Severe Acute Respiratory Syndrome Coronavirus as an Agent of Emerging and Reemerging Infection

Vincent C. C. Cheng, Susanna K. P. Lau, Patrick C. Y. Woo, and Kwok Yung Yuen*

State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong Special Administrative Region, China

INTRODUCTION

Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) is a novel virus that caused the first major pandemic of the new millennium (89, 180, 259). The rapid economic growth in southern China has led to an increasing demand for animal proteins including those from exotic game food animals such as civets. Large numbers and varieties of these wild game mammals in overcrowded cages and the lack of biosecurity measures in wet markets allowed the jumping of this novel virus from animals to human (353, 376). Its capacity for human-to-human transmission, the lack of awareness in hospital infection control, and international air travel facilitated the rapid global dissemination of this agent. Over 8,000 people were affected, with a crude fatality rate of 10%. The acute and dramatic impact on health care systems, economies, and societies of affected countries within just a few months of

early 2003 was unparalleled since the last plague. The small reemergence of SARS in late 2003 after the resumption of the wildlife market in southern China and the recent discovery of a very similar virus in horseshoe bats, bat SARS-CoV, suggested that SARS can return if conditions are fit for the introduction, mutation, amplification, and transmission of this dangerous virus (45, 190, 215, 347). Here, we review the biology of the virus in relation to the epidemiology, clinical presentation, pathogenesis, laboratory diagnosis, animal models or hosts, and options for treatment, immunization, and infection control.

TAXONOMY AND VIROLOGY OF SARS-CoV

SARS-CoV is one of 36 coronaviruses in the family *Coronaviridae* within the order *Nidovirales*. Members of the *Coronaviridae* are known to cause respiratory or intestinal infections in humans and other animals (Fig. 1). Despite a marked degree of phylogenetic divergence from other known coronaviruses, SARS-CoV together with bat SARS-CoV are now considered group 2b coronaviruses (190, 282). Primary isolation of SARS-CoV was achieved by inoculation of pa-

* Corresponding author. Mailing address: State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong Special Administrative Region, China. Phone: (852) 2855 4892. Fax: (852) 2855 1241. E-mail: hkumicro@hkucc.hku.hk.
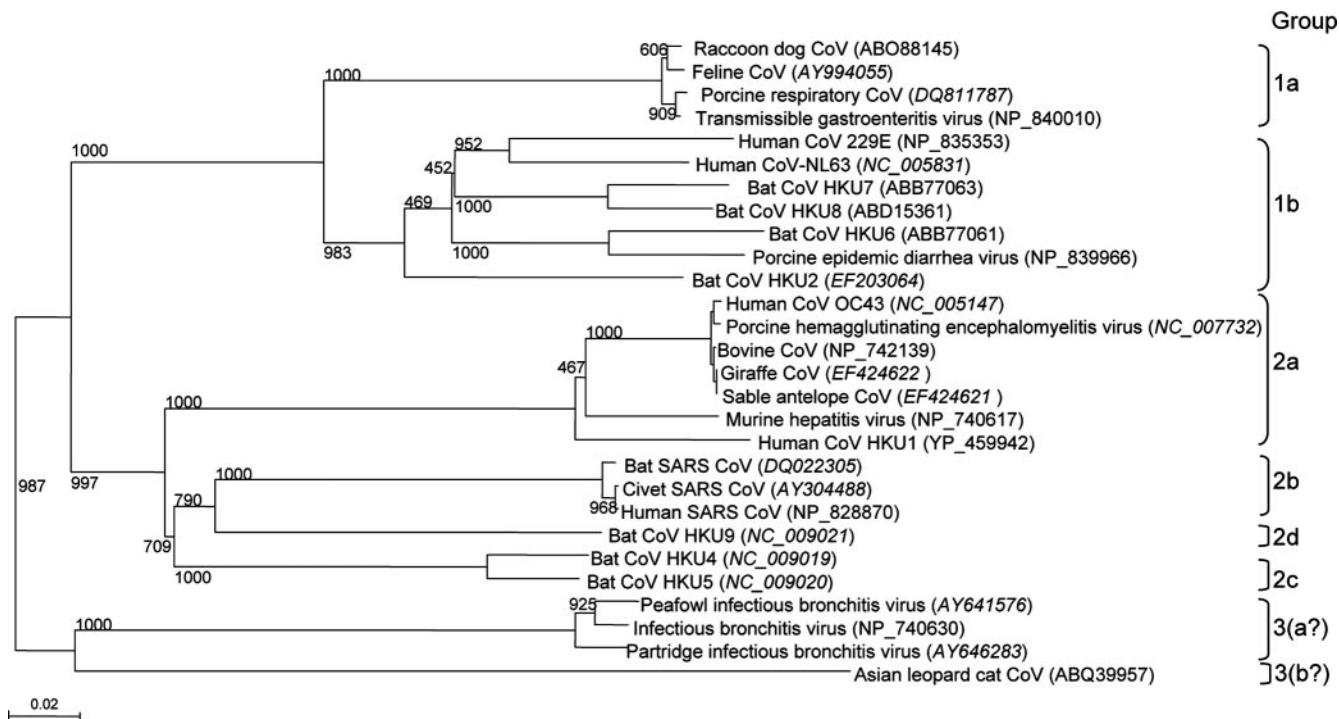
FIG. 1. Phylogenetic tree of 28 coronaviruses with complete protein sequences of helicase. Their accession numbers are shown in parentheses. Italic type indicates the complete genome accession numbers since helicase protein sequence accession numbers of these coronaviruses are not available. The helicase of another eight coronaviruses of spotted hyena, cheetah, ferret, puffinosis, rat, pigeon, goose, and duck are not included because no complete protein sequence is available. The classification of Asian leopard cat coronavirus is undefined. The tree was constructed by the neighbor-joining method using clustalX 1.83. The scale bar indicates the estimated number of substitutions per 50 nucleotides. (Data are from references 265, 326, 339, 367, 368, and 375.)

tients' specimens into embryonal monkey kidney cell lines such as FRhK-4 or Vero E6 cell lines, which produced cytopathic changes at foci, where cells become round and refractile within 5 to 14 days (259). These initial cytopathic changes spread throughout the cell monolayers, leading to cell detachment within 24 to 48 h. Subcultures can be made on Vero (monkey kidney), Huh-7 (liver cancer) (301), CACO-2 (colonic carcinoma) (79) or other colorectal cancer, MvLu (mink lung epithelial) (104), and POEK and PS (pig) cell lines (122). Transmission electron microscopy of infected cell lines showed characteristic coronavirus particles within dilated cisternae of rough endoplasmic reticulum and double-membrane vesicles. Clusters of extracellular viral particles adhering to the surface of the plasma membrane were also seen. Negatively stained electron microscopy showed viral particles of 80 to 140 nm with characteristic surface projections of surface proteins from the lipid envelope (89, 180, 259). SARS-CoV has a higher degree

of stability in the environment than other known human coronaviruses (91, 276). It can survive for at least 2 to 3 days on dry surfaces at room temperature and 2 to 4 days in stool (276). The electron microscopic appearance and genome order of 5′-replicase (Orf1ab)-structural proteins (spike [S]-envelope [E]-membrane [M]-nucleocapsid [N])-poly(T)-3′ are similar to those of other members of the *Coronaviridae* (236). Similar to other coronaviruses, it is an enveloped positive-sense single-stranded RNA virus with a genome size of almost 30 kb (Fig. 2). The genome is predicted to have 14 functional open reading frames (ORFs) (290). Their functions and putative roles are outlined in Table 1. Two large 5′-terminal ORFs, ORFs 1a and 1b, encode 16 nonstructural proteins, 7 of which are likely to be involved in the transcription and replication of the largest genome among all RNA viruses (92, 95, 158, 166, 242, 284, 309, 316, 343, 414). The two proteases are involved in posttranslational proteolytic processing of the viral polyprotein (5, 15,
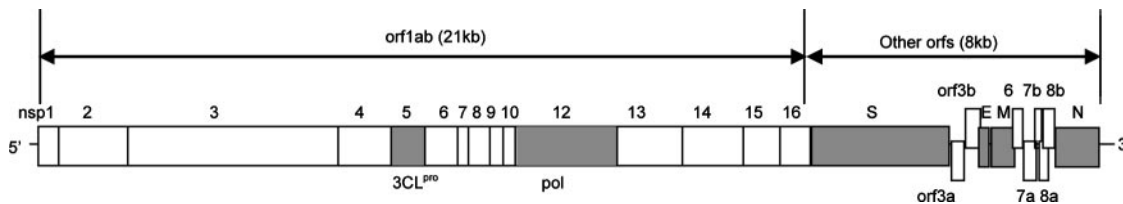

FIG. 2. Genome arrangement of SARS-CoV. Gray boxes indicate 3CL protease (3CL^{pro}), polymerase (pol), spike (S), envelope (E), membrane (M), and nucleocapsid (N) genes.

TABLE 1. Nomenclature and functional characteristics of SARS-CoV gene products and their interactions with host cells
in disease pathogenesis

Gene nomenclature (no. of amino acid residues in product)	Gene product and/or characteristic(s) (reference[s])	Effect on cellular response of host (reference[s])
Orf1a/b		
nsp1 (180)	Expression promoted degradation of host endogenous mRNAs, which may inhibit host protein synthesis and prevented endogenous IFN-β mRNA accumulation (167)	Induce CCL5, CXCL10 (IP10), and CCL3 expression in human lung epithelial cells via activation of NF-κB; increases cellular RNA degradation, which might facilitate SARS-CoV replication or block immune responses (81, 192)
nsp2 (638)	Deletion attenuates viral growth and RNA synthesis (106)	
nsp3 (1,922)	Papain-like protease 2; proteolytic processing of the viral polyprotein at 3 sites and participation in synthesis of subgenomic RNA segment (15, 121, 224) ADP-ribose 1-phosphatase; dephosphorylates Appr-1″-p, a side product of cellular tRNA splicing, to ADP-ribose (271)	Putative catalytic triad (Cys1651-His1812-Asp1826) and zinc-binding site have deubiquitinating activity; this unexpected activity in addition to its papain-like protease suggests a novel viral strategy to modulate the host cell ubiquitination machinery to its advantage (15, 224, 279)
nsp4 (500)	Not known	
nsp5 (306)	3C-like protease; proteolytic processing of the replicative polyprotein at 11 specific sites and forming key functional enzymes such as replicase and helicase (5, 394)	Growth arrest and apoptosis via caspase-3 and caspase-9 activities demonstrated in SARS-CoV 3CLpro-expressing human promonocyte cells with increased activation of the nuclear factor-κB-dependent reporter (222)
nsp6 (290)	Not known	
nsp7 (83)	Three-dimensional structure by nuclear magnetic resonance study found potential sites for protein-protein interactions (261)	
nsp8 (198)	Putative RNA-dependent RNA polymerase; crystal structure of the hexadecameric nsp7-nsp8 possesses a central channel with dimensions and positive electrostatic properties favorable for nucleic acid binding; it is probably another unique RNA-dependent RNA polymerase for its large genome (158, 414)	
nsp9 (113)	Three-dimensional crystal structure of a dimer which binds viral RNA and interacts with nsp8 (92, 316)	
nsp10 (139)	Crystal structure suggests a nucleic acid binding function within a larger RNA binding protein complex for viral gene transcription and replication (166, 309)	Interacts specifically with the NADH 4L subunit and cytochrome oxidase II with depolarization of inner mitochondrial membrane of transfected human embryo lung fibroblast and extensive cytopathic effect (210)
nsp11 (13)	Not known	
nsp12 (932)	RNA-dependent RNA polymerase; replication and transcription to produce genome- and subgenome-sized RNAs of both polarities (158)	
nsp13 (601)	Helicase (dNTPase and RNA 5′-triphosphatase activities) (95)	
nsp14 (527)	3′→5′-exoribonuclease; this unusual 3′→5′-exoribonuclease activity supplements the endoribonuclease activity in the replication of the giant RNA genome (242)	
nsp15 (346)	Uridylate-specific endoribonuclease; RNA endonuclease that is critically involved in the coronavirus replication cycle (284)	
nsp16 (298)	Putative 2′-O-ribose methyltransferase (343)	
Orf2 (1,255)	Spike protein; binds to the host cell receptor ACE2 and other coreceptors, mediates viral entry into host cells as a type 1 viral fusion protein; required acidification of endosomes for efficient S-mediated viral entry; proteolytic cleavage by abundantly expressed infected cell membrane-associated factor Xa into S1 and S2; protease activation required for cell-cell fusion (159, 162, 206, 214, 227, 301, 334)	293 T cells transfected with ACE2 can form multinucleated syncytia with cells expressing the spike; intraperitoneal injections of spike protein into mice reduced ACE2 expression in lungs and worsened acute lung failure in vivo that can be attenuated by blocking the renin-angiotensin pathway (181); recombinant baculovirus expressing different deletion and insertion fragments identified the functional region of S protein from amino acids 324–688, which can induce the release of IL-8 in lung cells (43); induces unfolded protein response in cultured cells as SARS-CoV with a substantial amt of S protein accumulation in the endoplasmic reticulum, which may modulate viral replication (30)
Orf3a (274)	Forms potassium-sensitive ion channel, may promote virus budding and release (234)	Overexpression in cell line may trigger apoptosis; its expression in A549 lung epithelial cells up-regulates mRNA and intracellular and secreted levels of all three subunits, alpha, beta, and gamma, of fibrinogen, which is also observed in SARS-CoV-infected Vero E6 cells; it is highly immunogenic and induces neutralizing antibodies (193, 321); 3a/X1 and 7a/X4 were capable of activating NF-κB and c-Jun N-terminal kinase and significantly enhanced IL-8 promoter activity in A549 cells; enhanced production of inflammatory chemokines that were known to be up-regulated in SARS-CoV infection (169)

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TABLE 1—*Continued*

Gene nomenclature (no. of amino acid residues in product)	Gene product and/or characteristic(s) (reference[s])	Effect on cellular response of host (reference[s])
Orf3b (154)	Predominately localized to the nucleolus in different transfected cells (409)	Vero E6 but not 293T cells transfected with a construct for expressing Orf3b underwent necrosis as early as 6 h after transfection but underwent simultaneous necrosis and apoptosis at later time points; Orf3b inhibits expression of IFN-β at synthesis and signaling (175, 178)
Orf4 (76)	Envelope protein; synthetic peptides form ion channels in planar lipid bilayers, which are more permeable to monovalent cations than to monovalent anions; putatively involved in viral budding and release (359)	Induced apoptosis in transfected Jurkat T cells especially in the absence of growth factors; a novel BH3-like region was located in the C-terminal cytosolic domain of SARS-CoV E protein can bind to Bcl-xL, whose overexpression can antagonize apoptosis; this may explain the consistent lymphopenia found in SARS patients (397)
Orf5 (221)	Membrane protein; surface protein responsible for viral assembly and budding	M protein induced apoptosis in HEK293T cells, which could be suppressed by caspase inhibitors (29)
Orf6 (63)	Novel membrane protein that accelerates replication and virulence of a recombinant mouse coronavirus expressing Orf6; an important virulence factor in vivo demonstrated in a mouse model (327)	Inhibits both IFN synthesis and signaling; inhibited nuclear translocation but not phosphorylation of STAT1 (178); Orf6 is localized to the endoplasmic reticulum/Golgi membrane of infected cells; it binds and disrupts nuclear import complex formation by tethering karyopherin alpha 2 and karyopherin beta 1 to the membrane; this retention of the complex at the endoplasmic reticulum/Golgi membrane leads to a loss of STAT1 transport into the nucleus despite viral RNA-induced IFN signaling; thus, it blocks the expression of STAT1-activated genes, which are essential for establishing an antiviral state (100)
Orf7a (122)	Unique type I transmembrane protein; involved in viral assembly by interacting with M and E, which are essential for virus-like particle formation when coexpressed with S and N (97, 150, 245)	Expression of *Orf7a* induces apoptosis via a caspase-3-dependent pathway and in cell lines derived from different organs including lung, kidney, and liver (179, 320, 408)
Orf7b (44)	Not known	
Orf8a (39)	Not known	Orf8a was localized in mitochondria, and overexpression resulted in increases in mitochondrial transmembrane potential, reactive oxygen species production, caspase-3 activity, and cellular apoptosis; Orf8a enhances viral replication and induces apoptosis through a mitochondrion-dependent pathway (49)
Orf8b (84)	May modulate viral replication; expression of E was down-regulated by Orf8b but not Orf8a or Orf8ab (172)	
Orf9 (422)	Nucleocapsid protein; binding and packaging of viral RNA in assembly of the virion (147)	N antagonized IFN by inhibiting synthesis of IFN-β (130); NF-κB activation in Vero E6 cells expressing the N protein is dose dependent (220); N may cause inflammation of the lungs by activating COX-2 gene expression by binding directly to the promoter, resulting in inflammation through multiple COX-2 signaling cascades (393); induced apoptosis of COS-1 monkey kidney but not 293T cells in the absence of growth factors; induced actin reorganization in cells devoid of growth factors (315)
Orf9b (98)	Crystal structure of *Orf9b*, an alternative ORF within the N gene, may be involved in membrane attachment and associates with intracellular vesicles, consistent with a role in assembly of the virion (241)	

121, 224, 394). The surface S protein is involved in the attachment and entry of the host cell and is therefore the main target for neutralizing antibody and antiviral peptides (159, 206, 227, 301, 334). N together with M, E, and Orf7a are involved in the assembly of the virion (97, 147, 150, 245, 359). Orf3a is an ion channel protein that is likely to be involved in viral budding and release (234). Analysis of genome sequences of many isolates of SARS-CoV from humans with civet SARS-CoV and bat SARS-CoV showed that the most variable genes with nucleotide homologies of less than 90% are the S gene, *Orf3*, *Orf8*, *nsp2*, *nsp3*, and *nsp4* (190, 215, 282). Deletions of 82 and 415 nucleotides in *Orf8* were found in some human isolates, whereas a unique 29-nucleotide signature insertion in *Orf8* can be found in animal isolates (64, 117). Therefore, the more

conserved *Orf1b* is generally chosen to be the molecular target for the design of clinical diagnostic tests rather than these less conserved regions.

