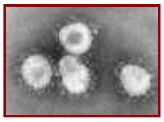
VIROLOGY - CHAPTER TWENTY FIVE

CORONA VIRUSES, COLDS AND SARS

Dr Richard Hunt
Professor
Department of Pathology, Microbiology and Immunology
University of South Carolina School of Medicine



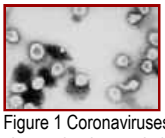


Figure 1 Coronaviruses are a group of viruses that have a halo or crown-like (corona) appearance when viewed under a microscope
CDC/Dr. Fred Murphy (top) CDC/Dr. Erskine Palmer (bottom)

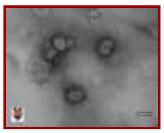


Figure 2 Torovirus © Queen's University, Belfast

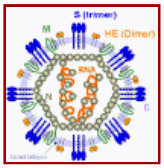


Figure 3 Coronavirus structure. Adapted from Lai and Homes. In Fields' Virology. Lippencott

Coronaviruses, which are about 100nm in diameter, are the largest positive strand RNA viruses (indeed they have the largest genomes of any RNA virus). They infect humans and animals in which they cause respiratory and enteric disease. The coronaviruses, along with the toroviruses and arteriviruses, belong to a group, the nidovirales, that produce a nested set of mRNA with a common 3' end (see below). The coronaviruses and the toroviruses (which together make up the Coronaviridae) have helical nucleocapsids while the arteriviruses have icosahedral nucleocapsids. Coronaviruses have an envelope that is derived from intracellular membranes and not the plasma membrane. In electron micrographs they have spikes sticking out of their surfaces (due to a large glycoprotein), leading to their name (corona = crown) (Figure 1 and 2).

This chapter will only discuss the Coronaviruses since they are particularly important in human respiratory disease, causing about one third of "common colds" and the newly recognized severe acute respiratory syndrome (SARS).

PROTEINS

S (spike) protein (150k)

This is a transmembrane glycoprotein with three domains (figure 3): the large external domain (with two sub domains), the transmembrane sequence and the small internal domain. The external domain (N-terminal) folds to a globular shape and forms the spike structures in electron micrographs. This region gives the virus its antigenic properties and contains the binding site for the cell surface receptor. The inner part of the external domain is probably coiled-coil and contains heptad repeats. There is a fatty acyl molecule here which may stabilize the protein in the lipid bilayer. The inner part of the external domain forms a stalk-like structure that associates with other S proteins to form a trimer. In some coronaviruses, the external domain is cleaved but the two parts of the glycoprotein remain associated by ionic interactions (in a similar manner to the gp120 and gp41 of HIV). The inner part of the S protein, which may become exposed on binding to the host cell, is responsible for membrane fusion. Interestingly, the S protein has a region that is similar to the Fc-gamma receptors for immunoglobulins allowing the virus to coat itself with these proteins and protect itself from immune attack (herpes viruses have a similar strategy). S protein can bind to sialic acid (9-O-acetyl neuraminic acid) on the host cell surface which gives the virus a hemagglutinating ability. Antibodies against S protein are neutralizing.

HE protein (65kD)

Only some coronaviruses have a hemagglutinin-esterase protein. This also forms spikes (shorter than S spikes) on the virus surface. It is a dimer and does not appear to be essential for replication in those types that possess it. This protein also binds sialic acid. . The esterase activity of HE protein can cleave the sialic acid from a sugar chain, which may aid the virus in escaping from the cell in which it was replicated. Antibodies against HE protein can also neutralize the virus.

M (membrane) protein

This is another membrane-spanning glycoprotein but most of the protein is internal with only a small external N-terminal domain. M protein spans the viral membrane three times. This protein helps in the attachment of the nucleocapsid to the membranes of internal structures such as the Golgi Body and is not found on the plasma membrane of the cell (unlike the other glycoproteins)

E (envelope) protein (9-12kD)

This small protein is also on the viral membrane. In the infected cell it is found around the nucleus and at the cell surface.

N (nucleocapsid) protein (60kD)

The nucleocapsid protein binds to the genomic RNA via the leader sequence and to the M protein on the inner surface of the viral membrane. N protein is phosphorylated

Unlike many other RNA viruses, coronavirus do not incorporate the RNA polymerase into the virus particle; rather the polymerase is made after infection by using the positive sense genomic RNA as an mRNA. This is possible because the pol gene is at the 5' end of the genome.

