection [54]. However, the IgA response is short [44, 54]. In the lower respiratory tract, the IgG response has been described as more efficient. The role of antibodies as a down-modulator of clinical symptoms has been confirmed by the clinical experience with palivizumab.

In temperate regions, RSV circulates quickly during winter/early spring, but timing varies more elsewhere. Effective vaccines are not available and effective therapies are not available. However, an RSV-neutralizing human-

ized monoclonal antibody, palivizumab, reduces RSV-associated hospitalization if used as a passive immunoprophylaxis [78].

RSV infection is assumed to be frequently misdiagnosed, particularly in adults [56], because the symptoms are similar to those caused by other respiratory viruses like influenza. Laboratory diagnostic tests are usually performed on secretion samples obtained from the nasopharynx. Novel rapid ELISA-based or RT-PCR-based tests are useful particularly in a hospital setting to identify outbreaks, prevent further transmission, initiate therapies or reduce inappropriate use of antibiotics [56].

Experimental models

Animal models are comprehensively reviewed by Moore and Stokes Peebles [79]. RSV is species specific; however, some animal species exhibit semi-permissive infection with RSV. Chimpanzees were productively infected with RSV and exhibited upper respiratory tract illness, whereas adult squirrel monkeys, newborn rhesus monkeys, and infant cebus monkeys did not show symptoms but shed low levels of virus [80]. Bonnet monkeys, which are more widely available than chimpanzees, can be infected with RSV [81]. Non-human primate models of RSV infection, especially chimpanzee, have advantages but certainly limitations associated with the high cost and genetic variability, which limits the reproducibility of results.

The cotton rat which is susceptible to both URTI and LRTI with RSV is seen as one of the best animal models of RSV infection and disease [79, 82]. In these animals, RSV infection led to histologically confirmed proliferative rhinitis, bronchiolitis, and the pulmonary infiltration of lymphocytes and neutrophils [82–84]. The cotton rat model was used to study the mechanism of antibody-mediated clearance of RSV [84].

The advantages of mouse models are obvious: they are inexpensive, inbred strains are available and a wealth of reagents (e.g., antibodies), arrays, probes or information (e.g., the genome sequence) is available. Although there is variability between the strains regarding susceptibility and viral load, viral load does not vary much within the strains [79]. BALB/c is the most widely used inbred mouse strain to study RSV infection [79]. RSV-infected BALB/c mice show signs of clinical illness including weight loss, ruffled fur and ataxia peaking at day 8 after infection [85]. Interestingly, the susceptibility to RSV replication in nose and lung increased with age. The predominant histological findings in RSV-infected 3-week-old BALB/c mice were peribronchiolar and perivascular accumulations of mononuclear cells [86].

The role of Th1 and Th2 cell response to RSV infection has been the dominant focus of the BALB/c mouse studies in this context [79]. RSV infection induces a Th1 response dominated by high IFN-γ levels in the lungs of infected mice, abundant IFN-γ-producing cells in the bronchoalveo-

lar lavage fluid (BALF) and RSV-specific cytotoxic T cell (CTL) response [87, 88].

STAT1$^{(-/-)}$ mice with a BALB/c background have been described as having an excellent RSV disease phenotype (reviewed in [79]). Although this model has the limitation that it probably does not exactly mirror the complexity of a natural infection in humans, it is regarded as an attractive tool to study RSV pathogenesis and evaluating novel therapies.

In addition to other mouse models and the infection in chinchillas [89], infection of natural hosts has been studied in detail. Bovine respiratory syncytial virus is a major cause of respiratory illness in calves and has been studied in this context. The clinical signs after experimental infection include cough, lung sound, dyspnea, fever, increased respiratory rate and pulmonary resistance. Prominent histological findings include proliferative bronchiolitis, alveolitis, syncytia, and, to some extent, emphysema [90]. RSV infection of calves might be useful for evaluating vaccination strategies; however, it is not an animal model for the evaluation of novel therapies.

The list of experimental models also includes pneumonia virus infection of mice (PVM) [91]. Although the PVM model of respiratory disease is interesting because it shows the phenotype of a natural infection, PVM differs from RSV including the G and the NS1 proteins that fulfill important functions during RSV infection.

