5. The Severe Acute Respiratory Syndrome

KWOK-YUNG YUEN, SAMSON S.Y. WONG, AND J.S. MALIK PEIRIS

5.1. Introduction

Severe acute respiratory syndrome (SARS) was the first major pandemic of the new millennium. It had dramatic impact on the health care systems, economies, and societies of countries directly affected. Since its emergence in 2002–2003, much has been learned about the disease and the causative agent, and this chapter summarizes the current understanding of the SARS coronavirus and the disease it causes.

5.2. Sequence of Events

In the past century, Southern China has been the epicenter for emergence of some notable infectious diseases. The Hong Kong Special Administrative Region (HKSAR), being part of southern China, which has enjoyed a relatively more advanced public health infrastructure and international connectivity, often became the first place where such emerging infectious diseases were discovered and from where they disseminated globally. The first such example occurred in 1894 when an outbreak of plague started in Canton (now the Guangdong Province) of China. A similar outbreak, this time an acute community-acquired atypical pneumonia syndrome, occurred in the Guangdong Province in late 2002. Retrospective investigations showed that severe cases of atypical pneumonia had been recorded in five cities around Guangzhou over a period of 2 months. The index case was reported on 16 November 2002 in Foshan, a city 24 km away from Guangzhou. The second case appeared in December 2002 when a chef from Heyuan who worked in a restaurant in Shenzhen was affected. He had an occupational history of regular contact with wild-game food animals. He transmitted the disease to his wife, two sisters, and seven hospital staff who had contact with him. From 16 November 2002 to 9 February 2003, 305 cases of atypical pneumonia

were reported in mainland China with 105 of them being health care workers. The link between the outbreak in mainland China and the devastating epidemics in HKSAR and the rest of the world was a professor of nephrology from a teaching hospital in Guangzhou, China. He had contact with patients suffering from this unusual atypical pneumonia between 11 and 13 February 2003, then developed flu-like symptoms, including a fever and cough, before coming to Hong Kong. He arrived in Hong Kong on 21 February and within a day transmitted infection to 16 other people in the hotel that he stayed in. Thorough investigation of this patient failed to reveal any significant pathogens. One of the secondary cases, his brother-in-law who subsequently became infected, underwent an open lung biopsy that was pivotal in the discovery of the etiology this disease. The virus was a novel coronavirus, subsequently called the severe acute respiratory syndrome (SARS) coronavirus.

Infected contacts in the hotels unknowingly carried the disease to other hospitals in the HKSAR and to other countries and continents including Vietnam, Canada, Singapore, the Philippines, the United Kingdom, the United States, and back again to China. Dr. Carlo Urbani, a physician stationed at the World Health Organization (WHO) office in Hanoi, was the first to notify the WHO of the occurrence of this disease outside Guangdong after witnessing an explosive nosocomial outbreak in a hospital in Hanoi. The index case of this outbreak was one of those who acquired infection on 21–22 February in the hotel in Hong Kong. The WHO named this "atypical pneumonia" as severe acute respiratory syndrome (SARS). Dr Urbani's description of the disease, to which he later succumbed, alerted health authorities throughout the world and started an unprecedented collaboration of 11 research laboratories in 9 countries to identify the etiological agent responsible.

5.3. Epidemiologic Characteristics

SARS is essentially an acute community-acquired or nosocomial pneumonia that does not respond to conventional antimicrobial therapy for known pneumonia pathogens. As of mid-2003, 8096 cases with 774 deaths occurred on five continents (World Health Organization, 2003a). Interpersonal transmission has occurred in health care facilities, workplaces, homes, and public transports (World Health Organization, 2003b). From September 2003 to May 2004, three laboratory-associated outbreaks were reported (Lim et al., 2004; Orellana, 2004). The laboratory-acquired cases in Singapore and Taiwan were each limited to one affected person, whereas that in Beijing was associated with secondary and tertiary cases (World Health Organization, 2004).

SARS-coronavirus (SARS-CoV) is most likely zoonotic in origin and then transmitted to humans. The virus may have been circulating among wild game animals in the wet markets of southern China. The palm civet is now considered to be the most likely amplification host and one of the likely candidates for introduction to humans (Guan et al., 2003). A seroprevalence of about 80% was recently reported for civets in animal markets in Guangzhou (Tu et al., 2004). The initial human cases of the 2003 pandemic and those of the 2004 Guangdong outbreak were epidemiologically linked to game animals either because of occupational contact or eating (Zhong et al., 2003). The most important route of interpersonal spread appears to be direct or indirect contact of the mucosae with infectious respiratory droplets or fomites (World Health Organization, 2003b). SARS-CoV has been detected in respiratory secretions, feces, urine, and tears (Chan et al., 2004a; Loon et al., 2004). Nosocomial transmission of SARS could be facilitated by the use of nebulizers, suction, intubation, bronchoscopy, or cardiopulmonary resuscitation on SARS patients by generation of large numbers of infectious droplets (Lee et al., 2003b; Varia et al., 2003; World Health Organization 2003b; Christian et al., 2004). Almost half of the SARS cases in Hong Kong acquired the infection within health care facilities and institutions (Leung et al., 2004a). The overall attack rate among hospital workers is 1.2% and the attack rate was correlated with the number of SARS patients admitted (Lau et al., 2004c). Airborne transmission is considered uncommon given the relatively low attack rates. A unique form of airborne transmission was considered the likely explanation for a large community outbreak in a private housing estate: Negative pressure generated by exhaust fans coupled with dry U traps of sewage drains was thought to facilitate the dissemination of contaminated aerosols between toilets. It is suggested that the plume of contaminated warm air along the light well was then carried by wind to other blocks of the housing complex (Yu et al., 2004). The finding of culturable viruses in stool and the high fecal viral load shown by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) (Peiris et al., 2003a) suggested the possibility of fecal-oral transmission though this has not been proved conclusively.