VIRAL LIFE CYCLE

Trimers of the S protein form the peplomers that radiate from the lipid envelope and give the virus a characteristic corona solis-like appearance under an electron microscope. S is a class I fusion protein that consists of the amino-terminal S1 and carboxyl-terminal S2 subunits connected by a fusion peptide. The two subunits are indispensable for receptor binding and membrane fusion, respectively. The receptor binding domain of S1 has been mapped to residues 318 to 510 (9, 365). The binding of S1 to the cellular receptor will trigger conformational changes, which collocate the fusion peptide upstream of the two heptad repeats of S2 to the transmembrane domain, and, finally, fusion of the viral and cellular lipid envelopes. Moreover, this process could be facilitated by the infected cell membrane-associated protease, such as factor Xa, which can cleave S into S1 and S2. This proteolytic cleavage is specifically inhibited by a protease inhibitor, Ben-HCl (90).

The key receptor of the host cell attached by S is angiotensin-converting enzyme 2 (ACE2), which is a metalloprotease expressed in the cells of the lung, intestine, liver, heart, vascular endothelium, testis, and kidney (119). Since ACE2 was shown to protect against acute lung injury in a mouse model and since the binding of the S protein to host cells results in the downregulation of ACE2, this mechanism may contribute to the severity of lung damage in SARS (181). Cells expressing some lectins, including DC-SIGN, L-SIGN, and LSECtin, have been shown to augment the cellular entry of pseudotype virus expressing S but only in the concomitant presence of ACE2 (40, 107, 162, 398). Nonsusceptible cells expressing these lectins in the absence of ACE2, such as dendritic cells, were able to promote the cell-mediated transfer of SARS-CoV to susceptible cells (40). Although lysosomotropic agents can block viral entry, which indicates that endosomal acidification is required for entry, the activation of the S protein by protease can bypass this inhibition and result in cell-to-cell fusion. Despite the role of the pH-sensitive endosomal protease cathepsin L in the entry pathway (151, 300), viral culture does not require pretreatment with trypsin. However, this pH-sensitive cathepsin L may be a target for agents such as chloroquine, which elevates endosomal pH (174, 341).

The process of viral disassembly in the cytoplasm for the release of viral RNA for translation and replication remains elusive. Translation starts with two large polyproteins from *Orf1a* and *Orf1ab*, which are posttranslationally cleaved by the two viral proteases into nsp1 to nsp16. These cleavage products form the replication-transcription complex, which replicates the viral genome and transcribes a 3′-coterminal nested set of eight subgenomic RNAs. It is therefore conceivable that infected cells contain a higher number of transcripts containing genes towards the 3′ terminus of the viral genome. On this basis, reverse transcriptase PCR (RT-PCR) using the N gene may have a better sensitivity than those using the other genes.

As in other coronaviruses, SARS-CoV may attach by the hydrophobic domains of their replication machinery to the limiting membrane of autophagosomes and form double-mem-

brane vesicles. Once sufficient viral genomic RNA and structural proteins are accumulated, viral assembly by budding of the helical nucleocapsid at the endoplasmic reticulum to the Golgi intermediate compartment occurs. Here, the triple-membrane-spanning M protein interacts with the N protein and viral RNA to generate the basic structure. It also interacts with the E and S proteins to induce viral budding and release. Unlike other coronaviruses, the M protein of SARS-CoV also incorporates another triple-membrane-spanning protein of *Orf3a* into the virion (161). The N protein is the most abundantly expressed viral protein in infected cells in which the mRNA levels were amplified 3 to 10 times higher at 12 h postinfection than other structural genes (138) and is therefore an important target for immunohistochemistry and antigen detection in clinical specimens. Various diagnostic tests, antiviral agents, and vaccines are designed on the basis of our understanding of the structure and function of the various viral proteins involved in the life cycle of this virus.

SEQUENCE OF THE SARS EPIDEMIC AND MOLECULAR EVOLUTION OF THE VIRUS

Sequence of Events

SARS was the first known major pandemic caused by a coronavirus. During the epidemic in 2003, 8,096 cases with 774 deaths had occurred in over 30 countries among five continents (89, 117, 144, 180, 182, 197, 236, 250, 259, 260, 270, 290, 292, 303, 336, 377). The disease emerged in late 2002, when an outbreak of acute community-acquired atypical pneumonia syndrome was first noticed in the Guangdong Province (Table 2). Retrospective surveillance revealed severe cases of the disease in five cities around Guangzhou over a period of 2 months (431). The index case was reported in Foshan, a city 24 km away from Guangzhou. The second case involved a chef from Heyuan who worked in a restaurant in Shenzhen. The patient had regular contact with wild game food animals. His wife, two sisters, and seven hospital staff members who had contact with him were also affected. From 16 November 2002 to 9 February 2003, a total of 305 cases were reported in mainland China, with 105 of those cases involving health care workers. The devastating pandemic started in Hong Kong, Special Administrative Region (HKSAR), when a professor of nephrology from a teaching hospital in Guangzhou who had acquired the disease from his patients came to HKSAR on 21 February 2003. Within a day, he transmitted the infection to 16 other people in the hotel where he resided. His brother-in-law, one of the secondary cases, underwent an open lung biopsy from which the etiological agent was discovered and first isolated (259). It was a novel coronavirus, named SARS-CoV.

The secondary cases unknowingly carried the disease to 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. Carlo Urbani, a physician working at the World Health Organization (WHO) office in Hanoi, Vietnam, was the first to notify the WHO of cases outside Guangdong after witnessing an explosive nosocomial outbreak of SARS in a hospital in Hanoi, which resulted from a person who had returned from the hotel in HKSAR. Carlo Urbani's description of the disease,

TABLE 2. Sequence of events and molecular evolution of SARS-CoV throughout the epidemic[a]

Phase and date	Important event, phase of evolution, and genotypic marker(s)[b]
Early	Most isolates had SNV genotypic marker of the GZ02 reference nucleotide at positions 17564, 21721, 22222, 23823, and 27827 of G:A:C:G:C; some initial cases had the 29-bp insertion or 82-bp deletion at *Orf8*; avg K_a/K_s ratio of >1, which was higher than that of the middle phase, which indicates strong positive selection
16 November 2002	First case that fulfilled the WHO definition of SARS at Foshan, Guangdong Province, China
17 December 2002	Chef from Heyuan who worked at a restaurant in Shenzhen had atypical pneumonia
26 December 2002 to 20 January 2003	Outbreak of similar cases at Zhongshan
Middle	SNV genotypic marker of G:A:C:T:C; avg K_a/K_s ratio was higher than that of the late phase but was <1, which indicates purifying selection
12 January 2003	Outbreak in Guangzhou resulted in complicated SARS cases transferred to the major hospitals in Guangzhou
31 January 2003	Outbreak in Guanzhou hospitals involving patients and health care workers
Late	SNV marker of T:G:T:T:T; avg K_a/K_s ratio shows stabilization of nonsynonymous mutation rate; some isolates had 415-bp deletion at *Orf8*
21 February 2003	65-yr-old doctor from Guangdong Province resided at "hotel M" in Hong Kong (index patient); unwell since 15 February and admitted to the hospital on 22 February; infected 17 residents at hotel M, some of whom traveled to Vietnam, Singapore, and Toronto, where they started new local clusters of cases
26 February 2003	Hotel M contact was admitted to a hospital in Hanoi and started a nosocomial outbreak
4 March 2003	Another hotel M contact was admitted to Prince of Wales Hospital in Hong Kong and started a nosocomial outbreak
5 March 2003	Another hotel M contact died in Toronto; five family members were affected
12 March 2003	WHO issued a global alert
14 March 2003	Clusters of atypical pneumonia were reported in Singapore and Toronto, which were epidemiologically linked to hotel M outbreak
15 March 2003	WHO named this new disease SARS after receiving reports of more than 150 cases; WHO issued emergency travel advice in response to SARS
21 March 2003	A novel coronavirus was identified in two patients with SARS in Hong Kong; the agent, isolated in rhesus monkey kidney cells (fRhk4), produced a cytopathic effect; in an immunofluorescence antibody assay, sera from SARS patients had rising antibody titers against the virus-infected cells
22 to 27 March 2003	Isolation of a novel coronavirus was confirmed in laboratories of the United States and Germany
12 April 2003	Sequencing of the full genome of SARS-CoV was completed
16 April 2003	WHO announces that SARS-CoV is the causative agent of SARS
June 2003	A virus with 99.8% nucleotide identity with SARS-CoV was isolated from palm civets and other game food mammals
5 July 2003	Absence of further transmission in Taiwan signaled the end of human-to-human transmission
Aftermath	
3 September 2003	Laboratory-acquired SARS-CoV infection was reported in Singapore
16 December 2003 to 8 January 2004	4 symptomatic cases and 1 asymptomatic case of SARS due to animal-to-human transmission occurred in the city of Guangzhou, Guangdong Province, China; all isolates had a 29-bp signature sequence insertion for animal SARS-CoV in *Orf8*
17 December 2003	Second laboratory-acquired SARS-CoV infection reported in Taiwan
25 March and 17 April 2004	Third and fourth laboratory-acquired SARS-CoV infection reported in Beijing, China
16 September 2005	Finding of SARS-CoV-like virus in horseshoe bats; all isolates sequenced had a 29-bp signature sequence for bat SARS-CoV

[a] See references 27, 89, 117, 182, 190, 197, 215, 218, 221, 236, 251, 252, 259, 277, 304, 377, 378, 422, and 431.
[b] K_a/K_s ratio refers to the ratio of nonsynonymous nucleotide substitutions to synonymous nucleotide substitutions during the molecular evolution of SARS-CoV.

to which he later succumbed, alerted health authorities throughout the world and accelerated collaborative research to identify the virus and combat the disease (281).

Molecular Evolution

Soon after the isolation of SARS-CoV, SARS-CoV-like viruses were found in palm civets and a raccoon dog from wild-animal markets in the Guangdong Province of China (117), suggesting that these animals could be the source of human infections. As a result, massive numbers of palm civets were culled to remove sources for the reemergence of SARS in

Guangdong in January 2004. The virus was found in many civets and raccoon dogs from the wildlife market prior to culling but not in over 1,000 civets later sampled at 25 farms in 12 provinces (168). The evolutionary starting point was a prototype group consisting of three viral genome sequences of animal origin. This prototype group representing low-pathogenicity virus has seven single-nucleotide variation (SNV) sites that caused six amino acid changes, at positions 147, 228, 240, 479, 821, and 1080 of the S protein, which were involved in generating the early phase of the 2002 and 2003 epidemic. One of these was found in the first SARS patient in the subsequent epidemic of 2003 to 2004. A further 14 SNVs caused 11 amino

acid residue changes, at positions 360, 462, 472, 480, 487, 609, 613, 665, 743, 765, and 1163. This resulting high-pathogenicity virus group caused the middle phase of the epidemic of 2003. Finally, the remaining six SNVs caused four amino acid changes, at positions 227, 244, 344, and 778, which resulted in the group of viruses responsible for the late phase and the global epidemic (168). The neutral mutation rate of this virus during the epidemic in 2003 is almost constant, at around 8×10^{-6} nt^{-1} day^{-1}, which is similar to those of most known RNA viruses (64, 304). The most recent common ancestor was estimated to be present around mid-November, which is epidemiologically compatible with the first case of SARS found in Foshan.

After the epidemic was over, a second interspecies-jumping event occurred in late 2003 to early 2004, resulting in the reemergence of four human cases in China (45, 347). These four cases were believed to be due to an independent interspecies transmission event, instead of residual cases of the major epidemic, because of the much lower affinity for human ACE2 (hACE2) of the S proteins of SARS-CoV isolated from these patients and palm civets than that of the major 2003 epidemic isolates from SARS patients, which utilized both human and palm civet ACE2 efficiently (216). Since S contains the receptor binding domain for the host receptor and is immunogenic, it is under selection in the host and becomes the most rapidly evolving protein, with most mutations located in the S1 domain and especially the receptor binding domain. Bioinformatic analysis has identified three key amino acid residues at positions 360, 479, and 487 that are responsible for host-specific binding (17). Most human isolates in the 2003 epidemic have N479 and T487 in their S, whereas most civet isolates have K/R479 and S487. The low affinity of the S proteins bearing K479 and S487 combinations for hACE2 was confirmed by pseudotype binding assays. However, the human and civet isolates of the outbreak of 2003 to 2004 had N479 and S487, which suggested that this is an intermediate stage of mutation of the S protein. Further change to the N479 and T487 combination will allow efficient human-to-human transmission (275). Apart from the subsequent minor outbreak, three laboratory-associated outbreaks were reported in Singapore, Taiwan, and Beijing from September 2003 to May 2004 (221, 251, 252, 256). In Beijing, the outbreak also involved secondary and tertiary cases.

