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Review

The severe acute respiratory syndrome (SARS)

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The world was shocked in early 2003 when a pandemic of severe acute respiratory syndrome (SARS) was imminent. The outbreak of this novel disease, caused by a novel coronavirus (the SARS-coronavirus), hit hardest in the Asian Pacific region, though eventually it spread to five continents. The speed of the spread of the SARS epidemic was unprecedented due to the highly efficient intercontinental transportation. An international collaborative effort through the World Health Organization (WHO) has helped to identify the aetiological agent about 1 month after the onset of the epidemic. The power of molecular biology and bioinformatics has enabled the complete decoding of the viral genome within weeks. Over 1000 publications on the phylogeny, epidemiology, genomics, laboratory diagnostics, antiviral, immunization, pathogenesis, clinical disease, and management accumulated within just 1 year. Although the exact animal reservoir of virus and how it evolved into a human pathogen are still obscure, accurate diagnosis and epidemiological control of the disease are now possible. This article reviews what is currently known about the virus and the disease. *Journal of NeuroVirology* (2005) **11,** 455–468.

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Epidemiology

Severe acute respiratory syndrome (SARS) is the first pandemic of the new millennium caused by a hitherto unknown virus. The disease manifests primarily as an acute community- or hospital-acquired pneumonia that does not respond to antimicrobial coverage for typical and atypical pathogens. At the end of the first wave of the outbreak in mid-2003, 8096 cases with 774 deaths occurred in five continents (World Health Organization, 2003a). Human-to-human transmission has occurred in hospitals, clinics, hotels, workplace, homes, taxis, and airplanes (World Health Organization, 2003b). After the initial pandemic ended in June 2003, four other outbreaks occurred from September 2003 to May 2004. Three of these four instances were associated or likely to be associated with laboratory-acquired infections (Orellana, 2004; Lim *et al*, 2004). The laboratory-acquired cases in Singapore and Taiwan were each limited to one affected personnel whereas the latest instance in Beijing was associated with secondary and tertiary cases (World Health Organization, 2004).

The virus—now named SARS-coronavirus (SARS-CoV)—probably circulated among wild mammals, which was subsequently transmitted to humans. The virus probably originated from wild game food animals caged in the wet markets of southern China, of which the palm civet is the most likely amplification host and one of the likely candidates for the introductory host (Guan *et al*, 2003). The first few cases of the 2003 pandemic and the 2004 Guangdong outbreak had history of eating or occupational contacts with wild game animals (Zhong *et al*, 2003). However, the most important route of transmission appears to be direct or indirect contact with infectious respiratory droplets or fomites with the mucous membrane of the eye, mouth, or nose (World Health Organization, 2003b). Respiratory droplet transmission to health care workers might have been augmented by a nebulizer used by SARS patients (Lee *et al*, 2003b; Varia *et al*, 2003; World Health Organization, 2003b). In addition, airborne transmission to health care workers was suspected during the manipulation of patients' airway by suction, intubation, bronchoscopy, or cardiopulmonary resuscitation (Christian *et al*, 2004). Epidemiological and environmental investigations of

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a common source outbreak at a housing complex in Hong Kong also suggested that airborne transmission may have occurred in a previously unreported fashion (Yu *et al*, 2004). Virus-laden aerosols generated by toilet flushing may have been sucked back from the sewage drain through dry U traps into the toilet as a result of a strong negative pressure generated by exhaust fans. A plume of contaminated warm air ascending the light well to other floors was then carried by wind to other blocks of the housing complex. Cultivable viruses could be found in stool from which quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) showed a high viral load (Peiris *et al*, 2003a), which suggested that fecooral transmission could be another possible mode of transmission. Food-, water-, vector-, or pest-borne transmission could not be documented despite the hypothesis that rats may be alternative explanation for the outbreak in this housing complex (Ng, 2003).

The incubation period is estimated to be 2 to 14 days (World Health Organization, 2003b). Epidemiological control measures based on the World Health Organization–recommended maximal incubation period of 10 days was found to be effective in interrupting transmission. Overall the average number of secondary cases resulting from a single case is two to four (World Health Organization, 2003b). Asymptomatic or mildly symptomatic infections do not appear to be associated with transmission or positive viral culture. Seroepidemiological studies showed a highly variable seroprevalence (0%, 0.43%, 1.2%) for normal individuals and 1% for health care workers, ca. 1% for asymptomatic family contacts under quarantine, depending on the type of serological assay being used and the cohort of population (Lee *et al*, 2003a; Peiris *et al*, 2003a; Woo *et al*, 2004; Zheng *et al*, 2004). Transmission from symptomatic patients tended to occur on the fifth day or later after the onset of symptoms and especially from those who were severely ill. When patients have been afebrile for more than 10 days, no transmission could be documented. Although the initial cases of outbreaks in both 2003 or 2004 were retrospectively identified in November and December, respectively, a definite seasonality of the disease cannot be demonstrated thus far as a result of the intensive and successful intervention of the pandemic.