Human parainfluenza virus

Human parainfluenza viruses (HPIV) are important causes of respiratory diseases in infants and children. They usually cause URTI of which 30–50% may be accompanied by otitis media. HPIV may also cause LRTI, about 0.3% of which require hospitalization. HPIV1–3 infections are second to RSV infections as the viral cause of serious acute respiratory infections in young children that occur primarily in the first 6 months of life [92, 93]. HPIV3 may cause severe diseases. Approximately 80% of infants and children infected with HPIV3 developed febrile illness and one third of these infected individuals developed LRTI, resulting in bronchitis or pneumonia [93–95]. Most children have been infected with HPIV3 by the age of 2 years.

Croup is the main clinical manifestation of infection with parainfluenza viruses, especially HPIV1 and 2, and these infections may extend to the lower respiratory tract and result in pneumonia [94]. HPIV4 mainly causes mild URTI in children and adults [93]. Along with RSV, HPIV are also a leading causative agent of serious acute respiratory infections and community-acquired respiratory disease requiring hospitalization in adults.

The proportions of hospitalizations associated with HPIV infection vary widely in hospital-based studies. According to the WHO, HPIV1 is estimated to account for 5800–28 900 annual hospitalizations in the USA,

HPIV2 for 1800–15 600 hospitalizations, and HPIV3 for 8700–52 000 hospitalizations [92].

Taxonomy, structure and replication

Parainfluenza viruses belong to the order of *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*. HPIV1 and 3 belong to the genus Respirovirus, and HPIV2 and 4 to the genus Rubulavirus [96]. The virions are spherical enveloped particles of approximately 150–250 nm in diameter with an internal helical nucleocapsid. Virions are enveloped by a lipid bilayer membrane that bears spike-like projections composed of hemagglutinin-neuraminidase (HN) and fusion (F) protein [97].

As for other paramyxoviruses, all HPIVs contain a negative strand, ~15 500-nucleotide-long non-segmented RNA genome [93] encoding two envelope glycoproteins, the HN, and the F protein, a matrix protein (M), a nucleocapsid protein (NP) and several nonstructural proteins including a polymerase-associated protein (P/V) and the viral replicase (L) (reviewed in [93]). The replication of PIV is similar to that of other paramyxoviruses with the RNA genome serving as a template for the transcription of the mRNAs.

Binding of the HN glycoprotein to its cell receptor initiates the infection [98]. In addition to having this function, HN is thought to enhance the fusion activity of F, which, following virus attachment to the host cell, mediates the fusion of virus and subsequent penetration. F also mediates fusion of infected and uninfected cells, allowing virus to spread. F is synthesized as an inactive precursor (F_0) that is post-translationally cleaved by a host cell protease to yield two subunits, F_1 and F_2 that remain linked by a disulfide bond [93].

Pathogenesis, immunology and clinical symptoms

The mucous membranes of the upper respiratory tract are the common sites of infection. Prominent clinical symptoms of HPIV infection can be characterized by rhinitis, pharyngitis and bronchitis. The incubation period is about 4 days [95]. Coughing, hoarseness and fever that last for approximately 2–3 days are frequent. Involvement of the trachea results in croup and extension to the lower respiratory tract may lead to pneumonia. Severe disease characterized as bronchopneumonia or bronchiolitis has been observed with HPIV3 infections [93–95].

Specific virus and host properties that determine the severity of HPIV-related disease are not yet understood. Infection with PIV induces an immune response to HN and F. Neutralizing antibodies to PIV correlate with partial resistance to infection or clinical symptoms but usually do not

prevent re-infection [99]. Secretory IgA neutralizing antibodies are more important in adults than in children [100].

Epidemiology, diagnosis and treatment

As mentioned above, the HPIVs are important causes of respiratory tract diseases in infants and children. Epidemics of HPIV3 usually occur in the early spring [101]. Viruses do not persist for a long time in the environment [93]. The seasonal peak of HPIV1 and 2 infections is reflected in the seasonality of croup, which is the highest in autumn in the USA [102]. Croup during the winter months is more likely to be caused by other viruses, such as influenza virus or RSV [93].

The diagnosis of PIV infection is mainly clinical, and molecular diagnostic procedures are usually not performed. There is no specific antiviral treatment against PIV available, and therapeutic intervention is mainly targeted against symptoms of croup. Early treatment will reduce the severity of the symptoms, the rates at which patients return to a health care practitioner for additional medical attention, visits to the emergency department, and admission to the hospital [103].

