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Severe Acute Respiratory Syndrome (SARS)

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Glossary

Bioavailability A measurement of the proportion of the orally administered dose of a therapeutically active drug that reaches the systemic circulation and is available at the site of pathology.
Coryza A runny nose.
Desquamation Shedding of epithelialium.
Lymphopenia Reduction of lymphocytes in the circulating blood below the normal range for age.
Myalgia Muscle pain.
Pathognomonic Characteristic and diagnostic of a particular disease.
PEGylated An adjective for describing molecules conjugated with polyethylene glycol (PEG).
Radiological abnormalities Atypical findings observed by medical imaging procedures (e.g., chest X-ray).

History

From November 2002 to January 2003, cases of an unusually severe atypical pneumonia were being observed in Guangdong Province, China. The disease was characterized by the lack of response to conventional antibiotic therapy and the occurrence of clusters of cases within a family or healthcare setting. In retrospect, these were the first known cases of the disease that was later to be called severe acute respiratory syndrome (SARS). Through January, the numbers of cases of this unusual 'atypical pneumonia' continued to increase with examples of 'super-spreading incidents' that were to punctuate the course of the subsequent SARS epidemic. Between 16 November and 9 February, 305 cases were identified, one-third of them in healthcare workers (Table 1).

On 21 February 2003, a 65-year-old doctor working in a hospital in the city of Guangzhou, the provincial capital of Guangdong, arrived in Hong Kong and checked into Hotel M. He had treated patients with 'atypical pneumonia' in Guangzhou and had been ill himself since 15 February. His 1-day stay on the ninth floor at this hotel led to the infection of at least 17 other guests or visitors, some of whom traveled on to Hanoi, Toronto, Vancouver, Singapore, USA, Philippines, Guangzhou, and Australia. Five of these secondary cases initiated clusters of infection in Hanoi, Singapore, Toronto and two clusters of infection within Hong Kong. This was the most significant single event in the global spread of SARS, and arguably the most dramatic known event in the global spread of any infectious disease. However, because the secondary cases had largely dispersed outside of Hong Kong, this cluster of cases remained 'invisible' until the epidemiological linkages were reconstructed in mid-March.

Between 26 February and 10 March, disease outbreaks were recognized in the Hanoi-French Hospital in Vietnam and in Prince of Wales Hospital in Hong Kong. Dr. Carlo Urbani, a World Health Organization (WHO) communicable diseases expert stationed in Vietnam, examined the first cases of the disease outbreak in Hanoi and provided WHO with the first case descriptions of this new disease. Later, Dr. Urbani was himself one of the victims who succumbed to this disease. On 12 March, the WHO issued a Global Health Alert regarding an atypical pneumonia that was a particular risk to healthcare workers. Subsequently, Singapore and Toronto also reported clusters of cases. On 15 March, the WHO issued a Travel Advisory. The new disease was named SARS and a preliminary case definition was provided. The WHO set up virtual networks of virologists, clinicians, and epidemiologists to rapidly collate, evaluate, and disseminate information about the new disease.

Within weeks, SARS had spread to affect 8096 patients in 29 countries across five continents with 744 fatalities, an overall case–fatality rate of 9.6%. Healthcare facilities served as a major amplifier of infection, constituting 21% of all reported cases.

By 21–24 March, the etiological agent of SARS was identified to be a novel coronavirus, subsequently termed SARS coronavirus (SARS CoV). Serological tests demonstrated that the human population had no prior evidence of infection with SARS CoV, indicating that this virus had newly emerged in humans and implying a likely zoonotic origin.

Early case detection and isolation of infected individuals reduced and interrupted SARS CoV transmission across the world. By 5 July 2003, the WHO announced that all chains of human transmission of SARS were broken and the outbreak was at an end. This was indeed a historic triumph for global public health. Although SARS was subsequently to re-emerge to cause limited human disease (and in one instance, limited human-to-human transmission) as a result of laboratory escapes and zoonotic transmission from the live game animal markets of Guangdong in December 2003–January 2004 (Table 1), the human outbreak of SARS had been controlled.