Of all the mysteries surrounding this new disease, two epidemiological questions were particularly intriguing. The first pertains to the natural reservoir of SARS-CoV, its transmission dynamics, amplification, and recombination in game animals caged in the markets that lead to interspecies jumping. This is very important in the prevention of further transmission of similar diseases from animals to human (Guan *et al*, 2003). The other unsolved issue is the so-called "superspreading" event in which a few SARS patients had led to a disproportionately large number of transmissions. The understanding of the viral multiplication, infectious droplet formation, and social behavior of such hosts will be another important area for further research.

Virology

The SARS-CoV is an enveloped positive-sense single-stranded RNA virus that was identified independently in three laboratories in different continents at almost the same time (Peiris *et al*, 2003b; Drosten *et al*, 2003; Ksiazek *et al*, 2003). Primary viral isolation was successful in embryonal monkey cell lines including the Vero E6 and fRhK-4 cell. It can be subcultured onto other Vero cells, a liver cancer cell line Huh-7 (Simmons *et al*, 2004) and a colonic carcinoma cell line called CACO-2. The mink lung epithelial cells (Mv1Lu) is also permissive to the growth of SARS-CoV (Gillim-Ross *et al*, 2004). Unlike other human coronaviruses, the virus proliferates rapidly and causes obvious cytopathetic effects in Vero E6 within 48 h of inoculation. Compared to other human coronaviruses, the SARS-CoV appears to have a higher degree of stability in the environment (Duan *et al*, 2003; Rabenau *et al*, 2005). As in the case of other human coronaviruses, SARS-CoV is capable of infecting human and rat neural cell lines, though with a much lower level of viral production and no cyopathic effects can be observed (Yamashita *et al*, 2005). On dry surfaces at room temperature, SARS-CoV could survive for at least 2 to 3 days, whereas in stool samples, survival for 2 to 4 days can be expected (Table 1). Resistance to heat and chemical disinfectants are similar to other enveloped viruses.

The appearance on electron microscopy and genome order of 5′-replicase-structural (spike-envelope-membrane-nucleocapsid)-polyT-3′ on complete genomic sequencing are characteristic of Coronaviridae. Of all the 16 known coronaviruses, this is the only coronavirus affecting human which seems to be phylogenetically distinct from any of the three groups. It is now shown to be distantly related to the group II viruses and currently classified as group IIb. The other human coronaviruses 229E and NL63 belong to antigenic group I (van der Hoek *et al*, 2004; Fouchier *et al*, 2004), whereas human coronavirus OC43 and the newly described HKU1 belongs to group IIa (Woo *et al*, 2005). Group III contains all the avian coronaviruses. Using the relatively rapid mutating surface spike protein as the phylogenetic marker, analysis of 139 SARS-CoV isolates in the Hong Kong outbreak showed that several introductions had occurred, but only one of them was associated the major outbreak in Hong Kong and the rest of the world (Guan *et al*, 2004). Some of the strains found in the beginning of the outbreak were phylogenetically distinct from the major cluster and closer to some of the Guangdong and Beijing strains. This concurred with the epidemiological study that identified that the index patient of the Hong Kong outbreak is a Guangzhou medical

Table 1 Comparison of all known human coronavirus.

	HCoV-229E[a]	*HCoV-NL63*[b]	*HCoV-OC43*[c]	*HCoV-HKU1*[d]	*SARS-CoV*[e]
Antigenic group	I	I	IIa	IIa	IIb
Clinical disease	URTI, LRTI	Bronchiolitis, URTI	URTI, LRTI, perimyocarditis	LRTI	SARS, asymptomatic infection
Primary isolation in tissue/cell cultures	Human foetal tracheal/intestinal organ cultures	Tertiary monkey kidney cells	Human foetal tracheal/intestinal organ cultures	NA	Foetal rhesus kidney cells (FRhK4), Vero E6
Passage in tissue/ cell cultures	Vero E6, human embryonic kidney cell lines, human diploid fibroblast cell lines (MA-177), human rhabdomyosarcoma cells, neural cell lines (astrocytoma, neuroblastoma, neuroglioma, and oligodendrocytic cell lines)	Monkey kidney cell line (LLC-MK2)	Vero E6, human rhabdomyosarcoma cells, neural cell lines (astrocytoma, neuroblastoma, neuroglioma, oligodendrocytic, and immortalized foetal microglial cell lines)	NA	Vero E6, Vero, human colonic cancer cell line (CACO2), MA-104, PK-15, CL14, HPEK, Huh7, mink lung epithelial cells
Presence of hemagglutinin esterase glycoprotein	No	No	Yes	Yes	No
Presence of proteolytic cleavage site between S1 and S2	No	Unknown	Yes (potential proteolytic cleavage site (RRSR) between amino acid residues 758 and 759)	Yes	No
Spike receptor binding site	Human aminopeptidase N	Unknown	*N*-acetyl-9-*O*-acetylneuraminic acid	Unknown	ACE-2
Genome size (kb)	27317	27553	30738	29926	29751
G+C content	0.38	0.34	0.37	0.32	0.41
Environmental survival	Up to 3 hours on dry surfaces	NA	Up to 1 hour on dry surfaces	NA	Up to 4 days on dry surfaces

HCoV-229E = human coronavirus 229E; HCoV-NL63 = human coronavirus NL63; HCoV-OC43 = human coronavirus OC43; HCoV-HKU1 = human coronavirus HKU1; SARS-CoV = SARS coronavirus; URTI = upper respiratory tract infections; LRTI = lower respiratory tract infection.