ATTACHMENT OF THE VIRUS TO THE HOST CELL

As noted above, the major attachment protein is the S protein and this binds to sialic acid. HE protein also binds sialic acid. However, sialic acid is found on the surfaces of all cells and coronaviruses have a restricted tissue tropism and so binding must be more complicated. Moreover, some coronaviruses do not bind to sialic acid at all. S protein can bind to other more specific receptors. In the case of murine hepatitis virus, the receptor appears to be a member of the immunoglobulin superfamily and antibodies against this protein block virus attachment. Other coronaviruses including human respiratory coronavirus use a membrane bound metalloproteinase (aminopeptidase N) as their receptor.

PENETRATION

Fusion of the viral membrane with a cellular membrane, a prerequisite for viral replication in the cytoplasm, can occur at the plasma membrane or in acidic endosomes.

VIRUS ASSEMBLY

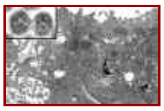


Figure 4. Coronavirus within cytoplasmic membrane-bound vacuoles and cisternae of the rough endoplasmic reticulum. This thin section electron micrograph of infected Vero E6 cell, shows particles of coronavirus, suspected as the cause of severe acute respiratory syndrome (SARS). CDC/C.S. Goldsmith/T.G. Ksiazek/ S.R. Zaki

There is a sequence of 61 nucleotides near the 3' end of the genome that is only found in the positive sense genomic RNA. This interacts with N protein to form the nucleocapsid. The nucleocapsid interacts with M protein that is exposed on the cytoplasmic surfaces of intracellular membranes (endoplasmic reticulum, Golgi Body and especially a budding compartment that is between the endoplasmic reticulum and the Golgi Body, perhaps the cis-Golgi network). M protein is not on the cytoplasmic surface of the plasma membrane. It seems that the N protein must be associated with RNA for this to happen. In addition, E protein is required for budding of the nucleocapsid into the membrane where it may alter membrane curvature as part of the budding process. It is probably the E protein that attaches to the M protein. The E protein is found in the mature virus, but only in small amounts so it may be a scaffold protein that is needed for assembly initiation but can be dispensed with thereafter. S and HE protein also interact in the plane of the lipid bilayer of the budding compartment with the M protein. The S-M and HE-M complexes then associate and the sugar chains are processed as the virus passes through the Golgi Body. The virus matures morphologically in the Golgi Body and accumulates in membrane-bound vesicles in the cytoplasm which subsequently fuse with the plasma membrane (figure 4).

GENOME

Coronaviruses have a very large (for RNA viruses) single strand genome, in fact the largest of all of the RNA viruses. The genome is positive sense (that is, the same sense as the mRNA) and is non-segmented (c.f. the orthomyxoviruses). The genomic RNA is capped and polyadenylated and ranges in size from 27 to 32kB. It is the large size of the genome coupled with the lack of proof reading in RNA polymerases that leads to the high mutation frequency in coronaviruses. Several coronaviruses have been sequenced, including the SARS virus. The order to the genes is always the same. At the 5' end is the polymerase (pol) and this is followed by four structural proteins that are found in all coronaviruses:

- The spike protein (S), so called because it sticks out of the surface of the virus
- The envelope protein (E)
- The membrane protein (M), that is incorporated into intracellular membranes of the host cells (particularly the Golgi Body)
- The nucleocapsid protein (N)

Some coronaviruses also have a gene between the pol gene and the S gene that may have been picked up from a paramyxovirus, the hemagglutinin-esterase (HE) gene. There are also additional open reading frames (ORFs) which are not highly conserved among different coronaviruses. These genes likely code for proteins but their function is unknown. In addition to the protein-coding genes in the genomic RNA, all coronaviruses have 7 base sequences called intergenic sequences that are at the 5' end of each gene. If the intergenic sequence is altered (mutated), the sub-genomic mRNA that starts at this point is not made.

REPLICATION

Figure 5. Messenger RNAs of corona viruses. A nested set of RNAs with a common 3' end are formed. The mRNA for the polymerase (pol) is the same length as the genomic RNA. The remainder are truncated at the 5' end although all have a common leader sequence

Most of the genomic length RNA in the infected cell is destined for packaging into virus particles. These molecules are presumably made by continuous synthesis (in contrast to the mRNAs), although there is evidence for separate production of the leader sequence in manner similar to that used for mRNA synthesis (see below).

Messenger RNAs

All coronaviruses make a nested set of mRNAs that have a common 3' end but lack the 5' end (except for the mRNA that codes for gene 1 protein, the polymerase) (Figure 5). Like the genomic RNA, these sub-genomic mRNAs are capped and polyadenylated. Only one protein is translated from each sub-genomic mRNA, that is the protein encoded in the 5'-most open reading frame (orf), even though all of these molecules, except the smallest, have more than one protein coding sequence. Each mRNA also has a common leader sequence of about 70 bases at the 5' end. This is also found at the 5' end of the genomic RNA but not elsewhere in this molecule, although the intergenic sequence is similar to a region of the leader.