Table 1 A chronology of events in the emergence of SARS

Date	*Key events*
16 November 2002	45-year-old man in Foshan city, Guangdong Province, mainland China becomes ill with fever and respiratory symptoms and transmits the disease to four other relatives.
10 December 2002	35-year-old restaurant chef working in Shenzhen is admitted to Heyuan City People's Hospital. Transmits disease to eight healthcare workers.
January 2003	Pneumonia outbreaks in Guangzhou (capital city of Guangdong Province). These include number of healthcare workers infected through the care of patients with the disease.
11 February 2003	Guangdong health authorities report an outbreak of respiratory disease in Guangdong with 305 cases and five deaths, one-third of the cases being in healthcare workers caring for patients with the disease. Cases were reported from Foshan, Heyuan, Zhongshan, Jiangmen, Guangzhou, and Shenzhen municipalities of Guangdong Province.
21 February 2003	A 65-year-old doctor from Guangdong arrives and checks in at Hotel M in Hong Kong. His stay of 1 day at this hotel leads to the infection of at least 17 other guests or hotel visitors who initiate clusters of infection within Hong Kong, Vietnam, Singapore, and Toronto.
26 February 2003	A 48-year-old 'Hotel M contact' is admitted to Hanoi-French Hospital in Vietnam and is the source of an outbreak there. Seven healthcare workers were ill by 5 March.
1 March 2003	A 22-year-old 'Hotel M contact' is admitted to Tan Tock Seng Hospital, Singapore. She will pass on infection to 22 close contacts.
4 March 2003	A 26-year-old Hotel M contact admitted to Prince of Wales Hospital, Hong Kong. His illness is relatively mild and is not categorized as severe pneumonia. He transmits infection to 143 persons including 4 members of his family, 67 healthcare workers or medical students, and 30 other patients.
5 March 2003	A 78-year-old 'Hotel M contact' dies at home in Toronto, Canada. Four family members are infected. They are the source for the subsequent Toronto outbreak.
5–10 March 2003	Outbreaks are recognized in Hanoi and Hong Kong.
12 March 2003	WHO issues a Global Alert about atypical pneumonia in Guangdong, Hong Kong, and Vietnam that appears to place healthcare workers at high risk.
13–14 March 2003	Singapore and Toronto report clusters of atypical pneumonia. In retrospect, both groups have an epidemiological link to Hotel M. One of the doctors who had treated develops symptoms while traveling and is quarantined on arrival in Germany on 15 March.
15 March 2003	WHO has received reports of over 150 cases of this new disease, now named severe acute respiratory syndrome (SARS). An initial case definition is provided. Travel advisory issued.
17 March 2003	WHO multicenter laboratory network on SARS etiology and diagnosis is established.
21–24 March 2003	A novel coronavirus is identified in patients with SARS.
12 May 2003	The genome sequence of the SARS coronavirus is completed.
June 2003	A virus related to SARS CoV is detected in civets and other small mammals in live game-animal markets in Guangdong.
5 July 2003	Lack of further transmission in Taiwan, the last region to have SARS transmission, signals the end of the human SARS outbreak.
September 2003	Laboratory-acquired SARS coronavirus infection in Singapore.
December 2003–January 2004	Re-emergence of SARS infecting humans from animal markets in Guangdong. Laboratory-acquired SARS coronavirus infections in Taiwan.
February 2004	Laboratory-acquired SARS leads to community transmission in Beijing and Anhui in China.

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Virology

SARS Virus

SARS coronavirus is a member of the genus *Coronavirus* within the family *Coronaviridae* and the order *Nidovirales*. Coronaviruses are classified on genetic and antigenic characteristics into three groups and SARS CoV is presently regarded as a group 2b coronavirus. It is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approx 29.7 kbp. The virus particle is approximately 100–160 nm in diameter with a distinctive corona of petal-shaped spikes on the surface which is comprised of the spike glycoprotein (S). The S protein is in a trimeric form on the viral surface. It has an N-terminal variable subdomain (S1) which contains the motifs responsible for receptor binding. A more conserved subdomain (S2), which contains heptad repeats and a coiled-coil structure, is important in the membrane fusion process. The S1–S2 subdomains remain in a noncleaved form in the intact SARS CoV virion and cleavage is believed to occur within the endocytic vesicle during the viral entry process. The envelope also contains a transmembrane glycoprotein M and in much smaller amounts, an envelope (E) protein. The M protein is a

triple-spanning membrane protein and has a key role in coronavirus assembly. The hemagglutinin-esterase (HE) glycoprotein, found in some group 2 coronaviruses, is absent in SARS CoV. The nucleocapsid protein (N) interacts with the viral genomic RNA to form the viral nucleocapsid. Viral replication complexes are believed to be localized within double-membraned vesicles or autophagosomes.

SARS CoV Genome

The genome of SARS CoV is that of a typical coronavirus. The viral genomic RNA has at least 14 open reading frames (ORFs) (**Figure 1**). The genome codes for 16 nonstructural proteins (nsp1–16), 5 structural proteins, and 7 accessory proteins. The genomic RNA encoding the replicase gene functions as mRNA to generate polyproteins 1a and 1ab. The translation of ORF1b is directed by a –1 ribosomal frameshift (RFS) signal that contains a nucleotide slippery sequence (5′-UUUAAAC-3′) and an RNA pseudoknot. By contrast, the structural and accessory proteins are products derived from subgenomic RNA (sgRNA 2–9) which are synthesized by discontinuous RNA transcription. Translated products from ORF2 (S), ORF4 (E), ORF5 (M), and ORF9a (N) are viral structural proteins as described above. Recently, it was reported that the protein encoded by the ORF3a, which is able to interact with S and contains ion channel activity, is also a structural protein. However, the full function of this protein is yet to be determined.