[a]References for information on HCoV-229E: Arbour *et al*, 1998, 1999a; Holmes, 2001; Sizun *et al*, 2000; Yeager *et al*, 1992.

[b]References for information on HCoV-NL63: van der Hoek *et al*, 2004.

[c]References for information on HCoV-OC43: Arbour *et al*, 1998, 1999b; Holmes, 2001; Krempl *et al*, 1995; Kunkel *et al*, 1993; Mounir *et al*, 1993; Sizun *et al*, 2000; Zhang *et al*, 1992.

[d]References for information on HCoV-HKU1: Woo *et al*, 2005.

[e]References for information on SARS-CoV: Li *et al*, 2003c; Peiris *et al*, 2003a, 2003b; Rota *et al*, 2003; World Health Organization, 2003c; Yao *et al*, 2004.

doctor who had travelled to Hong Kong. A similar molecular epidemiological study of an outbreak in Guangdong also suggested that the disease appeared to spread from Guangdong to Hong Kong and the rest of the world and the index case was a chef who handled game food animals (Zhong *et al*, 2003). Subsequent animal surveillance recovered strains of coronavirus having a nucleotide homology of 99.8% with the human virus (Guan *et al*, 2003). A characteristic 29-base insertion between open reading frames (ORFs) ORF10 to ORF11 (also named as ORF8a and ORF8b) was found in these animal isolates (Guan *et al*, 2003; Snijder *et al*, 2003). This 29-nucleotide segment was deleted after transmission to humans, or before crossing species to humans. The biological effect of this deletion worths further research. However, a number of human viruses in the later stages of the SARS epidemic had larger deletions around this site (The Chinese SARS Molecular Epidemiology Consortium, 2004). A significantly higher seroprevalence of SARS-CoV antibody is found in wild animal traders and slaughterers also supported that the virus has jumped from these animals to human. Indeed two independent molecular epidemiological studies by complete genome comparison of 12 and 63 virus isolates have found evidence of a strong positive selection at the beginning of the epidemic, which is followed by a purifying selection, as indicated by the amino acid substitution rate at the spike and Orf1 a (Yeh *et al*, 2004; The Chinese SARS Molecular Epidemiology Consortium, 2004). Both studies suggested that the findings are

compatible with molecular adaptation by the virus jumping from animals into humans. In the small outbreak of Guangzhou in 2004, all the four human isolates belong to a separate sublineage with the concurrent animal, predominantly civet cat, isolates that are distinct from the human pandemic or animal virus of the 2003. Though the SARS-CoV is rather distinct from the three existing groups of coronaviruses, it may be closer to group II because 19 out of 20 cysteines found in the S1 domain of the spike protein are spatially conserved when compared with the group II consensus sequence, whereas only 5 cysteine residues are conserved when compared with that of the groups I and II (Eickmann *et al*, 2003). Because coronaviruses are believed to have co-evoluted with their animal hosts, it was suggested that rats, mice, and cattle harboring group II coronaviruses are more likely to be the animal host for SARS-CoV than cats that harbor group I coronavirus. However, when a comparison of the phylogenetic trees for 11 known host-species and nucleocapsid sequences of 36 coronaviruses was done using an interference approach with sliding window analysis, there are statistical incongruence that indicates multiple host-species shifts between the coronaviruses of many animals that are phylogenetically distant (Rest and Mindell, 2003). Thus even if the civet cats or other related mammals are the true animal reservoir rather than mice and rats, it would not be too big a surprise. Moreover, the civet cats and other related mammals had at least served as a major amplification mechanism in the markets of southern China irrespective of the original animal reservoir. The control of these animals and the markets plays a pivotal role in the epidemiological control of SARS.

Besides molecular epidemiology, the genomic sequence has provided invaluable information on pathogenesis and targets for diagnostics, immunization, and antiviral therapy. A metalloprotease angiotensin-converting enzyme 2 (ACE2) from Vero E6 cells was found to bind the S1 domain of the spike protein of the virus (Li *et al*, 2003c). The 293T cells transfected with ACE2 can form multinucleated syncytia with cells expressing the spike. ACE2 expression is present on enterocytes, pneumocytes, vascular endothelial and smooth muscle cells (Hamming *et al*, 2004; Leung *et al*, 2003). This is consistent with the clinical and histopathological manifestations of SARS which include diffuse alveolar damage, colonic mucosal presence of abundant viral particles in a patient with diarrhoea, pulmonary vasculitis and thrombosis. ACE2 has been shown to be the crucial cellular receptor for SARS-CoV *in vivo* (Kuba *et al*, 2005). The lack of virus infection in some cell lines which expresses ACE2 has led to a search for alternative cellular receptors, and one such candidate receptor is called dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin-related (DC-SIGNR), a type II C-type lectin receptor, also known as L-SIGN, CD209L (Jeffers *et al*, 2004). The significance of this alternative receptor still awaits further confirmation. The fragment of S1 containing amino acids 270 to 510 of the S1 domain was localized to be the minimal receptor-binding region found by truncation and binding assays (Babcock *et al*, 2004). ("S1" rather than "SI".) The helicase and 3CL proteinase were cloned and characterized (Anand *et al*, 2003; Chou *et al*, 2003; Tanner *et al*, 2003; Yang *et al*, 2003). A molecular model of the RNA-dependent RNA polymerase has also been published (Xu *et al*, 2003), which provides clues to the functional aspect of the enzyme and therefore may facilitate the design of new antivirals in the future. Other putative targets such as the nsp13 (nonstructural protein), a putative mRNA cap-1 methyltransferase, and nsp9 are being investigated intensively by bioinformatics and crystal structure studies (Campanacci *et al*, 2003; Egloff *et al*, 2004; von Grotthuss *et al*, 2003). A full-length cDNA of the viral genome was shown to cause lytic infection in cell line with good viral titre and antigen expression (Yount *et al*, 2003). This would provide the tool to study function of many nonstructural protein by reverse genetics.