Effective vaccines are currently not available. Attenuated strains have been studied and a virosomal formulation of an HPIV3 vaccine is currently under development [92]. The National Institute of Allergy and Infectious Diseases (NIAID) is studying the safety and immunogenicity of a recombinant live-attenuated chimeric bovine/human parainfluenza type 3 virus, rB/hPIV3, vaccine in a Phase I study. The test vaccine is delivered as nose drops to adults 18–49 years of age, HPIV3-seropositive children 15–59 months of age, and HPIV3-seronegative infants and children 6–36 months of age [104]. HPIV vaccines are also developed by some companies [105].

Adenovirus

Adenoviruses cause infections of the respiratory and gastrointestinal tract, kidney, eye and other organs, the latter mostly as a consequence of immunosuppression [106]. They are known to frequently cause respiratory infections among people in institutional environments – outbreaks among children are reported at boarding schools and summer camps [107]. Outbreaks have also been reported in military camps [108]. Most infections with adenovirus result in infections of the upper respiratory tract. In addition, adenovirus infections may result in conjunctivitis, tonsillitis, ear infection or croup [106–109]. Adenoviruses are responsible for approximately 5% of acute respiratory infections in children under the age of 5 [107, 109]. The Centers for Disease Control (CDC) reported in the November 16, 2007, issue of the Morbidity and Mortality Weekly Report [MMWR 56(45):1181–1184] an unusual num-

ber of cases of severe pneumonia and deaths caused by adenovirus serotype 14 (Ad14) infection among civilian and military communities.

Taxonomy, structure and replication

Adenoviruses belong to the family *Adenoviridae*, genus Mastadenovirus. There are 6 species including human adenovirus A–F with 51 immunologically distinct human adenovirus serotypes [110]. The most common human adenoviral pathogens belong to the C adenoviruses and those mainly infect the upper respiratory tract [107].

The virions are not enveloped. They consist of a capsid and a core with proteins associated to it. The icosahedral capsid has a diameter of 70–100 nm [111, 112]. All capsids consist of 252 capsomers. The surface structure reveals a regular pattern with distinctive features. Surface projections are often lost during preparation. Distinct filaments protrude from the 12 vertices/pentons [112–114].

The genome is not segmented and contains a single molecule of linear double-stranded DNA with terminally redundant sequences, which have inverted terminal repetitions (ITR). The complete genome of mastadenoviruses is approximately 31–36 kpb long and has a guanine + cytosine content of 48–61%. The genome has a terminal protein, which is covalently linked to the 5'-end of each DNA strand [114, 115].

The viral genome encodes structural proteins and non-structural proteins. Virions consist of 11 proteins located in the capsid, fibers, and core. The capsid is comprised of seven polypeptides, polypeptide II which is the basis for the hexon (three tightly associated proteins). Polypeptides VI, VIII, IX are associated with the hexon, polypeptides VI and VIII serve as bridge between the capsid and the core. Five polypeptide III copies are the basis for the penton. Polypeptide IV forms the trimeric fiber [116], which has a knob domain that serves as a viral receptor for the target host cell. The cell receptor is the receptor for coxsackie B virus and adenovirus (CAR) for adenoviruses A, C, D, E and F and CD46 for adenoviruses B with the exception of serotypes 3 and 7 [117]. The core consists of four proteins (V, VII, mu and the terminal protein, which is covalently linked to the 5´ end of the DNA) and the DNA (reviewed in [112]).

The replication cycle of adenoviruses is divided into two phases. The early phase includes adsorption of the virus to the host cell, penetration, transcription and translation of early genes. The early gene products mediate gene expression and DNA replication, block apoptosis and promote the cell cycle progression. In addition, they possess potent immunomodulatory functions (reviewed in [112]).

The viral E1A gene product should briefly be mentioned: In the nucleus, E1A activates the expression of a number of genes by interacting with cellular transcription factors and other cellular regulatory proteins [112]. E1A

has been postulated to play role in the pathogenesis of chronic obstructive pulmonary disease (COPD) [118–120].

