The Immunobiology of SARS*

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Key Words

SARS-CoV, ACE2, immune response, chemokines, cytokines

Abstract

Severe acute respiratory syndrome (SARS) presented as an atypical pneumonia that progressed to acute respiratory distress syndrome in ~20% of cases and was associated with a mortality of about 10%. The etiological agent was a novel coronavirus (CoV). Angiotensin-converting enzyme 2 is the functional receptor for SARS-CoV; DC-SIGN and CD209L (L-SIGN) can enhance viral entry. Although the virus infects the lungs, gastrointestinal tract, liver, and kidneys, the disease is limited to the lungs, where diffuse alveolar damage is accompanied by a disproportionately sparse inflammatory infiltrate. Pro-inflammatory cytokines and chemokines, particularly IP-10, IL-8, and MCP-1, are elevated in the lungs and peripheral blood, but there is an unusual lack of an antiviral interferon (IFN) response. The virus is susceptible to exogenous type I IFN but suppresses the induction of IFN. Innate immunity is important for viral clearance in the mouse model. Virus-specific neutralizing antibodies that develop during convalescence prevent reinfection in animal models.

INTRODUCTION

SARS: severe acute respiratory syndrome

CoV: coronavirus

Acute respiratory distress syndrome (ARDS): a clinical syndrome of acute lung injury and severe acute respiratory failure caused by a variety of processes that directly or indirectly injure the lung. ARDS is characterized by pulmonary edema, respiratory distress, and hypoxemia. The underlying pathological process is termed diffuse alveolar damage (DAD)

Severe acute respiratory syndrome (SARS) was unprecedented in the rapidity and extent of its spread, in the magnitude of its impact on health systems and economies, and in the effectiveness of public health measures that were implemented for its control. However, progress in studying this new viral disease was also rapid. Investigators described the clinical syndrome (1, 2), identified the etiological agent (3–6), devised diagnostic tests (6), and completely sequenced the genome (7, 8) within weeks of the first reports of the illness. Previous reviews have addressed issues on the clinical presentation (9), etiology and laboratory diagnosis (10), epidemiology and virology (11), animal models (12), vaccines and therapeutics (13, 14), and public health (15).

Although much has been learned in the three years since its discovery, many aspects of the pathogenesis of the disease are still not fully understood because there has not been an outbreak of SARS since 2003 and because no single animal model accurately reflects the spectrum of human disease. Our understanding of the pathogenesis of SARS is based largely on the lessons from the 2002–2003 outbreak that are constrained by several important caveats. First, relatively little is known about the early events in SARS. Second, few studies focused on the primary site of disease, the lung. Third, although several studies report cytokine and chemokine responses in the plasma or serum, these findings may not reflect what is happening in the lung. Fourth, clinical evaluations were carried out at different time points in the course of the disease. Finally, each investigator did not necessarily look for (or find) the same immune mediators in the samples that they tested, so some observations have not been confirmed. We have attempted to synthesize the available information, and we focus this review on the source of the virus and evidence of adaptive change, cellular entry of the virus, pathology seen in SARS, consequences of the virus-host interaction, proposed mechanisms of acute lung injury, the host immune response, and the use of animal models for the evaluation of immunoprophylaxis and vaccines.

THE DISEASE AND THE VIRUS

SARS emerged in Guangdong Province, China, in November 2002 and spread rapidly to several countries; within weeks, the disease had spread to infect more than 8,000 people in 29 countries across 5 continents, with 774 deaths reported by the World Health Organization (WHO, **http://www.who.int/csr/sars/country/table2003_09_23/en/**) (13). The etiological agent of the disease was identified as a previously unrecognized coronavirus (CoV) that likely entered the human population from an animal reservoir by way of the wet markets in southern China (16). The virus was transmitted from person to person via airborne droplets and close contact (17, 18).

Clinically, patients with SARS presented with an atypical pneumonia (1–3, 6, 9, 19). The illness was characterized by fever, dyspnea, lymphopenia, and rapidly progressing changes on radiography. Upper respiratory tract symptoms were not prominent, but watery diarrhea was reported. Approximately 40% to 70% of patients developed diarrhea during their illness, with virus detected in the feces, potentially providing a route of virus spread (**http://www.info.gov.hk/info/ap/pdf/amoy_e.pdf**) (20). There was no response to conventional antibiotics used to treat pneumonia (21). In two-thirds of infected patients, the disease progressed, and chest radiography revealed changes compatible with viral pneumonitis. Respiratory insufficiency leading to acute respiratory distress syndrome (ARDS) and respiratory failure was the main cause of death among fatal cases of SARS. The virus was isolated or was identified by reverse transcription polymerase chain reaction (RT-PCR) (22, 23). Lymphopenia, decreased platelet counts,

prolonged coagulation profiles, and mildly elevated serum hepatic enzymes were also detected in affected individuals during the acute phase of the disease (24).

Age was a determinant of disease severity and mortality in SARS. During the outbreak, mortality rates among infected individuals in Hong Kong who were 0 to 24, 25 to 44, 45 to 64, and older than 65 years of age were 0%, 6%, 15%, and 52%, respectively (**http://www.who.int/csr/sars/archive/2003_05_07a/en/**) (11). None of the children younger than 12 years of age who were infected with SARS-CoV in Hong Kong had disease severe enough to require intensive care or mechanical ventilation (25, 26). The underlying biological basis of this pattern of age-specific morbidity and mortality remains unclear. Several possible explanations have been suggested, such as the possibility that age-associated severity of disease may be related to interferon (IFN) levels, that the course of infection is influenced by coinfection with other pathogens that interfere with the IFN-mediated antiviral response (27), or that immunopathology is caused by prior cross-reactive immunity.

At the outset, the cause of SARS was not known, and specific therapy was not available. In Guangdong, many of the early sporadic cases had epidemiological links to the live animal market trade, but in the outbreaks associated with person-to-person spread, healthcare workers were affected in large numbers. The outbreak was ultimately brought under control through a concerted global effort coordinated by the WHO, including patient isolation, intensive infection control in hospitals, quarantine measures, and travel advisories. By July 5, 2003, no further human-to-human transmission was reported, and the global outbreak was declared over (10). Four cases of community-acquired SARS occurred in 2003–2004, but these cases were mild, and the infection did not spread to contacts. There were four cases of laboratory-acquired SARS-CoV infection since 2003; the illness was mild in three of these cases, but one was severe and was associated with secondary spread to care providers.

THE SOURCE OF SARS-CoV

Different CoVs infect avian and mammalian species, but none of the previously identified human CoVs caused severe illness in humans. The lack of serological evidence of previous infection in healthy humans prompted speculation that interspecies transmission from animals to humans was the most likely explanation for the emergence of SARS-CoV. The hypothesis was strengthened by evidence that the early cases of SARS reported in Guangdong in November and December 2002 had epidemiological links to the wild animal trade (28–30).

Wet markets are commonplace across Southeast Asia. In parts of southern China, Guangdong province in particular, increasing affluence has resulted in large markets that house a diverse range of animals, including reptile and mammalian species, to supply the restaurant trade with exotic meats. These wet markets in Guangdong likely provided the interface for transmission to humans.

Studies conducted on animals sampled from live animal markets in Guangdong, China, showed that masked palm civets (*Paguma larvata*), raccoon dogs (*Nyctereutes procyonoides*), and Chinese ferret badgers (*Melogale moschata*) had been infected by SARS-CoV (16). The animal CoV identified in civet cats showed high sequence identity with, but was distinct from, SARS-CoV. Compared with animal SARS-CoV-like viruses and early human SARS-CoV strains, viruses isolated later during the outbreak had a 29-nucleotide deletion in open reading frame (ORF) 8 (16). Serologic evidence of infection was also found in animal workers without a history of a SARS-like disease (16, 28), suggesting that the live animal markets were a site where the interspecies transfer of an animal precursor virus to humans could occur. However, subsequent surveys failed to find evidence of widespread infection in farmed or wild civets

zoonotic virus: a virus that infects animals and is transmitted from animals to humans

RBD: receptor-binding domain

(31), while experimental infection of palm civets with two different human isolates of SARS-CoV resulted in overt clinical illness (32). Although palm civets in the wet markets may have been the source of the human infection that precipitated the SARS outbreak, this and other animal species in the markets may not be the reservoirs of the virus in nature. The presence of virus in palm civets in the wet markets and the absence of the virus in those on farms suggest that palm civets became infected in the market or during transportation to the market from other infected animals or by reactivation of a latent infection.

In September 2005, two research groups independently identified a virus from Chinese horseshoe bats that was genetically closely related to human SARS-CoV, suggesting that bats may be a reservoir from which the SARS-CoV that infected humans and palm civets emerged (33, 34). Bats are reservoir hosts of several zoonotic viruses, including the Hendra and Nipah paramyxoviruses that have recently emerged in Australia and East Asia, respectively, and that cause encephalitis and respiratory disease in humans (35, 36). Bats are well suited to transmit zoonotic diseases: They are genetically diverse, live longer than most small mammals, roost in clusters, and fly long distances. Bats can be persistently infected with many viruses but rarely display clinical symptoms (37). These characteristics and the increasing presence of bats and bat products in food and traditional medicine markets in southern China and elsewhere in Asia provide a plausible route of infection to palm civets that led investigators to survey bats in the search of the natural reservoir of SARS-CoV.

Nucleic acids of SARS-CoV-like viruses were identified from the fecal samples of bats from the wild and Chinese markets. Antibodies against human SARS-CoV were also detected in blood samples from the animals. Sequence analysis of the bat SARS-CoV showed that these members of the CoV family displayed great genetic variation and that some were closely related to the SARS-CoV identified from humans and palm civets. However, attempts to isolate the virus from bats have not been successful, so current information is limited to genetic sequence data and serology. The ability of CoVs to recombine and the high rate of mutation of RNA viruses may allow the generation of variant viruses that can adapt to new hosts and cross the species barrier. Palm civets conceivably became infected from bats or another animal host in the wild or in the live animal markets of southern China, where they are sold as food. Further surveillance in animals will help us better understand the animal reservoir of SARS-CoV-like viruses in nature.

THE PATHOGENESIS OF SARS

Cellular Entry of the Virus

Spike protein: the surface antigen of SARS-CoV. Coronaviruses, including SARS-CoV, associate with cellular receptors to mediate infection of target cells via the surface spike protein (S protein) (38). Studies using pseudotyped lentiviruses, carrying the S, membrane (M), and envelope (E) proteins of SARS-CoV separately and in combination, demonstrated that the S protein is both necessary and sufficient for virus attachment to susceptible cells (38, 39). The SARS-CoV S protein uses a mechanism similar to that of class I fusion proteins (40), such as HIV gp160, influenza virus hemagglutinin, and paramyxovirus F protein, in mediating membrane fusion. The N-terminal half of the S protein (S1) contains the receptor-binding domain (RBD), and the C-terminal half (S2) is the membrane-anchored subunit that contains a putative fusion peptide and two heptad repeat regions (HR1 and HR2) (41, 42). Entry of SARS-CoV requires three stages: receptor binding, a conformational change in the S protein, followed by cathepsin L–mediated proteolysis within endosomes (43–46).

Apart from direct membrane fusion at the target cell surface, SARS-CoV might also gain

entry into cells through pH-dependent endocytosis mediated by the S protein (39). S protein–driven, cell-to-cell fusion can also occur in the absence of low pH (47, 48). Thus, the S protein of SARS-CoV might be able to mediate membrane fusion in both pH-dependent and -independent fashions.