Clinical disease

SARS is generally manifested as a viral pneumonia with rapid respiratory deterioration in about two thirds of the affected individuals (World Health Organization, 2003b). It affects all age groups with a slight bias for females. Preliminary genetic susceptibility study suggested association of HLA-B*4601 with severity of SARS and perhaps susceptibility to infection in the Chinese populations (Lin *et al*, 2003). In a study involving the Chinese population in Hong Kong, association of susceptibility and resistance to SARS have been found with HLA-B*0703 (OR 4.1, 95% CI 2.0–8.2) and HLA-DRB1*0301 respectively (Ng *et al*, 2004). The co-inheritance of B*0703 and B60 was significantly higher in patients with SARS than in the general population. No association of HLA-B*4601 was found with disease severity in this later study. The patient typically presents with fever, chills, myalgia, malaise, and a nonproductive cough (Tsang *et al*, 2003). Upper respiratory tract symptoms such as runny nose and sore throat are less prominent. Patients are usually admitted because of persistent fever, worsening of cough, and shortness of breath. Such deterioration is especially prominent 1 week after the onset of symptoms and often coincides with the appearance of watery diarrhoea. The physical findings on chest examination are disproportionally mild when compared with the changes on chest radiography. The radiographic abnormalities include ground glass opacities and focal consolidations that are expected from the histological findings of interstitial pneumonia and alveolar exudation on lung biopsy or postmortem examination of the early stage of SARS. These changes are typically found

over the peripheral and often subpleural regions of the lower zones in the initial chest radiographs. As the disease progresses, extensive involvement of both lungs occurred. Other late but characteristic radiological changes include shifting radiographic shadows and pneumomediastinum without preceding positive pressure ventilation. By far the commonest extrapulmonary manifestation is diarrhoea (Cheng *et al*, 2004), followed by hepatic dysfunction (Chau *et al*, 2004), dizziness that may be related to diastolic cardiac impairment (Li *et al*, 2003b), abnormal urinalysis, petechial skin rash (Wu and Sung, 2003), myositis (Wang *et al*, 2003b), and epileptic fit (Lau *et al*, 2004). In elderly patients, SARS may present with a fall and fracture leading to the so-called afebrile or "hidden SARS" (Wong *et al*, 2003; Chow *et al*, 2004). Viral replication at different sites is apparently important in the pathogenesis of the clinical and laboratory abnormalities of SARS (Hung *et al*, 2004). Serum viral load was associated with oxygen desaturation, mechanical ventilation, and mortality; stool viral load was associated with diarrhoea; and urine viral load was with abnormal urinalysis.

Peripheral blood examination often revealed lymphocytopenia and sometimes thrombocytopenia with increases in D-dimers and activated partial thromboplastin time. The hepatic parenchymal enzymes, muscle enzymes and lactate dehydrogenase may be increased. About 20% to 30% of the patients will continue to deteriorate and required mechanical ventilation in the intensive care unit. The overall mortality is 15%, which is mainly related to uncontrolled respiratory failure, sepsis, or precipitation of underlying medical illness. Poor prognostic factors include age, comorbidities such as heart diseases and diabetes mellitus, increased lactate dehydrogenase level, and a high neutrophil count at the time of admission. For those who recovered from SARS, most have residual ground glass opacifications on follow up chest radiographs, probably related to fibrosis. About 20% of these cases have restrictive lung function test abnormalities which were attributed to residual lung fibrosis and muscle weakness (Chan *et al*, 2003). Depression and post-traumatic stress disorder are especially common in patients with affected family members and health care workers. Biochemical evidence of adrenal insufficiency and magnetic resonance imaging (MRI) evidence of avascular bone necrosis were also noted in patients who were treated with corticosteroids.

