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Review

Pathology and Pathogenesis of Severe Acute Respiratory Syndrome

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Severe acute respiratory syndrome (SARS) is an emerging infectious viral disease characterized by severe clinical manifestations of the lower respiratory tract. The pathogenesis of SARS is highly complex, with multiple factors leading to severe injury in the lungs and dissemination of the virus to several other organs. The SARS coronavirus targets the epithelial cells of the respiratory tract, resulting in diffuse alveolar damage. Several organs/cell types may be infected in the course of the illness, including mucosal cells of the intestines, tubular epithelial cells of the kidneys, neurons of the brain, and several types of immune cells, and certain organs may suffer from indirect injury. Extensive studies have provided a basic understanding of the pathogenesis of this disease. In this review we describe the most significant pathological features of SARS, explore the etiological factors causing these pathological changes, and discuss the major pathogenetic mechanisms. The latter include dysregulation of cytokines/chemokines, deficiencies in the innate immune response, direct infection of immune cells, direct viral cytopathic effects, down-regulation of lung protective angiotensin converting enzyme 2, autoimmunity, and genetic factors. It seems that both abnormal immune responses and injury to immune cells may be key factors in the pathogenesis of this new disease. ***(Am J Pathol 2007, 170:1136–1147; DOI: 10.2353/ajpath.2007.061088)***

Severe acute respiratory syndrome (SARS) first emerged in China's Guangdong Province in November 2002. During the following 3 months, it spread rapidly across the world, infecting individuals in several countries and thus resulting in the first human pandemic of the 21st century. At the end of the initial epidemic in August 2003, 8096 probable SARS cases had been reported, with a fatality rate of ~10% (World Health Organization: *http://www.who.int/csr/sars/country/table 2004_04_21/en/*). Additional sporadic cases occurred in the period between the winter of 2003 and early spring of 2004 (World Health Organization: *http://www.who.int/csr/don/archive/disease/severe_acute_respiratory_syndrome/en/index.html*).

A novel coronavirus was identified as the etiological agent of SARS.[1,2] This virus (SARS-CoV) belongs to a family of large, positive, single-stranded RNA viruses.[3] Nevertheless, genomic characterization showed that the SARS-CoV is only moderately related to other known coronaviruses.[1,3] In contrast with previously described coronaviruses, SARS-CoV infection typically causes severe symptoms related to the lower respiratory tract. The virus has been isolated from several animals, including civet cats and raccoon dogs, although neither of these animals is regarded as the true source.[4] Recently, certain bat species have been reported as potential natural reservoirs.[5] SARS is transmitted to and among humans by direct contact, droplet, and airborne routes.[6] Viral isolation from fecal and urinary samples suggests additional routes of transmission.[6,7]

SARS has a characteristic clinical course. Patients present with flu-like symptoms including fever, chills, cough, and malaise.[8] Approximately 70% of the patients subsequently suffer from shortness of breath and recurrent or persistent fever, whereas the remaining 30% show clinical improvement after the first week.[6] Approximately 20 to 30% of patients require intensive care treatment including mechanical ventilation.[6] Increased alanine aminotransferase, lactate dehydrogenase, thrombocytopenia, and lymphopenia have all been frequently detected in SARS patients.[6,8–11] In patients younger than 60 years of age the estimated fatality rate amounts to 6.8% and in older patients attains an estimated 43%.[8]

A number of complete and partial autopsies of SARS patients have been reported since the first outbreak in 2003. The predominant pathological finding in these

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Table 1. Major Pathological Findings in Various Organs and Tissue