Phylogenetic analysis of the S protein of 139 SARS-CoV isolates in the Hong Kong outbreak showed that several introductions of viruses had occurred but that only one of them was associated with the major outbreak in HKSAR and the rest of the world (116). 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 HKSAR outbreak was a Guangzhou medical doctor who had traveled to HKSAR. Another molecular epidemiological study of the Guangdong outbreak suggested that the disease spread from Guangdong to HKSAR and the rest of the world, and the index case was a chef who handled game animals (431). Subsequent animal surveillance in China recovered coronavirus isolates that had 99.8% nucleotide identity with SARS-CoV (117). A characteristic 29-bp insertion between *Orf8a* and *Orf8b* (also initially known as *Orf10* and *Orf11*) was found in these animal isolates (117, 302). This 29-nucleotide segment was deleted either before or soon after crossing the species barrier to humans. The biological effect of this deletion remains elusive. A number of SARS-CoV isolates in the later stages of the epidemic showed larger deletions around this site (64). Two independent molecular epidemiological studies comparing the complete genomes of 12 and 63 virus isolates also found evidence of strong positive selection at the beginning of the epidemic, which was followed by a purifying selection, as indicated by the amino acid substitution rate at S, Orf3a, and nsp3 (64, 304, 402). Both studies suggested that molecular adaptation of the virus had occurred after interspecies transmission from animals to humans. In the small outbreak in Guangzhou in 2004, all four human isolates belonged to a separate sublineage of the concurrent animal isolates that were distinct from the human pandemic or animal viruses in 2003. Although SARS-CoV is 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 S protein are spatially conserved compared with the group II consensus sequence, whereas only five cysteine residues are conserved compared with those of groups I and III (93, 302). Since coronaviruses are believed to have coevolved with their animal hosts, it is possible that rats, mice, and cattle harboring group II coronaviruses are more likely to be the animal host for SARS-CoV than cats, which 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 inference approach with sliding-window analysis, there was statistical incongruence, which indicates multiple host species shifts between the coronaviruses of many animals that are phylogenetically distant (283). Thus, it would not be too unexpected if other mammals are the true animal reservoir rather than mice and rats. Nevertheless, civets and other related mammals had at least served as a major amplification host in the markets of southern China irrespective of the original animal reservoir. The control of these animals and the markets played a pivotal role in the epidemiological control of SARS (304). In view of the low rate of detection of SARS-CoV in wild and farm civets (338), in contrast to a very high rate in caged civets in wildlife markets, efforts were made to find the natural reservoir of SARS-CoV in birds, pig, cattle, sheep, mice, and rats, which all turned out to be negative. However, SARS-CoV-like viruses with around 90% genomic identity with SARS-CoV were independently discovered in horseshoe bats (*Rhinolophus* spp.) in HKSAR and mainland China (190). The high seroprevalence and viral load of infected Chinese horseshoe bats, *Rhinolophus sinicus*, strongly suggested that bats are the natural reservoir of SARS-CoV-like viruses, similar to the situation of fruit bats carrying Hendra virus or Nipah virus (363).

EPIDEMIOLOGICAL CHARACTERISTICS

The epidemiological linkage of the initial human cases of the 2003 pandemic to wild game animals suggested that SARS-CoV is zoonotic in origin (431). The isolation of SARS-CoV-like viruses from palm civets and subsequently horseshoe bats further supported this contention (117, 190). It was reported that a seroprevalence rate of about 80% was found in civets in

animal markets in Guangzhou (338). However, person-to-person transmission has been the primary mode of spread of the epidemic, which has occurred in health care facilities, workplaces, homes, and public transportation. The most important route of person-to-person spread appears to be direct or indirect contact of the mucosae with infectious respiratory droplets or fomites (296). SARS-CoV has been detected in respiratory secretions, feces, urine, and tears of infected individuals (42, 229). Nosocomial transmission of SARS was facilitated by the use of nebulizers, suction, intubation, bronchoscopy, or cardiopulmonary resuscitation on SARS patients, when large numbers of infectious droplets were generated (70, 197, 340). In fact, almost half of the SARS cases in HKSAR were nosocomial infections that were acquired within health care facilities and institutions (202). The attack rate among health care workers was higher where the number of SARS patients was greater (187). Although airborne transmission is considered uncommon, a unique form of airborne transmission was considered a likely explanation for a large community outbreak in a private housing estate called Amoy Garden in HKSAR. Contaminated aerosols generated in toilets by exhaust fans coupled with dried U traps of sewage drains, which ascended the light well connecting different floors, caused an explosive outbreak affecting hundreds of people (71, 405). The presence of viruses in stool, often with high viral loads (156, 258), also suggested the possibility of feco-oral transmission, although this has not been proven conclusively. It was suggested that SARS was transmitted in commercial aircraft during the epidemic. Out of a total of 40 flights investigated, 5 were associated with probable in-flight SARS transmission, affecting 37 passengers (254). Most of the affected passengers sat within five rows of the index case. The overall risk of transmission appears to be low, at around 1 in 156 (358). In the largest incident, during a 3-h flight carrying 120 passengers traveling from HKSAR to Beijing, a super-spreading event (SSE) infected 22 passengers (254). The pattern of involvement was atypical, considering the short duration of exposure of 3 h and the widespread involvement of patients sitting within seven rows in front of and five rows behind the index case. Although airborne transmission was considered to be a possible explanation, other potential modes of transmission, such as contact of passengers with the index case before or after the flight, cannot be excluded, especially since 17 out of the 22 people infected were from two tourist groups (254). In another study, a SARS patient traveled between HKSAR and European countries during the presymptomatic and early symptomatic period, and no transmission among passengers seated in close proximity to the index patient was found, suggesting that in-flight transmission of SARS is not common (23). Symptomatic SARS patients appeared to transmit infections on board much more readily than presymptomatic ones (23, 254, 358). Initiation of screening procedures to detect people with fever prior to boarding has been used in an attempt to reduce the risk of in-flight transmission of SARS, but the efficacy is still uncertain (342).

In 17 studies that reported on seroepidemiology, the seroprevalence varied from 0 to 1.81% for the general population, 0 to 2.92% for asymptomatic health care workers, 0 to 0.19% for asymptomatic household contacts, and 12.99 to 40% for asymptomatic animal handlers (28, 37, 45, 69, 117, 141, 198, 201, 203, 207, 209, 228, 352, 369, 387, 406, 429). The last finding

is quite expected, since frequent zoonotic challenges by low-level-pathogenic strains of SARS-CoV before 2003 in animal handlers of southern China would probably have caused such a high seroprevalence in this at-risk group. Genuine asymptomatic infection with antigenemia detected by enzyme immunoassay (EIA) and seroconversion confirmed by neutralization antibody assay was documented in a restaurant worker who worked in the same restaurant as the index case of the outbreak of 2003 to 2004 (45). However, in 2003, sustained exposure of the animal handlers to these infected civets and other wild animals would result in the introduction of a moderately transmissible and more virulent SARS-CoV strain, which would have mutated from the animal strain and adapted to infect humans more efficiently. The result was a massive global outbreak, but the overall asymptomatic infection rate was still relatively low with this more virulent human-adapted virus in the general population, health care workers, and household contacts. A meta-analysis gave overall seroprevalence rates of 0.1% for the general population and 0.23% for health care workers (203). It is also important to remember that these seroprevalence studies are not directly comparable since different serological methods of various sensitivities or specificities were used with or without confirmation by another test. Thus, the true incidence of asymptomatic infection remains elusive.

The incubation period of SARS is 2 to 14 days, although occasional cases with longer incubation periods have been reported (41). The average number of secondary cases resulting from a single case was two to four (225, 285). Unlike influenza virus, where the patients were most infectious in the first 2 days of illness, transmission from symptomatic SARS patients usually occurred on or after the fifth day of onset of disease, which is in line with the rising viral load in nasopharyngeal secretions that peaked at around day 10 (258). There have been speculations about the incidence of SARS and ambient temperature (319), but a definite seasonality could not be concluded. SSEs have been noted to play an important role in the propagation of the SARS outbreak, which gives rise to a disproportionate number of secondary cases, as in the Amoy Garden of HKSAR. A study comparing the clinical and environmental features of SSE and non-SSE cases showed that SSEs were likely to be related to a combination of factors including delayed isolation, admission to a nonisolation ward, and severe disease at the time of isolation (53).

CLINICAL FEATURES

The typical clinical presentation of SARS is that of viral pneumonia with rapid respiratory deterioration (Table 3). Fever, chills, myalgia, malaise, and nonproductive cough are the major presenting symptoms, whereas rhinorrhea and sore throat are less frequently seen (7, 21, 37, 149, 197, 258, 259, 270, 278, 336, 411, 425). Clinical deterioration, often accompanied by watery diarrhea, commonly occurs 1 week after the onset of illness (58, 258). Similar to other causes of atypical pneumonia, physical signs upon chest examination are minimal compared with the radiographical findings. Chest radiographs typically show ground-glass opacities and focal consolidations, especially in the periphery and subpleural regions of the lower zones. Progressive involvement of both lungs is not uncommon

TABLE 3. Correlation between clinical, virological, immunological, and histopathological findings

Clinical and laboratory features (% positive isolates [no. of isolates studied/total no.]) (reference)[a]	Viral load for indicated day(s) after onset of symptoms (reference)	Blood immune profile or histopathological feature (reference)
Systemic involvement Fever (99.9 [751/752]) Chill or rigors (51.5 [377/732]) Malaise (58.8 [317/539])	Mean 1.1 log copies/ml between days 10 and 15 in serum (156)	Increased mean serum concentrations of IL-16, TNF-α, and transforming growth factor β1 but decreased IL-18 between days 3 and 27 (16); increased IFN-γ and inflammatory cytokines IL-1, IL-6, and IL-12 for at least 2 wk; chemokine profile demonstrated increased neutrophil chemokine IL-8, MCP-1, and Th1 chemokine IP-10 (360); increased serum concn of IP10, MIG, and IL-8 during the first wk was associated with adverse outcome or death (325)
Respiratory involvement Rhinorrhea (13.8 [50/362]) Sore throat (16.5 [91/552]) Cough (65.5 [460/702]) Dyspnea (45.9 [282/614])	Mean 2.4 log copies/ml between days 10 and 15 for NPA (156), 9.58×10^2–5.93×10^6 copies/ml for throat swab and 7.08×10^2–6.38×10^8 copies/ml for saliva between days 2 and 9 (349), and 2×10^4–1×10^{10} copies/ml between days 5 and 51 for lung tissue (96)	IP10 highly expressed in both lung and lymphoid tissues, with monocyte-macrophage infiltration and depletion of lymphocytes (163); increased alveolar macrophages and CD8 cells, decreased CD4-to-CD8 ratio, and increased TNF-α, IL-6, IL-8, RANTES, and MCP-1 levels in bronchoalveolar lavage samples (124, 344); IP10 was increased in lung tissue from patients who died of SARS (325); increased differential expression of cytokines within these pulmonary tissues, including Stat1, IFN-regulatory factor 1, IL-6, IL-8, and IL-18, often characteristic of patients with acute respiratory distress syndrome (8)
Cardiovascular involvement Tachycardia (46.1 [71/154]) Bradycardia (14.9 [18/121]) (403) Hypotension (50.4 [61/121]) (403)	1×10^4–2.8×10^7 copies/ml between days 5 and 23 for cardiac tissue (96)	Subclinical diastolic impairment without systolic involvement but no interstitial lymphocytic infiltrate or myocyte necrosis in histology (211); gross pulmonary thromboemboli and marantic cardiac valvular vegetations in some autopsies (67)
Gastrointestinal involvement Diarrhea (20.1 [130/647])	Mean 6.1 log copies/ml between days 10 and 15 for stool (156), with higher mean viral load in NPA obtained on day 10 significantly associated with diarrhea (58); 2.7×10^3–2.7×10^9 copies/ml between days 10 and 29 for small intestinal tissue and 5.3×10^3–3.7×10^8 copies/ml between days 10 and 43 for large intestinal tissue (96)	Minimal architectural disruption despite active viral replication in enterocytes of both terminal ileum and colonic biopsy specimens; no villous atrophy or inflammation (205); atrophy of mucosal lymphoid tissue (298)
Other symptoms Myalgia (48.5 [365/752])		Focal myofiber necrosis with scanty macrophage infiltration may be related to steroid treatment (204)
Headache (38.8 [292/752]) Dizziness (27.3 [163/597])	RT-PCR positive for some cerebrospinal fluid (188)	Necrosis of neuron cells and broad hyperplasia of gliocytes (389)
Hematological involvement Anemia (12.6 [17/135]) Leukopenia (24.2 [114/472]) Lymphopenia (66.4 [296/446]) Thrombocytopenia (29.7 [140/472])		Prolonged lymphopenia with nadir during days 7–9 returning to normal after 5 wk; death and severity are associated with profound CD4$^+$ and CD8$^+$ lymphopenia; little change in CD4/CD8 ratio (136)
Biochemical involvement Increased serum alanine aminotransferase levels (44.1 [208/472])	Positive RT-PCR for liver tissue (44), 6×10^3–5×10^4 copies/ml between days 2 and 9 for liver tissue (96)	Ballooning of hepatocytes and mild to moderate lobular lymphocytic infiltration (44)
Impaired serum creatinine (6.7 [36/536]) (76)	Mean 1.3 log copies/ml between days 10 and 15 for urine (156) and 4.3×10^3–7.4×10^5 copies/ml between days 11 and 27 for kidney tissue (96)	Acute tubular necrosis (76)
Decreased serum tri-iodothyronine and thyroxine		Extensive cell apoptosis and exfoliation of the follicular epithelium into distorted, dilated, or collapsed follicles (354)
Other Histological orchitis (388)		Widespread germ cell destruction, few or no spermatozoa in the seminiferous tubule, thickened basement membrane, and leukocyte infiltration with T lymphocytes and macrophages in the interstitial tissue (388)

[a] See references 7, 21, 37, 149, 197, 258, 259, 270, 278, 336, and 425 for clinical and laboratory features unless otherwise specified in the table.

(113, 148, 184, 362). Shifting of radiographic shadows and spontaneous pneumomediastinum may occur (74, 258). A retrospective analysis of serial chest radiographs in all SARS patients from HKSAR showed that the initial extent and progression of radiographic opacities may be useful for prognostic prediction (6).

Diarrhea is the most common extrapulmonary manifestation, followed by hepatic dysfunction; dizziness, which may be related to diastolic cardiac impairment and pulmonary arterial thrombosis; abnormal urinalysis; petechiae; myositis; neuromuscular abnormalities; and epileptic fits (44, 58, 188, 211, 248, 335, 346, 383). The elderly may present atypically without fever or respiratory symptoms (68, 361). While infections in children appear to be milder than those in adults (20, 144, 183), SARS in pregnant women carries a significant risk of mortality (364, 410). Higher nasopharyngeal and serum viral loads were asso-

ciated with oxygen desaturation, mechanical ventilation, and mortality; higher stool viral loads were associated with diarrhea; and higher urine viral loads were associated with abnormal urinalysis (58, 75, 156). The significant correlation of the viral loads in these specimens to the severity of clinical or laboratory findings suggested that extrapulmonary viral replication was contributing to clinical manifestations (156).

As for hematological parameters, peripheral blood lymphopenia and elevated hepatic parenchymal enzymes are common with or without thrombocytopenia or increases in D dimers and activated partial thromboplastin time (197). About 20% to 30% of patients developed respiratory failure requiring mechanical ventilation, and the overall mortality rate was around 15%. Age, presence of comorbidities, increased lactate dehydrogenase level, hypouricemia, acute renal failure, more extensive pulmonary radiological involvement at presentation, and a high neutrophil count at the time of admission are poor prognostic indicators (153, 197, 385). Restrictive lung function abnormalities due to residual lung fibrosis and muscle weakness are common in the convalescent phase (34, 247, 255). Among survivors of SARS in HKSAR 1 year after illness, significant impairment in diffusion capacity was noted in 23.7% of studied subjects. The exercise capacity and health status of SARS survivors were also remarkably lower than those of the healthy population (154). A study on the pathological changes of testes from six patients who died of SARS indicated that orchitis was also a complication and suggested that reproductive functions in male patients who recovered from SARS should be monitored (388). Depression and posttraumatic stress disorder are especially common among health care workers and patients with affected family members (57, 66, 238, 310). Complications due to the use of corticosteroids including psychosis, adrenal insufficiency, and avascular osteonecrosis were also reported (36, 112, 145, 195, 200).

HISTOPATHOLOGICAL CHANGES OF SARS

Histological Changes

Acute diffuse alveolar damage with air space edema was the most prominent feature in patients who died before the 10th day after onset of illness (99, 250). 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 (157). Patients who died after the 10th day of illness exhibited a mixture of acute changes and those of the organizing phase of diffuse alveolar damage. There was 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 giant 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 (345). 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 (87).