Laboratory diagnosis

SARS is clinically not distinguishable from other causes of acute pneumonia at the initial stage. Most of the cases are initially defined by clinical and epidemiological criteria and later confirmed by virological testing. Besides a positive viral culture from the respiratory, fecal and occasionally urine specimens, a fourfold rise of neutralizing antibody titre in the serum taken on admission and 28 days afterwards remains the gold standard for confirming the diagnosis. Viral culture is insensitive and neutralizing antibody testing are both difficult and offers only retrospective confirmation. For clinical management, real-time quantitative RT-PCR with a modified RNA extraction protocol is able to achieve a sensitivity of 80% with good specificity in nasopharyngeal specimens collected within the first 3 days of illness (Poon *et al*, 2003). Serum specimens can be tested but fecal specimens are especially useful after the first week of illness. A recent study showed that the quantitative reverse transcription–polymerase chain reaction has higher sensitivities than polyclonal and monoclonal antibody-based nucleocapsid antigen capture enzyme-linked immunosorbent assays on both fecal and urine samples (Lau *et al*, 2005). All initially positive RT-PCR results must be confirmed by testing a second clinical specimen, re-extraction, and testing the original specimen and repeating the RT-PCR targeting on different parts of the genome that is conserved. In general, the polymerase gene is most conserved whereas the nucleocapsid gene has the highest copy number in infected cells due to the unique transcription strategy of coronavirus by subgenomic RNA formation. Confirmed SARS cases may have persistently positive RT-PCR for more than 30 days—particularly in the stool specimens—but no virus can be cultured 21 days after the onset of symptoms. In view of the hazards associated with performing the neutralizing antibody test, most laboratories opt for the indirect immunofluorescent antibody test using inactivated whole virus–infected cell line. Recombinant nucleocapsid enzyme immunoassay (EIA) can be used as a rapid screening test but results should be confirmed by the neutralizing antibody or indirect immunofluorescent antibody test. Convalescent sera from patients infected by human coronavirus OC43 may have cross-reactions with this recombinant nucleocapsid EIA and rarely even with the indirect immunofluorescent test. Viral load study on different clinical specimens showed that viral load in nasopharyngeal specimens or serum even on admission is highly correlated with respiratory failure and mortality (Cheng *et al*, 2004). Similarly, viral load in stool and urine at day 10 after onset of symptoms is correlated with the presence of diarrhea and abnormal urinalysis respectively. Longitudinal study of nasopharyngeal aspirates from SARS patients showed that the viral load peaked at around day 10 after the onset of symptoms (Peiris *et al*, 2003a). The viral load then decreased irrespective of clinical deterioration or improvement. This viral load profile is distinct from influenza A or respiratory syncytial virus for which the viral load peaks in the first few days after the onset of symptoms. The three laboratory outbreaks of SARS has hastened the use of pseudotype viruses for research and neutralization antibody testing. Studies using pseudotyped retroviral vector carrying the S, M, or E proteins showed

that the S protein is both necessary and sufficient for virus attachment on susceptible cells (Wang *et al*, 2004a; Simmons *et al*, 2004). Virus entry occurred via a pH-dependent receptor-mediated endocytic pathway (Yang *et al*, 2004). Pseudotyped virus carrying the S protein binds to DC-SIGN on dendritic cells, without causing cell death or replication but this dendritic cell may serve as a conduit for infection of susceptible host cells as in the case of HIV.