The polyproteins 1a and 1ab generated from the replicase gene are cleaved by a papain-like proteinase (part of nsp3) and a 3C-like proteinase (nsp5) to generate 16 nonstructural proteins (**Figure 1(b)**). Nsp12 is a primer-dependent RNA-dependent RNA polymerase (RdRp), whereas nsp8 is a noncanonical RdRp (nsp8) synthesizing primers utilized by nsp12. In addition, eight nsp7 and eight nsp8 subunits are able to form a hexadecamer with a hollow, cylinder-like structure. RNA-binding studies and the overall architecture of this nsp7–nsp8 complex suggest that it might encircle RNA and confer processivity of nsp12. The nsp9 is a single-stranded RNA-binding protein and is able to interact with nsp8. The nsp13 is a helicase and unwinds duplex RNA (and DNA) in a 5′-to-3′ direction. The nsp3, nsp14, nsp15, and nsp16 have been shown to have ADP-ribose 1′-phosphatase, 5′-to-3′ exonuclease, endoribonuclease, and 2′-*O*-ribose methyltransferase activities, respectively. These four proteins are distantly related to cellular enzymes involved in RNA metabolism. These observations may be relevant to viral RNA processing. The nsp10 contains two zinc finger motifs and is suggested to be a regulator of vRNA synthesis. The biological functions of nsp1, nsp2, nsp4, nsp6, and nsp11 are largely unknown. The nsp1 is reported to induce chemokine dysregulation and host mRNA

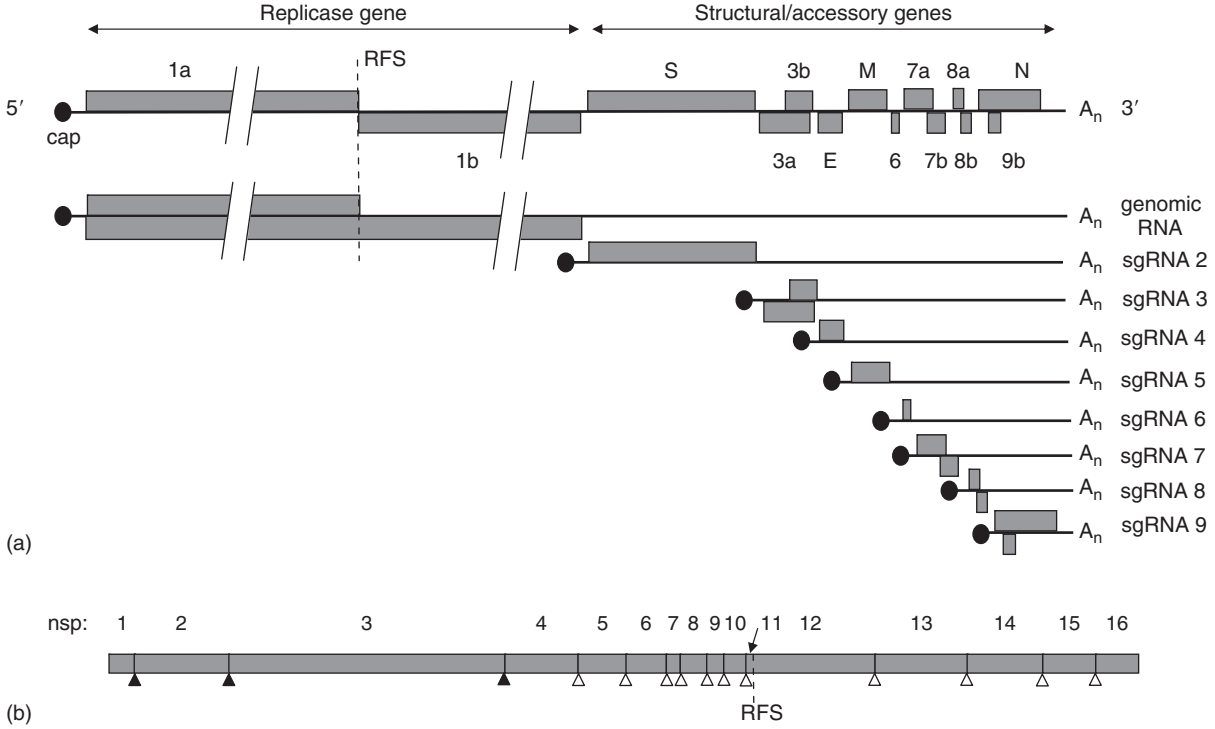

Figure 1 SARS CoV genome. (a) Genomic organization of SARS CoV. The 14 ORFs are expressed from the genome RNA and a nested set of subgenomic mRNA (sgRNA 2–9) that all have a common leader sequence derived from the 5′ end of the genome. The genomic RNA and all sgRNA contain a 5′ cap and a polyadenylated tail at the 3′ end. (b) Domain organization of the proteins for ORF1ab. Black and white arrow heads represent the sites cleaved by papain-like and 3C-like proteinases, respectively. The ribosomal frameshift (RFS) site is highlighted by a broken line.

degradation. The nsp2 is dispensable for virus replication. The nsp4 and nsp6 each contain a putative transmembrane domain.