The incubation period is estimated to be 2 to 14 days. Epidemiologic control measures based on the World Health Organization recommended maximal incubation period of 10 days was effective in most cases to interrupt transmission, though there had been occasionally cases in which the incubation period was found to be longer (Chan et al., 2004b). The average number of secondary cases resulting from a single case is two to four (World Health Organization, 2003b). Most of the transmissions resulted from contacts with patients with overt disease rather than asymptomatic

or mildly symptomatic cases. Seroprevalence appeared to be low (0%, 0.43%, 1.2%) for normal individuals and about 1% for health care workers, around 1% for asymptomatic family contacts under quarantine, and 0.19% for asymptomatic contacts overall (Lee et al., 2003a; Peiris et al., 2003a; Leung et al., 2004b; Woo et al., 2004; Zheng et al., 2004a; Chen et al., 2005). Transmission from symptomatic patients tended to occur on or after the fifth day of onset of disease, which could be explained by the rising viral load in the nasopharyngeal secretion, which peaked at around day 10. The median time for RT-PCR to convert to negative is 30 days after onset of illness and 13 days after discharge from hospital (Chu et al., 2005).

A definite seasonality of SARS could not be documented at this stage, though there have been correlations between the incidence of the disease and ambient temperature (Tan et al., 2005). There was also definite geographic clustering of cases upon retrospective analysis by the geographic information system (Lai et al., 2004).

5.4. General Virology

The family Coronaviridae consists of a group of large, enveloped, positive-sense, single-stranded RNA viruses that are of human and veterinary importance (Table 5.1). They have the largest genome of all the RNA viruses. The SARS-CoV is one of the newly described members of the genus Coronavirus (Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003b). Primary viral isolation can be achieved by inoculating embryonal monkey cell lines such as Vero E6 and FRhK-4 cells. Subcultures can be made on other Vero cells, Huh-7 (a liver cancer cell line; Simmons et al., 2004) and CACO-2 (a colonic carcinoma cell line), and two pig cell lines (POEK, PS) (Hattermann et al., 2005). In contrast with other human coronaviruses, SARS-CoV can be maintained in cell culture easily and it produces obvious cytopathetic effects in Vero E6 within 48 h. SARS-CoV has a higher degree of stability in the environment as compared with other known human coronaviruses (Duan et al., 2003; World Health Organization 2003c; Rabenau et al., 2005): it survives for at least 2 to 3 days on dry surfaces at room temperature and 2 to 4 days in stool (Rabeneu et al., 2005).

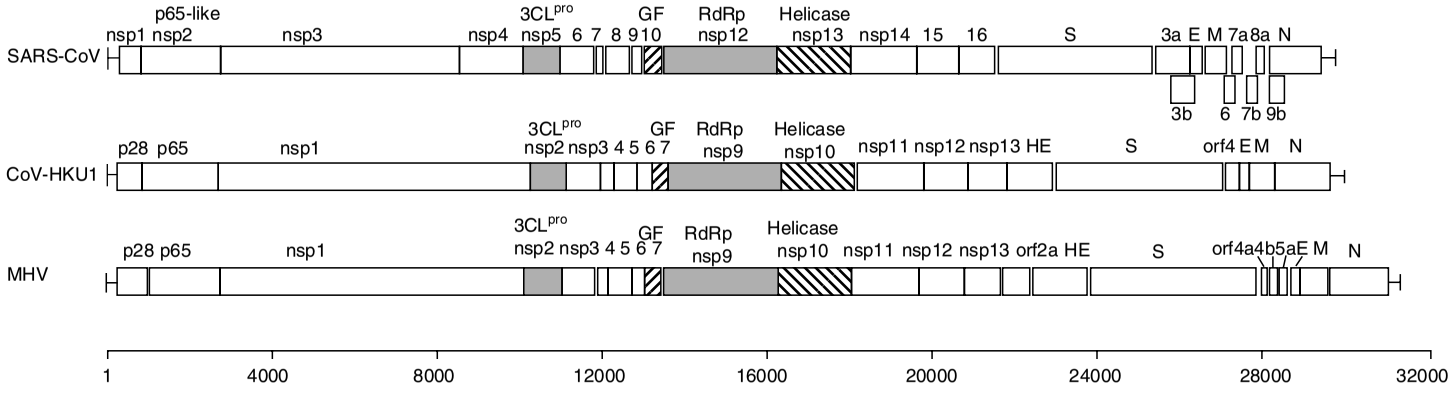
Electron microscopy appearance and genome order (Fig. 5.1) of 5′-replicase-structural (spike-envelope-membrane-nucleocapsid)-polyT-3′ on complete genomic sequencing are similar to other members of Coronaviridae. SARS-CoV is phylogenetically distinct from any of the three groups of known coronaviruses. Human coronaviruses 229E and NL63 belong to group 1 (Fouchier et al., 2004; van der Hoek et al., 2004); human coronavirus OC43 belongs to group 2, and group 3 consists of all