Pathogenesis and clinical symptoms

Human adenovirus A–F can cause human infections ranging from respiratory disease, and conjunctivitis (B and D), to gastroenteritis (F serotypes 40 and 41) [106, 112]. The most common clinical picture after adenovirus infection of the respiratory tract is mild self-limiting upper respiratory disease with nasal congestion, coryza and cough [106, 113]. Some patients develop exsudative tonsillitis that is clinically indistinguishable from streptococcus tonsillitis [120]. These infections are commonly caused by serotype 1, 2, 5 and 6 C adenoviruses and serotype 3 B adenovirus [107, 109]. Respiratory symptoms may be accompanied by systemic manifestations including generalized malaise, chills, fever and headache [106]. However, adenoviruses may infect alveolar and bronchiolar epithelial cells [121] and cause pneumonia, bronchiolitis or bronchiolitis obliterans [122–124]. Adenoviruses account for approximately 10% of pneumonias in children [106].

In contrast to many other respiratory viruses, both persistent and latent infections have been described, particularly in lymphocytes [106]. Adenoviral DNA may persist in the nuclei of infected cells or even integrate into the host DNA. Adenoviral E1A protein has been postulated to play a role in the pathogenesis of COPD [121]. Adenoviral DNA was found in the lungs of COPD patients and expression of E1A correlated with disease severity [125–127]. In response to inflammatory stimuli, E1A increases ICAM-1 and IL-8 expression along with nuclear factor-κB (NF-κB) activation in lung epithelial cells ([128], reviewed in [113]). While these factors support emphysema, E1A up-regulates transforming growth factor-β1 (TGF-β1) in bronchiolar epithelial cells [129], supporting a role for E1A in airway remodeling [130].

In summary, adenoviruses are pathogens that frequently cause mild or severe acute infections of the respiratory tract. The importance of adenovirus infections, however, goes beyond acute airway disease.

Epidemiology, diagnosis and treatment

Adenoviruses are non-enveloped pathogens and thus very stable to chemical or physical agents and adverse pH conditions. It is believed that respiratory adenoviruses are mainly spread *via* aerosols; however, other routes (fecal, waterborne) also frequently lead to infection. Antibodies to one or more adenoviruses are found in approximately 50% of infants and nearly 100% of adults and since there are many different types of adenovirus, repeated adenoviral infections can occur [109, 131, 132].

Although adenovirus infections can occur at any time of the year, respiratory tract disease caused by adenovirus is more common in late winter, spring, and early summer [106].

Virological diagnosis can be performed using a variety of molecular or immunological approaches. This is, however, only important in the context of severe or epidemic diseases. Development efforts for vaccines were discontinued [133]. Antiviral therapy is only important in infected immunocompromised patients. In these patients, cidofovir has shown some promise [122].

Human metapneumovirus

Human metapneumovirus (HMPV) was identified in 2001 [134], and is today considered as a major cause of acute respiratory infections worldwide, especially in children. Virtually all children have experienced an infection with HMPV by the age of 5–10 years (reviewed in [135]).

Taxonomy, structure and replication

HMPV is a member of the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Pneumovirinae* (as is RSV), genus Metapneumovirus [136]. There are two major groups and at least four subgroups of HMPV [137–140]. HMPV particles are enveloped, pleomorphic, filamenteous and spherical and have a mean diameter of approximately 210 nm [137]. The genome consists of a single-stranded negative RNA of approximately 13.3 kb and contains eight genes in the order 3'N-P-M-F-M2-SH-G-L-5' coding for a nucleoprotein (N), a phosphoprotein (P), matrix protein (M), fusion protein (F), a transcription elongation factor (M2-1), a protein regulating RNA synthesis (M2-2), a small hydrophobic protein (SH), attachment protein (G), a polymerase subunit (L) and probably additional proteins [141, 142]. The F protein is the major immunogenic viral protein [143]. Replication is generally comparable to that of other members of *Mononegavirales*.

Pathogenesis, immunology and clinical symptoms

This virus infection occurs primarily during winter months or early spring and can manifest as both upper and lower respiratory tract disease [144]. After a severe HMPV infection, virus was detected in alveolar and airway epithelial cells [145]. It was also reported in this study that the virus caused acute organizing lung injury, tissue damage and, which is not observed in other paramyxovirus infections, induction of smudge cell formation.

