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# Coronavirus in severe acute respiratory syndrome (SARS)

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**Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus (SARS-CoV). Future research on the molecular virology of SARS-CoV will be important in the understanding of the epidemiology and the natural history of SARS. This will also facilitate the development of sensitive and accurate diagnostic tests, as well as vaccination and other therapeutics to combat SARS.**

In November 2002, an outbreak of a life-threatening ‘atypical pneumonia’ for which no etiological agent could be identified occurred in Guangdong Province, China. In March 2003, a similar outbreak was reported in Hong Kong [1]. Cases of similar respiratory illness were subsequently reported globally including China, Taiwan, Singapore, Vietnam, Canada and USA. This syndrome is a new clinical entity and has been designated ‘severe acute respiratory syndrome’ (SARS). Up to the time of writing, the World Health Organization had reported > 6200 cases and 435 deaths in 30 countries as a result of SARS (see [http://www.who.int/csr/sarscountry/2003\\_05\\_03/en](http://www.who.int/csr/sarscountry/2003_05_03/en)). Vigorous research has been carried out worldwide to understand the cause and the possible preventive measures for this disease, which was believed to be caused by a highly contagious virus.

In less than four weeks after the global outbreak, a novel coronavirus [SARS-coronavirus (SARS-CoV)] was identified in the blood, respiratory specimens (oropharyngeal wash, nasopharyngeal aspirate, sputum and lung biopsy) and stools of SARS patients by various research groups [2–4]. Typical coronavirus particles of 80–140 nm in diameter, with 20–40 nm complex surface projections surrounding the periphery, were seen under electron microscopy [2]. Direct cytopathic effects could be demonstrated on inoculating the viral isolates into African Green Monkey Kidney (Vero E6) cells, indicating pathogenic properties of this novel coronavirus [2,4]. Comparing the nucleotide sequences at limited regions of the SARS-CoV genome has found ~40–50% difference from that of other human and animal coronaviruses [2,3]. Recently, the sequencing of the entire genome of many SARS-CoV strains has been completed [5]. It is a single-stranded, plus-sense RNA virus, ~30 kb in length, with a genomic sequence that does not closely resemble any of the previously characterized coronaviruses [6,7]. All these

data support an etiological role of this novel coronavirus in the pathogenesis of SARS.

## Virology

The SARS-CoV genome contains 11 open reading frames coding for the replicase, four major structural proteins [nucleocapsid (N), envelop (E), membrane (M) and spike (S)] and several proteins with unknown functions [6]. The replicase is cleaved to form many proteins, including the viral proteases, the RNA-dependent RNA polymerase and the RNA helicase. The RNA-dependent RNA polymerase is an error-prone polymerase and therefore high mutation and recombination rates of the coronavirus genome can be expected. The N-proximal and C-proximal regions of the replicase are processed by the papain-like and 3C-like proteases, respectively. Recently, the crystal structures for human coronavirus (HCoV) 229E (3CL<sup>pro</sup>) have been determined [8]. Because the SARS-CoV 3CL<sup>pro</sup> displays 40% amino acid sequence identity to the HCoV 229E 3CL<sup>pro</sup>, the three-dimensional model for SARS-CoV 3CL<sup>pro</sup> has been constructed by bioinformatics. This model can be used as a basis for the design of antiviral inhibitors against the 3CL<sup>pro</sup>.

N protein binds to the viral RNA forming the helical nucleocapsid in the interior cavity of the SARS-CoV virion, whereas M and E proteins are important for the assembly of the viral envelope. S protein is a membranous glycoprotein with prominent petal-shaped spikes on the surface of the virion. This glycoprotein is important for viral entry and might define host range, tissue tropism and virulence [9]. Mutations of the S protein gene have previously been correlated with altered pathogenesis and virulence on other coronaviruses [10]. Although carcinoembryonic antigen cell-adhesion molecules [11] and aminopeptidase N [12] were identified as receptors of other coronaviruses, the human receptor of SARS-CoV still remains to be elucidated. Study of the variations and mutations of this spike glycoprotein gene could add more clues to the understanding of the biology and pathogenesis of this novel SARS-CoV.