No tissue destruction or severe inflammatory process associated with viral infection was noted in other organs or tissues, but viral particles could be detected in pneumocytes and enterocytes by in situ hybridization (331). Inflammation, cellular apoptosis, or microvillus atrophy of a significant degree was not found in the intestinal mucosa to account for the watery diarrhea. Immunohistochemical staining showed the presence of viral nucleoproteins in type II pneumocytes and occasionally pulmonary macrophages. Necrosis or atrophy in the lymphoid tissue of lymph nodes and white pulp of the spleen are commonly observed extrapulmonary pathologies.

Immunological Profiles

Flow cytometric examination of the peripheral blood at the time of admission before the use of steroid showed decreases in levels of dendritic cell subsets, natural killer cells, CD4$^+$ and CD8$^+$ T lymphocytes, and B lymphocytes (82, 213, 420). 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 minus-strand RNA, the replicative intermediate of the virus during the initial week of illness (208). Studies of the cytokine profile of SARS patients showed conflicting results, which may be due to the use of many immunomodulators including steroids. However, those studies generally showed consistent and significant elevations of the plasma chemokines gamma interferon (IFN-γ)-inducible protein 10 (IP10 [CXCL10]), monocyte chemotactic protein 1 (MCP-1 [CCL2]), and interleukin-8 (IL-8). In some studies, levels of the Th1-related cytokines IFN-γ and IL-12 and the inflammatory cytokines IL-1β and IL-6, which can induce an intense inflammatory response, were also increased (63, 152, 163, 165, 325, 360). In one study, patients with severe disease tended to have increased plasma levels of IFN-α, IFN-γ, and CXCL10 and decreased levels of IL-12p70, IL-2, and tumor necrosis factor alpha (TNF-α) during the acute phase. In the late phase, patients with severe disease had significantly increased plasma chemokine levels of IL-8, CXCL10, and CCL2 but decreased cytokine levels of IL-12p70, IL-2, TNF-α, and IFN-γ compared with mild cases of SARS (26). These host responses 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, respectively. Since SARS-CoV appears to evade the triggering of IFN-α and IFN-β in human macrophages in vitro (61, 280), the lack of an antiviral innate immune response may permit uncontrolled viral replication with progressive increases in viral load and the accompanying proinflammatory systemic response. This situation continues into the second week of illness until the appearance of the adaptive immune response, which brings viral replication under control. Moreover, comparative transcriptomal microarray analysis showed that SARS-CoV rather than CoV-229E markedly upregulated genes associated with apoptosis, inflammation, the stress response, and procoagulation during the early phase of infection of a human liver cancer cell line (Huh7) (322). Both observations help to explain the clinical severity of SARS in relation to the high

viral load at up to 2 weeks of illness and the intense inflammatory response as evident from serum cytokine profiles and histopathology. The majority of SARS patients resolved the proinflammatory cytokine and chemokine responses at the acute phase and expressed adaptive immune genes. In contrast, patients who later succumbed showed deviated IFN-stimulated gene and immunoglobulin gene expression levels, persistent chemokine levels, and deficient anti-SARS spike antibody production. It was speculated that unregulated IFN responses during the acute phase may lead to a malfunction of the switch from innate immunity to adaptive immunity. Indeed, recovered patients were found to have higher and sustainable levels of N-specific antibody and S-specific neutralizing antibody responses, whereas patients who later succumbed had an initial rise and then a fall in antibody levels just before death, suggesting that antibody response is likely to play an important role in determining the ultimate disease outcome (417).

PATHOGENESIS, IMMUNE RESPONSE, AND HOST SUSCEPTIBILITY

Interaction between Viral and Cellular Factors

The exact mechanism of how the virus produces damage at cells, tissue, and organs to clinical levels remains elusive. Similar to other viruses such as influenza A virus, Nipah virus, or Ebola virus, SARS-CoV must possess the ability to evade the innate antiviral response of the cells in order to replicate efficiently in the host. Transfection experiments with Orf3b, Orf6, and N in 293T cells showed that these viral proteins are IFN antagonists that can interfere with the synthesis of IFN and its downstream signaling pathways (178). However, this cannot explain the apparent discrepancy of IFN-β/α production in infected human intestinal Caco-2 cell line (253) and the lack of such production in SARS patients' peripheral blood mononuclear cells or in human primary macrophages abortively infected with SARS-CoV despite the activation of several IFN-stimulated genes in the latter case (61). On the other hand, this may explain the increased serum level of IFN of some SARS patients, which may have an intestinal source. Due to the lack of a type 2 pneumocyte cell line that is susceptible to SARS-CoV, the relevance of these findings cannot be ascertained for lung epithelial cells.

Once the virus can overcome the innate immune response at the cellular level, it can take over the host metabolic apparatus through the degradation of host mRNA by nsp1 and the modulation of the ubiquitination pathway of the host by nsp3 (15, 81, 192, 224, 279). Efficient viral replication ensues, and cell damage occurs by virus-induced cytolysis or immunopathology. Infected cell lines and postmortem lung tissues have shown cytopathic changes due to apoptosis, necrosis, or occasionally syncytium formation. Expression of nsp5, nsp10, Orf3a, Orf3b, Orf7a, Orf8a, E, M, and N in different cell lines by transfection can cause cellular apoptosis (Table 1). Expression of S in transfected cells can lead to syncytium formation with cells expressing ACE2 (181). Paradoxically, little cytopathic effect or inflammation was found in intestinal biopsy specimens of SARS patients despite marked viral replication seen with electron microscopy (205). The transcriptomal profile of infected Caco-2 cells showed a marked upregulation of the potent immunosuppressive cytokine transforming growth factor β and the antiapoptotic host cellular response, which may explain the noninflammatory secretory diarrhea and huge amount of viral shedding in stool (79). Therefore, the clinical or histopathological manifestations at various organs or tissues do not depend solely on the presence of the relevant receptor and coreceptors or the viral productivity as reflected by the viral load. The inflammatory and apoptotic responses of the cell triggered by the virus and the compensatory regenerative power or functional reserve of that organ may be equally important in determining the manifestations and the outcome of infection. nsp1 expression in human lung epithelial A549 cells can increase the expression of the chemokines IP10, CCL3, and CCL5 through the NF-κB pathway (192). This correlated well with the plasma chemokine profile of SARS patients and the immunohistochemical staining of infected lungs. IP10 expressed on pneumocytes is a potent chemoattractant for activated cytotoxic T lymphocytes, natural killer cells, and monocytes, which may therefore infiltrate the interstitium and alveoli of lungs of SARS patients. Administration of a recombinant S fragment between positions 324 and 688 and Orf3a expression in lung cells can excite the production of IL-8 (43, 169). The expression of N in transfected cells can also activate the Cox2 inflammatory cascade (393). If SARS-CoV can indeed suppress the early innate immune response of IFN-β/α in type 2 pneumocytes without activating the IFN-stimulated genes and therefore also allowing an uncontrolled viral replication in the adjacent cells, the concomitant activation of proinflammatory chemokines and cytokines would explain the dominant and highly fatal manifestation of SARS in the lungs.

Adaptive Immune Response

In general, specific serum antibody against whole SARS-CoV by indirect immunofluorescence or neutralization tests starts to appear at around day 7, 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 was not detected after 2 to 3 months (371). Serum testing by recombinant nucleocapsid EIA can detect such an antibody as early as the fifth day after the onset of symptoms (46). The virus-specific T-cell-mediated immune response is not clearly defined. In one study, S-specific cell-mediated immunity mediated by CD4 and CD8 cells was found to last for more than 1 year (395).

Host Susceptibility

Some studies suggested a possible association of HLA-B*4601 with susceptibility to and severity of SARS among the Chinese population in Taiwan (223), but the finding was not confirmed in HKSAR SARS cases. Among the Chinese population in HKSAR, similar associations with HLA-B*0703 and the genetic variant ICAM3 Gly143 have been found (35, 249). Low-mannose-binding lectin producing the YB haplotype has an increased risk of acquiring SARS (160, 416). On the other hand, individuals with HLA-DRB1*0301 or that are homozygous for CLEC4M tandem repeats were found to be less susceptible to SARS-CoV infection (40, 249). However, the latter

finding was strongly disputed in two subsequent studies (324, 430).

LABORATORY DIAGNOSIS OF SARS-CoV INFECTION

No pathognomonic signs or symptoms of SARS can be used to differentiate SARS from other causes of community- or hospital-acquired pneumonia. Etiological diagnosis and differentiation from other causes of atypical pneumonia can be made only by laboratory confirmation. A positive viral culture from respiratory, fecal, and, occasionally, urine or tissue specimens or a fourfold rise in the neutralizing antibody titer in serum samples taken upon admission and 28 days afterward is the most definitive evidence of infection. However, both viral culture and neutralizing antibody testing required a biosafety level 3 laboratory, which is not available in most hospitals. Rapid detection by nucleic acid amplification such as RT-PCR or antigen detection by EIA is the alternative. It is important that most of these rapid tests have never been thoroughly investigated in prospective field trials due to the short-lasting nature of the SARS epidemic. Thus, most of our data on these assays came from evaluations of stored clinical specimens. As for the collection of clinical specimens, although bronchoalveolar lavage fluid and lung biopsy tissue should be the ideal specimens at the onset of illness, such procedures are invasive and can be hazardous to health care workers. Nasopharyngeal aspirates and throat washings, taken with respiratory precautions and preserved in viral transport medium, remain the most important diagnostic specimens.

Nucleic Acid Amplification Assays

Most nucleic acid amplification tests are designed with the *Orf1b* or nucleoprotein gene (32, 56, 88, 108, 155, 189, 264, 266, 268, 349, 384, 391, 413). The latter gene has the theoretical advantage of being more abundant in infected cells and therefore of higher sensitivity, but this has not been clearly proven in clinical studies. Of these methods, real-time quantitative RT-PCR (Table 4) of the nasopharyngeal aspirate is the most sensitive and rapid method for aiding in clinical diagnosis and may achieve a sensitivity of 80% with good specificity even if it is collected within the first 5 days of illness (266). In-house qualitative RT-PCR tests are generally less sensitive and prone to contamination. Positive test results from a single sample must be confirmed by a repeat test detecting a different region of the SARS-CoV genome on the same sample. If possible, another repeat sample should also be tested to exclude false-positive results due to amplicon carryover. Since the viral load in nasopharyngeal aspirate usually peaked on the 10th day after the onset of symptoms, suspected SARS cases must have the tests repeated as the disease evolves to avoid false-negative results (32, 258). Stool specimens should also be routinely sent for testing since a very high percentage of patients develop diarrhea and shed virus during the second week of illness (58). Viral load determination of nasopharyngeal specimens or serum upon presentation might have clinical value, as it is an important prognostic factor (72, 73, 75, 156). Longitudinal monitoring of viral load would be an important part of any treatment trials in the future.

Antigen Detection Assays

Antigen detection with monoclonal antibodies or monospecific polyclonal antibody against the N protein was found to be a sensitive and specific test for the diagnosis of SARS (Table 5). In a large study with sera collected from 317 SARS patients at different time points of illness, EIA detection of SARS N was performed using a panel of three monoclonal antibodies (46). Over 80% of SARS cases can be detected within the first 7 days after the onset of illness. As serum antibody levels started to rise at day 7, the sensitivity of the serum antigen assay progressively decreased to 0% at day 21 (46). Antigen detection with EIA in nonserum specimens is generally less sensitive than RT-PCR because the cutoff value is usually set at a much higher level than that of serum specimens to overcome the high background optical density values in nonserum specimens (189, 191).

Antibody Detection Assays

For antibody testing (Table 6), the indirect immunofluorescent antibody test is more commonly performed than the neutralizing antibody test since the former involves minimal manipulation of infectious virus and therefore carries less risk of a biohazard. The test is generally not useful during the first week of illness. Single low-titer positive results can be related to cross-reactions with other human coronaviruses (31, 47). A recombinant nucleocapsid EIA may be used as a rapid screening test and possesses a higher sensitivity, with detection as early as day 5 after onset of illness (46), but again, false-positive results due to cross-reactions with HCoV-O43 and HCoV-229E can occur and require confirmation by Western blotting against the S polypeptide of SARS-CoV (372). Serum IgG, IgM, and IgA appeared at around the same time, between days 5 and 17 after the onset of symptoms, and paralleled the appearance of neutralizing antibody activity, but one study reported that IgM appeared 3 days earlier using an IgM capture EIA against nucleoprotein (404). The titer of neutralizing antibody peaked at days 20 to 30 and was sustained for a long time. It is interesting that the neutralizing antibody level of those who died peaked at day 14 and then started to fall, whereas those who survived had a sustained level of antibody (417). A new immunofluorescence assay using the S protein and a recombinant N-S fusion protein as an antigen has been described. The results are comparable to those obtained with whole-virus-based immunofluorescence assays (128, 235). The three laboratory outbreaks of SARS prompted the use of pseudotype viruses for research and neutralization antibody testing, but data on systematic evaluation are lacking.

CLINICAL MANAGEMENT AND ANTIVIRALS

Since there is no proven effective antiviral agent by randomized placebo control trial (Table 7), clinical management of SARS has relied largely upon supportive care. Broad-spectrum antimicrobial coverage for community-acquired pneumonia should be given while virological confirmation is pending. Such antibiotics should be stopped once the diagnosis of SARS is confirmed, but nosocomial infections as a result of prolonged

