Recently Discovered Human Coronaviruses

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Coronaviruses (CoVs) are a large and diverse group of positive-stranded RNA viruses in the Coronaviridae family, which also comprises members of the Torovirus genera. Together with the Arteriviridae and Roniviridae families, Coronaviridae are grouped in the order of Nidovirales, based on their conserved genome organization and mechanism of replication. The name Nidovirus is derived from their unique transcription strategy involving formation of nested (in Latin: nidus) mRNA molecules with identical 3’ ends during an infection. Coronavirus particles are enveloped and measure 120 to 160 nm in diameter, containing a linear, single and positive stranded RNA genome with an average length of 27 to 31 kb, the largest RNA genome described so far. The viral RNA molecule is organized together with multiple copies of the nucleocapsid (N) protein to form a flexible core inside the viral membrane that constitutes the spike (S), envelope (E), and membrane (M) proteins. In certain isolates, an additional structural protein is present on the virion: hemagglutinin esterase (HE). The heavily glycosylated S proteins are crucial for CoVs to establish and maintain an infection cycle, by interacting with specific cellular entry molecules to initiate a fusion between viral and cellular membranes.

In the 1930s, isolation of the first CoV, avian bronchitis virus (IBV), was reported. Ever since, many CoVs have been discovered in a broad range of hosts, including mammals and birds. CoVs are transmitted by means of respiratory aerosols and the fecal–oral route of infection, and primarily target mucosal surfaces of respiratory and intestinal tracts, causing illnesses of varying severity. In addition, manifestations...
of neurologic, hepatic, and systemic disorders are induced after infection with certain CoVs.8

Human CoV (HCoV) infections initially seemed to be associated primarily with mild and self-limiting upper respiratory tract infections, such as the common cold, and were not thought to be connected to severe human illnesses.9 Coronaviral pathogens, however, gained renewed scientific interest in 2003, when a novel HCoV was proven to be the etiologic agent of the worldwide severe acute respiratory syndrome (SARS) epidemic.10–14 Infection with SARS-CoV resulted in severe lower respiratory tract infections, causing high morbidity and mortality within a short period of time.15 Soon thereafter, numerous new CoVs were discovered, including two species with the potential to infect humans: HCoV-NL63 and HCoV-HKU1.16–18

HUMAN CORONAVIRUS 229E AND HUMAN CORONAVIRUS OC43: COMMON COLD AGENTS

For a long time, only two HCoV species were known: HCoV-229E and HCoV-OC43, both isolated in the mid-1960s. HCoV-229E was recovered from medical students in Chicago who had clinical symptoms of upper respiratory tract infection. The virus could be propagated on primary human kidney cells and human embryonic lung cells.19 Shortly thereafter, a distinct CoV was isolated using human embryonic tracheal organ cultures, and termed OC43 for organ culture number 43.20 Inoculation of volunteers at the Common Cold Unit in Salisbury, United Kingdom, demonstrated a causal relationship between HCoV-229E and HCoV-OC43 infections and common cold symptoms.21

The common cold is a typical self-limiting upper respiratory tract disease, characterized by mild clinical symptoms, including nasal obstruction and rhinorrhea, sneezing, sore throat, and cough. There is no single cause for this heterogeneous group of upper respiratory tract illnesses; in fact numerous viruses from several different families function as etiologic agents.22 Although rhinoviruses account for the largest proportion of all upper respiratory tract infections, HCoV-229E and HCoV-OC43 now are known to be responsible for a high number of these cases, which occur mainly during winter and early spring seasons in temperate climate countries.23–25 Although coryza occurs more often during HCoV-229E infections, there are indications that sore throat manifestations are observed more frequently in patients who have HCoV-OC43 infections.26 Infants, elderly, and immunocompromised individuals are thought to be vulnerable for more severe upper and lower respiratory tract infections, including pneumonia, caused by infections with HCoV-229E and HCoV-OC43.9,27,28