## Epidemiology

The origin of the SARS-CoV is not certain but the possibility of zoonoses cannot be excluded. Epidemiological studies demonstrate that most human transmissions are by close person–person contact. Healthcare workers and household contacts are at highest risk. Droplets and

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formites are believed to be the major route of transmission. Although Guangdong Province in China has been publicized as the source of the global SARS outbreak, new cases that are not epidemiologically traceable to any SARS patients in China have been reported in various parts of the world. To study the epidemiological origins and relationships of different SARS patients, comparative genome sequence analysis of 14 SARS-CoV isolates has been performed [5]. In total, 129 sequence variations and 16 recurrent variant sequences have been identified. Common variant sequences define two distinct genotypes of the SARS-CoV, one linked with infections originating in a hotel in Hong Kong and another from isolates from Hong Kong, Guangzhou and Beijing with no association with the hotel. Because mutation(s) arisen in a particular generation will appear in the following generations, recurrent sequence polymorphisms might serve as genetic signatures to trace the contact source of the SARS-CoV. The forthcoming task would be to investigate the possible effects of these sequence variations on the infectivity, tissue tropism and pathogenicity of the virus.

### Natural history

The natural history of SARS in relation to the viral dynamics of SARS-CoV is poorly understood. SARS is primarily a respiratory disease and the highest concentration of virus can be found in the respiratory tract during the febrile phase [3]. Logically, the infectivity of respiratory secretions at the febrile stage is very high. The infectivity at the incubation period that ranges from 2–16 days before fever occurs is uncertain [1]. Most patients have fever settled within two weeks, and this is accompanied by resolution of chest symptoms and radiological changes [1,13]. However, SARS-CoV can still be found in the stool samples by polymerase chain reaction (PCR) after two weeks of convalescence [3]. This indicates a delayed fecal viral shedding and a potential fecal–oral route of transmission. Droplets of SARS-CoV-contaminated sewage in the soil stack extracted by the bathroom exhaust fans might be responsible for the outbreak of SARS among the residents of Amoy Gardens in Hong Kong [see the Hong Kong Department of Health Report (2003) Outbreak of severe acute respiratory syndrome (SARS) at Amoy Gardens, Kowloon Bay, Hong Kong. Main findings of the investigation; <http://www.info.gov.hk/dh/ap.htm>]. To understand the viral load and infectivity at different stages of SARS, quantification of serial viral titer by real-time PCR to study the viral dynamics in the blood, respiratory secretions and excreta is required. This information will be important in the implementation of public health measures to prevent the spreading of SARS in the community.

### Diagnosis

Currently, the diagnosis of SARS is based on the clinical features, radiological findings and history of SARS contact (see the WHO report on the case definition for surveillance of SARS; <http://www.who.int/csr/sars/caedefinition/en>). This is often inconclusive, particularly at the early stage of disease and among those elderly patients with nonspecific symptoms. Asymptomatic patients in the

incubation phase will also be overlooked. Currently, 10–14 days quarantine for travelers from areas with SARS outbreak is practiced by many countries, and this causes major inconvenience and economic loss. Therefore, a sensitive and accurate laboratory diagnostic test is urgently required. Most PCR-based tests using SARS-CoV-specific primers can achieve good specificity and can therefore be used as confirmatory tests (see the WHO report on laboratory diagnostic tests for SARS; <http://www.who.int/csr/sars/diagnostictests/en>). Unfortunately, the high false-negative rates, which might be related to the low viral load at the particular site or time of sample collection or possible genomic variations of different SARS-CoV strains, limits the use of these tests as a diagnostic tool. Appropriate selection of sampling sites at different phases of the disease and the use of primers at well-conserved regions of different SARS-CoV variants will form the basis of developing a more accurate diagnostic test. The availability of a reliable screening test will be useful to detect SARS at the incubation phase among the asymptomatic close contacts of the index patients. Serological assay will be an alternative for the diagnosis of SARS, but the antibody titer is usually low at the incubation phase and initial phase of illness, and convalescent antibody titer might sometimes be necessary to confirm the diagnosis.