TABLE 4. Clinical evaluation of molecular diagnostic tests for SARS-CoV

Diagnostic method and target gene	Clinical specimen	Diagnostic gold standard	Collection time after onset of symptoms (no. of samples)	% Sensitivity (viral load [copies/ml])	Reference
In-house RT-PCR					
RNA pol[a]	NPA	Laboratory confirmed[b]	Days 1–5[c] (72)	59.7[d]	391
RNA pol	NPA	WHO criteria, probable SARS	Days 1–5 (98)	29.6	32
			Days 0–5 (501)	41.1	56
			Days 6–11 (211)	58.8	
			Days 12–20 (62)	37.1	
			Day >21 (15)	13.3	
RNA pol	Throat swab	WHO criteria, probable SARS	Days 1–13 (590)	37.5	384
RNA pol[a]	Nose and throat swab	Laboratory confirmed[b]	Days 1–5[c] (54)	61.1[d]	391
			Days 1–5 (53)	28.3	32
RNA pol	Upper respiratory[e]	WHO criteria, probable SARS	Days 0–5 (212)	31.1	56
			Days 6–11 (73)	37	
			Days 12–20 (45)	31.1	
			Day >21 (159)	5.7	
RNA pol[a]	Respiratory[f]	Laboratory confirmed[b]	1 wk (243)	26.3	39
			2 wk (134)	30.6	
			3–4 wk (94)	18.1	
RNA pol	Stool	Laboratory confirmed[b]	Days 5–10[c] (19)	57.9[d]	391
			Days 1–5 (25)	20	32
			1 wk (21)	42.9	39
			2 wk (25)	68	
			3–4 wk (80)	42.5	
RNA pol	Stool	WHO criteria, probable SARS	Days 0–5 (77)	23.4	56
			Days 6–11 (86)	57	
			Days 12–20 (72)	52.8	
			Day >21 (297)	15.2	
RNA pol	Urine	Laboratory confirmed[b]	Days 5–10[c] (78)	50[d]	391
			Days 1–5 (15)	0	32
			1 wk (75)	2.7	39
			2 wk (82)	6.1	
			3–4 wk (54)	11.1	
RNA pol	Urine	WHO criteria, probable SARS	Days 0–11 (16)	12.5	56
			Days 12–20 (21)	4.8	
			Days >21–23 (161)	1.9	
RNA pol	Blood (serum)	WHO criteria, probable SARS	Days 0–5 (64)	17.2	56
			Days 6–11 (14)	35.7	
			Days 12–20 (9)	11.1	
Nested RNA pol	Blood (plasma)	Laboratory confirmed[b]	Days 1–3 (24)	79.2	108
In-house quantitative PCR					
ORF 1b[a]	NPA	Laboratory confirmed[b]	Days 1–3 (32)	50	263
			Days 4–6 (35)	31.4	
			Days 7–10 (31)	51.6	
ORF 1b[g]	NPA	Laboratory confirmed[b]	Day 1 (8)	62.5[d]	264
			Day 2 (16)	87.5[d]	
			Day 3 (26)	80.1[d]	
ORF 1b	NPA	WHO criteria, probable SARS or laboratory confirmed[b]	Days 10–15 (142)	42.3 (2.4 log [mean])	156
1-step ORF 1b[g]	NPA	Laboratory confirmed[b]	Days 1–3 (29)	96.6[d]	266
			Days 4–9 (57)	80.7[d]	
1-step ORF 1b	Throat wash	WHO criteria, laboratory-confirmed SARS	Days 2–9 (17)	9.58×10^2 (min)–5.93×10^6 (max)	349
N	Saliva	WHO criteria, laboratory-confirmed SARS	Days 2–9 (17)	7.08×10^2 (min)–6.38×10^8 (max)	88
N	Respiratory[h]	WHO criteria, laboratory-confirmed SARS	Days 2–54 (31)	74.2	
N	NPA and stool	WHO criteria, probable SARS	Days 1–4 (32)	18.8	155
			Days 5–10 (37)	35.1	
RNA pol	Stool	Laboratory confirmed[b]	Days 1–10 (8)	75	189
			Days 11–20 (27)	81.5	
			Days 21–30 (5)	80	
ORF 1b	Stool	WHO criteria, probable SARS or laboratory confirmed[b]	Days 1–3 (6)	66.7	263
			Days 4–6 (15)	80	
			Days 7–10 (16)	62.5	
N	Stool	WHO criteria, probable SARS	Days 10–15 (94)	87.2 (6.1 log [median])	156
			Days 1–10 (37)	27	413
			Days 11–20 (71)	26.8	
			Days 21–30 (77)	15.6	
			Days 31–40 (67)	17.9	
			Day >40 (74)	9.5	
N	Stool	WHO criteria, laboratory-confirmed SARS	Days 2–54 (23)	87 (5.5×10^4 [median])	88
RNA pol	Urine	Laboratory confirmed[b]	Days 1–10 (14)	7.1	189
			Days 11–20 (86)	31.4	
			Days 21–30 (21)	14.3	
			Days 31–60 (12)	16.7	
ORF 1b	Urine	WHO criteria, probable SARS or laboratory confirmed[b]	Days 10–15 (111)	28.8 (1.3 log [mean])	156
	Blood (serum)		Days 10–15 (53)	41.5 (1.1 log [mean])	

TABLE 4—*Continued*

Diagnostic method and target gene	Clinical specimen	Diagnostic gold standard	Collection time after onset of symptoms (no. of samples)	% Sensitivity (viral load [copies/ml])	Reference
1-step ORF 1b[i]	Blood (plasma)	WHO criteria, laboratory-confirmed SARS	Days 2–4 (20) Days 5–7 (12) Days 8–11 (11) Days 12–16 (8)	50 (2.36×10^3 [median])[d] 75 (6.91×10^3 [median])[d] 63.6 (1.74×10^4 [median])[d] 37.5 (3.83×10^3 [median])[d]	349
N	Blood (plasma)	WHO criteria, laboratory-confirmed SARS	Days 2–54 (7)	42.9	88
N	Blood (whole blood)	WHO criteria, probable SARS	Days 1–10 (62) Days 11–20 (41) Days 21–30 (165) Days 31–40 (158)	45.2 48.8 13.3 16.5	413
In-house LAMP[j]					
ORF 1b[g]	NPA	Laboratory confirmed[b]	Days 1–3 (15) Days 4–7 (37) Day >7 (7)	60[d] 70.3[d] 100[d]	267
1-step replicase	Respiratory specimens[k]	WHO criteria, probable SARS	Day 3 (49)	26.5[d]	146
Commercial quantitative PCR					
Roche kit[l]	Respiratory[h]	WHO criteria, laboratory-confirmed SARS	Days 2–54 (30)[m]	73.3	88
Roche kit	NPA and throat swab	Laboratory confirmed[b]	Days 1–5[c] (47)	85[d]	390
Roche kit	Stool	WHO criteria, laboratory-confirmed SARS	Days 2–54 (23)	78.3 (4.3×10^4 [median])	88
Roche kit	Stool	Laboratory confirmed[b]	Days 1–5[c] (32)	88[d]	390
Commercial kit[n]	Stool	WHO criteria, probable SARS	Day ≤7 (13) Days 8–21 (12) Day >21 (31)	46–62[d] 58–75[d] 13–19[d]	230
Roche kit	Urine	Laboratory confirmed[b]	Days 1–5[c] (22)	82[d]	390
Roche kit	Blood (plasma)	WHO criteria, laboratory-confirmed SARS	Days 2–54 (7)	28.6	88
Artus kit[o]	Respiratory[h]	WHO criteria, laboratory-confirmed SARS	Days 2–54 (31)	74.2	88
Artus kit	NPA and throat swab	Laboratory confirmed[b]	Days 1–5[c] (47)	87[d]	390
Artus kit	Stool	WHO criteria, laboratory-confirmed SARS	Days 2–54 (22)[g]	81.8	88
Artus kit	Stool	Laboratory confirmed[b]	Days 1–5[c] (32)	91[d]	390
Artus kit	Urine	Laboratory confirmed[b]	Days 1–5[c] (22)	82[d]	390
Artus kit	Blood (plasma)	WHO criteria, laboratory-confirmed SARS	Days 2–54 (7)	14.3	88
Artus kit	Lung tissue	WHO criteria, probable SARS	Days 5–51 (19)	100 (2×10^4 [min]–1×10^{10} [max])	96
Artus kit	Small bowel tissue	WHO criteria, probable SARS	Days 10–29 (15)	73 (2.7×10^3 [min]–2.7×10^9 [max])	96
Artus kit	Large bowel tissue	WHO criteria, probable SARS	Days 10–43 (15)	73 (5.3×10^3 [min]–3.7×10^8 [max])	96
Artus kit	Lymph node tissue	WHO criteria, probable SARS	Days 14–34 (13)	69 (1.5×10^4 [min]–8.9×10^8 [max])	96
Artus kit	Liver tissue	WHO criteria, probable SARS	Days 10–24 (17)	41 (6×10^3 [min]–5×10^4 [max])	96
Artus kit	Cardiac tissue	WHO criteria, probable SARS	Days 5–23 (18)	40 (1×10^4 [min]–2.8×10^7 [max])	96
Artus kit	Kidney tissue	WHO criteria, probable SARS	Days 11–27 (16)	38 (4.3×10^3 [min]–7.4×10^5 [max])	96

[a] For the RNA extraction protocol, 140 μl of nasopharyngeal aspirate (NPA) was used.

[b] A rise of fourfold or more in antibody titer against SARS-CoV.

[c] Day after admission.

[d] Specificity of the test was 100%.

[e] Upper respiratory specimens consisted of throat and nasal swabs ($n = 216$), throat swabs ($n = 164$), nasopharyngeal swabs ($n = 47$), and nasal swabs ($n = 62$).

[f] Respiratory specimens consisted of tracheal aspirate ($n = 7$), pooled throat and nasal swabs ($n = 25$), nasal swabs ($n = 58$), NPA ($n = 192$), throat swabs ($n = 43$), and throat washing ($n = 146$).

[g] For the modified RNA extraction protocol, 560 μl of NPA was used.

[h] Respiratory specimens consisted of saliva ($n = 3$), nasopharyngeal swabs ($n = 16$), sputum ($n = 8$), endotracheal aspirate ($n = 2$), and bronchoalveolar lavage fluid ($n = 2$).

[i] The test adopted the SARS1S_AS TaqMan assay design (Applied Biosystems, Foster City, CA), and 280 μl of plasma was used for RNA extraction.

[j] LAMP, real-time loop-mediated amplification.

[k] Respiratory specimens consisted of throat wash ($n = 15$), throat swabs ($n = 13$), and throat and nasal swabs ($n = 21$).

[l] Roche kit indicates a LightCycler SARS-CoV quantification kit (b-Test_Lot) (Roche Diagnostics, Germany).

[m] There was no result for one sample due to failed internal control. The sample was omitted from the sensitivity evaluation of this assay.

[n] Commercial kit indicates a RealArt HPA-Coronavirus LC RT PCR kit (Artus GmbH, Hamburg, Germany) and a SARS-CoV POL assay (EraGen Biosciences, Madison, WI) with different extraction methods (QIAGEN viral RNA Minikit, bioMerieux miniMg, and Cortex MagaZorb).

[o] Artus kit indicates a RealArt HPA-Coronavirus LC RT-PCR kit (Artus GmbH, Hamburg, Germany).

intubation and the use of corticosteroids should be appropriately managed.

The correlation between viral loads and clinical outcome suggests that suppression of viral replication by effective antiviral drugs should be the key to preventing morbidity and mortality. However, in vitro susceptibility test results were often conflicting, as in the case of IFN-β1a (78, 137, 318) and IFN-α2b (308, 318). Nevertheless, it appears that IFN-β, IFN-αn1, IFN-αn3, and leukocytic IFN-α have some potential activity and warrant evaluation by clinical trials (50, 305, 426). Although a very high 50% cytotoxic concentration exceeding 1,000 mg/liter has been demonstrated for

TABLE 5. Clinical evaluation of antigen detection for SARS-CoV

Diagnostic method and detection target	Diagnostic gold standard	Collection time after onset of symptoms (days)[a] (no. of samples)	Sensitivity (%)	Overall specificity (%)	Reference
EIA					
N protein	WHO criteria, probable SARS	3–5 (8)	50	98.5	48
		6–10 (14)	71.4		
		11–20 (9)	44.4		
N protein	WHO criteria, probable SARS	1–5 (84)	92.9	100	86
		6–10 (63)	69.8		
		11–20 (52)	30.8		
N protein	WHO criteria, probable SARS	1–5 (85)	94	99.9	46
		6–10 (60)	78		
N protein	WHO criteria, probable SARS	NM (18)	100	100	127
N protein	Laboratory confirmed[b]	6–24 (66) (NPA)	52	96.7	191
		11–31 (94) (urine)	5	99	
		8–32 (65) (stool)	55	96	
Immunofluorescence assay					
N protein	WHO criteria, probable SARS	2–9 (17) (throat wash)	65	100	226

[a] Serum samples collected unless specified. NM, not mentioned.
[b] Positive for IgG antibodies against SARS-CoV by indirect immunofluorescence assay in serum sample during the course of illness.

ribavirin (77), and although its low level of in vitro activity against SARS-CoV was initially attributed to cellular toxicity (318), ribavirin has good activity when tested in other human Caco-2 and pig kidney cell lines despite its lack of activity in Vero cells (243). The use of different cell lines, testing conditions, and virus strains may have contributed to these discrepancies.

Numerous other potential antiviral agents have been identified using different approaches (Table 8). Replication of SARS-CoV requires proteolytic processing of the replicase polyprotein by two viral cysteine proteases, a chymotrypsin-like protease (3CL^{pro}) and a papain-like protease (PL^{pro}). These proteases are important targets for the development of antiviral drugs. Protease inhibitors (especially nelfinavir) (386, 392), glycyrrhizin (77), baicalin (50), reserpine (381), aescin (381), valinomycin (381), niclosamide (380), aurintricarboxylic acid (129), mizoribine (293), indomethacin (4), chloroquine (174), and many herbal formulations, have also been found to possess some antiviral activity against SARS-CoV in vitro. In addition, an organic nitric oxide donor, S-nitro-N-acetylpenicillamine, appeared to have inhibitory activity against SARS-CoV (2), which has formed the basis for the use of nitric oxide inhalation as an experimental form of rescue therapy for SARS (52). Several agents with good in vitro antiviral activities, including ACE2 analogues, helicase inhibitors, and nucleoside analogues, were also reported to have some activity in vitro (14, 332). Antiviral peptides designed against the S protein and especially those derived from heptad repeat region 2 of S2 were shown to inhibit membrane fusion and cell entry (22, 177, 227). Small interfering RNA (siRNA) also demonstrated activities in reducing cytopathic effects, viral replication, and viral protein expression in cell lines (125, 232, 351, 418, 419, 428). Screening of chemical libraries has identified several inhibitors of protease, helicase, and spike-mediated cell entry (170). Most of the above-mentioned chemicals or approaches have not been evaluated in human or animal models. In mouse models, nelfinavir, β-D-N^4-hydroxycytidine, calpain inhibitor VI, 3-deazaneplanocin A, human leukocyte IFN-αn3, and anti-inflammatory agents including chloro-

quine, amodiaquin, and pentoxifylline did not significantly reduce lung virus titers in mice. When not given in combination with other antivirals, the IMP dehydrogenase inhibitors, including ribavirin, suppress the proinflammatory response while augmenting viral replication in this mouse model (13).

Before the demonstration of viral load as an important factor in determining clinical outcome, immunomodulators were empirically used for the treatment of SARS during the initial epidemic (59). These immunomodulators include corticosteroids, intravenous immunoglobulins, pentaglobulin, thymosin, thalidomide, and anti-TNF (140, 421). Corticosteroids were previously found to reduce mortality in patients with pneumonia due to varicellazoster virus and influenza virus (1, 109). High-dose hydrocortisone was shown to reduce the expression of the proinflammatory chemokines CXCL8 and CXCL10 in infected Caco-2 cells (80). However, without an effective antiviral agent, the early use of high doses of corticosteroids for prolonged periods could be detrimental. It may increase the plasma viral load and the risk of nosocomial infections and avascular osteonecrosis (196). Pegylated IFN-α2a 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 (118). Among clinical treatments studied, combinations of steroid with either alfacon-1, a recombinant consensus IFN-α (231), or protease inhibitors and ribavirin were found to improve outcomes in two different treatment trials using historical controls (33, 72). Due to the very short time course of this epidemic and the initial lack of suitable animal models, randomized control treatment trials are difficult to be organized and executed despite the finding of some commercially available candidate agents that appeared to be active in vitro.