Pathology and immunology

Acute diffuse alveolar damage with air space edema were the most prominent features in patients who died before the tenth day after onset of illness (Nicholls *et al*, 2003; Franks *et al*, 2003). In addition, hyaline membranes, interstitial oedema, interstitial infiltrates of inflammatory cells, bronchiolar injury with loss of cilia, bronchiole epithelial denudation, and focal deposition of fibrin on the exposed basement membranes were also noted. Patients who died after the 10th day of illness exhibited a mixture of acute changes and the organizing phase of diffuse alveolar damage. There were interstitial and airspace fibroblast proliferation, type II pneumocyte hyperplasia, and squamous metaplasia of bronchial epithelium. The alveolar spaces contained a combination of macrophages, desquamated pneumocytes, and multinucleated cells. Hemophagocytosis in the alveolar exudates and thrombosis of venules were noted in some cases. Other pulmonary changes include secondary bacterial bronchopneumonia and invasive aspergillosis (Wang *et al*, 2003a). Systemic vasculitis involving the walls of small veins with edema, fibrinoid necrosis, and infiltration by monocytes, lymphocytes, and plasma cells were noted in one report (Ding *et al*, 2003). No tissue destruction or inflammatory process in association with viral infection were noted in other organs or tissues. Virus can be detected by in situ hybridization in pneumocytes and enterocytes of the small intestine (To *et al*, 2004). However, no remarkable histological changes can be found in the intestinal mucosa that can account for the watery diarrhea. Immunohistochemical staining showed the presence of viral proteins in pneumocytes and occasional macrophages. Another common extrapulmonary pathology is the presence of necrosis or atrophy in the lymphoid tissue of lymph nodes and white pulp of the spleen. Peripheral blood examination by flow cytometry at the time of admission before the use of steroid had shown decreases in levels of dendritic cell subsets, natural killer cells, CD4+ and CD8+ T lymphocytes and B lymphocytes (Cui *et al*, 2003; Li *et al*, 2004; Zhang *et al*, 2004b). A study of three SARS patients suggested that a self-limiting or abortive infection of peripheral blood mononuclear cells can occur as evident by the presence of the minus-RNA, the replicative intermediate of the virus during the initial week of the illness (Li *et al*, 2003a). Study of the cytokine profile of SARS patients showed significant elevation of the plasma chemokines interleukin (IL)-8, monocyte chemoattactant protein (MCP)-1, and inducible protein-10 (IP-10); T-helper1 (Th1)-related cytokines interferon (IFN)-γ and IL-12; and inflammatory cytokines IL-1β and IL-6, which can induce an intense inflammatory response (Wong *et al*, 2004; Jones *et al*, 2004). This may account for the recruitment and accumulation of alveolar macrophages and polymorphs, and the activation of Thl cell–mediated immunity by the stimulation of natural killer and cytotoxic T lymphocytes. In general, specific serum antibody by indirect immunofluorescent or neutralization test start to appear at around day 10, plateaus at around the second month, and is maintained for over 12 months. Immunoglobulin M (IgM) and IgG appeared at around the same time but the former is not detected after 2 to 3 months. Serum testing by recombinant nucleocapsid EIA can detect antibody as early as the fifth day after the onset of symptom. Because the rise in antibody titre coincides with the reduction of viral load and the continued deterioration in about one third of all cases, researchers are diligently searching for markers of immunopathology such as autoantibodies, antiphospholipid antibodies, and chemokine and cytokine markers. The pathology and clinical manifestations of SARS most likely represent a mixture direct cellular invasion and destruction by SARS-CoV and immunolopathological damage as a result of the infection. Interaction between different viral components and host molecules have been shown to facilitate viral invasion and promote the inflammatory responses. Examples include the liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin) which is co-expressed with DC-SIGNR on hepatic and lymph node sinusoidal endothelial cells and may augment the infection by SARS-CoV in these tissues (Gramberg *et al*, 2005). Antibodies to the S2 domain of the SARS-CoV spike protein can act as an autoantibody which induces cellular cytotoxicity to A549 (a human lung adenocarcinoma cell line), which may partly account for the lung injury seen in SARS infection (Lin *et al*, 2005). Although SARS-CoV causes only a non-permissive infection in human macrophages and dendritic cells, physiological and immunological modulations of these immune cells can still be demonstration which could result in enhanced pro-inflammatory responses (Tseng *et al*, 2005). In addition to being the crucial cellular receptor for SARS-CoV, interactions with ACE2 may also play a key role in the genesis of acute respiratory distress syndrome (ARDS). During SARS-CoV infection, the viral spike protein downregulates the expression of ACE2 (Kuba *et al*, 2005). Since ACE2 is protective against the development of ARDS in a mice model (Imai *et al*, 2005), the depression in ACE2 may therefore have detrimental effects in the pulmonary injury seen in SARS. The role of ACE gene polymorphism and SARS (with or without ARDS) has been investigated

in both Hong Kong and Vietnam and both studies failed to show any significant association (Chan *et al*, 2005; Itoyama *et al*, 2005), although another study showed that ACE1 polymorphism could be associated with the development of hypoxemia in SARS patients (Itoyama *et al*, 2004). Polymorphism of two interferon-inducible genes (2′,5′-oligoadenylate synthetase and myxovirus resistance-A) have also been suggested to affect disease progression (Hamano *et al*, 2005).

Animal models and Koch's postulates

Despite the consistent isolation of SARS-CoV without other known respiratory pathogens from SARS patients who subsequently had seroconversion against this virus in different continents, the causative role of this virus was challenged by *Chlamydia* and human metapneumovirus. The issue was finally settled by a primate model of infection that demonstrated viral excretion, diffuse alveolar damage, and subsequent seroconversion after cynomolgus macaques (*Macaca fascicularis*) were inoculated with SARS-CoV (Kuiken *et al*, 2003). Inoculation of the monkeys by human metapneumovirus alone cannot reproduce the pathology and does not aggravate the disease when the two virus are inoculated together. Later, serological and virological surveillance in markets of southern China found that Chinese ferret badgers (*Melogale moschata*), masked palm civets (*Paguma larvata*), and raccoon dogs (*Nyctereutes procyonoides*) were infected with another lineage of SARS-CoV and investigation of the outbreak in a Hong Kong housing complex also found that domestic cats were infected with the prevalent SARS-CoV. Therefore ferrets (*Mustela furo*) and domestic cats (*Felis domesticus*) were experimentally inoculated and shown to be susceptible to infection by SARS-CoV (Martina *et al*, 2003). The cats had asymptomatic infection but some of the ferrets died from the infection. Furthermore asymptomatic infection associated with viral replication in lungs and nasal turbinates of BALB/c mice can also be established by intranasal inoculation. The Golden Syrian hamster is also susceptible to SARS-CoV infection with high levels of viral replication in the respiratory tract but without development of clinical disease (Roberts *et al*, 2005). It is therefore a suitable alternative to other animal models in the study of SARS-CoV. Recently, the common marmoset (*Callithrix jacchus*) is found to be another nonhuman primate model that develops pneumonitis and multi-organ infection following SARS-CoV infection (Greenough *et al*, 2005). Because distantly related carnivores can be infected with this virus, the animal reservoir for this pathogen may be broader than initially expected. The rapid development of these animal models have important implication in the understanding of the pathogenesis of SARS and the testing of putative antiviral or vaccines.