ACE2: a functional receptor for SARS-CoV and its role in acute lung injury. Identification of virus receptors can provide insight into mechanisms of virus entry, tissue tropism, pathogenesis, and host range. Investigators have identified the receptors for different groups of coronaviruses: The mouse hepatitis virus receptors are the murine carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) and the related murine glycoproteins in the carcinoembryonic antigen family, and both are members of the Ig superfamily (49). The receptors for group I CoV, including HCoV-229E, transmissible gastroenteritis virus of swine, and feline CoV, are aminopeptidase N glycoproteins (50–52).

A metallopeptidase, ACE2 (angiotensin-converting enzyme 2), that binds to the S protein of SARS-CoV is a functional receptor for SARS-CoV (53, 54). The RBD lies between amino acids 270 and 510 of the S protein (55). ACE2 protein is reportedly present in human lung alveolar epithelial cells (type I and type II pneumocytes), enterocytes of the small intestine, the brush border of the proximal tubular cells of the kidney, and the endothelial cells of arteries and veins and arterial smooth muscle cells in several organs (56). The localization of ACE2 may explain the tissue tropism of SARS-CoV for the lung, small intestine, and kidney (57).

Notably, ACE2 is not expressed on T or B cells or macrophages in the spleen or lymphoid organs (56) and there are conflicting reports on the expression of ACE2 in the upper respiratory tract. Hamming et al. (56) reported that only the basal layer of the nonkeratinized squamous epithelium of the upper respiratory tract expresses ACE2, but Sims and colleagues (58) found abundant ACE2 expression on the luminal surface of ciliated cells in freshly excised human nasal and tracheobronchial tissue. SARS-CoV infects ACE2-expressing ciliated cells of well-differentiated human airway epithelium in culture (58, 59). These cells can be used as an in vitro model to study SARS-CoV replication and pathogenesis.

The presence of ACE2 does not fully explain findings in different organs in SARS. For example, although colonic enterocytes and liver cells reportedly lack ACE2 protein expression, virus has been detected in the colon and hepatocytes (56, 60). In contrast, although ACE2 is expressed on the endothelial cells of small and large arteries and veins and the smooth muscle cells of the intestinal tract, there is no evidence of virus infection in any of these cells (56). The absence of virus infection in tissues that express the putative receptor prompts the question of whether a coreceptor or other cellular properties are required for successful virus infection.

ACE2 is a homolog of ACE. It is thought to be an essential regulator of cardiac function and blood pressure control that negatively regulates the renin-angiotensin system by inactivating angiotensin II (Ang II) (61, 62). In a murine model of acute lung injury (ALI), Imai et al. (63) reported a protective role of ACE2 that was mediated by inactivation of Ang II. Loss of ACE2 expression precipitates ALI, and ACE2-knockout mice were more severely affected by acid aspiration, a method of experimentally inducing ALI, than were wild-type mice. The receptors for Ang II in mice are Ang II type 1a (AT1a), type 1b (AT1b), and type 2 (AT2) receptors, of which AT1a and AT2 are present in lungs of mice. AT1a and AT2 receptors have opposite functions in controlling severity of lung injury; loss of the AT1a receptor improves lung function, and loss of the AT2 receptor aggravates lung injury. In models of ALI, there was a marked downregulation of ACE2, whereas ACE levels remained constant. The resulting increase of Ang II drives severe lung failure through

ACE2: angiotensin-converting enzyme 2

ALI: acute lung injury

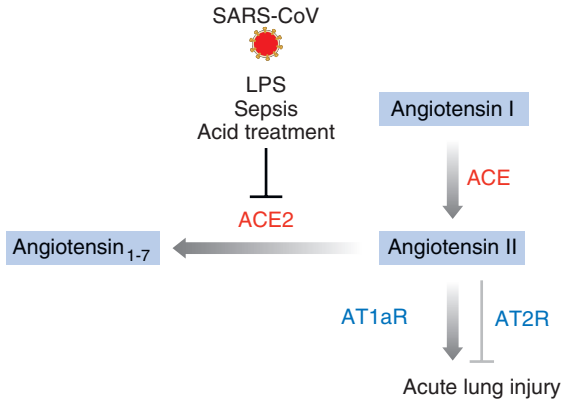

Figure 1

Role of ACE2 in acute lung injury. ACE converts angiotensin I to angiotensin II, which binds either to angiotensin II receptor 1a (AT1aR), leading to tissue damage and lung edema, or to angiotensin II receptor 2 (AT2R), reducing tissue damage in the lung. ACE2 converts the potent angiotensin II to a less damaging angiotensin $_{1-7}$. SARS-CoV binds to ACE2 and downregulates ACE2 on infected cells. ACE levels are not affected. This results in an increase in angiotensin II, which enhances lung injury through AT1aR.

the AT1a receptor. ACE promotes lung injury, whereas ACE2 alleviates it (**Figure 1**).

Experimental SARS-CoV infection of mice resulted in considerably reduced ACE2 expression in the lungs. Intraperitoneal injection of recombinant SARS-CoV S protein aggravated ALI in mice, and the effect of S protein on ALI was ACE2 specific (64). These observations provide a possible molecular explanation for the severe lung failure and lethality associated with SARS. Notably, this mechanism has been shown in a mouse model using SARS-CoV S protein but has not yet been confirmed in humans or animals infected with SARS-CoV (65).

Nicholls & Peiris (66) have reviewed the questions that remain despite these insights into the pathogenesis of SARS. Conflicting results obtained by RT-PCR, northern blotting, and immunohistochemical (IHC) detection of ACE2 in human tissues leave the organ and cell expression of ACE2 unresolved (56, 67). Furthermore, SARS-CoV infection in mice does not produce the typical diffuse alveolar damage (DAD) seen in human disease (68). Moreover, the newly discovered human CoV NL63 also binds ACE2 (69). Unlike SARS-CoV, NL63 is a ubiquitous human pathogen that is not generally associated with severe lung damage. Thus, the use of ACE2 as a viral receptor does not always result in ALI, leading to DAD.

The catalytic domain of ACE2 participates in binding to S protein and in SARS-CoV infection. In particular, lysine at amino acid 353 and, to a lesser extent, residues 82 to 84 are important residues in this interaction (70). Li and colleagues (70) studied the affinity of binding of S protein derived from human and palm civet SARS-CoV isolates with cognate ACE2 proteins (**Table 1**). The S protein of human SARS-CoV (TOR2) bound equally well to human ACE2 (hACE2) and palm civet ACE2, whereas the palm civet S protein (SZ3) preferentially bound palm civet ACE2. The RBD of the S protein of human SARS-CoV isolates differed from those isolated from apparently healthy palm civets in the wet markets in China at four residues, K344R, F360S, N479K, and T487S. Of these residues, 479 and 487 were critical for high-affinity association with hACE2. Residue 479 of the S protein RBD interacts with residues along α-helix 1, particularly lysine 31 of ACE2, which is present in human but not palm civet ACE2 (**Table 1**). Threonine at residue 487 was absolutely conserved in human SARS-CoV isolates from 2002–2003, whereas animal SARS-CoV isolates have serine at this position. Mutagenesis of amino acid 487 from serine to threonine increased binding of the SZ3 S protein to hACE2 (71).

Additional receptors for SARS-CoV entry into cells: DC-SIGN and L-SIGN. Dendritic cells (DCs) are crucial in host defense against pathogens. Invading pathogens are recognized by Toll-like receptors (TLRs) and receptors such as C-type lectins expressed on the surface of DCs. Some pathogens, including viruses such as HIV-1 and nonviral pathogens such as *Mycobacterium tuberculosis*, subvert functions of DCs to escape immune surveillance by targeting the

Diffuse alveolar damage (DAD): the pathologic injury underlying ARDS that results from severe injury to the alveolar-capillary unit. DAD occurs in three overlapping phases: exudative, proliferative, and fibrotic

Table 1 The amino acid sequence of RBD of the S protein of SARS-CoV determines the interaction with human and palm civet ACE2

ACE2 from indicated species	Critical residues for S protein binding	Binding of specific residues in the RBD of the S protein of indicated virus with critical residues of ACE2			
		TOR2		SZ3	
		479N/S	487T	479K	487S
Human	K31	++	−	−	−
	K353	−	++	−	+
Palm civet	T31	++	−	++	−
	K353	−	++	−	+++

C-type lectin, DC-specific intercellular adhesion molecule–grabbing nonintegrin (DC-SIGN/CD209) (72). DC-SIGN is a type II transmembrane adhesion molecule with a C-type lectin domain that recognizes carbohydrate residues on a variety of pathogens. It is expressed on dermal DCs as well as in the mucosal tissues by interstitial DCs in the lungs, intestine, rectum, cervix, and placenta and in lymph nodes (73, 74).

DC-SIGN functions as a receptor or coreceptor for several viruses, including HIV-1, Ebola virus, cytomegalovirus (CMV), hepatitis C virus, and dengue virus, by interaction with viral envelope glycoproteins that contain a relatively large number of N-linked carbohydrates (75–79). DC-SIGN serves as a receptor in *trans* for SARS-CoV (39). Unlike ACE2 on pneumocytes and enterocytes, binding to DC-SIGN does not facilitate viral infection of the DCs but allows the cells to transfer infectious SARS-CoV to susceptible target cells. A similar mechanism has been described for dengue virus, HIV-1, and CMV, and it may be relevant in SARS pathogenesis (75, 77, 80). Adaptation of pathogens to target DC-SIGN supports virus survival, and these viruses likely have devised distinct mechanisms to misuse DC-SIGN to circumvent antigen processing or alter TLR-mediated signaling (72).

Human liver/lymph-node-specific-SIGN (L-SIGN/CD209L), also known as DC-SIGNR, is a DC-SIGN homolog, with which it shares 77% amino acid identity (81). L-SIGN is expressed in the liver, lymph node, and placenta (82, 83) and can facilitate SARS-CoV infection in conjunction with liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin) (84). Several enveloped viruses, including Ebola and Sindbis, use L-SIGN as a portal of entry.

L-SIGN can bind to SARS-CoV S protein, mediating viral entry and thus serving as an alternate receptor for SARS-CoV (85). Chinese hamster ovary cells that do not express ACE2 became susceptible to SARS-CoV infection when transfected with L-SIGN. Expression of L-SIGN is found in type II alveolar cells and endothelial cells in human lungs and is consistently expressed along with SARS-CoV antigen and ACE2 in cells of the lung and small bowel of fatal cases of SARS. L-SIGN may facilitate SARS-CoV infection in these tissues. However, the role of L-SIGN in initiating productive virus replication remains to be clarified.

Viral Replication

Damage to the lungs of SARS patients seems to occur directly by viral destruction of alveolar and bronchial epithelial cells and macrophages, as well as indirectly, through production of immune mediators, although the relative contribution of these mechanisms to disease remains controversial. Quantitative studies of viral shedding in SARS patients provide some hints into the pathogenesis of the

disease. Compared with other respiratory viral infections, such as influenza, SARS had a longer incubation period (mean 4.6 days, variance 15.9 days) (25), and the viral load in the upper respiratory tract, including nasopharyngeal aspirates and throat swabs, was low during the first 4 days and peaked at $10^{5.8}$ copies/mL in nasopharyngeal aspirates 10 days after the onset of disease (19, 86, 87). However, nasopharyngeal viral titers did not always accurately reflect viral load in the lungs. Viral load in the lower respiratory tract, including bronchoalveolar lavage, sputum, and endotracheal aspirates, was higher than in the upper airways (86, 88). Virus was detected in multiple tissues at autopsy, including the lungs, intestine, liver, kidneys, brain, spleen, and lymph nodes (21, 23, 57, 89–93). Evidence for viral infection in the lungs and extrapulmonary sites is summarized in **Table 2**. It should be noted that disease in SARS results primarily from infection of the respiratory tract with a systemic component and extrapulmonary dissemination that results in viral shedding in respiratory secretions, feces, and urine. The amount of virus and the number of virus-infected cells present in extrapulmonary sites were not clearly quantified.