INFECTION CONTROL AND LABORATORY SAFETY

Because of the physical stability of SARS-CoV in the environment, the absence of protective immunity in the general population, and the lack of effective antivirals or vaccines, infection control against SARS remains the primary means to prevent person-to-person transmission in future epidemics. Early recognition, triage, and prompt iso-

TABLE 6. Clinical evaluation of antibody detection for SARS-CoV

Diagnostic method and detection target	Diagnostic gold standard	Collection time (days) after onset of symptoms[a] (no. of samples)	Sensitivity (%)	Overall specificity (%)	Reference
EIA					
Anti-N protein antibodies	WHO criteria, probable SARS	1–5 (27)	14.8	100	299
		6–10 (38)	68.4		
		11–61 (135)	89.6		
Anti-N protein antibodies	WHO criteria, probable SARS	10 (16)	81.3	100	330
		20 (16)	100		
		30 (16)	100		
Anti-N protein antibodies	WHO criteria, probable SARS	12–72 (280)	89.3	NM	273
Anti-N protein antibodies	Laboratory confirmed[b]	12–43 (106)	95.3/96.6/96.6[f]	94.3/59.4/60.4[f]	373
Anti-N protein antibodies	Laboratory confirmed[b]	NM[c] (106)	94.3	100	369
Anti-N protein antibodies	Laboratory confirmed[d]	First wk (36) (IgM)	33	100	404
		Second wk (36) (IgM)	97		
		Third wk (36) (IgM)	100		
Anti-N protein antibodies	WHO criteria, probable SARS	NM (407)	70.2	99.9	54
Anti-N protein antibodies	Neutralization antibody assay	NM (276)	92	92	294
Anti-S protein antibodies	Laboratory confirmed[b]	11–45 (95)	58.9/74.7[g]	98.6/93.9[g]	374
Anti-S protein antibodies	WHO criteria, probable SARS	NM (51)	80.4	100	423
Anti-S2 protein antibodies	WHO criteria, probable SARS	NM (20)	85	100	350
Anti-SARS-CoV antibodies	WHO criteria, probable SARS	NM (56)	96.4	100	297
Antibodies against Gst-N and Gst-U274	WHO criteria, probable SARS	16–65 (74)	100	99.5	115
Antibodies against Gst-N and Gst-U274	WHO criteria, probable SARS	1–10 (31)	58	99.5	114
		11–20 (120)	70		
		21–30 (57)	75.4		
		>30 (19)	94.7		
Immunochromatography					
Antibodies against Gst-N and Gst-U274	WHO criteria, probable SARS	1–10 (31)	54.8	97.7	114
		11–20 (120)	68.3		
		21–30 (57)	80.7		
		>30 (19)	78.9		
Western blot assay					
Anti-N protein antibodies	WHO criteria, probable SARS	4–76 (44)	90.9	98.3	126
Immunofluorescence assay					
Anti-SARS-CoV antibodies	True SARS[e]	1–5 (64) (IgG)	0	100	38
		6–10 (35) (IgG)	34.3		
		11–15 (23) (IgG)	78.3		
		16–20 (43) (IgG)	97.7		
		21–37 (47) (IgG)	100		
Anti-S protein antibodies	WHO criteria, probable SARS	7–14 (6)	100	100	235
		14–76 (15)	100		
Antibodies against N-S fusion protein	WHO criteria, probable SARS	>17 (23)	95.6	100	128

[a] Serum samples were collected.
[b] Positive for IgG antibodies against SARS-CoV by indirect immunofluorescence assay in serum sample during the course of illness.
[c] NM, not mentioned.
[d] Details not specified.
[e] True SARS is defined as WHO criteria for probable cases of SARS and/or at least one specimen positive for SARS-CoV by RT-PCR.
[f] IgG/IgM/IgA.
[g] IgG/IgM.

lation of suspected cases are the principal measures against nosocomial transmission (142). Although respiratory droplet and contact precautions are effective under most circumstances (296), airborne precautions should be considered for aerosol-generating procedures such as bronchoscopy, tracheostomy, and suctioning of the airway. The virus can be easily inactivated by commonly used disinfectants such as household bleach, which reduced the viral load by more than 3 logs within 5 min (185). In a study on the survival of SARS-CoV, fecal and respiratory samples were shown to be infectious for 4 and >7 days at room temperature, respectively. Survival was found to be longer on disposable gowns than on cotton gowns. Therefore, absorbent material such as cotton is preferred over nonabsorptive material for personal protective clothing in routine patient care. In contrast, the virus cannot be recovered after the drying of a paper request form even with a high inoculum. Therefore, the risk of infection via contact with droplet-contaminated paper is small (185). When managing patients, oxygen delivery by low-flow nasal cannula instead of high-flow face masks

TABLE 7. Antiviral agents and immunomodulators against SARS-CoV in vivo

Antiviral agent and/or immunomodulator (no. of subjects) (study design)	Main findings[a]	Reference
Ribavirin (144 patients) (retrospective case series)	126 patients (88%) treated; side effects of hemolysis (76%) and lowered hemoglobin of 2 g/dl (49%)	21
Ribavirin (229 patients) (retrospective uncontrolled cohort analysis)	97 patients (42.2%) treated; crude death rate of 10.3% (treatment) vs 12.9% (control) ($P = 0.679$)	199
Ribavirin and corticosteroids (75 patients) (prospective case series)	9 patients (12%) had spontaneous pneumomediastinum; 20% developed ARDS in wk 3	258
Ribavirin and MP (31 patients[b]) (retrospective case series)	No patient required intubation or mechanical ventilation; no mortality noted in this series	303
Ribavirin and corticosteroids[c] (71 patients[d]) (prospective cohort study)	Crude mortality rate of 3.4% (only in patients aged >65 yr); none of the discharged survivors required continuation of oxygen therapy	186
Ribavirin and corticosteroids[e] (138 patients) (prospective uncontrolled study)	None responded to antibacterials; 25 patients (18.1%) responded to ribavirin and low-dose corticosteroid; 107 patients required high-dose MP, 88.8% of whom responded; 21 patients (15.2%) required mechanical ventilation; mortality rate, 10.9%	314
Ribavirin and MP (72 patients[f]) (retrospective uncontrolled study)	Patients treated with initial pulse MP therapy had no better rate on mechanical ventilation (5.9% vs 9.1%) (NS) and mortality (5.9% vs 5.5%) (NS)	139
Lopinavir-ritonavir and ribavirin (41 patients) (retrospective study with historical control)[g]	ARDS and death were lower in treatment group than in historical control (2.4% vs 28.8%) ($P < 0.001$) at day 21 after symptom onset	72
Lopinavir-ritonavir as initial therapy (44 patients) (retrospective matched cohort study)[h]	Intubation rate of 0% vs 11% ($P < 0.05$); mortality rate of 2.3% vs 15.6% ($P < 0.05$)	33
Lopinavir-ritonavir as rescue therapy (31 patients) (retrospective matched cohort study)[i]	Intubation rate of 9.7% vs 18.1% (NS); mortality rate of 12.9% vs 14% (NS)	33
IFN-alfacon-1 and corticosteroids (22 patients) (open-label study)	9 patients (40.9%) were treated; 1 (11.1%) patient required mechanical ventilation, and no patient died; of 13 patients (59.1%) treated with corticosteroid alone, 3 (23.1%) required mechanical ventilation and 1 (7.7%) died	231
Pentaglobin, an IgM-enriched immunoglobulin (12 patients[j]) (retrospective analysis)	Improvement in radiographic scores compared with day 1 (median, 9.5) on days 6 (median, 6) ($P = 0.01$) and 7 (median, 6) ($P = 0.01$) and in oxygen requirement compared with day 1 (median, 2.5 liters/min) on days 6 (median, 1 liter/min) ($P = 0.04$) and 7 (median, 0.5 liters/min) ($P = 0.04$) after commencement of pentaglobin treatment	140
Convalescent plasma (1 patient[k]) (case report)	Convalescent plasma (200 ml) was given at day 15 after onset of illness without adverse reaction; patient recovered uneventfully	366
Convalescent plasma (3 patients)	Viral load decreased from 4.9×10^5–6.5×10^5 copies/ml to undetectable 1 day after transfusion	401
Convalescent plasma (80 patients)	A higher day 22 discharge rate was observed in patients treated before day 14 of illness (58.3% vs 15.6%) ($P < 0.001$), and in patients with positive PCR, SARS-CoV antibodies were negative at the time of plasma infusion (66.7% vs 20%) ($P = 0.001$)	60
Two herbal formulas (Sang Ju Yin and Yu Ping Feng San) (37 healthy volunteers)	Given oral traditional Chinese medicine regimen daily for 14 days with transient increase in CD4/CD8 ratio	269

[a] ARDS, acute respiratory distress syndrome; MP, methylprednisolone; NS, P value was not significant.
[b] One patient recovered on antibacterial treatment alone.
[c] A 3-week step-down course of corticosteroids and pulsed methylprednisolone rescue for deterioration.
[d] Three patients recovered on antibacterial treatment alone.
[e] Low-dose corticosteroid and selective use of high-dose methylprednisolone.
[f] Initially treated with high-dose pulse ($n = 17$) versus nonpulse ($n = 55$) methylprednisolone.
[g] One hundred eleven patients treated with ribavirin as a historical control.
[h] Six hundred thirty-four patients selected as matched cohort.
[i] Three hundred forty-three patients selected as matched cohort.
[j] Patients who continued to deteriorate despite ribavirin and corticosteroid therapy.
[k] Patient who continued to deteriorate despite ribavirin and corticosteroid therapy.

should be used to reduce the risk of airborne transmission. Mechanical ventilation, including noninvasive modalities such as continuous positive airway pressure and bilevel positive airway pressure, should be carried out only in negative-pressure isolation rooms under strict airborne precautions (62). All health care personnel caring for patients with suspected or confirmed SARS must have daily temperature checks in the late afternoon and be quarantined after unprotected exposure to achieve early detection and to avoid nosocomial and community outbreaks. Upon discharge of patients, adherence to strict personal hygiene is important.

Clinical specimens of patients remained RT-PCR positive for a substantial period of time, although the clinical significance of this finding is unknown (73). At the community level, contact tracing and quarantine of contacts, temperature checks at borders, health declarations for travelers, social distancing by suspension of schools and closing of workplaces, public education, and effective communication of information have been used to control community spread. Although screening of suspected cases at international borders and airports was widely practiced during the epidemic, the value of doing so has been questioned (307). To prevent

TABLE 8. Antiviral agents and immunomodulators tested against SARS-CoV in animals and in vitro

Antiviral agent(s) and/or immunomodulator(s)	Study setting and methods (virus strain)	Main findings[a]	Reference
IFN-αB/D (hybrid IFN)	BALB/c mice (Urbani)	i.p. IFN-αB/D once daily for 3 days beginning 4 h after virus exposure reduced SARS-CoV replication in lungs by 1 $\log_{10}$ at 10,000 and 32,000 IU; at the highest dose of 100,000 IU, virus lung titers were not detectable	13
Ampligen [poly(I:C124)] (mismatched double-stranded RNA IFN inducer)	BALB/c mice (Urbani)	i.p. Ampligen at 10 mg/kg 4 h after virus exposure reduced virus lung titers to undetectable levels	13
Pegylated IFN-α as prophylactic treatment	Cynomolgus macaques (*Macaca fascicularis*) (patient 5668)	Significantly reduced viral replication and excretion, viral antigen expression by type 1 pneumocytes, and pulmonary damage; postexposure treatment with pegylated IFN-α yielded intermediate results	118
IFN-α2b (Intron A)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 4,950 (890) IU/ml (SI of >2) for FFM-1 isolate; mean (SD) EC_{50} = 6,500 (980) IU/ml (SI of >105) for HK isolate	78
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = 1,530 (220) IU/ml (SI of >6.5) for FFM-1 isolate; mean (SD) EC_{50} = 880 (130) IU/ml (SI of >11.4) for HK isolate	78
IFN-β1b (Betaferon)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 95 (17) IU/ml (SI of >105) for FFM-1 isolate; mean (SD) EC_{50} = 105 (21) IU/ml (SI of >95) for HK isolate	78
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = 21 (3.9) IU/ml (SI of >476) for FFM-1 isolate; mean (SD) EC_{50} = 9.2 (2.1) IU/ml (SI of >1,087) for HK isolate	78
IFN-γ1b (Imukin)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 2,500 (340) IU/ml (SI of >4) for FFM-1 isolate; mean (SD) EC_{50} = 1,700 (290) IU/ml (SI of >5.9) for HK isolate	78
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = >10,000 IU/ml (SI NA) for FFM-1 isolate; mean (SD) EC_{50} = >10,000 IU/ml (SI NA) for HK isolate	78
IFN-β1a	Vero E6 (Tor2, Tor7, and Urbani)	IFN with p.i. IC_{50} = 50 IU/ml; IFN added postinfection IC_{50} = 500 IU/ml	137
IFN-β, IFN-α, IFN-γ	Vero, MxA-expressing Vero (FFM-1)	SARS-CoV strongly inhibited by IFN-β (with p.i.) and less so with IFN-α and IFN-γ; MxA does not interfere with viral replication	305
IFN-α, IFN-β	FRhK-4 (NM[f])	↓ intracellular viral RNA copies; IFN-α IC_{50} = 25 IU/ml; IFN-β IC_{50} = 14 U/ml	426
IFN-α2b	Vero E6 (Tor2, Tor3, Tor7, and Tor684)	IC_{50} = ~500 IU/ml	308
Leu-IFN-α	FRhK-4 (HKU39849)	EC_{50} at 48 h = 5,000 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml	50
IFN-α (p.i. for 16 h before viral inoculation)	FRhK-4 (HKU39849)	EC_{50} at 48 h = 39 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml	50
IFN-β	FRhK-4 (HKU39849)	EC_{50} at 48 h = 200 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 106 μg/ml	50
IFN-β (p.i. for 16 h before viral inoculation)	FRhK-4 (HKU39849)	EC_{50} at 48 h = 625 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml	50
IFN-β1b (Betaferon)	Vero E6 (2003VA2774)	IC_{50} = 0.2 IU/ml; IC_{95} = 8 IU ml	318
IFN-αn3 (Alferon)	Vero E6 (2003VA2774)	IC_{50} = 0.8 IU/ml; IC_{95} = 200 IU/ml	318
Human leukocyte IFN-α (Multiferon)	Vero E6 (2003VA2774)	IC_{50} = 2 IU/ml; IC_{95} = 44 IU/ml	318
IFN-β	Vero E6 (FFM-1)	IC_{50} = 110 IU/ml at 10 $TCID_{50}$; IC_{50} = 625 IU/ml at 100 $TCID_{50}$	83
Multiferon	Vero E6 (FFM-1)	IC_{50} = 540 IU/ml at 10 $TCID_{50}$; IC_{50} = 2,400 IU/ml at 100 $TCID_{50}$	83
IFN-α2b	Vero E6 (FFM-1)	IC_{50} = >3,125 IU/ml at 10 $TCID_{50}$; IC_{50} = >3,125 IU/ml at 100 $TCID_{50}$	83
IFN-α2a	Vero E6 (FFM-1)	IC_{50} = >3,125 IU/ml at 10 $TCID_{50}$; IC_{50} = >3,125 IU/ml at 100 $TCID_{50}$	83
IFN-alfacon1 (Infergen)	Vero (Urbani)	IC_{50} = 0.001 μg/ml	257
IL-4 and IFN-γ	Vero E6 (HKU39849)	IL-4 and IFN-γ downregulated cell surface expression of ACE2; ACE2 mRNA levels were also decreased after treatment	84
IFN-β and ribavirin	Caco2 (FFM-1)	Mean (SD) CI = 0.45 (0.07)	243
HR2-8 (HR2-derived peptide)	Vero 118 (NM)	EC_{50} = 17 μM	22
CP-1 (HR2-derived peptide)	Vero E6 (WHU)	IC_{50} ≈ 19 μmol/liter	227
HR1-1 (HR1-derived peptide)	Vero E6 (BJ01 and pseudovirus)	EC_{50} = 3.68 μM for wild-type virus assay; EC_{50} = 0.14 μM for pseudotyped virus assay	407
HR2-18 (HR2-derived peptide)	Vero E6 (BJ01 and pseudovirus)	EC_{50} = 5.22 μM for wild-type virus assay; EC_{50} = 1.19 μM for pseudotyped virus assay	407
HR2	Vero E6 (WHU)	CPE inhibition IC_{50} = 0.5–5 nM (synthetic HR2 peptide) and 66.2–500 nM (fusion HR2 peptide)	432
Peptides representing various regions of ACE2	TELCeB6, HeLa, and VeroE6 (pseudovirus)	IC_{50} = 50 μM (peptide aa 22–44); IC_{50} = 6 μM (peptide aa 22–57); IC_{50} = 0.1 μM (peptide aa 22–44 and 351–357) artificially linked by glycine	120