Table 5.1. Classification of coronaviruses

Order	Family	Genus	Species
Nidovirales	Arteriviridae	Arterivirus	Equine arteritis virus
			Lactate dehydrogenase–elevating virus
			Porcine respiratory and reproductive syndrome virus
			Simian hemorrhagic fever virus
	Coronaviridae	Coronavirus	
		Group 1	Canine coronavirus
			Feline coronavirus
			Human coronavirus 229E
			Human coronavirus NL63
			Porcine epidemic diarrhea virus
			(Porcine) Transmissible gastroenteritis coronavirus
		Group 2	Bovine coronavirus
			Canine respiratory coronavirus
			Human coronavirus HKU1
			Human coronavirus OC43
			Murine hepatitis virus
			Porcine hemagglutinating encephalomyelitis virus
			Puffinosis virus
			Rat coronavirus
		Group 3	Turkey coronavirus
			(Avian) Infectious bronchitis virus
		Group 4	SARS coronavirus
		Torovirus	Bovine torovirus
			Equine torovirus
			Human torovirus
			Porcine torovirus
	Roniviridae	Okavirus	Gill-associated virus
			Yellow head virus

From the Universal Virus Database of the International Committee on Taxonomy of Viruses. Available at http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm.

the avian coronaviruses. Phylogenetic analysis of the spike protein of 139 SARS-CoV isolates in the Hong Kong outbreak showed that several introductions had occurred but only one of them was associated with the major outbreak in Hong Kong and the rest of the world (Guan et al., 2004). Some of the strains found in the early stages of the outbreak were phylogenetically distinct from the major cluster and were closer to some of the Guangdong and Beijing strains. This concurred with the fact that the index patient of the Hong Kong outbreak was a Guangzhou medical doctor who had traveled to Hong Kong. Another molecular epidemiological study of a Guangdong outbreak suggested that the disease spread from Guangdong to Hong Kong and the rest of the world, and the index case



GF: growth-factor-like protein
RdRp: RNA-dependent RNA polymerase

Figure 5.1. Comparison of the gene order of selected group 2 coronavirus genomes.

was a chef who handled game animals (Zhong et al., 2003). Subsequent animal surveillance in China recovered coronavirus isolates that have a nucleotide homology of 99.8% with the SARS-CoV (Guan et al., 2003). A characteristic 29-base insertion between open reading frames (ORF) 10 to 11 (also named as ORF8a and 8b) was found in these animal isolates (Guan et al., 2003; Snijder et al., 2003). This 29-nucleotide segment was deleted after or before crossing the species barrier to humans. The biological effect of this deletion remains elusive. A number of SARS-CoV in the later stages of the epidemic showed larger deletions around this site (The Chinese SARS Molecular Epidemiology Consortium, 2004). Two independent molecular epidemiological studies comparing the complete genome of 12 and 63 virus isolates also found evidence of a strong positive selection at the beginning of the epidemic that is followed by a purifying selection, as indicated by the amino acid substitution rate at the spike, Orf3a, and nsp3 (The Chinese SARS Molecular Epidemiology Consortium, 2004; Yeh et al., 2004; Song et al., 2005). Both studies suggested that molecular adaptation of the virus had occurred after interspecies transmission from animals to humans. In the small outbreak of Guangzhou in 2004, all four human isolates belonged 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 distinct from the three existing groups of coronaviruses, it may be closer to group 2 because 19 out of 20 cysteines found in the S1 domain of the spike protein are spatially conserved when compared with the group 2 consensus sequence, whereas only 5 cysteine residues are conserved when compared with that of the groups 1 and 3 (Eickmann et al., 2003; Snijder et al., 2003). Because coronaviruses are believed to have co-evolved with their animal hosts, it was possible that rats, mice, and cattle harboring group 2 coronaviruses are more likely to be the animal host for SARS-CoV than cats, which harbor group 1 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 is 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. Genomic evidence of another

animal-to-human interspecies transmission events was demonstrated in a small outbreak of four cases in early 2004 in Guangzhou (Song et al., 2005).

A metalloprotease angiotensin-converting enzyme 2 (ACE2) from Vero E6 cells was found to bind the S1 domain of the spike protein of the SARS-CoV (Li et al., 2003c). The 293T cells transfected with ACE2 can form multinucleated syncytia with cells expressing the spike. ACE2 expression is detected by immunohistochemical staining on enterocytes, pneumocytes, vascular endothelial and smooth muscle cells. (Leung et al., 2003; Hamming et al., 2004). This is compatible with the clinical and histopathological manifestations of SARS, which include diffuse alveolar damage, colonic mucosal presence of abundant viral particles in a patient with diarrhea, and pulmonary vasculitis and thrombosis. The lack of virus infection in some cell lines that express ACE2 has led to a rush for finding coreceptors for cell entry (To and Lo, 2004a). Recently, a C-type lectin called L-SIGN was shown to be an alternative receptor for binding and cell entry (Jeffers et al., 2004), although it is not clear whether this is a functional receptor for the virus that can lead to productive virus replication. Murine ACE2 binds less efficiently with the S1 domain of SARS-CoV, and even less well with rat ACE2, which suggests that efficient replication in the mouse or rat is limited by their ACE2 type (Greenough et al., 2005).