Clinical management

In the absence of data on randomized placebo controlled trials of antivirals or immunomodulators for SARS, respiratory support and intensive care remains the cornerstone of clinical management. Oxygen delivery by low-flow nasal cannula rather than the high-flow face mask should be used to reduce the risk of cross infection by aerosolization of infectious respiratory secretion. Other modes of noninvasive ventilation such as continuous positive airway pressure (CPAP) and bilevel positive airway pressure (BIPAP) should only be performed in negative pressure isolation room with adequate personal protective equipment for the health care workers. A low-tidal-volume strategy for lung protection is used for those who required mechanical ventilation. Broad-spectrum antibacterial therapy are often administered for the coverage of acute community-acquired typical or atypical pneumonic agents in the first week of the illness before the availability of virological confirmation. Diligent search for hospital-acquired sepsis should be performed for patients put on ventilator and especially for those who are given corticosteroids.

Antivirals and immunomodulators

The presence of viral particles in the diseased lungs and the strong association between initial and peak viral load with mortality suggested that an effective antiviral that can reduce the peak viral load may be the key objective for the treatment of SARS. This may decrease both the cytolytic damage and the degree of inflammatory or immunodysregulatory damage excited by the viral infection as a result of a decrease in viral burden.

The reports on findings of *in vitro* susceptibility testing were conflicting, which may reflect the use of different assay conditions and end-point determination. Contradictory results have been reported on interferon β-1a (Cinatl *et al*, 2003b; Hensley *et al*, 2004; Tan *et al*, 2004) and interferon α-2b (Tan *et al*, 2004; Stroher *et al*, 2004). Some reports noted the susceptibility towards ribavirin at a concentration of 200 to 1000 mg/L is related to cellular toxicity (Tan *et al*, 2004) whereas another reported that the 50% cytotoxic concentration of ribavirin exceeds 1000 mg/L in Vero cells (Cinatl *et al*, 2003a). However, much lower inhibitory concentrations which are clinically achievable have been demonstrated in other cell lines, including MA-104, PK-15, Caco2, CL14, and HPEK (Morgenstern *et al*, 2005). Furthermore, synergistic activity between interferon-β and ribavirin has also been shown in the same study. (Morgenstern *et al*, 2005).

Overall it appears that interferon β, interferon α-n1, interferon α-n3, and leukocytic interferon α do have some activity and should be considered for clinical trials. Many new compounds, especially protease inhibitors (Xiong *et al*, 2003), ACE2 analogues

(Towler *et al*, 2004), helicase inhibitor, and nucleoside analogues, screened from combinatorial chemical libraries are reported to possess good *in vitro* anti-SARS-CoV activities. Combinations of interferons or the above compounds with ribavirin can be synergistic. Because the mortality of SARS in China where traditional Chinese herbal medicine was used for treatment appeared to be lower, purified compounds extracted from these herbs have also been tested. Both glycyrrhizin and baicalin appeared to be active but the effective concentration of glycyrrhizin is unlikely to be clinically achievable (Cinatl *et al*, 2003a). Antiviral peptides designed to glue up the claw mechanics of the spike protein used for cell entry appeared to be useful *in vitro* (Kliger and Levanon 2003; Liu *et al*, 2004). Similarly, siRNA designed for sequence specific degradation of viral RNA generated during transcription also appeared to be useful (He *et al*, 2003; Zhang *et al*, 2004a). Due to the lack of a clinically effective antiviral, many clinicians resorted to the empirical use of immunodulators including corticosteroids, intravenous immunoglobulins, pentaglobin, thymosin, thalidomide, and anti-tumor necrosis factor during the pandemic. The use of corticosteroids for viral pneumonias due to varicella-zoster virus, influenza virus, and other viruses were reported as case series in the English literature (Greaves *et al*, 1981; Ahmed *et al*, 2002). Mortality in the first two conditions appeared to be lower with corticosteroids than supportive treatment alone. However in the absence of an effective antiviral, early use of high doses of corticosteroids for prolonged period is probably counterproductive and may increase the risks of hospital-acquired sepsis and avasuclar bone necrosis (Auyeung *et al*, 2005). Pegylated interferon α-2a was shown to be useful for prophylaxis and decreasing respiratory viral shedding and lung pathology when used as an early treatment in a monkey model (Haagmans *et al*, 2004). Combinations of steroid with either alfacon-1 (a recombinant consensus interferonα) (Loutfy *et al*, 2003) or lopinavir-ritonavir and ribavirin were shown to improve outcomes in two different treatment trials using historical controls (Chu *et al*, 2004).