Since their discovery, other pathologies have been connected occasionally to HCoV-OC43 and HCoV-229E. HCoV-OC43 initially was proposed to be involved in gastrointestinal (GI) disease development in children.29 This hypothesis, however, never was confirmed by inoculation studies with healthy individuals.9 In addition, presence of CoV RNA in brain tissue and antibody concentrations in serum of multiple sclerosis (MS) patients, led to the suggestion of CoV involvement in MS etiology.30–33 Although evidence for a significant correlation between presence of HCoV-229E and HCoV-OC43 RNA and MS has not been demonstrated,34,35 accumulating recent data from cell culture and animal models indeed confirm their neurotropic and neuroinvasive potential.36,37 Nevertheless, actual brain invasion in MS patients by HCoV-229E and HCoV-OC43 might be explained by a disrupted blood–brain barrier.9

SEVERE ACUTE RESPIRATORY SYNDROME

The first case of SARS, a severe lower respiratory tract illness with a mortality rate of 10%, emerged in November 2002 in Fushan City, China.38 Subsequently, SARS
spread rapidly throughout eastern Asia and to 28 other regions around the world, causing 774 deaths in 8098 infected individuals.\textsuperscript{39} In February 2003, a newly emerged HCoV, which originated from a wild animal reservoir, was demonstrated to be the etiologic agent of this syndrome.\textsuperscript{11} Of all HCoVs described thus far, SARS-CoV causes the most severe clinical symptoms. Of interest, SARS rarely is detected in young children, and if so, it seems to follow a less aggressive clinical course.\textsuperscript{40} The strongest predictor of poor disease outcome appears to be an advanced age (older than 60 years).\textsuperscript{41}

By means of droplet inhalation, SARS-CoV reaches the respiratory tract and invades epithelial cells of trachea, bronchi, bronchioles, and alveoli.\textsuperscript{42} SARS-CoV typically causes a broad spectrum of disease, starting with an influenza-like syndrome, including symptoms such as high fever, malaise, rigors, and fatigue.\textsuperscript{41} After disease onset, infections may progress to a nonsevere variant of disease or cough variant, characterized by relatively moderate symptoms.\textsuperscript{43} Generally, 2 to 7 days after SARS onset, a typical respiratory phase is initiated, including nonproductive cough and dyspnea. In two thirds of infected patients, disease deteriorates toward an atypical pneumonia, with shortness of breath and poor alveolar oxygen exchange.\textsuperscript{44} Symptoms may worsen even further into an acute respiratory distress syndrome (ARDS), as a result of progressive pulmonary immune infiltration, formation of hyaline membranes, diffuse alveolar damage (DAD), and a high viral burden.\textsuperscript{42,45,46} ARDS is the most severe form of acute lung injury (ALI) and is regarded as the leading cause of death in SARS-CoV infected individuals.\textsuperscript{47,48} Lung injury in patients who have SARS is supposed to occur directly, by viral-mediated destruction of alveolar and bronchial epithelial cells, as well as indirectly, through extensive production of immune mediators.\textsuperscript{41,44}