Pseudotyped retroviral vector carrying the S, M, or E proteins revealed that the S protein is both necessary and sufficient for virus attachment on susceptible cells (Simmons et al., 2004; Wang et al., 2004a). 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 human immunodeficiency virus (HIV) infection. 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).

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).The E (envelope) protein was found to form cation-selective ion channel in planar lipid bilayer (Wilson et al., 2004). 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 helicase (nsp13), a putative mRNA cap-1 methyltransferase (nsp16), a manganese-dependent endoribonulcease (nsp 15) (Bhardwaj et al., 2004), and a single-stranded RNA binding

protein (nsp9) are being investigated intensively by bioinformatics and crystal structure studies (Campanacci et al., 2003; von Grotthuss et al., 2003; Egloff et al., 2004). The nomenclature of these nonstructural proteins is summarized in Table 5.3. A full-length cDNA of the viral genome was shown to cause lytic infection in cell line with good viral titer and antigen expression (Yount et al., 2003). This would provide the tool to study function of many nonstructural proteins by reverse genetics.

Table 5.2. Putative genes and annotation of SARS coronavirus*

Open reading frames	Proteins	Function	Number of amino acids
Orf1a	nsp1	Unknown	180
	nsp2	Unknown	638
	nsp3	Papain-like cysteine protease/ adenosinediphosphate ribose phosphatase	1922
	nsp4	Unknown	500
	nsp5	3C-like cysteine protease(main)	306
	nsp6	Unknown (many transmembrane domain)	290
	nsp7	Unknown	83
	nsp8	Unknown	198
	nsp9	Single-strand RNA-binding protein	113
	nsp10	Unknown (growth factor-like domain present)	139
	nsp11	Unknown	13
Orf1b	nsp12	RNA-dependent RNA polymerase	932
	nsp13	Helicase (dNTPase and RNA 5′-triphosphatase activities)	601
	nsp14	Unknown. ?Exonuclease (homolog of ExoN)	527
	nsp15	Manganese dependent RNA endonuclease (homologue of XendoU)	346
	nsp16	?mRNA cap1 2′-*O*-ribose methyltransferase	298
Orf2		Spike protein	1255
Orf3a (X1/U274/Orf3)		Accessory protein associated with plasma membrane and trafficking of intracellular viral particles	274
Orf3b (Orf4)		Unknown	154
Orf4(X2)		Envelope (forms cation-selective ion channel)	76
Orf5(X3)		Membrane	221
Orf6 (X3/Orf7)		Unknown	63
Orf7a (X4/Orf8)		Unknown (antigenic protein expressed in infected cells)	122
Orf7b (Orf9)		Unknown	44
Orf8a (Orf10)		Unknown	39
Orf8b (X5/Orf11)		Unknown	84
Orf9a		Nucleocapsid	422
Orf9b (Orf13)		Unknown	98

* Mara et al. (2003); Rota et al. (2003); Thiel et al. (2003).

5.5. Clinical Findings

The typical manifestation of SARS in two-thirds of the cases is that of a viral pneumonia with rapid respiratory deterioration (World Health Organization, 2003b). The presenting symptoms are generally fever, chills, myalgia, malaise, and nonproductive cough (Tsang et al., 2003). Rhinorrhea and sore throat are less frequently seen. Clinical deterioration often occurs 1 week after the onset of illness and may be accompanied by watery diarrhea. Physical signs on chest examination are mild when compared with the radiographical abnormalities. The chest radiograph may show ground-glass opacities and focal consolidations, typically over the periphery and often in the subpleural regions of the lower zones. Progressive involvement of both lungs may occur, as may shifting radiographic shadows and pneumomediastinum without previous use of positive pressure ventilation.

Diarrhea is the commonest extrapulmonary manifestation (Cheng et al., 2004a), followed by hepatic dysfunction (Chau et al., 2004), dizziness that may be related to diastolic cardiac impairment (Li et al., 2003b), abnormal urinalysis, petechiae (Wu et al., 2003), myositis (Wang et al., 2003b), and epileptic fits (Lau et al., 2004a). The neuromuscular abnormalities are likely to be related to critical-illness polyneuropathy and/or myopathy (Tsai et al., 2004). The elderly patient may merely be admitted for a fall and fracture in the absence of fever or other symptoms and signs suggestive of SARS (Wong et al., 2003; Chow et al., 2004). Infections in children appear to be milder than in adults (Kwan et al., 2004), whereas pregnant women infected by SARS could pose a significant management problem and carries a high case-fatality rate, although no transmission to the neonate has been documented (Wong et al., 2004; Yudin et al., 2005). Viral replication at different sites appears to account for the various clinical and laboratory abnormalities of SARS (Hung et al., 2004; Farcas et al., 2005). Nasopharyngeal and serum viral load were associated with oxygen desaturation, mechanical ventilation, and mortality (Chu et al., 2004b); stool viral load with diarrhea; and urine viral load with abnormal urinalysis.