The mechanism of cell death in SARS-infected cells is an area of active research. The 3a protein of SARS-CoV induced apoptosis in Vero E6 cells (94). Overexpression of the 7a protein resulted in apoptosis via a caspase-dependent pathway in cell lines derived from different organs including lung, kidney, and liver (95). Investigators have suggested that the induction of apoptosis by the 7a protein relates to its demonstrated ability to inhibit cellular gene expression at the level of translation and to activate p38 mitogen-activated protein kinase (96).

Pathology in SARS Cases

A large proportion of SARS patients developed worsening lung disease and progressed to ARDS that was fatal in 10% of cases. Most of the data on the human pathology of SARS comes from autopsy studies of fatal cases, which reflect only the terminal stages of the disease. DAD is the primary pathology seen in the lungs of SARS patients. Tissues examined early after the onset of illness showed bronchial epithelial denudation, loss of cilia, squamous metaplasia, and enlarged pneumocytes (21). Different stages of DAD were seen, depending on the duration of illness. However, none of the characteristics of DAD in SARS cases were unique (97, 98). DAD in SARS cases examined within about 10 days of onset of illness was characterized by pronounced pulmonary edema, hyaline membrane formation, and interstitial thickening in lung tissue (98, 99). Desquamative alveolitis and bronchitis (57) and macrophage proliferation were seen in the lungs (21), accompanied by a disproportionately sparse inflammatory infiltrate, consisting mainly of histiocytes, lymphocytes, and occasional multinucleated giant cells (99). In later stages of disease, the proliferative phase of DAD was seen in the lung, with desquamation of epithelial cells, fibrin deposits in alveolar spaces, hyperplasia of type II pneumocytes, and increased mononuclear inflammatory cell infiltrates in the interstitium (91). At even later stages of disease, the lungs showed a predominantly acute fibrinous and organizing pneumonia with fibrin "balls" within airspaces and a pattern of an organizing pneumonia with fibrosis (98). Bronchiolitis obliterans organizing pneumonia (BOOP) has been described in subpleural areas (99).

Although SARS-CoV was identified in several extrapulmonary sites, such as urine and feces, no specific pathology was observed in the gastrointestinal tract (60), urinary system (100), or other organs (101). The only reproducible and remarkable extrapulmonary pathology reported was massive necrosis of splenic lymphoid tissue and localized necrosis in lymph nodes (21, 23, 57). As summarized in **Table 2**, IHC and in situ hybridization (ISH) demonstrated abundant multifocal SARS-CoV infection in the lung,

Table 2 Direct evidence of viral infection in tissues from SARS patients[a]

Reference	*n*	Target gene or protein	IHC		ISH		RT-PCR		EM	
			Lung	Other organs	Lung	Other organs	Lung	Other organs	Lung	Other organs
57	4	IHC: N ISH: SARS-CoV RNA polymerase gene	+: pneumocytes	+: small intestine, kidney, adrenal, liver, parathyroid, pituitary, cerebrum skin, pancreas	+: pneumocytes	+: small intestine, kidney, adrenal, liver, parathyroid, pituitary, cerebrum skin, pancreas	+	ND	+: pneumocytes	ND
90	32	IHC: N ISH: N, E, S	Early/+: bronchiolar epithelium, type I pneumocytes, alveolar macrophages	ND	+	ND	+	ND	+: ciliated cells	ND
			Late/–	ND	–	ND	+	ND	ND	ND
91	1	IHC: SARS-CoV ISH: N	+: type II pneumocytes, alveolar macrophages	ND	+: type II pneumocytes, alveolar macrophages	ND	ND	ND	+: pneumocytes macrophages	ND
23	8	IHC/ISH: ORF1a	ND	ND	+: lung/tracheal epithelial cells, macrophages, lymphocytes in hilar LNs	+: lymphocytes in spleen/LNs, mucosal epithelium of small intestine, tubular epithelium of kidney, neurons in the brain	+	+: blood	+: lung epithelium	+: circulating lymphocytes, mucosal cells in small intestine, tubular epithelium of kidney
89	6	ISH: M	ND	ND	+: pneumocytes	+: enterocytes in small intestine	ND	ND	ND	ND
21	6		ND	ND	ND	ND	Early/late: +	Early/late: + naso-pharygeal aspirate	Early/+: pneumocytes	ND

[a]Abbreviations: +: positive; –: negative; ND: not done; *n*: number of subjects studied; IHC: immunohistochemistry; ISH: in situ hybridization; RT-PCR: reverse transcription polymerase chain reaction; EM: electronic microscopy; N: nucleocapsid protein; E: envelope protein; S: spike protein; M: membrane protein.

Table 3 Comparison of pathology findings in cases of SARS and H5N1 influenza

	SARS		H5N1 influenza	
Infected organ	Pathology	Reference	Pathology	Reference
Lung	Acute pneumonia, DAD with fibrosis, occasional giant cells, enlarged pneumocytes, prominent macrophages	21, 57, 97–99, 103	Consolidated lungs with hemorrhage, DAD with interstitial fibrosis and organization, interstitial pneumonia, bronchiolitis	104–107
Intestinal tract	Minimal architectural disruption	60	No pathologic changes	108
Kidney	Acute tubular necrosis	100	Acute tubular necrosis	104, 105
Brain	Edema, demyelination of nerve fibers, and focal neuronal degeneration	57	Microglial nodules with demyelination in cerebral white matter, small foci of necrosis in brain	104, 107
Lymphoid organ	White pulp atrophy, lymphocyte depletion, and hemorrhagic necrosis in the spleen, necrosis in lymphoid nodes	21, 23, 57, 103	Lymphoid depletion in spleen, focal necrosis in lymph nodes, histiocytosis in lymphoid system	104–107
Other organs	Hemophagocytosis	21	Hemophagocytosis	104, 105

predominantly in type II pneumocytes and in association with intraalveolar necrotic debris (90, 91). Antigen was seen in enterocytes as well. There are conflicting reports regarding viral infection of alveolar macrophages (89–91). IHC staining was not found in bronchial tissues, multinucleated epithelial cells, DC-SIGN-expressing cells, endothelial cells, stromal cells, or lymphocytes (90).

When SARS cases first appeared in China in 2002–2003, patients presented with atypical pneumonia, leading to concerns that H5N1 influenza virus was the etiological agent. However, H5N1 infection was ruled out, and SARS-CoV was identified as the causative agent (3–6). SARS and H5N1 influenza infections share some similar pathology (**Table 3**): Pneumocytes are the primary target of viral infection, resulting in DAD (102). Reactive hemophagocytic syndrome and lymphoid depletion in the spleen and lymph nodes are commonly seen in both SARS (21, 23, 57, 103) and H5N1 (104–106) infections. Compared with H5N1, SARS-CoV tends to induce a more fibrocellular intraalveolar organization, with a BOOP-like pattern and the presence of multinucleated histiocytes and pneumocytes (97–99). In contrast, H5N1 causes a more fulminant, hemorrhagic, and necrotizing DAD, with patchy and interstitial paucicellular fibrosis without a BOOP-like pattern or the presence of multinucleated cells (107). Interestingly, diarrhea was commonly seen in SARS but not in H5N1 infection (108).

Immune Response to SARS-CoV

The immune system responds to viral infection with cellular and humoral responses. These responses are initiated by the innate immune system, which recognizes pathogens and induces proinflammatory cytokines and chemokines to initiate the immune response. This is followed by responses of the adaptive immune system, which consists of T cells that can directly kill virus-infected cells and of B cells that produce pathogen-specific antibodies. Initiation of the innate and/or adaptive immune response results in the

production of chemokines and other cytokines that induce an inflammatory response attracting proinflammatory cells, such as neutrophils and macrophages, to the sites of infection. Although these responses are crucial to clear viruses, they can also damage normal host tissues (65).

Although viremia occurred in SARS and the virus infected several organs, histopathologic changes were limited to the lungs, where DAD was accompanied by a disproportionately sparse inflammatory infiltrate. The reported data are summarized below, but, as stated in the introduction, it is difficult to understand all of the immunological consequences of SARS because of the limitations of the available data: Relatively little is known about the early events in SARS, especially in the lung; plasma and serum cytokine and chemokine responses may not reflect events in the lung; clinical evaluations were carried out at different time points in the course of the disease; and several of the observations cannot be confirmed because the same immune mediators were not sought or found by different investigators.

Innate immune response to SARS-CoV. Innate immunity is the first line of host defense against viral infection. Evidence from observational studies in SARS patients and experimental infection in mouse models of SARS-CoV infection provide evidence that innate immunity is important for viral clearance. The key components of this response include natural killer (NK) cells, molecules such as mannose-binding lectin (MBL) and surfactant, the IFN response, and chemokines and cytokines. NK cells can mediate suppression of viral replication by direct killing of virus-infected cells via perforin or indirectly via production of IFN-γ. Little is known about the role of NK cells in SARS; one group of investigators reported a decrease in the number of NK cells in the peripheral blood from SARS patients that correlated with the severity of disease and the presence of antibodies against the virus (109). In a mouse model of SARS, however, Glass et al. (110) demonstrated that NK cells were not required for the clearance of the virus.

Proteomic analysis of plasma from SARS patients revealed activation of innate immune responses by SARS-CoV, including increased acute-phase proteins such as serum amyloid A and MBL (111). MBL can bind SARS-CoV S protein through carbohydrate-recognition domains, resulting in protective biological effects in a calcium-dependent and mannan-inhibitable fashion. These observations suggest that MBL plays a protective role in the host innate response.

The antiviral IFN response is mediated by IFN production and signaling or direct inactivation of effector molecules. IFNs can induce several parallel antiviral pathways, and more than one pathway may operate (112). Like many viruses that modulate the expression of IFN-stimulated genes with antiviral activity, SARS-CoV has developed at least one mechanism to block activation of the IFN regulatory pathway at an early step following the nuclear transport of IFN regulatory factor (IRF)-3 (113). Findings in clinical studies were consistent with this observation; type I IFN was not detected in SARS patients, and it was not induced in vitro in SARS-CoV-infected cells. In a mouse model of SARS, the STAT1 signaling pathway was required for viral clearance, and the virus was shown to be susceptible to the antiviral effects of IFN-β in vitro (114) and in a cynomolgus monkey model (115).

Macrophages and DCs are potent producers of proinflammatory cytokines that are crucial components of innate immunity and potential mediators of immunopathology. Monocyte-derived macrophages, purified monocyte macrophages, DCs, and peripheral blood mononuclear cells (PBMCs) are only abortively infected by SARS-CoV (27, 116, 117), resulting in the production of certain cytokines and chemokines. SARS-CoV infection upregulates expression of chemokines IP-10 (IFN-inducible protein-10), MCP (monocyte chemoattractant protein)-1, MIP (macrophage inflammatory

protein)-1α, and RANTES by abortively infected macrophages and DCs. It is remarkable that IFN-α and -β are not produced following SARS-CoV infection (27, 116–118).