**HUMAN CORONAVIRUS NL63 AND HUMAN CORONAVIRUS HKU1 INFECTIONS**

Shortly after the SARS-CoV outbreak, an unknown respiratory virus was isolated in Amsterdam, The Netherlands, in a nasopharyngeal aspirate specimen (sample NL63) from a 7-month-old infant suffering from coryza, bronchiolitis, conjunctivitis, and fever. The infectious agent was identified as a distinct and fourth human member of the Coronaviridae family: HCoV-NL63, using a novel technique to amplify viral genomes without a priori knowledge of their sequence.\textsuperscript{17} Within a few weeks, a second research group from The Netherlands reported detection of essentially the same virus, initially designated HCoV-NL.\textsuperscript{16} Because similarity of these isolates is very high at the nucleotide level, they both represent the same species: HCoV-NL63. HCoV-NL63 is demonstrated to be genetically most closely related to HCoV-229E.\textsuperscript{49} HCoV-NL63 infections are recognized throughout the whole world, and are identified as nonfatal upper and lower respiratory tract infections in infants, the elderly, and immunocompromised adults.\textsuperscript{9} In addition, a clear association between HCoV-NL63 infections and trachea inflammation in children (laryngotracheitis or croup) has been demonstrated through population-based studies.\textsuperscript{50–53} In patients who have croup, HCoV-NL63 infections are detected as frequently as the parainfluenzaviruses, which initially were considered as the main causative agent for this illness.\textsuperscript{50} Although an additional fascinating disease association was proposed for HCoV-NL63 and Kawasaki disease (the most common form of childhood vasculitis),\textsuperscript{54} it could not be confirmed by subsequent investigations.\textsuperscript{55–60} Several current indications strongly suggest that HCoV-NL63, in addition to HCoV-229E and HCoV-OC43, is a common cold-causing virus in healthy adults. Actual evidence for this causal relationship is, unfortunately, still lacking.
In January 2005, a fifth HCoV was discovered in Hong Kong, China. HCoV-HKU1 was recovered from an adult who had chronic pulmonary disease, and it was only distantly related to HCoV-OC43. Clinical symptoms accompanying an HCoV-HKU1 infection include rhinorrhea, fever, cough, febrile seizure, wheezing, pneumonia, and bronchiolitis. Similar to HCoV-NL63, infections with HCoV-HKU1 have been detected worldwide; they presumably are associated with common colds, and most likely cause a more severe clinical spectrum of respiratory disease in young children, adults with underlying disease, or the elderly. Furthermore, there are indications that HCoV-HKU1 also might play a role in GI disease.

KOCH'S POSTULATES

Once novel viruses are identified, it is important to demonstrate their pathogenic potential and unravel a causal link with a specific disease. Proof of such a relationship ideally would imply fulfilling Koch’s postulates, which have been revisited for viral pathogens. These standard criteria propose that a causal connection between a new virus and an illness may be established if:

The organism is consistently present in patients who have disease at a higher prevalence than in control patients.

The disease is replicated in an appropriate animal model after viral challenge, and subsequently isolated from this animal.

A specific host immune response can be demonstrated.

In the case of HCoV-NL63 and HCoV-HKU1, application of all Koch’s postulates turned out to be impossible, and their role in disease therefore remains unconfirmed. Currently, HCoV-HKU1 cannot be maintained in cell culture systems, and animal models are unavailable for both NL63 and HKU1 CoVs. Animal model systems susceptible for HCoV-OC43 and SARS-CoV have been developed previously, and allow present studies of coronaviral tropism, replication, recombination, and accompanying immune modulatory mechanisms. Most recently, a very important technical achievement has been made for studying pathogenic mechanisms of HCoV-NL63, because infectious full-length cDNA clones of the HCoV-NL63 genome can be engineered. Nonetheless, thus far, the only option to identify pathogenic potential of HCoV-NL63 and HCoV-HKU1 is to determine a significant association with a disease through epidemiologic studies with proper control groups. An alternative strategy to gain novel insights in mechanisms of CoV pathogenesis is by extensive characterization of virus–host interactions and host cell invasion strategies. Viral receptor specificity and expression are generally important determinants of the pathogenic potential of a virus and the nature of the disease that it causes.

CELLULAR RECEPTOR MODULATION: A PATHWAY TO HUMAN CORONAVIRUS PATHOGENESIS

Viral receptors, components that actively promote host cell entry, differ greatly from one virus to the next and constitute a wide variety of proteins and carbohydrates, each with distinct physiologic functions. Although cellular receptors for HCoV-OC43 and HCoV-HKU1 remain to be elucidated, the family of membrane-associated proteases seems to be favored by HCoVs, because both neutral aminopeptidase (APN), the receptor for HCoV-229E, and angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV and HCoV-NL63, exist as prominent zinc-dependent peptidases on host cell plasma membranes. In fact, several structural features of zinc metalloenzymes probably facilitate targeting of APN and ACE2 and govern
cellular entry of HCoVs. Zinc peptidases are expressed abundantly on various cell types, because these proteases modulate activity of many proteins including membrane proteins and circulating regulatory peptides. Furthermore, both APN and ACE2 appear as heavy glycosylated ectoenzymes, with most of the protein, including catalytic domain, protruding into the extracellular space.