Peripheral lymphocytopenia is common with or without thrombocytopenia, increases in D-dimers, and activated partial thromboplastin time. Elevated hepatic parenchymal enzymes, muscle enzymes, and lactate dehydrogenase may be seen. About 20% to 30% of the patients will deteriorate and require mechanical ventilation and the overall mortality is 15%. Death is usually related to respiratory failure, concurrent sepsis, or deterioration of underlying medical illness. Age, presence of comorbidities such as cardiac problems and diabetes mellitus, increased lactate

dehydrogenase level, hypouricemia (Wu et al., 2005), acute renal failure (Chu et al., 2005), more extensive pulmonary radiological involvement at presentation (Hui et al., 2004), and a high neutrophil count at the time of admission are poor prognostic indicators. Most survivors have residual ground-glass opacifications on follow-up chest x-rays probably due to fibrosis. The radiological abnormalities improve with time, but advanced age, previous intensive care unit admission, mechanical ventilation, alternative treatment, higher peak lactate dehydrogenase, and the peak radiographic involvement during treatment have a positive correlation with overall reticulation and total parenchymal involvement at 6 months (Wong et al., 2004). Restrictive lung function abnormalities attributed to residual lung fibrosis and muscle weakness is commonly seen in the convalescent phase with an incidence of at least 20% in some studies (Chan et al., 2003b; Ng et al., 2004; Ong et al., 2004). Depression and posttraumatic stress disorder are especially common in patients with affected family members and among health care workers; psychosis could be related to the use of corticosteroids and underlying predispositions (Lee et al., 2004). As a result of treatment with corticosteroids, a patient may have biochemical evidence of adrenal insufficiency after recovery. There is also a cumulative dose-related risk of osteonecrosis, ranging from 0.6% to 13% (Griffith at al., 2005).

5.6. Laboratory Diagnostics

There are no pathognomonic signs or symptoms of SARS. Etiological diagnosis and differentiation from other causes of atypical pneumonia can only be made by laboratory confirmation. A positive viral culture from the respiratory, fecal, and occasionally urine specimens, a fourfold rise of neutralizing antibody titer in the serum taken on admission and 28 days afterwards are the most definitive evidence of infection. Of these methods, real-time quantitative RT-PCR of the nasopharyngeal aspirate is most practicable in aiding clinical diagnosis and can achieve a sensitivity of 80% with good specificity if it is collected within the first 3 days of illness (Poon et al., 2003). For antibody testing, the indirect immunofluorescent antibody test is more commonly performed than the neutralizing antibody test since the former does not involve manipulation of infectious virus and therefore carries less biohazard risk. Recombinant nucleocapsid enzyme immunoassay (EIA) can be used as a rapid screening test and possesses high sensitivity as early as 5 days after onset of illness (Che, 2004). EIA against the nucleocapsid antigen of SARS-CoV may occasionally yield false-positive results with HCoV-OC43 and HCoV-229E and may require confirmation by Western blot against the

spike polypepetide of SARS-CoV (Woo et al., 2004). A new immunofluorescence assay using recombinant nucleocapsid-spike fusion protein as antigen has been described (He et al., 2005). Viral load determination of nasopharyngeal specimens or serum on presentation might have clinical value as it is an important prognostic factor (Cheng et al., 2004a).

The three laboratory outbreaks of SARS have prompted the use of pseudotype viruses for research and neutralization antibody testing.

5.7. Pathology and Immunology

Acute diffuse alveolar damage with airspace edema were the most prominent features in patients who died before the tenth day after onset of illness (Franks et al., 2003; Nicholls et al., 2003). Hyaline membranes, interstitial edema, interstitial infiltrates of inflammatory cells, bronchiolar injury with loss of cilia, bronchiolar epithelial denudation, and focal deposition of fibrin on the exposed basement membranes were other observed features. Patients who died after the tenth 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 complications might 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. Viral particles can be detected by *in situ* hybridization in pneumocytes and enterocytes of the small intestine (To et al., 2004b). However, inflammation, cellular apoptosis, or microvilli atrophy are not found in the intestinal mucosa to account for the watery diarrhea. Immunohistochemical staining showed the presence of viral proteins in pneumocytes and occasional macrophages. Necrosis or atrophy in the lymphoid tissue of lymph nodes and white pulp of the spleen is a commonly observed extrapulmonary pathology. Flow cytometry examination of the peripheral blood 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 chemotactic protein 1 (MCP-1), and interferon-gamma-inducible protein 10 (IP-10), Th1-related cytokine interferon-gamma and IL-12, and inflammatory cytokines IL-1β and IL-6, which can induce an intense inflammatory response (Jones et al., 2004; Wong et al., 2004; Huang et al., 2005; Jiang et al., 2005). This may account for the recruitment and accumulation of alveolar macrophages and polymorphs and the activation of Th1 cell-mediated immunity by the stimulation of natural killer and cytotoxic T lymphocytes. In contrast, SARS-CoV appears to evade triggering an alpha and beta interferon response in human macrophages (Cheung et al., 2005). The lack of this antiviral innate immune response may permit uncontrolled viral replication with progressive increase in viral load continuing into the second week of illness until the appearance of the adaptive immune response brings the virus replication under control. Moreover, comparative transcriptomal microarray analysis showed that the SARS-CoV rather than the HCoV-229E markedly upregulates genes associated with apoptosis, inflammation, stress response, and procoagulation during the early phase of infection of a human liver cancer cell line (Huh7). Both findings help to explain the clinical severity of SARS in relation to the high viral load up to 2 weeks of illness and the intense inflammatory response as evident from the serum cytokine profile and the histopathology (Tang et al., 2005).