High-density oligonucleotide array analysis of gene-expression changes in PBMCs from normal healthy donors inoculated in vitro with SARS-CoV showed an early activation of the innate immunity pathway in the first 12 h, including enhanced expression of CD14, TLR9, CC chemokines (CCL4, CCL20, CCL22, CCL25, CCL27) and their receptors (CCR4, CCR7), IL-8, and IL-17 (119). The pattern indicates a rapid mobilization and increased trafficking of the monocyte-macrophage lineage into the lung very early in infection.

Cytokine storm: a potentially fatal immune reaction consisting of an uncontrolled feedback loop between cytokines and immune cells. When the immune system is activated, cytokines signal immune cells such as T cells and macrophages to travel to the site of infection, where they are activated and produce more cytokines

Cytokine and chemokine levels in the blood and lungs of SARS patients. Proinflammatory cytokines and chemokines, particularly IP-10, IL-8, and MCP-1, are elevated in the lungs (**Table 4a**) and peripheral blood (**Table 4b**) of SARS patients, with an unusual lack of an antiviral IFN response. IP-10 was identified in pneumocytes and alveolar macrophages by IHC (120, 121). Expression of IL-6, IL-8, and MCP-1 was also detected in the lungs of fatal cases of SARS (121) (**Table 4a**). TNF-α levels were not elevated in SARS patients in any of the reported case series (120–126). Contradictory findings were reported with respect to IFN-γ: IFN-γ was elevated along with IFN-γ-stimulated chemokines IP-10, MIG (monokine induced by IFN-γ), and MCP-1 in two series from Hong Kong (122, 123) and was implicated by Huang et al. (123) as evidence of a cytokine storm in the pathogenesis of lung injury. However, elevated IFN-γ levels were not reported in several other studies (120, 121, 124–126). In fact, Lee et al. (126) argued that proinflammatory cytokines do not play a role in the pathogenesis of SARS, but that the immunosuppressive cytokines TGF-β and PGE2 (prostaglandin 2) do. Unfortunately, PGE2 levels were not measured in any of the other case series, and TGF-β levels were not

Table 4a Cytokine and chemokine protein or RNA expression detected in the lungs of SARS patients[a]

Immune mediator	Method of detection (number of subjects studied)	
	qPCR, IHC (7)	RT-PCR, IHC (5)
Pro-inflammatory cytokines		
IL-6	ND	+
IL-1β	ND	–
IL-12	ND	–
TNF-α	ND	–
Inflammatory cytokines		
IFN-γ	ND	–
IL-2	ND	–
IL-4	ND	–
IL-10	ND	–
Chemokines		
IL-8/CXCL8	ND	+
MIG/CXCL9	ND	–
IP-10/CXCL10	+: pneumocytes, alveolar macrophages	+: pneumocytes, macrophages, lymphocytes
MCP-1/CCL2	ND	+
Reference	120	121

[a]Abbreviations: qPCR: quantitative PCR; IHC: immunohistochemistry; RT-PCR: reverse transcription polymerase chain reaction; +: positive; –: negative; ND: not done.

increased in the series of patients reported by Zhang and colleagues (125). Cheng et al. (127) speculated that an increase of the immunosuppressive cytokine TGF-β contributes to the absence of intestinal inflammation in SARS patients. Which cells are producing these cytokines and chemokines remains unclear, and further investigation would be valuable.

The profile of cytokine and chemokine responses to SARS has been compared to the profile of immune mediators during infection with different subtypes of influenza, including H5N1 influenza (105, 116, 128, 129). Although infection with both SARS-CoV and influenza induced production of chemokines, such as MIG, IP-10, and MCP-1, the remarkable difference between the two illnesses is that SARS did not induce type I IFN or TNF-α production, whereas influenza induced high levels of both (105, 116, 128, 129).

Adaptive cellular response to SARS-CoV. The cytotoxic T lymphocyte (CTL) response is the major specific defense against viral infection in adaptive cellular immunity. Two HLA-A2-restricted T cell epitopes in the S protein of SARS-CoV were immunogenic and elicited an overt specific T cell response in patients who survived SARS (130). Stimulation with inactivated SARS-CoV induced a memory CTL response in recovered SARS patients (131) and selective expansion of effector/memory Vγ9Vδ2 T cells. This expansion was associated with higher anti-SARS IgG levels. Stimulated Vγ9Vδ2 cells displayed IFN-γ-dependent anti-SARS-CoV activity and were able to kill SARS-CoV-infected target cells (132). Human memory T cell responses to the nucleocapsid (N) protein of SARS-CoV persisted for two years in the absence of antigen (133).

Table 4b Cytokine and chemokine responses detected in plasma or serum of SARS patients[a]

	Method of detection (number of subjects studied)						
Immune mediator	CBA (20)	CBA (8 children)	CBA/ELISA (88)	CBA/qPCR (255)	ELISA (228)	ELISA (15)	LiquiChip (23)
Proinflammatory cytokines							
IL-6	E	–	E	ND	E	–	E
IL-1β	E	E	ND	ND	ND	ND	–
IL-12	E	–	ND	ND	ND	–	–
TNF-α	–	–	–	ND	–	–	–
Inflammatory cytokines							
IFN-γ	E	ND	E	ND	–	ND	–
IL-2	–	ND	–	ND	ND	L	–
IL-4	–	ND	–	ND	–	ND	–
IL-10	–	–	–	ND	L	–	–
IL-13	ND	ND	–	ND	ND	ND	ND
IL-18	ND	ND	E/F	ND	ND	ND	ND
TGF-β	ND	ND	L	ND	–	E	ND
Chemokines							
IL-8/CXCL8	E	–	F	E	–	E	E
MIG/CXCL9	–	ND	E/F	E	ND	ND	ND
IP-10/CXCL10	E	ND	E/F	E	ND	ND	E
MCP-1/CCL2	E	ND	E/F	–	ND	ND	E
RANTES/CCL5	–	ND	–	–	ND	ND	–
PGE2	ND	ND	ND	ND	ND	E/L	ND
Reference	122	124	123	120	125	126	121

[a]Abbreviations: CBA: cytometric bead array; qPCR: quantitative PCR; E: elevated in early phase (<2 weeks); L: elevated in late or convalescent phase; F: elevated in fatal case; –: not elevated; ND: not done.

Antibody-dependent enhancement (ADE): a phenomenon in which virus-specific antibodies enhance the entry of virus into monocytes/macrophages through interaction with Fc and/or complement receptors, resulting in activation of macrophages, secretion of chemokines and cytokines, and enhanced severity of disease

One of the notable findings in SARS that was associated with an adverse outcome of disease was the rapid development of lymphopenia, with $CD4^+$ T cells being more severely reduced than $CD8^+$ T cells during acute infection (24, 134). Lymphopenia was prolonged, reaching a nadir at days 7–9 before returning to normal (134). The cause of lymphopenia in SARS is not known, but the proposed possibilities include virus-induced infection and destruction of lymphocytes, chemokine-mediated lymphocyte trafficking/redistribution and sequestration of lymphocytes in the lung, bone marrow suppression, or apoptosis. Neutrophilia may be due to steroid use (24).

Humoral immune response to SARS-CoV infection. Antibodies to SARS-CoV were found in patients and animals infected with SARS-CoV. Using immunofluorescence assays and ELISA against N protein, serum IgG could be detected as early as 4 days after onset of illness. Serum IgG, IgM, and IgA responses to SARS-CoV occurred around the same time, with most patients seroconverting by day 14 after onset of illness (135). In a follow-up study of 56 subjects, ELISA IgG and neutralizing antibodies peaked at 4 months and dropped progressively over time; 11% of the subjects did not have detectable ELISA IgG antibodies to SARS 24 months postinfection, but neutralizing antibodies were still detectable (136). In a large study of 623 SARS patients, antibodies were able to neutralize pseudotyped viruses bearing S proteins from four different SARS-CoV strains, suggesting that these antibodies were cross-reactive (137). Among the structural proteins, including M, E, and N, only the S protein elicits neutralizing antibody (138). The major immunodominant epitope in S protein lies between amino acids 441 and 700 (139).

Antibodies can also contribute to immunopathology. Given the evidence that SARS-CoV infection causes severe pulmonary inflammation with 10% mortality in adults but relatively mild symptoms and no fatal cases in children under age 12 (25, 26), antibody-dependent enhancement (ADE) may play a role in the immunopathogenesis of SARS. In ADE, virus-specific antibodies increase the uptake of the virus by macrophages, resulting in activation of macrophages and secretion of chemokines and cytokines. ADE has been observed after wild-type virus challenge in domestic cats that were immunized with vaccines against feline infectious peritonitis coronavirus (FIPV) (140). This experience with an animal CoV has raised concerns that a similar phenomenon could occur with SARS-CoV. However, there is no direct evidence that patients with SARS had previous exposure to a related virus. Also, macrophages are not productively infected by SARS-CoV, and there was no evidence of enhanced disease in animals that were infected with SARS-CoV following passive transfer of antibodies against SARS-CoV induced by infection or immunization (68, 141–144).

Animal Models of SARS-CoV Infection

With the lack of information on early events in SARS, our understanding of the disease is incomplete. The development of animal models of SARS is a key to the study of pathogenesis at different stages of disease and evaluation of effective antiviral drugs and vaccines (12). SARS-CoV causes pulmonary infection in nonhuman primates (NHPs) following intranasal and intratracheal inoculation (145–147). Both acute and organized stages of DAD were seen when the NHPs were sacrificed on days 2, 4, or 6 following virus infection. SARS-CoV was detected in the alveolar epithelial cells, macrophages, and syncytial cells. However, symptoms of SARS-CoV were not seen reproducibly in cynomolgus and rhesus macaques infected with SARS-CoV (146). Moreover, the disease in NHP models appears self-limiting and different from the human disease (148). The common marmoset model

seems promising and should be evaluated further (149).

Ferrets and cats support viral replication in the upper and lower respiratory tract, and the virus transmitted from infected cats and ferrets to naive cage-mates (150). Lung pathology associated with approximately 25% mortality was reported in ferrets (150).

Small animal models, such as rodents, are valuable for pathogenesis studies and for evaluating the efficacy of vaccines, immunotherapy, and antiviral drugs against SARS-CoV. SARS-CoV infection was established in Golden Syrian hamsters and mice (68, 110, 151, 152). The hamster model demonstrates high titers of virus in both the upper and lower respiratory tract, accompanied by pronounced lung pathology with pneumonic consolidation and prominent cellular infiltrates, robust antibody response, and extrapulmonary spread of the virus, although clinical illness and animal-to-animal transmission were not observed (151). Hamsters are small, outbred animals that are readily available, and findings in this species following SARS-CoV infection were more reproducible than those in NHPs or ferrets. In contrast to hamsters, SARS-CoV was of low virulence in young mice, and infection was cleared rapidly (68, 110). The absence of disease limits the application of the young mouse model for pathogenesis studies, but this model is valuable for vaccine and antiviral studies. Interestingly, however, intranasal inoculation of SARS-CoV to aged BALB/c mice (12–14 months) caused prolonged viral replication associated with significant weight loss and pneumonitis and dissemination to extrapulmonary organs (152). Pulmonary changes indicative of DAD, with multifocal, interstitial, and predominantly lymphohistiocytic infiltrates, proteinaceous deposits around alveolar walls, and intraalveolar edema were seen. Advanced age has been identified as a factor that correlated with adverse outcome of clinical illness and was a predictor of mortality in SARS. Therefore, the susceptibility of the aged mouse to SARS-CoV disease is of interest for the study of pathogenesis of SARS. Adaptation of SARS-CoV to mice, ferrets, and NHPs has been proposed to enhance the virulence of the virus for these animal models.