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TABLE 8—*Continued*

Antiviral agent(s) and/or immunomodulator(s)	Study setting and methods (virus strain)	Main findings[a]	Reference
Peptides analogous to viral spike protein	Vero E6, L2 (Urbani)	Inhibit viral plaque formation by 40–70% at 15–30 μM; peptides analogous to regions of the N terminus or the pretransmembrane domain of the S2 subunit; inhibit viral plaque formation by >80% at 15–30 μM (peptides analogous to the SARS-CoV loop region)	295
siRNA, RL004, RL005	Vero E6 (Y3)	siRNA (600 pmol/liter) targeting conserved regions of SARS-CoV, ↓ virally induced CPE at 67 h	418
siRNA	FRhK-4 (HKU66078)	siRNA duplexes targeting regions in entire viral genome, ↓ virally induced CPE and viral production at 72 h	428
siRNA targeting viral RP	Vero (NM)	↓ virally induced CPE, ↓ viral production, ↓ viral protein synthesis at 1.5 or 3 μg of siRNA	351
RNA interference targeting viral RP	Vero E6, 293, HeLa (SARS-CoV-p9)	↓ expression of RP (293 and HeLa cells); ↓ plaque formation at 1 μg of siRNA	232
siRNA targeting S gene	Vero E6, 293T (BJ01)	↓ S gene expression in SARS-CoV-infected cells at 2, 3, and 4 μg of siRNA	419
siRNAs targeting S gene and 3′ untranslated region	Vero E6 (HK strain)	↓ viral antigen synthesis of 64% (by siSARS-S2), 51% (siSARS-S3), 40% (siSARS 3′ untranslated region) at 100 pmol of siRNA	379
Glycyrrhizin	Vero (FFM-1, FFM-2)	CPE assay mean (SD) CC_{50} = >20,000 mg/liter; EC_{50} = 300 (51) mg/liter (SI of >67)	77
	FRhK-4 (HKU39849)	EC_{50} at 48 h = >400 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 100 μg/ml	50
Mizoribine	Vero E6 (FFM-1)	IC_{50} = 3.5 μg/ml; CC_{50} = >200 μg/ml	293
	Vero E6 (HKU39489)	IC_{50} = 16 μg/ml	293
Ribavirin	Vero E6 (FFM-1)	IC_{50} = 20 μg/ml; CC_{50} = >200 μg/ml	293
	Vero E6 (HKU39489)	IC_{50} = 80 μg/ml	293
	FRhK-4 (HKU39849)	EC_{50} at 48 h = 50–100 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = >200 μg/ml	50
Rimantidine	FRhK-4 (HKU39849)	EC_{50} at 48 h = 16 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 8–16 μg/ml	50
Lopinavir	FRhK-4 (HKU39849)	EC_{50} at 48 h = 16 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 8–16 μg/ml	50
Baicalin	FRhK-4 (HKU39849)	EC_{50} at 48 h = 12.5 μg/ml	50
	Vero E6 (HKU39849)	EC_{50} at 48 h = 100 μg/ml	50
Aurintricarboxylic acid	Vero (NM)	EC_{50} = 0.2 mg/ml; CC_{50} = 37.5 mg/ml; SI = 187	129
Reserpine	Vero E6 (HK strain)	EC_{50} = 3.4 μM; CC_{50} = 25 μM; SI = 7.3	381
Aescin	Vero E6 (HK strain)	EC_{50} = 6 μM; CC_{50} = 15 μM; SI = 2.5	381
Valinomycin	Vero E6 (HK strain)	EC_{50} = 0.85 μM; CC_{50} = 68 μM; SI = 80	381
Niclosamide	Vero E6 (Taiwan strain)	EC_{50} = 1–3 μM; CC_{50} = 250 μM	380
Nelfinavir	Vero E6 (FFM-1)	Mean (SD) EC_{50} = 0.048 (0.024) μM; CC_{50} = 14.75 (2.75) μM; SI = 302.1	392
Chloroquine	Vero E6 (FFM-1)	Mean (SD) IC_{50} = 8.8 (1.2) μM; CC_{50} = 261.3 (14.5) μM; SI = 30	174
	Vero E6 (Urbani)	Mean (SD) EC_{50} = 4.4 (1.0) μM; refractory to infection if pretreated with chloroquine (10 μM) for 20 h	341
Indomethacin	Vero E6 (Tor2)	IC_{50} = 50 μM	4
3C-like proteinase inhibitors			
Cinanserin (SQ 10,643)	Vero (NM)	IC_{50} = 5 μM	51
TG-0205221	Vero E6 (NM)	↓ viral load by 4.7 logs at 5 μM	396
Octapeptide AVLQSGFR	Vero (BJ01)	EC_{50} = 0.027 mg/liter; CC_{50} = >100 mg/liter; SI = >3,704	101
Peptidomimetic inhibitor	NM	IC_{50} = 45–70 μM	103
Calpain inhibitors, Val-Leu-CHO	Vero E6 (Urbani)	EC_{90} = 3 μM	14
Calpain inhibitors, Z-Val-Phe-Ala-CHO	Vero E6 (Urbani)	EC_{90} = 15 μM	14
Cyclopentenyl carbocyclic nucleosides	NM	EC_{50} = 47 μM for 1,2,3-triazole analogue (17c); EC_{50} = 21 μM for 1,2,4-triazole analogue (17a)	65
Nucleoside analogue inhibitor, β-D-N^4-hydroxycytidine	Vero E6 (Urbani)	EC_{90} = 6 μM	14
Nitric oxide, S-nitroso-N-acetylpenicillamine	Vero E6 (FFM-1)	Mean (SD) IC_{50} = 222 (83.7) μM; SI = 3	173
Pyridine N-oxide derivatives	Crandel feline kidney (CRFK) and Vero (FFM-1)	Among 192 compounds tested, the oxide part on pyridine moiety was indispensable for antiviral activity with CC_{50} of 50–100 mg/liter	11
Stilbene derivatives	Vero E6 (NM)	Inhibited by compounds 17 and 19 at 0.5 mg/ml, and no significant cytotoxic effects were observed in vitro	217
Peptide-conjugated antisense morpholino oligomers (P-PMO)	Vero E6 (Tor2)	Several virus-targeted P-PMO (AUG1, AUG2, AUG3, 1AFBS, and 3UTR) consistently reduced CPE at a concn of 20 μM	246
20-mer synthetic peptides (S protein fragments)	FRhK-4 (GZ50)	IC_{50} = 24.9–113 μg/ml; IC_{90} = 0.9–15.9 μg/ml	427
Diverse small molecules,[b] MP576, HE602, and VE607	Vero (HKU39849)	EC_{50} = <10 μM	170
Adamantane-derived compounds[c]	FRhK-4 (NM)	IC_{50} = 0.5–3 μM; CC_{50} = >300 μM	328

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TABLE 8—*Continued*

Antiviral agent(s) and/or immunomodulator(s)	Study setting and methods (virus strain)	Main findings[a]	Reference
Semisynthetic derivatives of glycopeptide			
Vancomycin	Vero E6 (FFM-1)	$EC_{50} = 22$–>100 μM; $CC_{50} = >80$ μM	10
Eremomycin	Vero E6 (FFM-1)	$EC_{50} = 14$–>100 μM; $CC_{50} = 45$–>100 μM	10
Teicoplanin, ristocetin A, and DA-40926	Vero E6 (FFM-1)	$EC_{50} = >80$ μM; $CC_{50} = >80$ μM	10
Lycoris radiata (Chinese medicinal herb)	Vero E6 (BJ001, BJ006)	Mean (SD) $EC_{50} = 2.4$ (0.2) μg/ml; $CC_{50} = 886.6$ (35.0) μg/ml	212
Artemisia annua (Chinese medicinal herb)	Vero E6 (BJ001, BJ006)	Mean (SD) $EC_{50} = 34.5$ (2.6) μg/ml; $CC_{50} = 1,035$ (92.8) μg/ml	212
Pyrrosia lingua (Chinese medicinal herb)	Vero E6 (BJ001, BJ006)	Mean (SD) $EC_{50} = 43.2$ (14.1) μg/ml; $CC_{50} = 2,378$ (87.3) μg/ml	212
Lindera sp. (Chinese medicinal herb)	Vero E6 (BJ001, BJ006)	Mean (SD) $EC_{50} = 88.2$ (7.7) μg/ml; $CC_{50} = 1,374$ (39.0) μg/ml	212

[a] i.p., intraperitoneal; EC_{50}, 50% effective concentration; SI, selectivity index; NA, not available; p.i., preincubation; NM, not mentioned; IC_{50}, 50% inhibitory concentration; $TCID_{50}$, 50% tissue culture infective dose; CI, combination index (combination index of <1 indicates synergism); CPE, cell culture cytopathic effect; aa, amino acids; RP, RNA polymerase; CC_{50}, 50% cytotoxic concentration; ↓, decreased.

[b] MP576, HE602, and VE607 were validated to be inhibitors of SARS-CoV M^pro, Hel, and viral entry, respectively.

[c] Bananin, iodobananin, vanillinbananin, and eubananin were effective inhibitors of the ATPase activity of the SCV helicase.

laboratory-acquired infections, all laboratories handling live SARS-CoV should strictly comply with WHO standards for biosafety level 3 laboratories.

PASSIVE IMMUNIZATION AND DEVELOPMENT OF A SARS-CoV VACCINE

Use of Convalescent-Phase Serum and Neutralizing Antibody

Passive immunization using convalescent plasma with high titers of neutralizing antibody has been used for SARS patients who continued to deteriorate. No significant adverse reactions were noted, with perhaps some clinical benefit in a retrospective analysis (60, 401). Currently, only hyperimmune globulin produced from plasma from convalescent patients and equine plasma produced by immunization with inactivated SARS-CoV are available for prophylactic trials in humans (233, 421). 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 (312). One of the single-chain variable region fragments, 80R, blocks spike-ACE2 receptor interactions through binding to the S1 domain. In a murine model of asymptomatic SARS infection, passive immunization by high titers of neutralizing antibody prevented viral replication in the lungs but was not as effective in nasal turbinates (311). Similarly, passive immunization of mice and ferrets with human IgG1 monoclonal antibody CR3014 was effective in preventing the development of lung pathology but less effective in reducing pharyngeal excretion (329). Recently, potent cross-reactive monoclonal antibodies against highly conserved sites within the spike protein, which can neutralize zoonotic or epidemic SARS-CoV, were reported (131, 434). These new weapons should be considered for clinical testing if SARS returns. Currently, there are no randomized placebo-controlled trials on the role of antibody therapy for pre- or postexposure prophylaxis in at-risk groups during the SARS epidemic.

Of all the surface proteins, only the ectodomains of S and Orf3a can induce significant neutralizing antibody with some augmentation from the M and E proteins (3, 24). The S1 fragment between amino acids 318 and 510 is the receptor binding domain for ACE2. This fragment induces the majority of the neutralizing antibody in convalescent SARS patients (135). The minor epitope for the neutralizing antibody is found at amino acids 1055 to 1192 around heptad repeat 2 of the S2 subunit. However, this minor neutralizing epitope was implicated in the induction of an infection-enhancing antibody (400). The risk of immune enhancement should not be underestimated because ferrets immunized by whole S protein carried in modified vaccinia virus Ankara developed hepatitis (355). Most of the highly immunodominant sites in S generate only nonneutralizing antibodies. It is important that only three to five amino acid changes in the receptor binding domain of S are found between the early and late isolates of human SARS (64), and even reverse-genetically-made isogenic viruses made with the spike protein from zoonotic variants and the early but not the late phase of the SARS epidemic can produce fatal disease in 1-year-old mice (289). Therefore, the receptor binding domain of S1 remains the best target for the development of a vaccine.

Active Immunization

As expected, the importance of the S protein was confirmed in the murine model using either intramuscular or intranasal administration of highly attenuated modified vaccinia virus Ankara carrying the S protein (18). Mucosal immunization of African green monkeys with recombinant attenuated parainfluenza virus–SARS-CoV S protein chimeric virus resulted in a good neutralizing antibody response and protection from viral replication in the upper and lower respiratory tracts following live SARS-CoV challenge (25). Other approaches to active immunization involved the use of an adenoviral vector carrying the S, M, and N proteins in rhesus macaques (102); subunit vaccine with S fragments in rabbits and mice (415); other vaccines derived from the SARS-CoV genome using reverse genetics, such as the attenuated rabies vector (94)-, attenuated vesicular stomatitis virus (171)-, or Venezuelan equine encephalitis virus (12, 85)-based vaccines; and S1 vaccine expressed in tomato and low-nicotine tobacco plants as a mucosal vaccine (262). A plasmid DNA vaccine carrying the S protein encoded by humanized codons was highly protective in a mouse model

TABLE 9. Passive and active immunization against SARS

Type of vaccine	Target (animal model)	Response[a]	Reference(s)
Passive immunization			
Human monoclonal antibody	S protein (ferret)	Decrease in lung viral titer, decrease in viral shedding, prevention of virally induced tissue pathology	329
	S protein (BALB/c mice)	Decrease in lung/nasal viral titers	333
	S protein (BALB/c mice)	Decrease in lung viral titers	313
Human monoclonal antibody from transgenic (HuMantibody) mice	S protein (BALB/c mice)	Decrease in lung/nasal viral titers	110
Active immunization			
Inactivated whole virus	Inactivated SARS-CoV (BALB/c mice)	Neutralizing antibodies	323
	Inactivated SARS-CoV (BALB/c mice)	Neutralizing antibodies; specific IgA in tracheal/lung wash fluid with adjuvant-added or PEG-precipitated vaccine only	274
	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 pseudotyped virus assay	134
	Inactivated SARS-CoV (BALB/c mice)	Neutralizing antibodies	306, 317
Recombinant protein fragment	S protein (BALB/c mice)	Neutralizing antibodies	19
	S protein (BALB/c mice, rabbits)	Neutralizing antibodies	415
	S protein (rabbits)	Neutralizing antibodies against receptor binding domain	133
	S protein (BALB/c mice)	Neutralizing antibodies against receptor binding domain	132
Adenoviral vector	S, M, and N proteins (rhesus macaques)	Neutralizing antibodies, T-cell responses	102
Modified vaccinia virus Ankara	S protein (BALB/c mice)	Neutralizing antibodies; decrease in lung/nasal viral titers, passive serum transfer protective	18
	S protein (BALB/c mice, rabbits, Chinese rhesus monkeys)	Neutralizing antibodies; decrease in lung/nasal viral titers	55
	S protein (ferrets)	Neutralizing antibodies; enhanced hepatitis in vaccinated ferrets when challenged with SARS-CoV	355
Recombinant attenuated HPIF3	S protein (African green monkey)	Neutralizing antibodies, decrease in viral shedding	25
Recombinant vesicular stomatitis virus	S protein (BALB/c mice)	Neutralizing antibodies, decrease in lung viral titer	171
Attenuated rabies virus	S or N protein (BALB/c mice)	Neutralizing antibodies specific to S but not with N protein	94
Recombinant Venezuelan equine encephalitis virus replicon particle	S or N protein (young and senescent BALB/c mice)	Good protection in young but partial protection in senescent mice with S vaccine; enhanced immunopathology with N vaccine	85
DNA vaccine	S protein (BALB/c mice)	Neutralizing antibodies, T-cell responses; decrease in lung/nasal viral titers; serum transfer protective	399
	S protein (BALB/c mice)	Neutralizing antibodies, T-cell responses	123, 412
	E, M, and N proteins (BALB/c mice)	DNA encoding N induced highest neutralizing antibodies and T-cell responses	164
	N protein (C3H/He mice)	Specific antibodies, T-cell responses	433
	N protein (C57BL/6 mice)	T-cell responses; decrease in surrogate viral titer in lungs	176
	S protein (rabbits)	Neutralizing antibodies	348
	N protein (mice)	Specific antibodies, N-specific splenocyte proliferation responses, DTH and CD8+ CTL responses	424
DNA vaccine boosted by recombinant protein produced in *E. coli*	S protein (BALB/c mice)	Very high S-specific neutralizing antibodies	370

[a] PEG, polyethylene glycol; RBD, receptor-binding domain; DTH, delayed-type hypersensitivity; CTL, cytotoxic T cell.

(412). The use of other targets such as inactivated whole virus in mice (323), DNA vaccine linking the N protein to calreticulin (176), DNA vaccination with the N gene in mice (433), and virus-like particles has also been reported. Only the inactivated whole-virus vaccine was tested in healthy Chinese volunteers, who showed good neutralizing antibodies with little side effects, but the data have not been published. However, the protective efficacy and risk of immune enhancement are still unknown in the situation of an epidemic.