The clinical symptoms associated with HMPV infection are indistinguishable from those of RSV infection [146] and range from common cold to pneumonia. Otitis media is observed in up to 50% of the infected individuals (reviewed in [135]). As for other respiratory viruses, HMPV may cause exacerbation of underlying chronic diseases like asthma, congestive heart disease or chronic obstructive pulmonary disease. The importance of coinfections with RSV or influenza viruses is not clear (reviewed in [135]). As for many other respiratory viruses, serious disease caused by HMPV is observed among immunosuppressed patients. Results from a recent retrospective study suggested that HMPV infection may be an important cause of idiopathic pneumonia syndrome after stem cell transplantation [147].

Epidemiology, diagnosis and treatment

HMPV is thought to be the second or third cause of severe acute respiratory tract infection in children, just ranking behind RSV and influenza virus [146, 148]. Infections occur very early in life and up to 100% of children have experienced an HMPV infection by the age of 10 years. Reinfections occur frequently. Incidences in hospitalized children with acute respiratory tract infection range from 5% to 10%. Incidence is up to 20% in patients consulting at an outpatient clinic (reviewed in [135]).

The routes of transmission are believed to be similar to those of RSV (respiratory droplets, hand-to mouth or hand-to eye contact) [149, 150].

The diagnosis of HMPV infection is mainly clinical. Molecular diagnostic procedures are usually not performed routinely but are possible using standard technologies like RT-PCR or immunological techniques.

There is no specific antiviral treatment available. However, ribavirin has shown some promise [151] and monoclonal antibodies are under development that could potentially be used to prevent HMPV infections [152]. Several vaccines are being developed but are still in an early stage.

Human bocavirus

Human bocavirus (HBoV), a parvovirus, was detected in children with LRTI in 2005 using random amplification methods [153]. HBoV infection is predominantly associated with respiratory and/or gastrointestinal symptoms in children at around 2 years of age [154]. Seroprevalence reaches 95% in adults [155]. However, the role of HBoV in the pathogenesis of human respiratory disorders is not yet fully understood – HBoV infections are frequently accompanied by coinfections with other viral and bacterial pathogens [154].

Taxonomy, structure and replication

HBoV is classified into the *Parvoviridae* family, subfamily *Parvovirinae*, genus Bocavirus. As other parvoviruses, HBoV virions are icosahedral non-enveloped particles with a diameter of 21–25 nm [154]. The HBoV genome (a linear single-stranded DNA that encompasses approximately 5.2 kb) is organized like that of other parvoviruses: conserved genes encoding for the two non-structural proteins are located in the 5' region and genes for two structural proteins are located in the 3' region of the genome [156]. The structural proteins VP1 and VP2 are identical in sequence but differ in an N-terminal extension that is only present in VP1 (VP1 unique region, VP1u) and possesses a phospholipase A2-like activity (PLA2) [157]. The functions of the two nonstructural proteins NS1 and NP1 of HBoV are not known; however, regulatory functions of NS1 of other parvoviruses have been described [154].

Pathogenesis, immunology and clinical symptoms

HBoV has been detected in children with respiratory disease. The range of clinical manifestations is broad. Diseases of the upper (rhinitis or coughing) and the lower respiratory tract (including pneumonia, bronchiolitis and wheezing) or even the gastrointestinal disease have been described (reviewed in [154]). Other symptoms include fever or rashes [158]. However, HBoV infections are linked frequently with coinfections with viral and bacterial pathogens in up to approximately 69% of HBoV DNA-positive individuals [159] which makes it rather difficult to distinguish between symptoms that have been caused by HBoV or by other pathogens. The role of vertical transmission, seen with other parvoviruses, is not known.

Antibodies against the viral structural protein VP1 have been detected in approximately 95% of children older than 2 years and adults [155]. In addition, IgG1 subclass antibodies against HBOV VP2-virus-like particles (VLP) were detected in approximately 98% of samples that were obtained from healthy adult blood donors [154]. The same authors found IgM antibodies in 41.7% of sera from HBoV DNA-positive children but not in samples from DNA-negative children. Cellular immunity also plays a role in HBoV infections and frequent $CD4^+$ T helper cell reactions have been observed against HBoV VLP [160].