All the animal models of SARS differ from human cases in that the incubation period between infection and peak of disease or viral load is shorter than in humans, and the disease is self-limited and rarely progresses to the fatal outcome that occurred in some patients. Although these animal models do not accurately represent the full spectrum of human disease, they can provide insights into the pathogenesis of SARS and are the only options presently available for addressing questions relevant to therapeutics and vaccine development.

The host response to SARS-CoV infection in mice. C57BL/6 mice infected with SARS-CoV showed increased production of proinflammatory chemokines, including IP-10, MIG, MCP-1, MIP-1α, and RANTES and the receptor for IP-10 and MIG, CXCR3, in the lung. Surprisingly, Th1 cytokines (IL-12p70 and IFN-γ) were not detectable, and there was little pulmonary infiltration (110). Moreover, beige, $CD1^{-/-}$, or $RAG1^{-/-}$ mice cleared SARS-CoV normally. These findings suggest that NK cells and adaptive cellular immunity are not required for viral clearance in this species and that proinflammatory chemokines coordinate a highly effective innate antiviral response in the lung (110). The importance of the innate immune response to SARS-CoV is further supported by the observation that STAT1-deficient mice develop a persistent SARS-CoV infection associated with severe morbidity and mortality (153). These findings in mouse models are consistent with the clinical observation that SARS infection in humans fails to elicit a type I IFN response, presumably as a result of a virally induced block in induction of type I IFNs. Mice and hamsters develop a neutralizing antibody response following SARS-CoV infection, and these antibodies protect animals from reinfection.

A Model of SARS Pathogenesis

The following sequence of events can be envisioned on the basis of available data (**Figure 2**). SARS-CoV infects pneumocytes expressing ACE2 in the airways. ACE2 expression on infected cells is downregulated, and the resulting increase of Ang II drives ALI. SARS-CoV-infected pneumocytes produce proinflammatory cytokines and chemokines and upregulate adhesion molecules on the cell surface, mediating an influx of monocytes/macrophages into the lung at the early phase of disease (154). The virus inhibits induction of type 1 IFN in infected cells, replicates to high titer in the lung, and spreads from the lung to extrapulmonary sites. Monocytes/macrophages interact with SARS-CoV through binding of DC-SIGN and transmit and promote infection of more susceptible cells, such as pneumocytes and monocytes (154). Although the infection of alveolar macrophages and DCs residing in

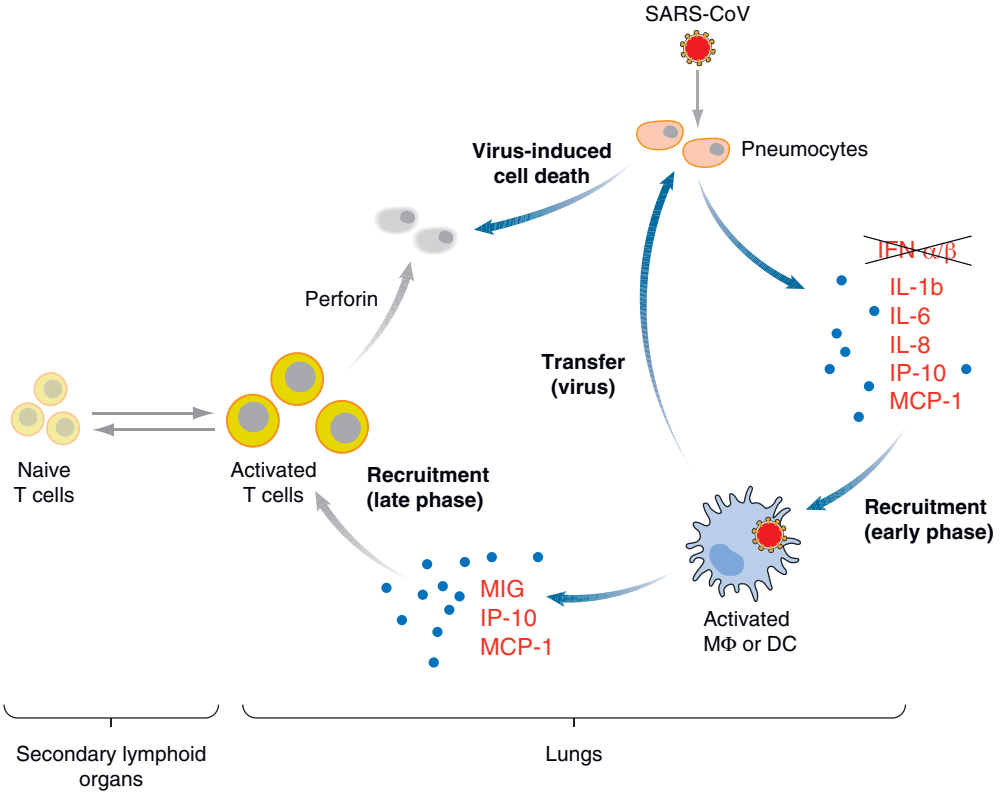

Figure 2

A model of SARS pathogenesis. Blue arrows are used where data are available to support the model, and gray arrows are used for pathways/steps that are proposed. SARS-CoV infects pneumocytes and induces the production of proinflammatory cytokines and chemokines that mediate an early recruitment of monocytes/macrophages (MΦ) into the lung. SARS-CoV inhibits induction of type 1 IFN in infected cells. Recruited MΦ interact with the virus through binding of DC-SIGN and transmit the virus to more susceptible cells. Activated MΦ produce a distinct set of chemokines, such as MIG, IP-10, and MCP-1, that can recruit neutrophils, monocytes, and T cells into the lung. DCs capture the virus at the site of infection and traffic to the secondary lymphoid organs, where they initiate T cell differentiation/activation. Activated T cells that express the receptor CXCR3 for chemokines MIG and IP-10 then migrate into the target lung and specifically destroy virus-infected pneumocytes and other permissive cells by the release of perforin, leading to extensive tissue damage to the lung parenchyma, resulting in ARDS.

the airway epithelium is not productive, functional alterations occur. The phagocytic capacity of the macrophages decreases, posing a threat for secondary infections (117). Activated monocytes/macrophages produce a distinct set of chemokines, such as MIG, IP-10, and MCP-1, that cause the migration of neutrophils, monocytes, and T cells into the lung (154). DCs capture the virus at the site of infection and traffic to the secondary lymphoid organs (spleen and lymph nodes), where they initiate T cell differentiation/activation through antigenic stimulation. Following chemotaxis, activated/effector T cells that express the receptor CXCR3 for chemokines MIG and IP-10 migrate to the lung and specifically destroy virus-infected pneumocytes and other permissive cells, leading to extensive tissue damage to the lung parenchyma, resulting in ARDS (117). B cells are activated and produce neutralizing antibodies against viral antigen, initiating a humoral immune response against the virus.

Host Genetic Susceptibility to SARS

Genetic factors associated with susceptibility to SARS have been investigated in several studies that were recently reviewed by Lau & Peiris (155). Association of HLA-B*4601 with SARS-CoV infection was reported in a study of 37 SARS patients in Taiwan (156) but was not confirmed in another study of 90 patients in Hong Kong (157). Instead, associations of HLA-B*0703 with disease susceptibility and of HLA-DRB1*0301 with resistance to development of SARS were found (157). The coinheritance of B*0703 and B60 was significantly higher in individuals with SARS than in the general population (157).

The distribution of MBL gene polymorphisms was significantly different between SARS patients and healthy controls, with a higher frequency of certain haplotypes in those developing SARS than in controls. These haplotypes were associated with low or deficient serum levels of MBL in SARS patients compared with controls (158). ACE2 gene polymorphisms were not associated with SARS susceptibility or outcome of disease in a study of 168 patients and 328 healthy controls (159). Identification of individuals who are at high risk for SARS infection may help in the selection of priority candidates for vaccination against SARS.

Predictors of Adverse Outcome in SARS

Predictors of an adverse outcome were sought in several case series. Advanced age, high concentration of lactate dehydrogenase (LDH) at presentation, and low $CD4^+$ and $CD8^+$ T cell counts on admission were predictive of an adverse outcome (2, 11, 19, 160). In 696 samples from 271 SARS-infected individuals compared with 51 controls, patients with severe or fatal illness had lower lymphocyte counts (134). In the first week of illness, age, plasma LDH concentration on admission, absolute neutrophil and lymphocyte count on admission, and plasma IP-10 concentration were identified as risk factors for adverse outcome by logistic regression (120, 121). These variables were not useful later in the course of disease. An IP-10 level in the first week of illness that was more than 1.5 times as high as the median value had an odds ratio of 3.7 (95% CI 1.5–9.2) for subsequent deterioration leading to an adverse outcome. The clinical utility of this marker was confirmed by ROC (receiver operating characteristic) analysis. In the second week, only MIG was associated with severe outcome. By day 12, only plasma LDH concentration and absolute neutrophil count were important predictive factors for adverse outcome by logistic regression (120).

PREVENTION OF SARS

Active Immunization with Vaccines

Natural human infection with SARS-CoV led to a long-lived neutralizing antibody response and immune sera cross-neutralized pseudotyped viruses bearing S proteins from diverse

but highly related human SARS-CoV isolates (137), suggesting that active immunization against SARS may be feasible. However, the next time a SARS-CoV or related virus infects the human population, it may be derived from another animal species and may be antigenically diverse from the virus derived from palm civets that led to the 2003 outbreak of SARS. Although there have been no known instances of human reexposure to SARS-CoV to confirm that a naturally acquired immune response confers protection from reinfection, a vaccine will be the most effective way to confer active and sustained protection against a future reemergence of SARS.

Several strategies for vaccine development have been pursued simultaneously. Recombinant virus vector vaccines have been developed that express SARS-CoV S, E, M, and N proteins. Among the structural proteins of SARS-CoV, S protein was the only protein that induced neutralizing antibody and protected hamsters from challenge when expressed in an attenuated parainfluenza type 3 vector (138). Mucosal immunization of African green monkeys with this parainfluenza type 3 S protein chimeric virus elicited neutralizing antibody and protection from viral replication in both the upper and lower respiratory tract after challenge with live SARS-CoV (161). S protein–encoding DNA vaccines and modified vaccinia Ankara (MVA) expressing the S protein stimulated neutralizing antibody production and provided protection from live virus challenge in mice (141, 143).

Other vaccine strategies including DNA vaccine (162–164), inactivated whole virus vaccine (142, 165–167), and recombinant protein vaccine (168, 169) were also immunogenic and efficacious in animal models. Many investigators have optimized the codon usage of the gene target to improve expression. Taken together, vaccines based on the S protein induce neutralizing antibody responses, confirming the observation that the S protein is the dominant protective antigen for SARS and that those carrying N protein can induce an enhanced antigen-specific T cell–mediated immune response (162, 170). There is a good correlation between neutralizing antibody titer and protection from wild-type virus challenge in animal models. Experiments of adoptive transfer and T cell depletion showed that protection from a DNA vaccine expressing the S protein was mediated by a humoral but not a T cell–dependent immune mechanism (143). An inactivated vaccine with alum adjuvant, which induced neutralizing antibody in mice, is scheduled to enter phase 2 human clinical trials in China, and so far no clinical complications have been observed (**http://my.tdctrade.com/airnewse/index.asp?id=8856**).