In general, specific serum antibody by indirect immunofluorescence or neutralization test starts to appear at around day 10, plateaus at around the second month, and is maintained for more than 12 months. 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 symptoms.

Some studies have suggested a possible association of HLA-B*4601 with susceptibility to and severity of SARS among the Chinese population in Taiwan (Lin et al., 2003). Among the Hong Kong Chinese population, similar associations have been found with HLA-B*0703 (Ng et al., 2004).

5.8. Animal Models and Koch's Postulates

The Koch's postulates for SARS-CoV as a causative agent of SARS was fulfilled by a primate model using cynomolgus macaques (*Macaca*

fascicularis), which demonstrated clinical and pathological features similar to those found in humans (Kuiken et al., 2003). On the contrary, African green monkeys (*Cercopithecus aethiops*) did not develop significant lung pathology after inoculation with the SARS-CoV (Bukreyev et al., 2004). The lack of consistency in primate animal models (rhesus, cynomolgus, and African green monkey) for experimental SARS disease was noted in a recent study (McAuliffe et al., 2004). Ferrets (*Mustela furo*) and domestic cats (*Felis domesticus*) are also susceptible to infection by SARS-CoV (Martina et al., 2003). The cats remained asymptomatic, but some of the infected ferrets died from the disease. BALB/c mice demonstrated asymptomatic infections in lungs and nasal turbinates by intranasal inoculation, in contrast with golden Syrian hamsters in which high levels of viral replication can be seen (Roberts et al., 2005). Recently, palm civets (*Paguma larvata*) were shown to be susceptible to symptomatic infection by SARS coronavirus with or without the 29-bp signature sequence (Wu et al., 2005). Experimental infection of SARS-CoV has also been demonstrated in pigs (Weingartl et al., 2004a). Because different SARS-CoV isolates were used by each of the groups, it is therefore uncertain whether one particular animal would be better than others as a model for SARS-CoV. However, the diverse range of mammalian species that are susceptible to experimental infection by SARS-CoV is a cause for concern. Indeed, our first report on animal SARS coronavirus showed that Chinese ferret badgers (*Melogale moschata*) and raccoon dogs (*Nyctereutes procyonoides*) could also be infected with SARS-CoV (Guan et al., 2003).

5.9. Clinical Management

Clinical management of SARS relies on respiratory support and intensive care because there are no randomized clinical trials on the value of potential antiviral agents. Oxygen delivery by low-flow nasal cannula rather than the high-flow facemask should be used to minimize the risk of nosocomial airborne spread. 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 rooms with adequate personal protective equipment for the health care workers (Cheung et al., 2004). Broad-spectrum antimicrobial coverage for community-acquired pneumonia should be given at presentation while pending virological confirmation. Nosocomial infections are important complications after prolonged intubation, hospitalization, and the use of corticosteroids.

5.9.1. Antivirals and Immunomodulators

The association between viral loads and clinical outcome raises the possibility that effective suppression of viral replication may be beneficial. *In vitro* susceptibility testing results were conflicting, which may partly be due to the use of different assay conditions and end-point determination. There have been contradictory results on the *in vitro* activities of interferon β-1a (Cinatl et al., 2003b; Hensley et al., 2004; Tan et al., 2004) and interferon α-2b (Stroher et al., 2004; Tan et al., 2004). The antiviral effects of interferon β appeared to be mediated by mechanisms other than MxA protein (Spiegel et al., 2004). Overall, it appears that interferon β, interferon α-n1, interferon α-n3, and leukocytic interferon α do have some activity and could be considered for further studies in clinical trials (Chen et al., 2004; Spiegel et al., 2004; Zheng et al., 2004b).

Some reports noted the sensitivity to 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 (Cinatl et al., 2003a). It is interesting to see that the same group reported good activity of ribavirin in other human and animal cell lines despite their initial report of the lack of activity of ribavirin in Vero cells (Morgenstern et al., 2005).

Glycyrrhizin, baicalin (Cinatl et al., 2003a), reserpine (Wu et al., 2004b), niclosamide (Wu et al., 2004a), aurintricarboxylic acid (He et al., 2004), chloroquine (Keyaerts et al., 2004), and the protease inhibitors, especially nelfinavir (Xiong et al., 2003; Yamamoto et al., 2004), were found to have antiviral acitivity against SARS-CoV. It is also interesting to find that an organic nitric oxide donor, *S*-nitro-*N*-acetylpenicillamine, also appeared to have inhibitory activity against SARS-CoV (Akerstrom et al., 2005), which provides a rationale for the use of nitric oxide inhalation as a rescue therapy for SARS (Chen et al., 2004). Good *in vitro* antiviral activities have also been observed for ACE2 analogues (Towler et al., 2004), helicase inhibitor, and nucleoside analogues screened from combinatorial chemical libraries. Synergistic activities have been seen with combinations of interferons or the above compounds with ribavirin (Cinatl et al., 2003b; Chen et al., 2004) The SARS-CoV spike protein uses a mechanism similar to that of class 1 fusion proteins in mediating membrane fusion and hence cell entry (Bosch et al., 2004). Antiviral peptides designed to inhibit this claw mechanics of the spike protein might be useful *in vitro* (Kliger and Levanon, 2003; Liu et al., 2004). Peptides derived from the heptad repeat 2 region of the SARS-CoV S protein were able to block viral infections (Xu et al., 2004; Yuan et al., 2004; Zhu et al., 2004a). siRNA designed for sequence-specific degradation of viral RNA generated during transcription demonstrated activities in reducing

cytopathic effects, viral replication, and viral protein expression in cell lines. (He et al., 2003; Zhang et al., 2003; Lu et al., 2004; Wang et al., 2004b; Zhang et al., 2004a; Zheng et al., 2004c). Screening of combinatorial chemical libraries have identified inhibitors for protease, helicase, and spike-mediated cell entry (Kao et al., 2004).