Epidemiology, diagnosis and treatment

Many epidemiological aspects have been discussed above. However, it is noteworthy that the majority of the analyses have been performed in symptomatic individuals and more data from asymptomatic healthy chil-

dren and adults would add to the understanding of the HBoV epidemiology.

Diagnosis of HBoV infection is mainly done by PCR amplification of viral DNA or the detection of anti-HBoV antibodies by ELISA [154, 161].

At present, no specific treatment of HBoV infections is available.

Human Coronavirus

Coronaviruses are known to cause a variety of diseases in animals [161]. Human coronaviruses are mainly associated with respiratory disorders; some may cause enteric infections [162]. The human coronaviruses HCoV-229E and HCoV-OC43 were identified in the 1960s [163–165]. A coronavirus causing a severe acute human respiratory syndrome (SARS), the SARS-CoV, was first described in 2003 [166, 167] and two additional human coronaviruses, HCoV-NL61 and HCoV-HKU1 that were both linked to respiratory disorders have been identified recently [168, 169].

Because of the economic importance of coronaviruses in veterinary medicine (for instance in swine), development of vaccines is more advanced in veterinary medicine than in human medicine. However, with the appearance of the SARS coronavirus, human coronaviruses gained a greater share of interest.

Taxonomy, structure and replication

Coronaviruses belong to the order *Nidovirales*, family *Coronaviridae*, genus: Coronavirus. In addition to the five human coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL 63, HCoV-OC43 and SARS-CoV), a specific human enteric coronavirus has been reported [170].

Coronaviruses have been assigned to three groups based on antigenic relationships between species of different groups (reviewed in [171]). HCoV-229E and HCoV-NL63 are included in group 1, HCoV-HKU1 and HCoV-OC43 in group 2 and SARS-CoV represents an early split from group 2 [172].

Coronaviruses are enveloped viruses, approximately 120 nm in diameter with large (20 nm) club-shaped surface projections (spike protein, S). The structure and function of the S protein have been reviewed elsewhere [173]. Apart from S, coronaviruses have a smaller membrane protein, M (reviewed in [174]). In addition, coronaviruses have a third envelope protein, the very small, non-glycosylated E protein [175]. E and M have been found to be essential for virus particle formation (reviewed in [176]). Group 2 coronaviruses also have an HE (hemagglutinin esterase) protein that forms a layer of approximately 7 nm [175]. HE is a neuraminic acetylesterase that hydrolyses the 9-O-acetylated sialic acid on erythrocytes, thus potentially

destroying receptors [177]. Another coronavirus protein, N, is closely linked to the RNA genome (and forms a ribonucleoprotein, RNP). N, which may have a functional role in replication and transcription, is phosphorylated (reviewed in [178]).

Coronaviruses have positive-sense single-stranded RNA genomes of about 30 kb. The genome is generally organized in the following manner: 5'-UTR-polymerase gene-structural protein genes-UTR3' where the UTR are untranslated regions each up to 500 nucleotides. The structural proteins are encoded in the following order: HE (only group 2 coronaviruses) – S – E – M - N [171].

The infection is initiated by binding of the S protein to the cell receptor [179]. CD13 (human aminopeptidase N, APN), a metalloproteinase located on the surface of epithelial cells, has been identified as the cell receptor for HCoV-229E [180]. A metallopeptidase, angiotensin converting enzyme 2 (ACE 2) may be the cell receptor for SARS-CoV [181]. Binding of the S protein to the cell receptor induces conformational changes in S that triggers fusogenic activity [182]. The coronavirus genome can only be released into the cytoplasm after fusion of the envelope with the cell membrane which is mediated through the S2 region of the S protein [173, 183]. Replication occurs within the cytoplasm. Early during infection the genomic RNA is released and acts as an mRNA for the translation of the first gene, the polymerase. mRNAs for the other genes are generated subsequently. In general, coronaviruses have several 3' co-terminal subgenomic mRNAs, so-called 'nested set'. The unique part of each mRNA (which is not within the next smaller mRNA) is translated during the replication cycle. At the 5' end of each gene there is a sequence that is common to all genes, the so-called 'transcription-associated sequence', which, as the name implies, is associated with the discontinuous transcription process (reviewed in [171]). The various mechanisms that have been proposed for the production of subgenomic mRNAs have been reviewed elsewhere [184].