Passive Immunization

Although randomized placebo-controlled trials evaluating passive postexposure prophylaxis in at-risk groups have not been undertaken, a retrospective analysis of outcomes in a limited human study using plasma from convalescent SARS patients suggested that passive immunization may shorten hospitalization without obvious adverse effects in patients (171). Passive immunization has been examined in animal models. Passive transfer of immune serum protects naive BALB/c mice from SARS-CoV infection (68). By screening phage-display antibody libraries and immortalizing B cells from convalescent SARS patients, investigators have developed monoclonal antibodies (mAbs) with sufficient neutralizing antibody activity (172–174). Passive immunization of ferrets, hamsters, and mice with mAbs was effective in suppressing viral replication in lungs (172, 174). Administration of a mAb as immunotherapy to SARS-CoV-infected hamsters was effective in limiting viral replication and pneumonitis (175). There was no evidence of disease potentiation by antibody in these studies. Hyperimmune globulin with sufficient neutralizing activity for use in humans could be prepared from pooled convalescent human plasma or from horses immunized with an inactivated vaccine.

We do not know whether or when SARS will reemerge in the future. Passive immunization could be useful for protection of individuals who are occupationally exposed to SARS-CoV, such as laboratory and healthcare workers.

Potential for ADE Following the Use of SARS-CoV Vaccines

Experience with animal CoV vaccines is relevant for SARS vaccine development, and this underlies a major concern regarding the potential for ADE. This concern is based on the experience with FIPV, in which prior immunization led to enhanced disease rather than protection (140). There are two major mechanisms by which enhanced disease occurs when immunized hosts are subsequently infected: One is classical ADE, as was seen with FIPV, and the other is enhanced disease and immunopathology that results from a cell-mediated immune response in the absence of protective antibodies, as was seen following immunization with a formalin inactivated respiratory syncytial virus vaccine. ADE in FIPV occurs in the presence of antibodies and results from Fc-receptor-mediated uptake of virus by macrophages. ADE is not expected to be a complication of SARS vaccines because macrophages are not productively infected by SARS-CoV, and the cells that are infected in SARS are epithelial cells of the respiratory tract.

Yang and colleagues (143) compared entry of a pseudotyped virus bearing the S protein of SARS-CoV isolate into renal adenocarcinoma 786-0 cells in the presence or absence of antibodies against human SARS-CoV. Enhanced entry was demonstrated when the pseudotyped virus bearing the S protein of a civet SARS-CoV isolate was incubated with convalescent human serum containing antibodies to SARS-CoV, and it was not observed when the pseudotyped virus bore the S protein of a human SARS-CoV isolate (161). The relevance of this observation for ADE is not clear because this experimental system does not reproduce ADE, enhanced replication of the virus was not demonstrated, and there is no correlate of the observation in an animal model.

There is one report in which ferrets that were immunized with a poorly immunogenic S protein–expressing MVA vaccine that had no protective effect on viral load developed hepatitis one month after challenge (176). However, no pulmonary changes were reported in the ferrets. The hepatitis in the immunized ferrets occurred in the context of a poor neutralizing antibody response and may have been caused by a cell-mediated immune mechanism rather than ADE. Given the evidence that SARS-CoV does not productively infect macrophages and neither vaccination nor passive transfer of antibody has been associated with enhanced viral replication and enhanced disease, the potential for ADE following the use of SARS-CoV vaccines is low.

CONCLUSIONS

The SARS outbreak provided evidence of the global impact of emerging infectious diseases. Although much has been learned in the three years since discovery of the disease, aspects of its pathogenesis are still not fully understood because hypotheses based on studies conducted during the 2003 outbreak cannot be confirmed in the absence of further human cases of SARS or an animal model that accurately reflects the spectrum of human disease. Although the kinetics and protective role of the host antibody responses are better defined, the roles of the adaptive cell-mediated and the innate immune responses to SARS are not completely understood. Innate immunity appears to play a critical role in viral clearance, and the ability of the virus to block induction of an antiviral IFN response may be an important mechanism of immune evasion. This phenomenon and early events in SARS, particularly in the lung, are topics that warrant further study. Empiric approaches to vaccine development are progressing rapidly, but it is

important to better elucidate the mechanisms underlying pathogenesis of the disease. The development of the aged mouse model and common marmoset model and the adaptation of SARS-CoV to enhance virulence may permit pathogenesis studies in animal models.

DISCLOSURE STATEMENT

K. Subbarao is co-inventor on a patent for Purified Trimeric S protein as a vaccine against SARS virus infections.

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LITERATURE CITED

1. Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, et al. 2003. A cluster of cases of severe acute respiratory syndrome in Hong Kong. *N. Engl. J. Med.* 348:1977–85
2. Lee N, Hui D, Wu A, Chan P, Cameron P, et al. 2003. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N. Engl. J. Med.* 348:1986–94
3. Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, et al. 2003. Identification of severe acute respiratory syndrome in Canada. *N. Engl. J. Med.* 348:1995–2005
4. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, et al. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 348:1967–76
5. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, et al. 2003. A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 348:1953–66
6. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, et al. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 361:1319–25
7. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, et al. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 300:1394–99
8. Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, et al. 2003. The genome sequence of the SARS-associated coronavirus. *Science* 300:1399–404
9. **Peiris JS, Yuen KY, Osterhaus AD, Stohr K. 2003. The severe acute respiratory syndrome. *N. Engl. J. Med.* 349:2431–41**
10. Poon LL, Guan Y, Nicholls JM, Yuen KY, Peiris JS. 2004. The etiology, origins, and diagnosis of severe acute respiratory syndrome. *Lancet Infect. Dis.* 4:663–71
11. Donnelly CA, Fisher MC, Fraser C, Ghani AC, Riley S, et al. 2004. Epidemiological and genetic analysis of severe acute respiratory syndrome. *Lancet Infect. Dis.* 4:672–83
12. Subbarao K, Roberts A. 2006. Is there an ideal animal model for SARS? *Trends Microbiol.* 14:299–303
13. Peiris JS, Guan Y, Yuen KY. 2004. Severe acute respiratory syndrome. *Nat. Med.* 10:S88–97
14. Lau YL. 2004. SARS: future research and vaccine. *Paediatr. Respir. Rev.* 5:300–3
15. Weinstein RA. 2004. Planning for epidemics—the lessons of SARS. *N. Engl. J. Med.* 350:2332–34

9. This review summarizes clinical aspects of SARS.

16. **Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, et al. 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302:276–78**
17. Stadler K, Masignani V, Eickmann M, Becker S, Abrignani S, et al. 2003. SARS—beginning to understand a new virus. *Nat. Rev. Microbiol.* 1:209–18
18. Yu IT, Li Y, Wong TW, Tam W, Chan AT, et al. 2004. Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N. Engl. J. Med.* 350:1731–39
19. **Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, et al. 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361:1767–72**
20. Poon LL, Wong OK, Chan KH, Luk W, Yuen KY, et al. 2003. Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). *Clin. Chem.* 49:953–55
21. **Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, et al. 2003. Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 361:1773–78**
22. Ng EK, Hui DS, Chan KC, Hung EC, Chiu RW, et al. 2003. Quantitative analysis and prognostic implication of SARS coronavirus RNA in the plasma and serum of patients with severe acute respiratory syndrome. *Clin. Chem.* 49:1976–80
23. Gu J, Gong E, Zhang B, Zheng J, Gao Z, et al. 2005. Multiple organ infection and the pathogenesis of SARS. *J. Exp. Med.* 202:415–24
24. Wong RS, Wu A, To KF, Lee N, Lam CW, et al. 2003. Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. *BMJ* 326:1358–62
25. Leung GM, Hedley AJ, Ho LM, Chau P, Wong IO, et al. 2004. The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Ann. Intern. Med.* 141:662–73
26. Hon KL, Leung CW, Cheng WT, Chan PK, Chu WC, et al. 2003. Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet* 361:1701–3
27. Yilla M, Harcourt BH, Hickman CJ, McGrew M, Tamin A, et al. 2005. SARS-coronavirus replication in human peripheral monocytes/macrophages. *Virus Res.* 107:93–101
28. Zhong NS, Zheng BJ, Li YM, Poon, Xie ZH, et al. 2003. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362:1353–58
29. Breiman RF, Evans MR, Preiser W, Maguire J, Schnur A, et al. 2003. Role of China in the quest to define and control severe acute respiratory syndrome. *Emerg. Infect. Dis.* 9:1037–41
30. Xu RH, He JF, Evans MR, Peng GW, Field HE, et al. 2004. Epidemiologic clues to SARS origin in China. *Emerg. Infect. Dis.* 10:1030–37
31. Tu C, Crameri G, Kong X, Chen J, Sun Y, et al. 2004. Antibodies to SARS coronavirus in civets. *Emerg. Infect. Dis.* 10:2244–48
32. Wu D, Tu C, Xin C, Xuan H, Meng Q, et al. 2005. Civets are equally susceptible to experimental infection by two different severe acute respiratory syndrome coronavirus isolates. *J. Virol.* 79:2620–25
33. Li W, Shi Z, Yu M, Ren W, Smith C, et al. 2005. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 310:676–79
34. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, et al. 2005. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc. Natl. Acad. Sci. USA* 102:14040–45

16. This was the first demonstration that a precursor virus of SARS-CoV infected civet cats and other small mammals in a wet market in Southern China. Many animal handlers in the market had antibodies to the virus.

19. This important study demonstrated the clinical progression of SARS and reported viral load in SARS patients.

21. This paper reported the pathology seen in the lungs of SARS patients.

35. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, et al. 1995. A morbillivirus that caused fatal disease in horses and humans. *Science* 268:94–97
36. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, et al. 2000. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288:1432–35
37. Sulkin SE, Allen R. 1974. Virus infections in bats. *Monogr. Virol.* 8:1–103
38. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P. 2004. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. *Proc. Natl. Acad. Sci. USA* 101:4240–45
39. Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, et al. 2004. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. *J. Virol.* 78:5642–50
40. Kielian M, Rey FA. 2006. Virus membrane-fusion proteins: more than one way to make a hairpin. *Nat. Rev. Microbiol.* 4:67–76
41. Liu S, Xiao G, Chen Y, He Y, Niu J, et al. 2004. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. *Lancet* 363:938–47
42. Bosch BJ, Martina BE, van der Zee R, Lepault J, Haijema BJ, et al. 2004. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. *Proc. Natl. Acad. Sci. USA* 101:8455–60
43. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. 2005. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc. Natl. Acad. Sci. USA* 102:11876–81
44. Tripet B, Howard MW, Jobling M, Holmes RK, Holmes KV, Hodges RS. 2004. Structural characterization of the SARS-coronavirus spike S fusion protein core. *J. Biol. Chem.* 279:20836–49
45. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ. 2003. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *J. Virol.* 77:8801–11
46. Hofmann H, Pohlmann S. 2004. Cellular entry of the SARS coronavirus. *Trends Microbiol.* 12:466–72
47. Dimitrov DS. 2003. The secret life of ACE2 as a receptor for the SARS virus. *Cell* 115:652–53
48. Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. 2003. The SARS-CoV S glycoprotein: expression and functional characterization. *Biochem. Biophys. Res. Commun.* 312:1159–64
49. Dveksler GS, Dieffenbach CW, Cardellichio CB, McCuaig K, Pensiero MN, et al. 1993. Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59. *J. Virol.* 67:1–8
50. Delmas B, Gelfi J, L'Haridon R, Vogel LK, Sjostrom H, et al. 1992. Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV. *Nature* 357:417–20
51. Tresnan DB, Levis R, Holmes KV. 1996. Feline aminopeptidase N serves as a receptor for feline, canine, porcine, and human coronaviruses in serogroup I. *J. Virol.* 70:8669–74
52. Yeager CL, Ashmun RA, Williams RK, Cardellichio CB, Shapiro LH, et al. 1992. Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* 357:420–22
53. **Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, et al. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426:450–54**
54. Wang P, Chen J, Zheng A, Nie Y, Shi X, et al. 2004. Expression cloning of functional receptor used by SARS coronavirus. *Biochem. Biophys. Res. Commun.* 315:439–44

53. This paper identified ACE2 as a functional receptor for SARS-CoV. ACE2 efficiently binds the S1 domain of the SARS-CoV S protein.