During establishment of an infection, interaction of HCoV-229E and SARS-CoV spike proteins with APN or ACE2, respectively, causes a substantial modulation of these cellular entry receptors. SARS-CoV has been proven to induce a rapid down-regulation of ACE2 cell surface expression, preferably by means of internalization of the receptor–ligand complex. Alternatively, SARS-CoV possesses the capacity to abrogate ACE2 cell surface expression by means of activation of tumor necrosis factor-alpha converting enzyme (TACE). This enzyme mediates ectodomain shedding of ACE2. Whether HCoV-NL63 induces a similar down-regulation of ACE2 during infection is at present unknown.

It is assumed that cellular APN expression is altered during establishment of an HCoV-229E infection. Following HCoV-229E binding to the target cell, APN molecules aggregate and translocate to caveolin-enriched membrane domains, to activate a specialized endocytic route of virus particle internalization. Most importantly, these processes of receptor-mediated endocytosis often involve simultaneous internalization of the cellular entry molecule itself. Likewise, sequestration of porcine APN molecules into intracellular vesicles has been visualized during endocytosis of CoV strain porcine-transmissible gastroenteritis virus (TGEV). Thus, HCoV-229E-induced abrogation of APN expression is highly plausible to occur, although direct evidence is unavailable. Viral targeting of APN and its subsequent down-regulation is definitely a known phenomenon, as this cellular peptidase possibly is implicated in infection with human cytomegalovirus (CMV) also. Notwithstanding the fact that human APN is most likely not the primary receptor of the virus, CMV induces abrogation of APN expression.

The phenomenon of entry receptor suppression has been reported for several additional viruses, including HIV, measles virus (MV), influenza virus, and human herpes virus (HHV) type 6. Although it may seem contradictory, viruses strongly benefit from down-regulation of their own receptors, and this process correlates with an enhanced pathogenesis also. Abrogation of receptor expression prevents infection of cells by additional virus particles in which viral replication is already progressing. In addition to limiting superinfection, receptor down-regulation can facilitate efficient virion release, leading to a controlled and productive infection. At the same time, abrogation of receptor expression hampers natural physiologic activity of these cellular molecules and therefore may contribute to viral disease pathogenesis also. Internalization of CD4 after HIV-gp120 binding, for example, leads to specific impairment of immune cell functions. Moreover, MV hemagglutinin (HA)-induced CD46 receptor abrogation induces serious dysregulation of complement pathways and mechanisms of immunosuppression.

Down-regulation of APN and ACE2 during HCoV-229E, HCoV-NL63, and SARS-CoV infection may impair the normal physiologic function of the host cells severely and contribute to the development of clinical manifestations. Besides their classification as zinc-dependent peptidases, APN and ACE2 share important functional enzymatic characteristics. Both proteins are integral components of the renin–angiotensin system (RAS). This endocrine system is one of the most important regulators of human physiology, with a key role in maintenance of arterial pressure, fluid hemostasis, salt balances, cardiac function, cell proliferation and hypertrophy, angiogenesis, and apoptosis. Therefore, impaired expression of APN and ACE2 also might alter crucial
normal physiologic functionalities of the RAS. Most intriguingly, suppression of ACE2 protein expression during SARS-CoV infection actually causes severe imbalances within the enzymatic RAS cascade, which is proposed to be the main cause of severe acute pneumonia and acute lung failure observed during SARS-CoV infection. These findings raise the possibility that CoV-induced dysregulation of the RAS might be important for the clinical outcome of HCoV-229E and HCoV-NL63 infections also.

SUMMARY

CoVs are recognized human pathogens, associated with relatively mild upper respiratory tract infections in healthy adults and more serious respiratory complications in weakened patients. A virus-induced modulation of receptor expression could be involved in the onset of CoV-associated clinical symptoms, and future research should focus on elucidation of the physiologic consequences following virus-host interactions. Insight into these processes would contribute to the clarification of the strategies used by HCoVs to elicit specific diseases and might provide a definite demonstration of their etiology also. Eventually, a better understanding of HCoV pathogenesis may lead to development of new therapeutic strategies.

REFERENCES