Pathogenesis, immunology and clinical symptoms

Human coronaviruses are generally thought of as common cold agents. This role was confirmed when healthy volunteers were infected with HCoV-OC43 and HCoV-229E and developed classical common cold symptoms [185]. Whereas most infections result in mild disease or are even asymptomatic, additional factors like immunosuppression or coinfections might cause severe disease, even pneumonia [186, 187]. It is not clear whether HCoV-229E and HCoV-OC43 infect the lower respiratory tract in otherwise healthy people, because only URTI was observed in this population. It has been suggested that the lower respiratory tract is more susceptible to HCoV infection in children [187]. Most studies were performed in healthy adults and there is less information about the most susceptible and vulnerable

population, children and elderly. However, HCoV-229E and HCoV-OC43 nucleic acid were frequently detected in children with respiratory tract disease (11%) using RT-PCR, whereas in an otherwise healthy control group (asymptomatic bone marrow recipients) only one sample tested positive (0.37%, $p < 0.01$) [188]. This finding suggests that these coronaviruses cause upper and lower respiratory tract diseases in children that are more severe than in adults. It has been suggested that up to 30% of wheezing episodes in asthmatic children may be due to coronavirus infections [189].

HCoV-NL63 was detected in young hospitalized children with severe LRTI [190]. This virus has also been detected in elderly patients with fatal respiratory disease [191]. The risk of developing croup was about 6.6 times higher in children shown to be positive for HCoV-NL63 than in those who tested negative [192, 193]. HCoV-HKU1 was first detected in elderly and children with underlying disease [194]. Symptoms of HCoV-HKU1 include rhinorrhea, fever, coughing, and wheezing as well as bronchiolitis and pneumonia [195]. HCoV-HKU1 might also cause gastrointestinal disease [196].

HCoV infection results in antibody titers in serum. Secretory antibodies can be detected in the respiratory and enteric tracts (and in milk or colostrum) [197].

Epidemiology, diagnosis and treatment

Infections with HCoV peak during winter season [198]. It is estimated that approximately 25% of common cold cases are caused by coronaviruses [162]. Outbreaks of different human coronaviruses have found to alternate every 2–3 years [197]. Although early studies demonstrated that antibodies against coronaviruses are frequently present in adults [199], new studies suggest that there are differences with respect to the HCoV species. Hofmann et al. [200] demonstrated that infections with HCoV-229E occur less frequently than with HCoV-NL63 by measuring specific antibodies neutralizing either HCoV-NL63 or HCoV-229E. In addition, co-detection of coronaviruses with other viruses is common [187]. As with most coronaviruses, human coronaviruses are species specific. However, the SARS coronavirus probably originated from an animal reservoir, presumably bats [201, 202] but was transmitted to humans by civet cats.

The transmission of human coronaviruses from human to human occurs *via* secretions like aerosols and respiratory droplets (or, in case of enteric infection, feces) [197]. Adults with acute symptomatic or inapparent infection transmit the virus to infants who develop clinical disease [162].

Diagnosis of HCoV infection is more clinical; an etiological diagnosis, however, can be performed using molecular or immunological techniques.

Vaccines against coronavirus diseases have been developed for domestic animals because of the economic importance [197], but not for humans. Recent antiviral strategies against coronavirus infection have been reviewed

elsewhere [203]. These strategies explore small interfering RNA, blocking of viral entry (e.g., using carbohydrate-binding agents) or neutralizing antibodies. In addition, viral enzymes like protease or helicase are studied as potential targets for novel antivirals (reviewed in [203]).

Influenza virus

According to estimates from the WHO, the burden of influenza in the USA is currently estimated to be 25–50 million cases per year, leading to 150 000 hospitalizations and 30 000–40 000 deaths per year. If these numbers are extrapolated to the rest of the world, influenza virus would infect 5–15% of the world population, causing 3–5 million cases of severe disease and approximately 0.5 million deaths per year. Although this number is high, it would only characterize inter-pandemic influenza [204]. Epidemics and outbreaks of influenza follow a seasonal pattern, which differs according to the region in the world: in temperate climate zones, seasonal epidemics typically begin in the late fall with a peak in late winter. The seasonal pattern is less pronounced in tropical zones (reviewed in [205]).