55. Babcock GJ, Esshaki DJ, Thomas WDJ, Ambrosino DM. 2004. Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. *J. Virol.* 78:4552–60

56. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203:631–37

57. Ding Y, He L, Zhang Q, Huang Z, Che X, et al. 2004. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J. Pathol.* 203:622–30

58. Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ. 2005. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. *J. Virol.* 79:15511–24

59. Jia HP, Look DC, Shi L, Hickey M, Pewe L, et al. 2005. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J. Virol.* 79:14614–21

60. Leung WK, To KF, Chan PK, Chan HL, Wu AK, et al. 2003. Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* 125:1011–17

61. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, et al. 2002. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417:822–28

62. Yagil Y, Yagil C. 2003. Hypothesis: ACE2 modulates blood pressure in the mammalian organism. *Hypertension* 41:871–73

63. Imai Y, Kuba K, Rao S, Huan Y, Guo F, et al. 2005. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436:112–16

64. Kuba K, Imai Y, Rao S, Gao H, Guo F, et al. 2005. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11:875–79

65. Perlman S, Dandekar AA. 2005. Immunopathogenesis of coronavirus infections: implications for SARS. *Nat. Rev. Immunol.* 5:917–27

66. Nicholls J, Peiris M. 2005. Good ACE, bad ACE do battle in lung injury, SARS. *Nat. Med.* 11:821–22

67. Harmer D, Gilbert M, Borman R, Clark KL. 2002. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett.* 532:107–10

68. Subbarao K, McAuliffe J, Vogel L, Fahle G, Fischer S, et al. 2004. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J. Virol.* 78:3572–77

69. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pohlmann S. 2005. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc. Natl. Acad. Sci. USA* 102:7988–93

70. Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, et al. 2005. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *EMBO J.* 24:1634–43

71. Li W, Wong SK, Li F, Kuhn JH, Huang IC, et al. 2006. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. *J. Virol.* 80:4211–19

72. van Kooyk Y, Geijtenbeek TB. 2003. DC-SIGN: escape mechanism for pathogens. *Nat. Rev. Immunol.* 3:697–709

56. This paper reported the distribution of ACE2 in different organs, demonstrating ACE2 on alveolar epithelial cells in lung and enterocytes of the small intestine.

63. This paper identified a critical function for ACE2 in acute lung injury using a mouse model.

64. This paper presented evidence that ACE2 is a receptor for SARS-CoV in vivo and provided molecular insights to explain severe pulmonary disease seen in SARS.

65. An excellent review of the immunopathogenesis of coronavirus infection that suggests that disease in SARS may be partly immune mediated.

70. This important study describes the affinity of binding of the S proteins from SARS-CoV isolated from humans and palm civets with cognate ACE2 proteins.

73. Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, et al. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100:575–85
74. Kammerer U, Eggert AO, Kapp M, McLellan AD, Geijtenbeek TB, et al. 2003. Unique appearance of proliferating antigen-presenting cells expressing DC-SIGN (CD209) in the decidua of early human pregnancy. *Am. J. Pathol.* 162:887–96
75. Tassaneetrithep B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, et al. 2003. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J. Exp. Med.* 197:823–29
76. Alvarez CP, Lasala F, Carrillo J, Muniz O, Corbi AL, Delgado R. 2002. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in *cis* and in *trans*. *J. Virol.* 76:6841–44
77. Halary F, Amara A, Lortat-Jacob H, Messerle M, Delaunay T, et al. 2002. Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell *trans*-infection. *Immunity* 17:653–64
78. Pohlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, et al. 2003. Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. *J. Virol.* 77:4070–80
79. Simmons G, Reeves JD, Grogan CC, Vandenberghe LH, Baribaud F, et al. 2003. DC-SIGN and DC-SIGNR bind Ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* 305:115–23
80. Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, et al. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell* 100:587–97
81. Soilleux EJ, Barten R, Trowsdale J. 2000. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. *J. Immunol.* 165:2937–42
82. Soilleux EJ. 2003. DC-SIGN (dendritic cell-specific ICAM-grabbing nonintegrin) and DC-SIGN-related (DC-SIGNR): friend or foe? *Clin. Sci.* 104:437–46
83. Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, et al. 2001. A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J. Exp. Med.* 193:671–78
84. Gramberg T, Hofmann H, Moller P, Lalor PF, Marzi A, et al. 2005. LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. *Virology* 340:224–36
85. Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, et al. 2004. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. *Proc. Natl. Acad. Sci. USA* 101:15748–53
86. Cheng PK, Wong DA, Tong LK, Ip SM, Lo AC, et al. 2004. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *Lancet* 363:1699–700
87. Hung IF, Cheng VC, Wu AK, Tang BS, Chan KH, et al. 2004. Viral loads in clinical specimens and SARS manifestations. *Emerg. Infect. Dis.* 10:1550–57
88. Drosten C, Chiu LL, Panning M, Leong HN, Preiser W, et al. 2004. Evaluation of advanced reverse transcription-PCR assays and an alternative PCR target region for detection of severe acute respiratory syndrome-associated coronavirus. *J. Clin. Microbiol.* 42:2043–47
89. To KF, Tong JH, Chan PK, Au FW, Chim SS, et al. 2004. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: an in-situ hybridization study of fatal cases. *J. Pathol.* 202:157–63

90. Nicholls JM, Butany J, Poon LL, Chan KH, Beh SL, et al. 2006. Time course and cellular localization of SARS-CoV nucleoprotein and RNA in lungs from fatal cases of SARS. *PLoS Med.* 3:e27
91. Shieh WJ, Hsiao CH, Paddock CD, Guarner J, Goldsmith CS, et al. 2005. Immunohistochemical, in situ hybridization, and ultrastructural localization of SARS-associated coronavirus in lung of a fatal case of severe acute respiratory syndrome in Taiwan. *Hum. Pathol.* 36:303–9
92. Mazzulli T, Farcas GA, Poutanen SM, Willey BM, Low DE, et al. 2004. Severe acute respiratory syndrome-associated coronavirus in lung tissue. *Emerg. Infect. Dis.* 10:20–24
93. Farcas GA, Poutanen SM, Mazzulli T, Willey BM, Butany J, et al. 2005. Fatal severe acute respiratory syndrome is associated with multiorgan involvement by coronavirus. *J. Infect. Dis.* 191:193–97
94. Law PT, Wong CH, Au TC, Chuck CP, Kong SK, et al. 2005. The 3a protein of severe acute respiratory syndrome-associated coronavirus induces apoptosis in Vero E6 cells. *J. Gen. Virol.* 86:1921–30
95. Tan YJ, Fielding BC, Goh PY, Shen S, Tan TH, et al. 2004. Overexpression of 7a, a protein specifically encoded by the severe acute respiratory syndrome coronavirus, induces apoptosis via a caspase-dependent pathway. *J. Virol.* 78:14043–47
96. Kopecky-Bromberg SA, Martinez-Sobrido L, Palese P. 2006. 7a protein of severe acute respiratory syndrome coronavirus inhibits cellular protein synthesis and activates p38 mitogen-activated protein kinase. *J. Virol.* 80:785–93
97. Cheung OY, Chan JW, Ng CK, Koo CK. 2004. The spectrum of pathological changes in severe acute respiratory syndrome (SARS). *Histopathology* 45:119–24
98. Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. 2005. Pulmonary pathology of severe acute respiratory syndrome in Toronto. *Mod. Pathol.* 18:1–10
99. Tse GM, To KF, Chan PK, Lo AW, Ng KC, et al. 2004. Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS). *J. Clin. Pathol.* 57:260–65
100. Chu KH, Tsang WK, Tang CS, Lam MF, Lai FM, et al. 2005. Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. *Kidney Int.* 67:698–705
101. Leung TW, Wong KS, Hui AC, To KF, Lai ST, et al. 2005. Myopathic changes associated with severe acute respiratory syndrome: a postmortem case series. *Arch. Neurol.* 62:1113–17
102. Ng WF, To KF, Lam WW, Ng TK, Lee KC. 2006. The comparative pathology of severe acute respiratory syndrome and avian influenza A subtype H5N1—a review. *Hum. Pathol.* 37:381–90
103. Lang Z, Zhang L, Zhang S, Meng X, Li J, et al. 2003. Pathological study on severe acute respiratory syndrome. *Chin. Med. J.* 116:976–80
104. To KF, Chan PK, Chan KF, Lee WK, Lam WY, et al. 2001. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *J. Med. Virol.* 63:242–46
105. Peiris JS, Yu WC, Leung CW, Cheung CY, Ng WF, et al. 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 363:617–19
106. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, et al. 2005. Probable person-to-person transmission of avian influenza A (H5N1). *N. Engl. J. Med.* 352:333–40
107. Chokephaibulkit K, Uiprasertkul M, Puthavathana P, Chearskul P, Auewarakul P, et al. 2005. A child with avian influenza A (H5N1) infection. *Pediatr. Infect. Dis. J.* 24:162–66