When the genomic RNA enters the cytoplasm, it is copied into a complementary negative strand. This is then copied back into genomic positive strand and the sub-genomic mRNAs. Cells also contain sub-genomic negative strand (anti-sense) RNAs but these are always in double strand complexes with sense strands. It is not known how the sub-genomic mRNAs are made with their common leader sequence but several possibilities have been suggested. For example, the leader sequence of the negative strand may be copied to a positive sense stand of about 70 bases. The leader sequence could then dissociate from the genomic strand and recognize one of the intergenic sequences on the template. Here, it may prime positive stand synthesis as far as the end of the genomic RNA.. In support of this discontinuous model of mRNA synthesis is the observation that if an intergenic sequence is artificially inserted into the genome, a new mRNA starting at this point, including the leader sequence, is formed.

The polymerase (replicase)

The first gene at the 5' end of the genome is that which encodes the replicase or RNA polymerase. It takes up more than half of the genome (since it is about 20kB long). Sequencing shows that this gene actually contains two protein-coding sequences that are in different reading frames and overlap one another. However, the two sequences give rise to one protein, called a polyprotein, by ribosomal slippage when the ribosome comes to the beginning of the second sequence. As the huge polyprotein is being made, it is cut by proteases that are parts of the nascent protein. One of the proteins that is liberated is the RNA polymerase.

Mutation and recombination

Coronaviruses have large RNA genomes replicated by a virus-encoded replicase/polymerase. RNA polymerases have no proof-reading capability and typically have an error rate of about 1 in 10,000 nucleotides. Since the genome of an average coronavirus is about 30kB, this means that there will be several mutations in each progeny virus. There are also many deletion mutations formed in coronaviruses. There is a very high frequency of recombination in coronaviruses which is not typical of non-segmented RNA viruses. This may be due to the discontinuous mode of RNA replication in which the leader sequence is made and then the leader/polymerase may jump to another strand. This high rate of recombination results in rapid evolution of the virus and the formation of new strains.

COLDS AND OTHER ASPECTS OF CORONAVIRUS DISEASE

Pathogenesis

Coronaviruses cause respiratory and enteric disease in a variety of animals. In humans, the major site of virus replication is the epithelial cells of the respiratory tract and about one-third of colds are caused by coronaviruses. The symptoms are similar to those of rhinovirus colds (runny nose, sore throat, cough, headache, fever, chills etc.) with an incubation time of about 3 days. Viral spread is limited by the immune response of most patients but this immunity is short-lived. Symptoms may last about a week with considerable variation between patients. Often there are no apparent symptoms but the patient still sheds infectious virus

In contrast to the rhinoviruses, which are not enveloped, coronaviruses are rather unstable. Transmission is by transfer of nasal secretions such as in aerosols caused by sneezes. Viruses that infect epithelial cells of the enteric tract cause diarrhea. This can occur in human neonates but is common in many young animals where the infection can be fatal. Although coronavirus infections are usually local, they can spread. In humans, these viruses have been implicated in infections of the middle ear, in some pneumonias in immuno-suppressed patients and in myocarditis but again, in animals, systemic infections can be much more severe (e.g. feline infectious peritonitis).

Interestingly, coronaviruses, which can infect neural cells in the laboratory, can cause a disease in rodents that looks very like multiple sclerosis, leading to the suggestion of their involvement in the human disease; demyelination, a characteristic of multiple sclerosis in the rodent model, is linked to the S protein and it has been suggested that the disease results from molecular mimicry in which an immune response to the S protein results in immune attack on myelin. However, although the virus can be detected in the brain of patients, the link to multiple sclerosis remains unproven.

Epidemiology

Most people harbor anti-coronavirus antibodies but reinfection is common indicating that there are many circulating serotypes of the virus in the human population. There do not appear to be animal reservoirs for those viruses that infect humans.

As with most respiratory infections, coronavirus-caused colds are more common in the winter because of closer contact. Major outbreaks occur every few years with a cycle that depends on the type of virus involved.

WEB RESOURCES
WHO SARS Site
CDC SARS Site

Diagnosis

Most coronavirus infections go undiagnosed and the disease is self-limiting. Diagnosis can be carried out using immuno-electron microscopy and serology. There are no anti-virals for routine coronavirus infections but over-the-counter remedies to alleviate symptoms are useful.

SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

Figure 6A. Map of probable SARS cases. June 02, 2003 WHO

Figure 6B. Weekly new cases of SARS. © WHO/BBC

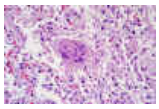


Figure 7. Pathologic cytoarchitectural changes indicative of diffuse alveolar damage, as well as a multinucleated giant cell with no conspicuous viral inclusions. CDC/Dr. Sherif Zaki

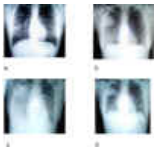


Figure 8. Chest radiographs of index patient with severe acute respiratory syndrome (SARS). a, day 5 of symptoms; b, day 10; c, day 13; d, day 15. Li-Yang Hsu, Cheng-Chuan Lee, Justin A. Green, Brenda Ang, Nicholas I. Paton, Lawrence Lee, Jorge S. Villacian, Poh-Lian Lim, Arul Earnest, and Yee-Sin Leo - Tan Tock Seng Hospital, Tan Tock Seng, Singapore. Emerging and Infectious Diseases

In late 2002, a new syndrome was observed in southern China (Guangdong Province). It was named severe adult respiratory syndrome (SARS). This disease, which has now been reported in Asia, North America, and Europe (figure 6A), is characterized by a fever above 38 degrees (100.4 degrees Fahrenheit) accompanied by headache, general malaise and aches. In fact, respiratory symptoms are initially usually mild but after a few days (or a week), the patient may develop a dry non-productive cough and breathing may become difficult (dyspnea). Respiratory distress leads to death in 3-30% of cases. Laboratory tests show a reduction in lymphocyte numbers and a rise in aminotransferase activity which indicates damage to the liver.

The initial outbreak of SARS peaked in April 2003 and by June had tailed off. By that time, there had been about 8,000 cases worldwide and 775 deaths. In addition, there were billions of dollars in economic losses.

The virus was grown on monkey Vero E6 cells in tissue culture and a new coronavirus (SARS-coV) was found to be associated with the disease. It has a genome of 29,727 bases and eleven open reading frames. The sequence, though similar to other coronaviruses, is sufficiently different to make this a member of a new coronavirus group. The organization of the genome is very similar to that of other coronaviruses (5' replicase (rep), spike (S), envelope (E), membrane (M), nucleocapsid (N)-3' and short untranslated regions at both termini). The replicase gene occupies the 5' two-thirds of the genome and has, like other coronaviruses, two overlapping open reading frames. It also codes for a protease in the pol polyprotein. There are nine possible open reading frames that are not found in other coronaviruses and may code for proteins that are unique to the SARS virus. Using antibody tests, SARS-coronavirus has been associated with SARS cases throughout the world.

Diagnosis

The Centers for Disease Control recommend a chest radiograph (figure 8), pulse oximetry, blood cultures, sputum Gram's stain and culture, and testing for viral respiratory pathogens, notably influenza A and B and respiratory syncytial virus. A specimen for Legionella and pneumococcal urinary antigen testing should also be considered. People with suspected SARS should be isolated and quarantined.

Treatment

There is no agreed treatment for SARS other than management of symptoms. Drugs are under development and of particular interest are drugs that may block the protease function since this is crucial to the virus. There is no vaccine against the SARS virus or any other human coronavirus. Veterinary vaccination programs of modest success exist for a number of economically important Coronaviruses. A major problem with live virus vaccine is antigenic shift and unpredictable outcomes.

What is the case definition for SARS?	What do the lung X-rays of a SARS patient look like? (External site)	Why are civet cats thought to be the vector for the SARS virus?	SARS and Bats: How did SARS enter the human population?

A NEW CORONAVIRUS - MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

BOOK
SARS REFERENCE
pdf

WEB RESOURCES
Middle East Respiratory Syndrome Coronavirus
CDC

In 2012, a disease caused by a novel Coronavirus appeared in the Middle East, particularly in Saudi Arabia. Initially, all patients lived in or had visited the Middle East even though some had subsequently migrated to Europe where cases appeared in France, the UK and Germany. After an initial infection, the virus spread to close contacts indicating human to human transmission. The patients develop pneumonia and sometimes kidney failure with a fatality rate of over 50% although this high fatality rate may reflect the failure to diagnose less virulent cases. The virus was first named Novel Corona Virus (nCoV) and then named "Middle East Respiratory Syndrome Coronavirus" (MERS-CoV) and is distinct from the SARS Coronavirus. It is treated with interferon α2b and ribavirin.

Once again the origin of this novel coronavirus is bats, specifically the pipistrelle bat and from phylogenetic analysis, it appears that the virus entered the human population around 2011.

Return to the Virology section of Microbiology and Immunology On-line

This page last changed on Wednesday, November 23, 2016
Page maintained by Richard Hunt

Please report any problems to Richard Hunt