Apart from the ORFs encoding the replicase and structural proteins, the viral genome contains additional ORFs that code for accessory proteins (3b, 6, 7a, 7b, 8a, 8b, and 9b). Genetically modified recombinant viruses without these accessory ORFs have been shown to be replication competent in cell cultures, indicating that the accessory ORFs may not be essential for virus replication *in vitro*. However, recombinant viruses with deletions in these regions are attenuated, suggesting that these proteins might have functions that are important for viral replication and pathogenesis *in vivo*. The accessory proteins from ORF3b and ORF7a induce apoptosis in transfected cells. There is also evidence suggesting that the 7a protein is incorporated into virions. The protein encoded in ORF6 has been shown to inhibit the nuclear import of STAT-1 and function as an interferon antagonist in infected cells. These properties might relate to virus virulence. Interestingly, comparative sequence analysis of SARS CoV isolated from palm civets (see below) and humans showed that all animal isolates contained a 29-nucleotide (nt) sequence which is absent from most human isolates obtained in the later phase of the SARS outbreak. As a result, the ORF8 in these human SARS CoVs encodes 8a and 8b proteins, whereas the corresponding ORF in the animal isolates encodes a single protein, known as the 8ab protein. These proteins from the animal and human ORF8 have differential binding affinities to various SARS CoV structural proteins. Furthermore, the expression of E can be downregulated by 8b but not 8a or 8ab in infected cells. These observations may suggest that the 29-nt deletion might modulate the replication or pathogenesis of the human SARS CoV. The crystal structure of the 9b protein suggests that it might be a lipid binding protein but its function is yet to be identified. Overall, these accessory proteins may play roles in viral replication and pathogenesis.

Ecology and Animal Reservoir

Until the end of January 2003, 39% of patients with SARS in Guangdong had handled, killed, or sold wild animals or prepared and served them as food. However, such risk factors were found in only 2–10% of cases from February to April 2003 when the virus had adapted to efficient human-to-human transmission. Thus, the early epidemiological evidence pointed to the live game animal trade as a potential source of the SARS CoV. SARS-like coronaviruses were identified in a number of small mammalian species sold in the live game animal markets in Guangdong, including the palm civet (*Paguma larvata*), raccoon dog (*Nyctereutes procyonides*), and the Chinese ferret badger (*Melogale moschata*). A high proportion of individuals working in these markets were observed to have developed antibodies to SARS CoV, although none of them had a history of the disease. Viruses isolated from the re-emergent SARS cases in Guangdong in December 2003–January 2004 were more similar to those found in civets in these markets, rather than to viruses causing the global outbreak in early 2003. These observations strongly implicated the live game animal trade as the interface for interspecies transmission of a precursor animal SARS-like coronavirus to humans.

SARS CoV can be shed for weeks in experimentally infected palm civets but many of the other species appear to clear the virus rapidly. While civets in live animal markets were often observed to be positive for SARS-like coronavirus RNA, civets tested in the farms that supply these markets and those caught in the wild rarely have evidence of infection. Thus, palm civets were believed not likely to be the natural reservoir of the precursor SARS CoV (see below). More recently, group 2b coronaviruses related to SARS CoV have been identified in *Rhinolophus* bats in Hong Kong and mainland China. Such bats are also sold live in these game animal markets. It is now believed that these or related bat coronaviruses may be the precursor from which SARS CoV originated (see below).

Phylogeny

SARS CoV and the SARS-like civet and bat coronaviruses form a distinct phylogenetic subgroup (2b) within the group 2 coronaviruses (**Figure 2**). Genetic and phylogenetic analysis indicates that the viruses associated with the early phase of the human SARS outbreak are more closely related to the viruses found in palm civets and other small mammals in the live game animal markets in Guangdong. The genomes of viruses in the early phase of the human outbreak in 2003 were observed to be under strong positive selective pressure, suggesting that the virus was rapidly adapting in a new host. Furthermore, virus in civets was also found to be under strong positive selective pressure, supporting the view that civets were not the natural host of the precursor SARS-like coronavirus. The search for the precursor of SARS CoV led to the discovery of a number of novel coronaviruses in bats which are related to group 1 and group 2 coronaviruses. Some of these bat coronaviruses are genetically related to SARS CoV (group 2b) and are likely to be the direct or indirect precursor of SARS CoV (see below).

Interestingly, considered overall, the recently discovered group 1 and group 2 (including SARS CoV-like) bat coronaviruses appear to be in evolutionary stasis while many other mammalian coronaviruses still appear to be under evolutionary selection pressure, raising the intriguing possibility that bats may in fact be the precursors, not only of SARS CoV, but also of most other mammalian coronaviruses.

Virus Receptors

The functional receptor for SARS CoV on human cells is the angiotensin-converting enzyme 2 (ACE-2) which