108. Uiprasertkul M, Puthavathana P, Sangsiriwut K, Pooruk P, Srisook K, et al. 2005. Influenza A H5N1 replication sites in humans. *Emerg. Infect. Dis.* 11:1036–41
109. National Research Project for SARS, Beijing Group. 2004. The involvement of natural killer cells in the pathogenesis of severe acute respiratory syndrome. *Am. J. Clin. Pathol.* 121:507–11
110. Glass WG, Subbarao K, Murphy B, Murphy PM. 2004. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. *J. Immunol.* 173:4030–39
111. Chen JH, Chang YW, Yao CW, Chiueh TS, Huang SC, et al. 2004. Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and mass spectrometry. *Proc. Natl. Acad. Sci. USA* 101:17039–44
112. Cinatl J Jr, Michaelis M, Scholz M, Doerr HW. 2004. Role of interferons in the treatment of severe acute respiratory syndrome. *Expert. Opin. Biol. Ther.* 4:827–36
113. Spiegel M, Pichlmair A, Martinez-Sobrido L, Cros J, Garcia-Sastre A, et al. 2005. Inhibition of beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. *J. Virol.* 79:2079–86
114. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. 2003. Treatment of SARS with human interferons. *Lancet* 362:293–94
115. Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Rimmelzwaan GF, et al. 2004. Pegylated interferon-α protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat. Med.* 10:290–93
116. Cheung CY, Poon LL, Ng IH, Luk W, Sia SF, et al. 2005. Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. *J. Virol.* 79:7819–26
117. Tseng CT, Perrone LA, Zhu H, Makino S, Peters CJ. 2005. Severe acute respiratory syndrome and the innate immune responses: modulation of effector cell function without productive infection. *J. Immunol.* 174:7977–85
118. Law HK, Cheung CY, Ng HY, Sia SF, Chan YO, et al. 2005. Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. *Blood* 106:2366–74
119. Ng LF, Hibberd ML, Ooi EE, Tang KF, Neo SY, et al. 2004. A human in vitro model system for investigating genome-wide host responses to SARS coronavirus infection. *BMC Infect. Dis.* 4:34
120. Tang NL, Chan PK, Wong CK, To KF, Wu AK, et al. 2005. Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. *Clin. Chem.* 51:2333–40
121. Jiang Y, Xu J, Zhou C, Wu Z, Zhong S, et al. 2005. Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. *Am. J. Respir. Crit. Care Med.* 171:850–57
122. Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, et al. 2004. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin. Exp. Immunol.* 136:95–103
123. Huang KJ, Su IJ, Theron M, Wu YC, Lai SK, et al. 2005. An interferon-γ-related cytokine storm in SARS patients. *J. Med. Virol.* 75:185–94
124. Ng PC, Lam CW, Li AM, Wong CK, Cheng FW, et al. 2004. Inflammatory cytokine profile in children with severe acute respiratory syndrome. *Pediatrics* 113:e7–14
125. Zhang Y, Li J, Zhan Y, Wu L, Yu X, et al. 2004. Analysis of serum cytokines in patients with severe acute respiratory syndrome. *Infect. Immun.* 72:4410–15

110. This in vivo study explored cellular and molecular mechanisms underlying the clearance of SARS-CoV in C57BL/6 mice.

113. This paper demonstrates that SARS-CoV blocks the induction of IFN by preventing the induction of IRF-3.

126. Lee CH, Chen RF, Liu JW, Yeh WT, Chang JC, et al. 2004. Altered p38 mitogen-activated protein kinase expression in different leukocytes with increment of immunosuppressive mediators in patients with severe acute respiratory syndrome. *J. Immunol.* 172:7841–47
127. Cheng VC, Hung IF, Tang BS, Chu CM, Wong MM, et al. 2004. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. *Clin. Infect. Dis.* 38:467–75
128. Reghunathan R, Jayapal M, Hsu LY, Chng HH, Tai D, et al. 2005. Expression profile of immune response genes in patients with severe acute respiratory syndrome. *BMC Immunol.* 6:2
129. Cheung CY, Poon LL, Lau AS, Luk W, Lau YL, et al. 2002. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 360:1831–37
130. Wang YD, Sin WY, Xu GB, Yang HH, Wong TY, et al. 2004. T-cell epitopes in severe acute respiratory syndrome (SARS) coronavirus spike protein elicit a specific T-cell immune response in patients who recover from SARS. *J. Virol.* 78:5612–18
131. Chen H, Hou J, Jiang X, Ma S, Meng M, et al. 2005. Response of memory $CD8^+$ T cells to severe acute respiratory syndrome (SARS) coronavirus in recovered SARS patients and healthy individuals. *J. Immunol.* 175:591–98
132. Poccia F, Agrati C, Castilletti C, Bordi L, Gioia C, et al. 2006. Anti-severe acute respiratory syndrome coronavirus immune responses: the role played by $V\gamma9V\delta2$ T cells. *J. Infect. Dis.* 193:1244–49
133. Peng H, Yang LT, Wang LY, Li J, Huang J, et al. 2006. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virology* 351:466–75
134. He Z, Zhao C, Dong Q, Zhuang H, Song S, et al. 2005. Effects of severe acute respiratory syndrome (SARS) coronavirus infection on peripheral blood lymphocytes and their subsets. *Int. J. Infect. Dis.* 9:323–30
135. Hsueh PR, Huang LM, Chen PJ, Kao CL, Yang PC. 2004. Chronological evolution of IgM, IgA, IgG and neutralization antibodies after infection with SARS-associated coronavirus. *Clin. Microbiol. Infect.* 10:1062–66
136. Liu W, Fontanet A, Zhang PH, Zhan L, Xin ZT, et al. 2006. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. *J. Infect. Dis.* 193:792–95
137. Nie Y, Wang G, Shi X, Zhang H, Qiu Y, et al. 2004. Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. *J. Infect. Dis.* 190:1119–26
138. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, et al. 2004. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. *Proc. Natl. Acad. Sci. USA* 101:9804–9
139. Lu L, Manopo I, Leung BP, Chng HH, Ling AE, et al. 2004. Immunological characterization of the spike protein of the severe acute respiratory syndrome coronavirus. *J. Clin. Microbiol.* 42:1570–76
140. Vennema H, de Groot RJ, Harbour DA, Dalderup M, Gruffydd-Jones T, et al. 1990. Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. *J. Virol.* 64:1407–9
141. Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, et al. 2004. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. *Proc. Natl. Acad. Sci. USA* 101:6641–46

142. Stadler K, Roberts A, Becker S, Vogel L, Eickmann M, et al. 2005. SARS vaccine protective in mice. *Emerg. Infect. Dis.* 11:1312–14
143. Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, et al. 2004. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* 428:561–64
144. Greenough TC, Babcock GJ, Roberts A, Hernandez HJ, Thomas WD Jr, et al. 2005. Development and characterization of a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody that provides effective immunoprophylaxis in mice. *J. Infect. Dis.* 191:507–14
145. Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, et al. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 362:263–70
146. Rowe T, Gao G, Hogan RJ, Crystal RG, Voss TG, et al. 2004. Macaque model for severe acute respiratory syndrome. *J. Virol.* 78:11401–4
147. Qin C, Wang J, Wei Q, She M, Marasco WA, et al. 2005. An animal model of SARS produced by infection of *Macaca mulatta* with SARS coronavirus. *J. Pathol.* 206:251–59
148. McAuliffe J, Vogel L, Roberts A, Fahle G, Fischer S, et al. 2004. Replication of SARS coronavirus administered into the respiratory tract of African green, rhesus and cynomolgus monkeys. *Virology* 330:8–15
149. Greenough TC, Carville A, Coderre J, Somasundaran M, Sullivan JL, et al. 2005. Pneumonitis and multi-organ system disease in common marmosets (*Callithrix jacchus*) infected with the severe acute respiratory syndrome-associated coronavirus. *Am. J. Pathol.* 167:455–63
150. Martina BE, Haagmans BL, Kuiken T, Fouchier RA, Rimmelzwaan GF, et al. 2003. Virology: SARS virus infection of cats and ferrets. *Nature* 425:915
151. Roberts A, Vogel L, Guarner J, Hayes N, Murphy B, et al. 2005. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. *J. Virol.* 79:503–11
152. Roberts A, Paddock C, Vogel L, Butler E, Zaki S, Subbarao K. 2005. Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. *J. Virol.* 79:5833–38
153. Hogan RJ, Gao G, Rowe T, Bell P, Flieder D, et al. 2004. Resolution of primary severe acute respiratory syndrome-associated coronavirus infection requires Stat1. *J. Virol.* 78:11416–21
154. Yen YT, Liao F, Hsiao CH, Kao CL, Chen YC, Wu-Hsieh BA. 2006. Modeling the early events of severe acute respiratory syndrome coronavirus infection in vitro. *J. Virol.* 80:2684–93
155. Lau YL, Peiris JS. 2005. Pathogenesis of severe acute respiratory syndrome. *Curr. Opin. Immunol.* 17:404–10
156. Lin M, Tseng HK, Trejaut JA, Lee HL, Loo JH, et al. 2003. Association of HLA class I with severe acute respiratory syndrome coronavirus infection. *BMC Med. Genet.* 4:9
157. Ng MH, Lau KM, Li L, Cheng SH, Chan WY, et al. 2004. Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. *J. Infect. Dis.* 190:515–18
158. Ip WK, Chan KH, Law HK, Tso GH, Kong EK, et al. 2005. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. *J. Infect. Dis.* 191:1697–704

159. Chiu RW, Tang NL, Hui DS, Chung GT, Chim SS, et al. 2004. ACE2 gene polymorphisms do not affect outcome of severe acute respiratory syndrome. *Clin. Chem.* 50:1683–86
160. Tsui PT, Kwok ML, Yuen H, Lai ST. 2003. Severe acute respiratory syndrome: clinical outcome and prognostic correlates. *Emerg. Infect. Dis.* 9:1064–69
161. Bukreyev A, Lamirande EW, Buchholz UJ, Vogel LN, Elkins WR, et al. 2004. Mucosal immunization of African green monkeys (*Cercopithecus aethiops*) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. *Lancet* 363:2122–27
162. Kim TW, Lee JH, Hung CF, Peng S, Roden R, et al. 2004. Generation and characterization of DNA vaccines targeting the nucleocapsid protein of severe acute respiratory syndrome coronavirus. *J. Virol.* 78:4638–45
163. Zeng F, Chow KY, Hon CC, Law KM, Yip CW, et al. 2004. Characterization of humoral responses in mice immunized with plasmid DNAs encoding SARS-CoV spike gene fragments. *Biochem. Biophys. Res. Commun.* 315:1134–39
164. Zhu MS, Pan Y, Chen HQ, Shen Y, Wang XC, et al. 2004. Induction of SARS-nucleoprotein-specific immune response by use of DNA vaccine. *Immunol. Lett.* 92:237–43
165. Tang L, Zhu Q, Qin E, Yu M, Ding Z, et al. 2004. Inactivated SARS-CoV vaccine prepared from whole virus induces a high level of neutralizing antibodies in BALB/c mice. *DNA Cell Biol.* 23:391–94
166. Takasuka N, Fujii H, Takahashi Y, Kasai M, Morikawa S, et al. 2004. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. *Int. Immunol.* 16:1423–30
167. Zhou J, Wang W, Zhong Q, Hou W, Yang Z, et al. 2005. Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys. *Vaccine* 23:3202–9
168. Bisht H, Roberts A, Vogel L, Subbarao K, Moss B. 2005. Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polypeptide containing an N-terminal segment of the spike glycoprotein. *Virology* 334:160–65
169. Zhang H, Wang G, Li J, Nie Y, Shi X, et al. 2004. Identification of an antigenic determinant on the S2 domain of the severe acute respiratory syndrome coronavirus spike glycoprotein capable of inducing neutralizing antibodies. *J. Virol.* 78:6938–45
170. Gao W, Tamin A, Soloff A, D'Aiuto L, Nwanegbo E, et al. 2003. Effects of a SARS-associated coronavirus vaccine in monkeys. *Lancet* 362:1895–96
171. Soo YO, Cheng Y, Wong R, Hui DS, Lee CK, et al. 2004. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. *Clin. Microbiol. Infect.* 10:676–78
172. ter Meulen J, Bakker AB, van den Brink EN, Weverling GJ, Martina BE, et al. 2004. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. *Lancet* 363:2139–41
173. Sui J, Li W, Murakami A, Tamin A, Matthews LJ, et al. 2004. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. *Proc. Natl. Acad. Sci. USA* 101:2536–41
174. Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, et al. 2004. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nat. Med.* 10:871–75

175. Roberts A, Thomas WD, Guarner J, Lamirande EW, Babcock GJ, et al. 2006. Therapy with a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody reduces disease severity and viral burden in golden Syrian hamsters. *J. Infect. Dis.* 193:685–92
176. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, et al. 2004. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J. Virol.* 78:12672–76