The focus of this book is on 'common cold'. For details on influenza and influenza viruses, further reading of standard literature is recommended.

Taxonomy, structure and replication

Influenza viruses are members of the *Orthomyxoviridae*, genus Influenzavirus and include influenza virus types A, B and C. The viruses are enveloped pleomorphic particles with a size ranging from 100 to >300 nm, their genome is organized on eight (influenza virus A and B) or seven (influenza virus C) negative-sense single-stranded RNA segments [206]. Spikes consist of hemagglutinin (HA) and neuraminidase (NA). The nomenclature for human influenza virus includes type, geographic location of the first isolation, isolate number and year of isolation. In addition, subtypes of influenza A are described by their HA and NA designations. To date, 16 HA and 9 NA types have been described. The viral envelope is also associated with a matrix protein (M) that, after infection, forms a tetrameric ion channel. Several polymerase proteins (PB1, PB2, PA) form, together with the nucleoprotein (NP) and the RNA, a ribonucleoprotein (RNP) complex (reviewed in [206]). Influenza viruses are transmitted *via* the respiratory route and bind to a cell receptor that consists of oligosaccharides and that is present on the surface of respiratory epithelial cells. The sialic acid-α2,6-galactose linkage (SAα2,6Gal) that is associated with binding to human influenza virus HA is present in the human respiratory tract (reviewed in [207]). After binding, the virus enters the host cell by endocytosis [208]. Further steps include fusion of the virus to the endosome [206] and the release of the RNP

into the nucleus through an ion channel that is formed by M2 [209]. The viral RNA is a template for complementary RNA and the mRNA. The non-structural NEP/NS2 as well as M1 play a role in the nuclear export of novel RNA. Assembly occurs at the apical surface of the cell, budding occurs and the novel virus is released (reviewed in [206]).

Pathogenesis, immunology and clinical symptoms

Influenza viruses are transmitted *via* the respiratory route [206]. Host specificity is largely determined by the availability of host cell receptors on the surface of epithelial cells [210]. Usually, influenza in humans is an URTI that is characterized by cough, headache, malaise and fever [211]. However, complications are frequent, and encephalitis, Reye's syndrome, myelitis [212] as well as muscular manifestations of the infection including myocarditis [213], disseminated intravascular coagulation and toxic and septic shock [214] may occur.

Major airway congestion, inflammation and necrosis have been found in histopathological examinations [215].

Although much progress in the understanding of the pathogenesis of human influenza has been made during the past decade, the molecular mechanisms responsible for the virulence of particular strains of influenza virus are not yet understood.

Epidemiology, diagnosis and treatment

Influenza A virus can infect humans as well as waterfowl and chickens, swine, horses, and other species. Influenza B virus, on the other hand, has a restricted host range and circulates predominantly in humans. However, influenza B virus was recently isolated from seals [216]. Different types of HA mediate species-specific binding of the virus [217, 218]. It would be beyond the scope of this book on common cold to review all the recent literature that has been published regarding the epidemiology of influenza viruses. However, one important aspect should be mentioned here. Influenza virus is a changing virus and the repetitive occurrence of the yearly epidemic is supported by 'antigenic drift', an accumulation of point mutations in the viral receptors (HA and NA). The drift is attributed to a low fidelity of the viral RNA polymerase [219]. These new variants then infect a population without pre-existing immunity (reviewed in [206]). A second process that contributes to the emergence of new influenza variants is called 'antigenic shift'. An antigenic shift may occur during co-infection with other influenza viruses. Such a co-infection may lead to a viral reassortment (an exchange of genome segments between different virus strains) [206]. If this process leads to a new virus strain that is able to effectively spread from individual

to individual, a worldwide outbreak, a pandemic, may occur [207]. There have been several pandemics in the past century including the so-called "Spanish flu" in 1918/1919, infecting about the half of the world population at that time and killing approximately 20–50 million people [204]. Current pandemic concerns are focused on the highly pathogenic variants of the strain A/H5N1 or H1N1. The epidemiology of influenza has been comprehensively reviewed [220, 221].

Influenza is typically diagnosed clinically, but laboratory diagnosis has been established and organized according to standardized procedures as part of national and global pandemic plans.

Several effective vaccines exist today (reviewed in [222]) and a pre-pandemic vaccine has been licensed recently.

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