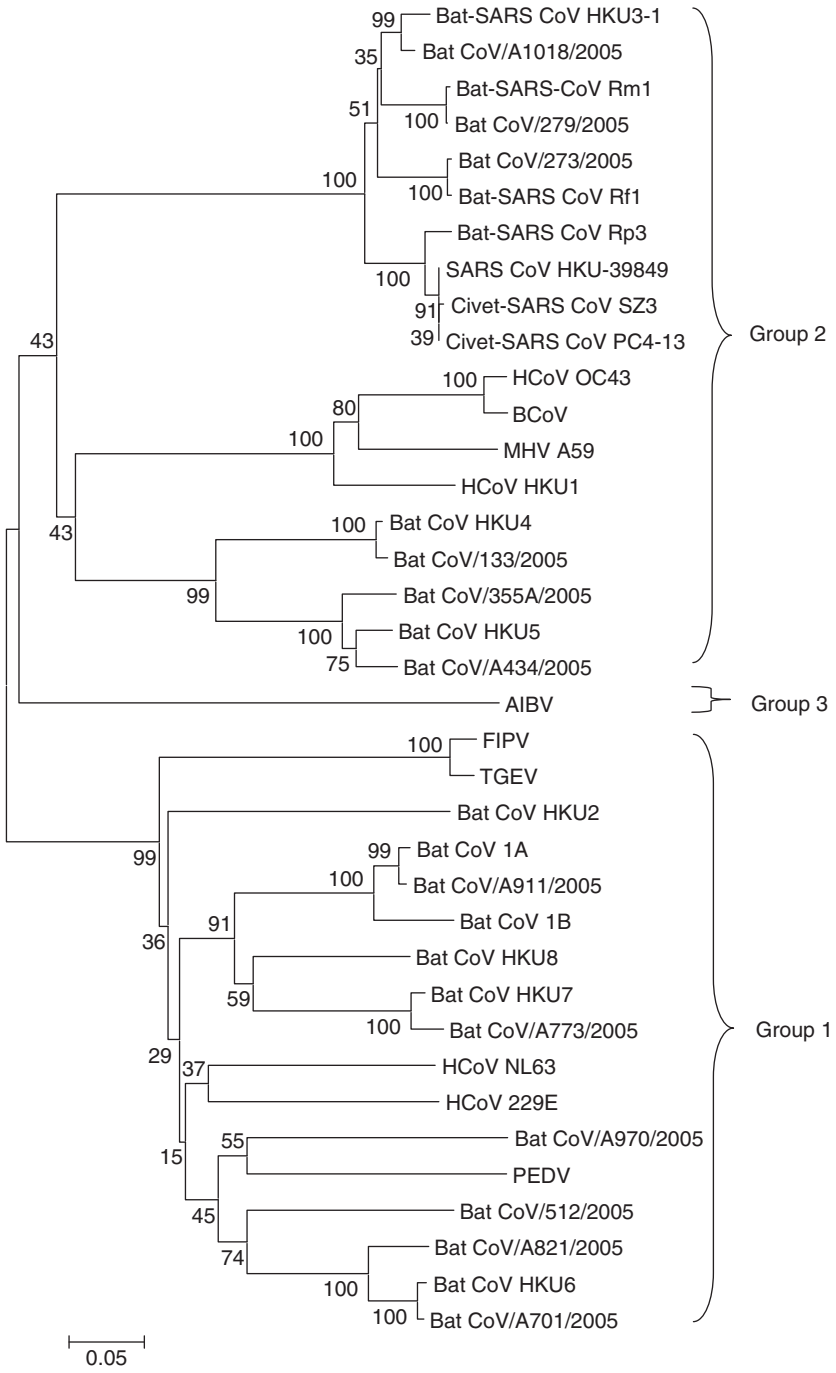

Figure 2 Phylogenetic analysis of RNA sequences coding for the RNA-dependent RNA polymerase (partial sequence). The phylogenetic tree was constructed by the neighbor-joining method and bootstrap values were determined with 1000 replicates. Human SARS CoV (GenBank accession AY278491.2), SARS CoVs isolated from palm civets in 2003 (AY304486.1) and 2004 (AY613948.1) and bat CoVs [Bat CoV 1A (DQ666337.1), Bat CoV 1B (DQ666338.1), Bat CoV HKU2 (DQ249235.1), Bat-SARS CoV HKU3–1 (DQ022305), Bat CoV HKU4 (DQ249214.1), Bat CoV HKU5 (DQ249217.1), Bat CoV HKU6 (DQ249224.1), Bat CoV HKU7 (DQ249226.1), Bat CoV HKU8 (DQ249228.1), Bat-SARS CoV Rp3 (DQ071615), Bat-SARS CoV Rm1 (DQ412043), Bat-SARS CoV Rf1 (DQ412042), Bat CoV/A434/2005 (DQ648819.1), Bat CoV/A701/2005 (DQ648833.1), Bat CoV/A773/2005 (DQ648835.1) Bat CoV/A821/2005 (DQ648837.1), Bat CoV/A970/2005 (DQ648854.1) Bat CoV/A911/2005 (DQ648850.1), Bat CoV/A1018/2005 (DQ648795.1), Bat CoV/133/2005 (NC_008315), Bat CoV/273/2005 (DQ648856), Bat CoV/279/2005 (DQ648857), Bat CoV/355A/2005 (DQ648809.1), Bat CoV/512/2005 (DQ648858)] were aligned with references sequences as indicated. Reference sequences are: transmissible gastroenteritis virus, TGEV (DQ811789); HCoV 229E (AF304460); HCoV NL63 (AY567487); HCoV-OC43 (AY391777); HCoV-HKU1 (DQ415903 HKU1); porcine epidemic diarrhea virus, PEDV (AF353511); avian infectious bronchitis virus, AIBV (AY646283); mouse hepatitis virus, MHV (AY700211); bovine coronavirus, BCoV (AF220295); feline infectious peritonitis virus, FIPV (AY994055).

binds the receptor-binding motif (amino acid residues 424 to 494) of the SARS CoV spike (S) protein. While the human SARS CoV S protein binds efficiently to both human and civet ACE-2, the civet-like SARS CoV S protein binds efficiently to ACE-2 from civets but poorly to human ACE-2. The spike protein of the bat SARS-like coronavirus lacks the ACE-2 receptor-binding motif and is therefore unlikely to bind to human ACE-2.