Passive and active immunizations

Because the usefulness of passive immunization by convalescent plasma containing high titer of neutralizing antibody had been demonstrated for the treatment of Lassa fever, similar treatment has been tried empirically for deteriorating SARS cases at the later stage and did not appear to be beneficial. There were, however, no obvious side effects being observed. At the moment, only hyperimmune globulin manufactured from convalescent patients' plasma and horses immunized by inactivated SARS-CoV are available for prophylactic trials in human. Another potential candidate is a human monoclonal IgGl produced from a single-chain variable region fragment against the S1 domain of from two nonimmune human antibody libraries (Sui *et al*, 2004). It has strong neutralizing activity and comparable binding affinity for spike to that by soluble ACE2. In the mouse model of asymptomatic SARS infection, passive transfer of immune serum containing high titers of neutralizing antibody prevented virus replication in the lungs but not as effective in the nasal turbinates (Subbarao *et al*, 2004). Using the same mice model, a plasmid DNA vaccine carrying the spike protein encoded by humanized codons was shown to be highly protective against challenge by SARS-CoV (Zeng *et al*, 2004). Moreover T-cell depletion with specific monoclonal antibodies against CD4 or CD8, alone or in combination with CD90, did not affect the protective immunity which was confirmed by adoptive T-cell transfer. Donor T lymphocytes alone did not inhibit pulmonary viral replication in recipient mice, whereas passive transfer of purified IgG from immunized mice achieve similar protection. At this juncture it appears that the humoral immune response against the viral spike antigen is the key factor for protection against a primary infection in the immunologically navie host. Similar findings were found in the same mouse model using either intramuscular or intranasal administration of a highly attenuated modified vaccinia virus Ankara carrying the spike protein (Bisht *et al*, 2004). Other approaches using adenoviral vector encoding S1 domain, membrane, and nucleoprotein or a DNA vaccine linking nucleocapsid protein to calreticulin were shown to induce virus specific immunity but were not tested in animal models (Kim *et al*, 2004). We are still uncertain about the relative importance of systemic or mucosal immunity in terms of neutralizing antibody or cytotoxic T-lymphocyte response against spike, nucleocapsid, or other targets in terms of recovery from SARS but neutralizing antibody against spike appears to be very important for prophylactic immunity. The use of live-attenuated virus is out of question because of the concern for reversion to virulence or recombination with wild strains to form new wild types. The most readily available vaccine to undergo clinical trial will be an inactivated SARS-CoV. However, laboratory safety is a major safety issue when cultivating huge stocks of virus. Recombinant proteins expressed in mammalian cells is an alternative for the induction of good neutralizing antibody response. Nonreplicating coronavirus particles will be safe and closely mimic the live virus in terms of imunogenicity. Irrespective of which approach is being used for immunization, it is important to note that immune enhancement of disease has occurred in feline peritonitis coronavirus infection. Atypical or more severe disease had occurred after the use of inactivated measles and respiratory syncytial virus vaccination. In a ferret model immunized with modified vaccinia virus Ankara-based recombinant vaccine, immunized animals developed more severe hepatitis following SARS-CoV challenge (Weingartl *et al*, 2004). Future vaccine studies must

take into account such potential adverse effects before going into clinical trials. Moreover, the economic viability of SARS vaccine depends on whether or not SARS is to come back and whether major antigenic variation will affect the effectiveness of this vaccine. The possibility of its use in farm setting for wild game food animals may worth further investigations.

Laboratory safety, community, and hospital infection control

The stability of SARS-CoV in the environment, the absence of protective immunity in the general population, and the lack of effective antivirals or vaccines demand a near-perfect compliance by health care or laboratory workers to infection control measures. In the health care setting, triage, early case detection, and isolation of suspected cases against nosocomial transmission are the key issues (Ho *et al*, 2003). Respiratory droplet and contact precautions have been shown to be effective in the prevention of nosocomial transmission of the disease under most circumstances (Seto *et al*, 2003). Additional measures of airborne precautions are necessary in situations where droplet nuclei are likely to be generated. Strict hand hygiene and a patrol nurse overseeing all gowning and degowning procedures in a SARS ward appear to be critical for the prevention of hospital transmission. In the community setting, contact tracing, quarantine of contacts to prevent community spread, temperature checks at borders, health declarations for travellers, public education, and effective risk communication with public media have proven to be effective for the control of the 2003 outbreak of SARS. All laboratories handling SARS specimens must comply to the World Health Organization standards. Accurate logging and secure storage of virus or infected samples are important to avoid further accidents. The amount of stored clinical specimens must be kept to a level just sufficient for research purpose. In institutions where live virus are being cultivated and manipulated, the laboratories must be regularly audited to meet the safety standards of a biosafety level 3 laboratory. Daily checking of temperature and reporting of sickness should be part of the monitoring protocol for laboratory workers handling live viruses. A culture of openness to mistakes, non-witch-hunting attitude for investigation of incidents, overseeing each other, and thus compliance can only be built if team members are not afraid of being punished after admission of accidents and lapses.

Globalization of infectious diseases

The density of human and domestic or wild food animals in southern China makes it an ideal incubator for emerging infections. Such novel infections usually have a limited epidemic potential among humans initially after crossing the species barrier. However, after accumulating appropriate genetic changes, they may acquire the ability to transmit efficiently between humans. Human infections by influenza A H5N1 and H9N2 viruses are the examples of the former whereas human immunodeficiency virus (HIV) and SARS-CoV are dramatic examples of the latter. Air travel provides the means for rapid globalization of such infectious disease. The horror, the social disruption, the paralysis to travel, and the loss in economy thus cause enormous damage to many countries in addition to the loss of human lives. Therefore the standard and capability in public health is no longer a national but an international issue. The failure of public health surveillance or the lack of transparency in communication of such critical data to the international community have been severely criticized by World Health Organization. The gravity of the damage by emerging infectious diseases have elevated the issue of public health to the level of those basic values or functions of governance such as democracy, human rights, national security, or economy.

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