Immunodulators have been empirically used in the treatment of SARS during the initial epidemic (Cheng et al., 2004b). These include corticosteroids, intravenous immunoglobulins, pentaglobin (Ho et al., 2004), thymosin, thalidomide, and anti-tumor necrosis factor. Corticosteroids have previously been used in the management of viral pneumonias due to varicella-zoster virus, influenza virus, and other viruses (Greaves et al., 1981; Ahmed et al., 2002). Mortality in the first two infections appeared to be lower with corticosteroids than supportive treatment alone. High-dose hydrocortisone can reduce the expression of the proinflammatory chemokines CXCL8 and CXCL 10 in coronavirus-infected CACO-2 cell lines (Cinatl et al., 2005). However, unless an effective antiviral agent is available, early use of high doses of corticosteroids for prolonged period could be detrimental and may increase the risks of nosocomial infections and avasuclar bone necrosis. One study has shown that the early use of corticosteroid actually increased the plasma viral load (Lee et al., 2004). Pegylated interferon α-2a was shown to be useful for prophylaxis and reducing 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 interferon-α) (Loutfy et al., 2003) or protease inhibitors and ribavirin improved outcomes in two different treatment trials using historical controls (Chan et al., 2003a; Chu et al., 2004a).

5.9.2. Passive and Active Immunization

Passive immunization using convalescent plasma with high titers of neutralizing antibody had been used for SARS patients who continued to deteriorate. No significant adverse reactions were noted, although this did not appear to be beneficial, either. Currently, only hyperimmune globulin produced from convalescent patients' plasma and the equine plasma immunized by inactivated SARS-CoV are available for prophylactic trials in human. A human monoclonal IgG1 produced from a single-chain variable region fragment against the S1 domain from two nonimmune human antibody libraries has also been produced (Sui et al., 2004). One of the single-chain variable region fragments 80R blocks the spike–ACE2 receptor interaction through binding to the S1 domain. In a murine model of asymptomatic SARS infection, passive immunization by high titers of

Table 5.3. Studies on active and passive immunization for SARS

Type of vaccine	Target	Animal model	Response	References
Protein fragment	S protein	Rabbits, BALB/c mice	Neutralizing antibodies	Zhang et al., 2004c
Inactivated whole virus	Inactivated SARS-CoV	BALB/c mice	Neutralizing antibodies	Tang et al., 2004
Inactivated whole virus	Inactivated SARS-CoV	BALB/c mice (intranasal)	Neutralizing antibodies; specific IgA in tracheal/lung wash fluid with adjuvant-added or PEG-precipitated vaccine only	Qu et al., 2005
Inactivated whole virus	Inactivated SARS-CoV	BALB/c mice, rabbits	Specific antibodies recognizing RBD of S1; blocked binding of RBD to ACE2; significant decrease in S protein–mediated cell entry in pseudo-typed virus assay	He et al., 2004
Adenoviral vector	S, M, and N proteins	Rhesus macaques	Neutralizing antibodies, T-cell responses	Gao et al., 2003
Modified vaccinia Ankara	S protein	BALB/c mice	Neutralizing antibodies; decrease in lung/nasal viral titers, passive serum transfer protective	Bisht et al., 2004
Modified vaccinia Ankara	S protein	BALB/c mice, rabbits, Chinese rhesus monkeys	Neutralizing antibodies; decrease in lung/nasal viral titers	Chen et al., 2005
Modified vaccinia Ankara	S protein	Ferrets	Neutralizing antibodies; enhanced hepatitis in vaccinated ferrets when challenged with SARS-CoV	Weingartl et al., 2004b
Recombinant attenuated HPIF3	S protein	African green monkey	Neutralizing antibodies, decrease in viral shedding	Bukreyev et al., 2004
DNA vaccine	S protein	BALB/c mice	Neutralizing antibodies, T-cell responses; decrease in lung/nasal viral titers; serum transfer protective	Yang et al., 2004

(*Continued*)

Table 5.3. Studies on active and passive immunization for SARS—Cont'd

Type of vaccine	Target	Animal model	Response	References
DNA vaccine	S protein	C57BL/6 mice	T-cell responses; decrease in surrogate viral titer in lungs	Kim et al., 2004
DNA vaccine	S protein	BALB/c mice	Neutralizing antibodies, T-cell responses	Zeng et al., 2004
DNA vaccine	N protein	C3H/He mice	Specific antibodies, T-cell responses	Zhu et al., 2004
DNA vaccine	S protein	Rabbits	Neutralizing antibodies	Wang et al., 2005
DNA vaccine	N protein	Mice	Specific antibodies, N-specific splenocytes proliferation responses, DTH and CD8+ CTL responses	Zhao et al., 2005
Human monoclonal antibody	S protein	Ferret	Decrease in lung viral titer, decrease in viral shedding, prevention of viral-induced tissue pathology	ter Meulen et al., 2004
Human monoclonal antibody	S protein	BALB/c mice	Decrease in lung/nasal viral titers	Traggiai et al., 2004
Human monoclonal antibody from transgenic (HuMantibody) mice	S protein	BALB/c mice	Decrease in lung/nasal viral titers	Greenough et al., 2005
Human monoclonal antibody from phage display library	S, N proteins		Decrease in binding of recombinant S fragment and neutralized SARS-CoV in Vero cell assay	van den Brink et al., 2005