These findings explain the increased human transmissibility of SARS CoV in the later stages of the SARS outbreak, the observation that human SARS CoV efficiently infects civets under experimental conditions, and the failure of civet SARS CoV or bat SARS-like CoV to replicate productively in primate (Vero-E6, FRhK4) cells that support replication of human SARS CoV. This finding also explains the poor virulence and transmissibility of re-emergent SARS in December 2003–January 2004 when humans are believed to have been infected with a civet-like SARS CoV.

Other cell-surface molecules such as L-SIGN, DC-SIGNR, DC-SIGN (CD209), and L-SECtin may serve as binding receptors but do not appear to be functional viral receptors in the absence of ACE-2. They may, however, promote cell-mediated transfer of the virus to other susceptible target cells. On the other hand, binding to L-SIGN appears to lead to proteasome-dependent viral degradation and it may function as a scavenger receptor (see below).

Human Disease

Transmission

Respiratory droplets are the major source of infectious virus for transmission of SARS. However, aerosol exposure has probably contributed to disease transmission, at least in some defined instances where aerosol-generating procedures (e.g., nebulizers, high-flow oxygen therapy, intubation) have been used. The unusual stability of SARS CoV also suggests that contaminated surfaces and fomites may contribute to disease spread. As SARS CoV is present in feces and urine (and possibly other body secretions), these body fluids may also play a part in disease transmission. The largest single outbreak of SARS at the Amoy Gardens apartment block in Hong Kong, where over 300 individuals were infected from a single index case, is believed to have been caused by aerosols generated from infected body secretions (e.g., feces).

The estimated incubation period for SARS is 2–14 days. During the 2003 outbreak, the majority of cases did not transmit disease at all and only a few patients accounted for a disproportionately large number of secondary cases. Host factors may have played a role in these super-spreading events but, in many cases, there was a unique combination of host factors and environmental circumstances that facilitated transmission. In contrast to the high transmission rates in these super-spreading events and within hospitals, there was less evidence of secondary transmission within the family or within households (e.g., 15% in Hong Kong). Notwithstanding the 'super-spreading phenomenon' that has characterized SARS, the basic reproduction number (Ro) of SARS is estimated to range from 2 to 4.

Seroepidemiological studies of contacts of SARS patients (both adults and children) have revealed that asymptomatic infection was uncommon. The absence of large numbers of asymptomatic transmitters and the paucity of transmission during the first 5 days of illness explain the success of the public health measures of aggressive case detection and isolation in interrupting transmission of human-adapted SARS CoV and the control of the global disease outbreak. These features of SARS have been attributed to the observation that, unlike many other acute viral respiratory infections, SARS transmission has mostly occurred only after the fifth day of illness. This is, in turn, probably related to the low viral load in the upper respiratory tract during the early phase of the illness (see below).

Clinical Features

As the clinical features of SARS are not pathognomonic, a contact history and virological evidence of infection are important for confirmatory diagnosis. SARS typically starts with myalgia and loose stools around the time of onset of fever without coryza or sore throat (seen in 70% of patients). The upper respiratory manifestations are less commonly observed. Radiological abnormalities have been observed in >60% of cases at initial presentation and preceded lower respiratory tract symptoms in approximately 41% of patients.

Children have had much milder illness than adults and mortality rates progressively increase with age. Some patients, particularly those with progressive lower respiratory tract involvement have had a watery diarrhea. Other extrapulmonary manifestations included hepatic dysfunction and a marked lymphopenia involving both B, T (CD4 and CD8 subsets), and natural killer (NK) cells. High serum levels of chemokines (interleukin 8 (IL-8), CCL2, and CCl10) and pro-inflammatory cytokines (IL-1, IL-6, IL-12) have been observed.

The overall case–fatality rate was 9.6% and the terminal events were severe respiratory failure associated with acute respiratory distress syndrome (ARDS) and multiple organ failure. Age, presence of co-morbidities, and viral load in the nasopharynx and serum during the first 5 days of illness correlated with an adverse prognosis.

Autopsy findings of those who died in the first 10 days of illness were diffuse alveolar damage, desquamation of pneumocytes, and hyaline membrane formation. Viral

RNA was detected by quantitative polymerase chain reaction (PCR) at high copy number in the lung, intestine and lymph nodes, and at lower levels in spleen, liver, and kidney. In lung biopsy tissue or in autopsy tissue of patients dying in the first 10 days after disease onset, viral antigen and viral nucleic acid were demonstrated by immunohistochemistry and *in situ* hybridization methods respectively, in alveolar epithelial cells and to lesser extent in macrophages. A few unconfirmed studies have also reported the detection of virus particles or viral RNA in multiple organs but these findings require independent confirmation.

Laboratory Diagnosis

Highly sensitive and specific real-time PCR assays for detection of viral RNA remain the best choice for early SARS diagnosis. Viral RNA has been detected in respiratory specimens, feces, serum, and urine. Specimens from the lower respiratory tract such as endotracheal aspirates have higher viral load than those from the upper respiratory tract and are better diagnostic clinical specimens. As viral load is low during the first 5 days of disease, a negative PCR result from specimens collected at this time does not exclude the diagnosis. Testing multiple specimens improves the detection rate of SARS. Virus culture on Vero E6 or FRhK-4 cells and viral antigen detection tests are much less sensitive than reverse transcriptase PCR (RT-PCR) for detecting the virus. While viral RNA remains detectable in the respiratory secretions and feces for many weeks after the onset of illness, specimens rarely yield a virus isolate after the third week of illness.