### Treatment

Vaccination will be one of the solutions to control SARS. The immunological target could be the spike glycoprotein that defines the virus–host interaction. As the predicted amino acid sequence of the spike glycoprotein of SARS-CoV has a low level of similarity (~25%) with other coronaviruses, it is likely that SARS-CoV has different receptor-binding specificity and antigenic properties [6]. Further research on the antigenicity of different motifs of the spike glycoprotein among different strains of SARS-CoV will be important. In other human coronavirus infections, re-infection is common owing to the presence of multiple antigenic coronavirus strains. Any mutations could affect the antigenic properties of the virus and the protectiveness of the vaccine developed.

Besides vaccination, several approaches might be considered for treating SARS patients in the future. Proteases and RNA polymerase are potential targets for the development of antiviral drugs that can decrease the rate of virus propagation. Antibodies against the S protein or the receptor might neutralize the virus and block the viral entry. Synthetic peptides might also block the cell fusion and viral entry, as in the case of treatment for HIV type 1 infection [14].

### Conclusions

In conclusion, to combat this dreadful disease, a better understanding of the molecular biology of SARS-CoV is essential. The application of this knowledge will aid the future diagnosis, management and prevention of SARS.

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# Severe acute respiratory syndrome: identification of the etiological agent

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**The severe acute respiratory syndrome (SARS) emerged in late 2002 in southern China and rapidly spread to countries around the globe. Three research groups within a World Health Organization (WHO)-coordinated network have independently and simultaneously shown that a novel coronavirus is linked to SARS. A fourth group has completed the Koch's postulates by infecting monkeys with the agent. Sequencing of the complete genome was achieved only weeks after the first isolate of the virus became available.**

The severe acute respiratory syndrome (SARS) was first reported as a new disease entity to the World Health Organization (WHO) by Carlo Urbani during his work in Hanoi, Vietnam, in February 2003. He succumbed to SARS himself on 29 March 2003. Retrospectively, reports from China on cases compatible with the disease date back to November 2002. The disease involves an initial febrile phase that is followed by interstitial pneumonia, leading to respiratory distress syndrome and death in a fraction of patients. The cumulative case fatality rate at the time of writing is 8.4% [1]. On the basis of gamma-distribution models, this number has recently been corrected to values of 13.2% in patients below 60 years of age and 43.3% in those above 60 [2]. When it became clear in mid-March 2003 that hospital outbreaks with high rates of transmission had simultaneously occurred in Hanoi, Singapore,

Hong Kong and Toronto (Canada), the WHO issued a global health alert and initiated studies in a network of laboratories, dedicated to clarifying the etiology of SARS [3]. Four recent articles describe how members of the network rapidly identified and confirmed a novel coronavirus as the causative agent in an independent, yet collaborative, manner [4–7].

## Laboratory identification of the etiological agent

In the beginning of the investigations, known causative agents of interstitial pneumonia were sought in patients from different sites of outbreaks, and anecdotal results from some laboratories pointed to the involvement of chlamydia [5], rhinoviruses [6] and paramyxoviruses [3,8]. However, other laboratories could not confirm the presence of these pathogens in their cohorts of patients [5–7].

The initial experimental step in all three groups that later succeeded in identifying the causative pathogen was to inoculate various cell culture lines with patient specimens. Whereas cells commonly used for respiratory pathogens (e.g. LLC-Mk2, RDE, Hep-2, MRC-5, NCI-H292, HELA, MDCK, HUT-292, LLC-MK2, B95-8) yielded no indicative results, the virus could be replicated in monkey kidney cells. A group from Hong Kong was the first to observe a cytopathic effect compatible with viral growth, 2–4 days after inoculating a lung biopsy specimen and a nasopharyngeal aspirate sample on fetal rhesus kidney cells (FRhK-4) [7]. The cytopathic effect consisted of cell rounding and detachment. A specific immune response

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