ACE, angiotensin-converting enzyme; CTL, cytotoxic T cell; DTH, delayed-type hypersensitivity; M, SARS-CoV membrane protein; N, SARS-CoV nucleoprotein; RBD, receptor-binding domain; S, SARS-CoV spike protein.

neutralizing antibody prevented virus replication in the lungs but not as effectively in the nasal turbinates (Subbarao et al., 2004). Similarly, passive immunization of mice and ferrets with a human IgG1 monoclonal antibody CR3014 was effective in preventing the development of lung pathology but less effective in reducing pharyngeal excretion (ter Meulen et al., 2004). Currently, there are no randomized placebo control trials on the role of antibody therapy for pre- or postexposure prophylaxis in at-risk groups during the SARS epidemic. Retrospective analysis of outcome in a human study using SARS convalescent plasma suggested that passive immunization had no major side effects and perhaps some clinical benefits (Soo et al., 2004).

It appears that the antibody response against the viral spike antigen is the key factor for protection against infection. Similar findings were found in the murine model using either intramuscular or intranasal administration of the highly attenuated modified vaccinia virus Ankara carrying the spike protein (Bisht et al., 2004). Mucosal immunization of African green monkeys with the recombinant attenuated parainfluenza virus–SARS-CoV spike protein chimeric virus resulted in a good neutralizing antibody response and protection from viral replication in the upper and lower respiratory tracts after live SARS-CoV challenge (Bukreyev et al., 2004). Other approaches to active immunization involved the use of an adenoviral vector (carrying S, M, and N proteins) in rhesus macaques (Gao et al., 2003), inactivated whole virus vaccine in mice (Tang et al., 2004), S protein fragments in rabbits and mice (Zhang et al., 2004c), DNA vaccination with the nucleocapsid gene in mice (Zhu et al., 2004b); adenoviral vector carrying the S1 domain, membrane and nucleoprotein or a DNA vaccine linking nucleocapsid protein to calreticulin (Kim et al., 2004). A plasmid DNA vaccine carrying the spike protein encoded by humanized codons was highly protective in a mouse model (Zeng et al., 2004). Furthermore, 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 cells alone did not inhibit pulmonary viral replication in recipient mice, whereas passive transfer of purified IgG from immunized mice achieved similar protection. In summary (Table 5.3), all vaccines based on the spike protein appeared to be capable of inducing neutralizing antibody responses, and those carrying nucleoprotein induced nucleoprotein-specific cell-mediated immunity.

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 is unknown. Nevertheless, neutralizing antibody against spike appears to be

crucial for prophylactic immunity. Live-attenuated virus is unlikely to be useful owing to the concern for reversion to virulence or recombination with wild strains to form new wild types. An inactivated SARS-CoV is the most likely candidate for clinical trials. Recombinant proteins expressed in mammalian cells can also be used to induce good neutralizing antibody response. Irrespective of the approach to immunization, one should bear in mind the phenomenon of immune enhancement of disease has occurred in feline peritonitis coronavirus infection. SARS-CoV vaccine may indeed lead to increased derangement in the liver function of challenged ferrets that had previously been vaccinated (Weingartl et al., 2004b). Atypical or a more severe form of disease had occurred after the use of inactivated measles and respiratory syncytial virus vaccination. The economic viability of SARS vaccine depends very much on the risk of having another SARS epidemic and future major antigenic variations that might appear.

5.10. Laboratory Safety, Community and Hospital Infection Control

Infection control against SARS is of paramount importance within health care facilities given the relative stability of SARS-CoV in the environment, absence of protective immunity in the general population, and the lack of effective antivirals or vaccines. Triage of suspected cases, early case detection, and prompt isolation of suspected cases are the principal measures against nosocomial transmission (Ho et al., 2003). Respiratory droplet and contact precautions are effective under most circumstances (Seto et al., 2003). Airborne precautions must be considered in situations where droplet nuclei are likely to be generated. Strict hand hygiene, preferably with a patrol nurse overseeing all gowning and degowning procedures, in a SARS ward are critically important. Contact tracing, quarantine of contacts to prevent community spread, temperature checks at borders, health declarations for travelers, public education and effective risk communication with public media were all used in the 2003 pandemic for effective control. All SARS laboratories must strictly comply with the World Health Organization standards, and live viruses should only be cultivated and handled in accredited biosafety level 3 laboratories. Daily checking of temperature and reporting of sickness should be part of the monitoring protocol for laboratory workers handling live viruses.

Although screenings at international borders and airports were widely practiced during the epidemic, the value of such screening has recently been questioned (St John et al., 2005). Nevertheless, travelers during the epidemic may be less vigilant on preventive measures against

the disease, and they remain an important target in the prevention of cross-border spread of the infection (Lau et al., 2004b).

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