Sero-conversion by immunofluoresence or neutralization occurs during the second week of illness and can provide reliable retrospective diagnosis. Enzyme-linked immunoassays using inactivated whole virus or recombinant antigens are convenient alternatives for serological screening, but any positive results must be confirmed by the more specific immunofluoresence or neutralization tests.

Pathogenesis

The primary mechanism of lung damage appears to be due to infection of type 1 and type 2 pneumocytes which are key target cells of the virus. Type 2 pneumocytes are important in the repair of lung injury and infection of these cells can potentially impair the regenerative responses of the lung and aggravate the respiratory impairment.

Whereas mice deficient in NK, T or B lymphocytes display similar kinetics of viral replication to normal mice, infection of mice with defects in the STAT1 signaling pathway results in more prolonged viral replication and more severe disease. These findings indicate the importance of innate immune responses in the control of infection, at least in the mouse. Infection of epithelial cells, macrophages, and myeloid dendritic cells fails to induce a type 1 interferon response although other interferon response genes are activated. Viral proteins expressed from ORF3b, ORF6, and the N gene have interferon antagonist effects *in vitro*. In contrast, macrophages and dendritic cells respond to infection *in vitro* with strong chemokine responses, including those (e.g., CCL10) that are elevated in the serum of SARS patients, and macrophage-chemoattractant chemokines (CCL2). This may explain the predominantly macrophage infiltrate in the lung.

There is evidence of viral replication within intestinal epithelial cells but there is minimal cellular infiltrate or disruption of intestinal architecture and the pathogenesis of diarrhoea in SARS remains unclear.

Treatment

As SARS emerged as a disease of unknown etiology, empirical therapeutic options were initially tested including broad spectrum antivirals and immunomodulators such as ribavirin, intravenous immune globulin, type 1 interferon, SARS convalescent plasma, and corticosteroids. However, in the absence of controlled clinical trials, no conclusions can be drawn on the efficacy of these interventions.

Anti-SARS CoV activity *in vitro* has been demonstrated for several therapeutics already in clinical use for other conditions, including lopinavir–nelfinavir, glycyrrhizin, baicalin, reserpine, and niclosamide. There are contradictory reports on the *in vitro* activity of ribavirin, interferon beta, and interferon alpha. In summary, taking into account bio-availability of these compounds and *in vitro* data, interferon alpha *n*1/*n*3, leukocytic interferon alpha, interferon beta and nelfinavir appear to be worthy of animal studies and randomized placebo-controlled clinical trials if SARS was to return.

A clinical trial of lopinavir 400 mg with ritonavir 100 mg orally every 12 h (added to an existing regimen of ribavirin and corticosteroid therapy) appeared to provide clinical benefit compared to historical controls. However, the lack of concurrent controls makes it difficult to draw conclusions. Similarly, a limited clinical trial of 13 patients using interferon alfacon-1 treatment showed a trend toward improved radiological and clinical outcomes, but without achieving statistical significance.

Studies in primate models have demonstrated prophylactic or therapeutic benefit from PEGylated recombinant interferon alpha-2b and from small interfering RNA therapy. More recently, screening of combinatorial chemical libraries *in vitro* has identified potential inhibitors of the viral protease, helicase, and spike protein-mediated entry.

Animal Models

Experimental SARS CoV infection leads to virus replication in a number of animal species including nonhuman primates (e.g., cynomolgous and rhesus macaques, African

green monkeys, and marmoset monkeys), mice (BALB/c, C57/BL6), Golden Syrian hamsters, ferrets, and cats. Only some of these develop pathological lesions in the lungs (cynomolgous macaques, ferrets, hamsters, marmosets, aged BALB/C mice).

Interestingly, whereas SARS CoV replicates in the lung of both young and aged (12–14 months) BALB/c mice, only aged mice manifest clinical symptoms and histological evidence of lung pathology. This is reminiscent of disease in humans in which children have mild illness (see above). Furthermore, few animal models reproduce the gastrointestinal manifestations of the illness.

While the ideal animal model for understanding SARS pathogenesis is lacking, those that support viral replication (with or without clinical disease) are adequate for evaluating the efficacy of vaccines.

Vaccines and Immunity

A wide range of strategies have been explored for development of SARS vaccines. These have included: inactivated whole virus vaccines; subunit vaccines including baculovirus expressed S1 subdomain or the complete trimeric spike protein of the virus expressed in mammalian cells; DNA vaccines expressing S (full-length and fragments), N, M, or E proteins; and vectored vaccines based on modified vaccinia Ankara (MVA) virus, vesicular stomatitis virus, adenoviral vectors carrying S, M, or N proteins, and attenuated parainfluenza virus type 3 vectored vaccines carrying S, E, M, and N proteins. Neutralizing antibody responses and, where appropriate, cell-mediated immune responses have been measured as correlates of immunity. Some of these vaccines have been evaluated in experimental models by challenging with infectious SARS CoV.