As for the key protective immune effector in the mouse model, T-cell depletion with specific monoclonal antibodies against CD4 or CD8, alone or in combination with CD90, did not affect protective immunity, which was confirmed by adoptive T-cell transfer (399). 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 9), all vaccines based on the S protein appeared to be capable of inducing neutralizing antibody responses, and those based on nucleoprotein can induce nucleoprotein-specific cell-mediated immunity. However, only vaccines based on the S protein were shown to be protective in animal models, whereas a DNA vaccine based on the N protein induced

TABLE 10. Animals tested for susceptibility to SARS-CoV in experimental and natural infection[a]

Animal species and age	Dose and route of inoculation (virus strain)	Point of evaluation (days)	Main findings	Reference
Cynomolgus macaques (*Macaca fascicularis*)	10^3 to 10^6 TCID$_{50}$; i.n., i.v., conjunctival (NM)	Up to 16	Lethargy from 3 dpi, respiratory distress from 4 dpi, died with severe multifocal pulmonary consolidation and histologically interstitial pneumonia, diffuse alveolar damage, necrosis of alveolar and bronchiolar epithelium, and alveolar edema with proteinaceous fluid admixed with fibrin, erythrocytes, alveolar macrophages, and neutrophils	98
Cynomolgus macaques (*Macaca fascicularis*); adult	10^6 TCID$_{50}$; i.n., IT, conjunctiva (strain from patient 5688)	6	Excreted SARS-CoV from nose, mouth, and pharynx from 2 dpi; diffuse alveolar damage with epithelial necrosis, serosanguineous exudate, hyaline membrane formation, type 2 pneumocyte hyperplasia, and syncytium formation	182
Cynomolgus macaques and rhesus macaques	10^7 PFU; IT, i.v. (Tor2)	12	Mild self-limited respiratory infection	291
African green, rhesus, and cynomolgus monkeys; juvenile	10^6 TCID$_{50}$; i.n., IT (Urbani)	Up to 28	SARS-CoV replicated in the respiratory tract but did not induce illness; moderate to high titers of SARS-CoV excretion with associated interstitial pneumonitis detected in lungs of African green monkeys on 2 dpi and resolved by 4 dpi	239
Rhesus macaques (*Macaca mulatta*); 1–3 yr	10^3, 10^5, 10^7 TCID$_{50}$; i.n. (PUMC01)	Up to 60	Transient fever occurred 2–3 dpi; SARS-CoV-specific IgGs detected in sera of macaques from 11 to 60 dpi; SARS-CoV RNA detected in pharyngeal swab samples 5 dpi; histopathological changes of interstitial pneumonia during the 60 dpi	272
Cynomolgus macaques (*Macaca fascicularis*); adult	1.25×10^6 PFU; i.n., i.v., conjunctiva (Urbani)	Up to 20	Mild to moderate symptomatic illness; evidence of viral replication and neutralizing antibodies; chest X-ray unifocal or multifocal pneumonia peaked between 8 and 10 dpi; inoculation by mucosal route produced more prominent disease than that with i.v. inoculation	194
BALB/c mice; 4 wk	2×10^5 TCID$_{50}$; i.n., oral (Urbani)	3, 5, 7, 10, and 28	Viral replication in lung and intestinal tissue but caused subclinical infection or very mild disease	357
BALB/c mice; 4–6 wk	10^5 TCID$_{50}$; i.n. (Urbani)	1, 2, 3, 5, 7, 9, and 11	Peak replication in the absence of disease on 1 or 2 dpi; viral clearance within a wk; viral antigen and nucleic acid detected in bronchiolar epithelial cells during peak viral replication	311
C57BL/6 mice; 5–6 wk	10^4 TCID$_{50}$; i.n. (Urbani)	Up to 15	Infected mice had transient infection with relative failure to thrive; viral replication to high levels in lungs of these mice, which peaked on 3 dpi and cleared by day 9 dpi	105
BALB/c mice; 12–14 mo	10^5 TCID$_{50}$; i.n. (Urbani)	1, 2, 5, 9, and 13	Viral replication in aged mice was associated with clinical illness and pneumonia demonstrating an age-related susceptibility to SARS disease	287
129SvEv mice (Stat1$^{+/-}$)	10^6 PFU; i.n. (Tor2)	1, 3, 5, 8, 11, 15, and 22	Stat1-deficient mice had viral replication of virus in lungs and progressively worsening pulmonary disease with inflammation of small airways and alveoli and systemic viral spread to livers and spleens	143
BALB/c mice; 6–8 wk	10^5 TCID$_{50}$; i.n. (Urbani of 15 passages [MA15] in BALB/c mice)	6	Lethality preceded by high-titer viral replication in lungs, viremia, and dissemination to extrapulmonary sites accompanied by lymphopenia, neutrophilia, and pathological changes in lungs; death from an overwhelming viral infection with extensive virally mediated destruction of pneumocytes and ciliated epithelial cells; the MA15 virus has six coding mutations (in *Orf1a*, *Orf1b*, S gene, and M gene) associated with adaptation and increased virulence	286
Transgenic mice expressing hACE2 (K18-hACE2)	2.3×10^2–2.3×10^4 PFU; i.n. (Urbani)	7[b]	Weight loss by 3–5 dpi and died by 7 dpi; infection begins in airway epithelia with subsequent alveolar involvement and extrapulmonary virus spread to the brain; infection results in macrophage and lymphocyte infiltration in the lungs and up-regulation of proinflammatory cytokines and chemokines in both the lung and the brain	240
Transgenic mice expressing hACE2 (AC70)	10^3 or 2×10^5 TCID$_{50}$; i.n. (Urbani)	8	Developed acute wasting syndrome and died within 4–8 dpi; high levels of virus replication detected in lungs and brain; pathological examination suggests that the extensive involvement of the central nervous system likely contributed to the death of mice, even though viral pneumonia was present	337
F344 rat; adult	100 μl[c]; i.n. (Frankfurt 1 after 10 passages)[d]	3, 5, 7, and 21	Adult rats showed respiratory symptoms and severe pathological lung lesions; inflammatory cytokine levels in sera and lung tissues were significantly higher in adult than in young rats	244
Golden Syrian hamsters; 5 wk	10^3 TCID$_{50}$; i.n. (Urbani)	2, 3, 5, 7, 10, and 14	Little clinical evidence of disease; viral replication in epithelial cells of the respiratory tract with early cellular necrosis, followed by an inflammatory response coincident with viral clearance; focal consolidation in lung; viremia and extrapulmonary spread of SARS-CoV to liver and spleen despite high levels of virus replication and associated pathology	288

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TABLE 10—*Continued*

Animal species and age	Dose and route of inoculation (virus strain)	Point of evaluation (days)	Main findings	Reference
Common marmosets (*Callithrix jacchus*), small nonhuman primate; juvenile or young adult	10^6 TCID$_{50}$; IT (Urbani)	2, 4, and 7	Mild clinical disease; multifocal mononuclear cell interstitial pneumonitis, multinucleated syncytial cells, edema, and bronchiolitis; viral antigen localized to infected alveolar macrophages and type 1 pneumocytes; viral RNA detected in all animals from pulmonary tissue and in some cases from tracheobronchial lymph node and myocardium with inflammatory changes; multifocal lymphocytic hepatitis with piecemeal necrosis	111
Cats	10^6 TCID$_{50}$; IT (patient 5688)	4	Asymptomatic shedding of virus from pharynx	237
Ferrets	10^6 TCID$_{50}$; IT (patient 5688)	4	3 out of 6 ferrets became lethargic from 2–4 dpi with viral replication and pneumonitis	237
Ferrets (*Mustela putorius furo*)	10^6 TCID$_{50}$; i.n. (Tor2)	Up to 29	Asymptomatic viral replication but significant hepatitis occurred only after vaccination with modified vaccinia virus Ankara carrying SARS-CoV spike protein	355
Masked palm civets (*Paguma larvata*); 1 yr	3×10^6 TCID$_{50}$; i.n., IT (BJ01[e] and GZ01[f])	0, 3, 8, 13, 18, 23, 28, and 33	Fever, lethargy, and loss of aggressiveness; infection was confirmed by virus isolation, detection of viral genomic RNA, and serum neutralizing antibodies	382
Guinea pigs	Up to $10^{4.7}$ TCID$_{50}$; i.p. (coinfected with SARS-CoV and reovirus)	NM	Guinea pigs infected with SARS-CoV developed only interstitial pneumonia without clinical diseases; death and severe pulmonary pathology were seen in those coinfected with reovirus	219
Chicken; 6 wk	10^6 PFU; i.n., i.v., oral, ocular (Tor3)	6, 7, 13, 14, 15, and 16	No disease, pathology, or viral shedding; no antibody response	356
Pigs; 6 wk	2×10^6 PFU; i.n., i.v., oral, ocular (Tor3)	6, 7, 13, 14, 15, and 16	No disease, pathology, or viral shedding; neutralizing antibodies present	356
Himalayan palm civet	Natural infection	NA	Viral shedding demonstrated by culture and RT-PCR in nasal and fecal samples; sequence analysis showed that all animal isolates retain a 29-nucleotide sequence not found in most human isolates	117
Raccoon dog (*Nyctereutes procyonoides*)	Natural infection	NA	Viral shedding demonstrated by culture and RT-PCR in nasal and fecal samples; sequence analysis showed that all animal isolates retain a 29-nucleotide sequence not found in most human isolates	117
Bats (*Rhinolophus sinicus*)	Natural infection	NA	Coronavirus closely related to SARS-CoV (bat SARS-CoV) in 39% of anal swabs by using RT-PCR with a viral load of 3.89×10^2–2.21×10^7; bat SARS-CoV genome is closely related to SARS-CoV from humans and civets; antibody against recombinant bat SARS-CoV nucleocapsid protein was detected in 84% of Chinese horseshoe bats	190
Bats (family Rhinolophidae)	Natural infection	NA	SARS-CoV antibody in 28.3% of *Rhinolophus pearsoni* and 33.3% of *Rhinolophus pussilus* isolates from Guangxi and 71% of *Rhinolophus macrotis* isolates from Hubei; RT-PCR positive for a SARS-like virus in 12.5% of *Rhinolophus ferrumequinum* and 12.5% of *R. macrotis* isolates from Hubei and 10% of *R. pearsoni* isolates from Guangxi	215

[a] TCID$_{50}$, 50% tissue culture infective dose; i.n., intranasal; i.v., intravenous; NM, not mentioned; dpi, day postinfection; IT, intratracheal. i.p., intraperitoneal; NA, not applicable.
[b] All mice died that day after infection.
[c] Concentration of virus inoculation was not mentioned.
[d] SARS-CoV Frankfurt 1 isolate was serially passaged in young F344 rats (4 weeks) 10 times before experimental inoculation.
[e] BJ01, with a 29-nucleotide deletion.
[f] GZ01, without the 29-nucleotide deletion.

immunopathology of lungs in mice after challenge with live virus (85).

The relative importance of systemic or mucosal immunity in terms of the neutralizing antibody or cytotoxic T-lymphocyte response against S, N, or other targets in terms of recovery from SARS is unknown. Nevertheless, neutralizing antibody against S1 appears to be crucial for prophylactic immunity. Live-attenuated virus is not a good choice because of the concern about reversion to virulence or recombination with wild strains to form new wild types. An inactivated SARS-CoV strain is the easiest and most likely candidate for clinical trials if SARS returns. Irrespective of

the approach to immunization, the phenomenon of immune enhancement of disease in feline peritonitis coronavirus infection is also a cause for concern in view of the immunopathology seen in immunized ferrets and mice after challenge with wild-type SARS-CoV.

ANIMAL MODELS AND ANIMALS SUSCEPTIBLE TO SARS-CoV

Reproducible and consistent animal models that mimic the clinical, viral load, and histopathological changes of SARS are essential for proving causation, studying pathogenesis, and

testing antivirals or immunization (Table 10). The Koch's postulates for SARS-CoV as a causative agent of SARS were fulfilled with a primate model using cynomolgus macaques (*Macaca fascicularis*), which demonstrated clinical and pathological features with some similarities to those found in humans (182). On the contrary, African green monkeys (*Cercopithecus aethiops*) did not develop significant lung pathology after inoculation with the SARS-CoV. The lack of consistency in primate animal models of rhesus, cynomolgus, and African green monkeys for experimental SARS was noted in another study (239). Moreover, these large mammals are expensive and difficult to handle. BALB/c mice demonstrated asymptomatic or mild infections in lungs and nasal turbinates by intranasal inoculation, which was not significantly different from the findings with inoculation of immunological Th1-biased C57BL/6 mice (105). BALB/c mice that were 12 to 14 months old developed symptomatic pneumonia, which correlated with the age-related susceptibility to acute SARS in humans (287). As expected, STAT-1 knockout-immunodeficient mice had fatal and disseminated disease (143). Transgenic mice expressing human ACE2 receptors also developed fatal disease, with extrapulmonary dissemination to many organs including the brain (240, 337). It is interesting that mouse-adapted SARS-CoV strains with six amino acid mutations can also cause fatal disseminated disease in young BALB/c mice (286). Adult F344 rats developed symptomatic disease after inoculation with passaged SARS-CoV strains containing one mutation in the receptor binding domain of S (244). Ferrets (*Mustela furo*) and domestic cats (*Felis domesticus*) were also susceptible to infection by SARS-CoV (237). The cats remained asymptomatic, and only some of the infected ferrets died of the disease. Very high levels of viral replication were found in infected golden Syrian hamsters, but they generally did not develop overt clinical disease (288). Similarly, inoculated common marmosets generally had mild clinical disease and histopathological changes of pneumonia with extrapulmonary dissemination and high levels of viral replication in affected tissues (111). As expected, palm civets (*Paguma larvata*) were shown to be susceptible to symptomatic infection by SARS-CoV with or without the 29-bp signature sequence (382). Pigs and chickens are not susceptible to SARS-CoV (356). Since different SARS-CoV isolates were used by different groups, it is therefore still uncertain whether one particular animal would be better than others as a model for SARS-CoV. It appears that the senescent BALB/c mouse is an inexpensive and relatively easily reproduced animal model for testing vaccines and antivirals for SARS. An important observation of this review is the diverse range of mammalian species that are susceptible to experimental infection by SARS-CoV, which again demonstrated that SARS-CoV is highly capable of jumping interspecies barriers and is an excellent candidate as an emerging or reemerging pathogen. Indeed, our first report on animal SARS-CoV showed that Chinese ferret badgers (*Melogale moschata*) and raccoon dogs (*Nyctereutes procyonoides*) were also infected with SARS-CoV (117). The recent discovery of a high proportion of Chinese horseshoe bats and subsequently other horseshoe bats shedding SARS-CoV-like viruses or being seropositive strongly suggested that the bats could be the natural reservoir of this group of viruses (190, 215).

SHOULD WE BE READY FOR THE REEMERGENCE OF SARS?

The medical and scientific community demonstrated marvelous efforts in the understanding and control of SARS within a short time, as evident by over 4,000 publications available online. Despite these achievements, gaps still exist in terms of the molecular basis of the physical stability and transmissibility of this virus, the molecular and immunological basis of disease pathogenesis in humans, screening tests for early or cryptic SARS cases, foolproof infection control procedures for patient care, effective antivirals or antiviral combinations, the usefulness of immunomodulatory agents for late presenters, an effective vaccine with no immune enhancement, and the immediate animal host that transmitted the virus to caged civets in the market at the beginning of the epidemic. Coronaviruses are well known to undergo genetic recombination (375), which may lead to new genotypes and outbreaks. The presence of a large reservoir of SARS-CoV-like viruses in horseshoe bats, together with the culture of eating exotic mammals in southern China, is a time bomb. The possibility of the reemergence of SARS and other novel viruses from animals or laboratories and therefore the need for preparedness should not be ignored.

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