Trials in hamsters of attenuated parainfluenza virus type 3-vectored vaccines individually expressing SARS CoV S, E, M, and N proteins have indicated that only the S protein construct elicits neutralizing antibody and protects against experimental challenge. Furthermore, passive transfer of serum containing S protein neutralizing antibody has been shown to be sufficient to induce protective immunity in mice. It is concluded that neutralizing antibody to the S protein is an important correlate of protection. The receptor-binding determinant of the S1 subdomain is an immuno-dominant epitope and a critical determinant for virus neutralization.

As antibody can enhance rather than protect against the coronavirus disease feline infectious peritonitis, antibody-dependent enhancement has been a concern for SARS-Co vaccine development. However, no evidence of vaccine-enhanced disease has been observed to date, with two possible exceptions. There is a report that a modified vaccinia Ankara virus S protein vaccine has led to hepatitis in vaccinated ferrets but this has not been independently confirmed. There is also a report that S protein antibody elicited by a subunit vaccine enhances entry of pseudo-particles carrying S spike into lymphoblastoic cell lines which lack ACE-2 and are not normally permissive to infection. However, in the challenge experiments in hamsters, the vaccine did not induce protection and there is no evidence of disease enhancement.

Passive immunization with human monoclonal antibodies to the S protein has been successful at protecting mice and ferrets from experimental challenge by reducing viral load in the lung but not in the nasopharynx.

Most of these active and passive immunization studies have evaluated protection from challenge using the homologous human-adapted SARS CoV. However, a newly emergent SARS outbreak will probably arise from the animal reservoir and it is therefore important to investigate cross-protection against animal SARS-like CoV. As none of the civet or bat SARS CoV has yet been successfully grown *in vitro*, the cross-reactive neutralizing antibody response has been studied using lentiviruses pseudotyped with CoV S protein from a civet virus (SZ3), a civet-like virus causing re-emergent SARS in humans in December 2003 (GD03), and from a human SARS CoV (Urbani-strain) isolated from the major human SARS outbreak in 2003. The viruses pseudotyped with human Urbani virus S protein were neutralized by antibodies to the civet SARS-like virus but pseudotypes with the civet-like S protein were not neutralized by antibodies to the human SARS CoV (Urbani). On the contrary, antibody to the Urbani virus appeared to enhance the infectivity of the GD03 and SZ3 pseudotyped viruses. These findings appear to reflect receptor usage of these viruses as it has been shown that GD03 and SZ3 bind poorly to human ACE-2 (see above). The development of vaccines that can prevent re-emergence of SARV CoV from its zoonotic reservoir remains a challenge.

Conclusion: Will SARS Return?

Like many recent emerging infectious diseases that threaten human health, SARS was a zoonosis. The SARS CoV that was responsible for the global outbreak in 2003 was well adapted to bind to human ACE-2 and was efficiently transmitted human-to-human. Laboratories remain a potential source of infection from such viruses and, as occurred in February 2004, laboratory escape can lead to a community outbreak.

The SARS-like coronavirus found in civets (and other mammals) in live game-animal markets is very closely related to SARS CoV, but it binds inefficiently to the human ACE-2 receptor (see above). Consequently, when human infection with the civet SARS-like CoV occurred in December 2003–January 2004, there was no human-to-human transmission and clinical disease was mild. While SARS-like coronaviruses have been found in bats, they are

genetically distinct to SARS CoV and the bat SARS-like CoV S protein appears unable to bind to human or civet ACE-2. Thus, it is likely that re-emergence of a virus capable of causing human disease from this source probably requires extensive adaptation in an intermediate host (e.g., small mammals such as civets). While it is difficult to assess the likelihood of SARS re-emergence, this possibility cannot be excluded.

The rapid expansion of the live game-animal trade and the development of large markets in southern China which house a diversity of wild and domestic animal species were probably important in facilitating the emergence of SARS CoV. It is therefore possible that, like Ebola, SARS may re-emerge at intervals in the future. However, a number of epidemiological characteristics of SARS (see above) should allow it to be contained by public health interventions, once the disease is diagnosed. Indeed, the chain of community transmission arising from a laboratory escape of SARS CoV in February 2004 was contained by such public health measures and community transmission was aborted. However, if the dynamics of transmission of a re-emergent virus are different, and particularly if transmission occurs earlier in the illness and there are more asymptomatic infections, the options for control and the ultimate consequences may be very different. It remains important, therefore, to understand better the ecological and viral factors that predispose to interspecies transmission and the emergence of animal viruses with efficient competence for transmission in humans. Attention should be directed toward the adaptation strategies and the ecological factors that are important in determining interspecies transmission, rather than focus on the disease itself (i.e., SARS). Efforts to understand better the molecular basis for interspecies transmission that led to the genesis of SARS CoV will help us to prepare better for the next emerging infectious disease challenge; whether this comes from SARS CoV, avian influenza H5N1, or a yet unknown virus.

See also: Coronaviruses: General Features; Coronaviruses: Molecular Biology.

Further Reading

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Shellfish Viruses

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Glossary

Aquaculture Cultivation of aquatic animals or plants.
Bivalve Marine or freshwater mollusks having a soft body with plate-like gills enclosed within two shells hinged together.
Gills Respiratory organ of aquatic animals that breathe oxygen dissolved in water.
Hatchery A place where eggs are hatched under artificial conditions.
Hemocyte Any blood cell especially in invertebrates.