Organs/tissue	Pathology	Number of cases	References
Respiratory tract	Diffuse alveolar damage with varying degrees of acute exudative features including edema and hyaline membranes, organization, and fibrosis. Macrophagic or mixed cellular infiltration, multinuclear giant cells, atypical reactive pneumocytes, and vascular injury. Positive *in situ* hybridization signals in pneumocytes, lymphocytes, and macrophages	63	12–16, 18–23
Spleen and lymph nodes	Lymphocyte depletion in spleen and lymph nodes with architectural disruption. Splenic white pulp atrophy. Positive *in situ* hybridization signals in immune cells	25	11–13, 15–17, 27
Digestive tract	Intestines: no obvious pathological changes/ nonspecific changes. Depletion of mucosal lymphoid tissue. Positive *in situ* hybridization signals in mucosal epithelial cells	19	12, 13, 39, 46
	Liver: no specific pathological changes. In some cases, necrosis and evidence of apoptosis	20	9, 12, 13, 17, 39, 48
Urogenital tract	Kidneys: acute tubular necrosis, in varying degrees and other nonspecific features. Positive *in situ* hybridization signals in the epithelial cells of the distal tubules	21	12, 13, 15, 17, 43, 44
Central nervous system	Edema and degeneration of neurons, several neurons *in situ* hybridization-positive	12	12, 15, 42
Bone marrow	In some cases, reactive hemophagocytosis	9	9, 12, 25
Skeletal Muscles	Myofiber necrosis and atrophy, few regenerative myofibers	13	12, 44, 46
Adrenal gland	Necrosis and infiltration of monocytes and lymphocytes	14	12, 13, 15
Thyroid gland	Destruction of follicular epithelial cells, several apoptotic cells	5	49
Testes	Germ cell destruction, apoptotic spermatogenetic cells	7	45
Heart	Edema and atrophy of myocardial fibers	22	12, 13, 15, 17

cases was diffuse alveolar damage (DAD). This severe pulmonary injury of SARS patients is caused both by direct viral effects and immunopathogenetic factors. Many important aspects of the pathology and pathogenesis of SARS have not yet been fully clarified. Here, we offer a comprehensive overview of the morphological and histopathological findings present in different organs and cells. In addition, we summarize the most important mechanisms that may play a role in the seemingly complex pathogenesis of this new disease.

Pathology

Certain organs of SARS victims, such as the lungs and intestines, have been extensively studied, and the pathological lesions of SARS in these organs are fairly well known. By contrast, the pathology of other organs is incompletely described, and imperfectly known. For ease of reference, the major pathological findings for each organ are summarized in Table 1. Table 2 lists the results of ancillary tests that have been used to confirm the diagnosis, including *in situ* hybridization, immunohistochemistry (IHC) with antibodies against viral antigens, reverse transcriptase-polymerase chain reaction (RT-PCR), electron microscopic (EM) examination, and viral culture.

Respiratory Tract

The pathological findings in the lungs of more than 60 autopsies of SARS cases have been reported. On gross examination, the lungs were edematous and increased in

Table 2. Results of Ancillary Tests, Used to Confirm SARS-CoV Infection in Lung and Intestinal Tissue

Additional test	Positive test results/total tests (lung tissue)	Positive test results/total tests (intestinal tissue)	References (lungs)	References (intestines)	Longest duration reported with positive test results in lungs/intestines
RT-PCR	47/55	12/23	18, 20, 22, 28	17, 37	51 days[37]/43 days[37]
In situ hybridization	31/67*	18/24	13, 15, 17, 22, 28–30 (23),† 31, 32	29, 30 (23),† 31, 39	62 days[15]/45 days[39]
IHC	12/47	9/11	24, 28–30 (23)†	29, 30 (23)†	20 days[31]/20 days[31]
EM	26/38	12/20	12, 14 (29),† 15, 16, 18, 22, 24	29, 39, 46	46 days[18]/21 days[39]
Viral culture	10/23	15/27	14 (29),† 17, 31	14 (29),† 17, 31, 46	20 days[16]/16 days[14]

For each test, the number of positive cases and the total number of cases are listed.

*In 63 SARS cases, the findings on general histopathology have been reported, whereas in 67 cases, the results of *in situ* hybridization have been reported. This difference is attributable to the fact that some recently published studies have only described *in situ* hybridization results without reporting general pathology.

†These results have been published in two different journals.

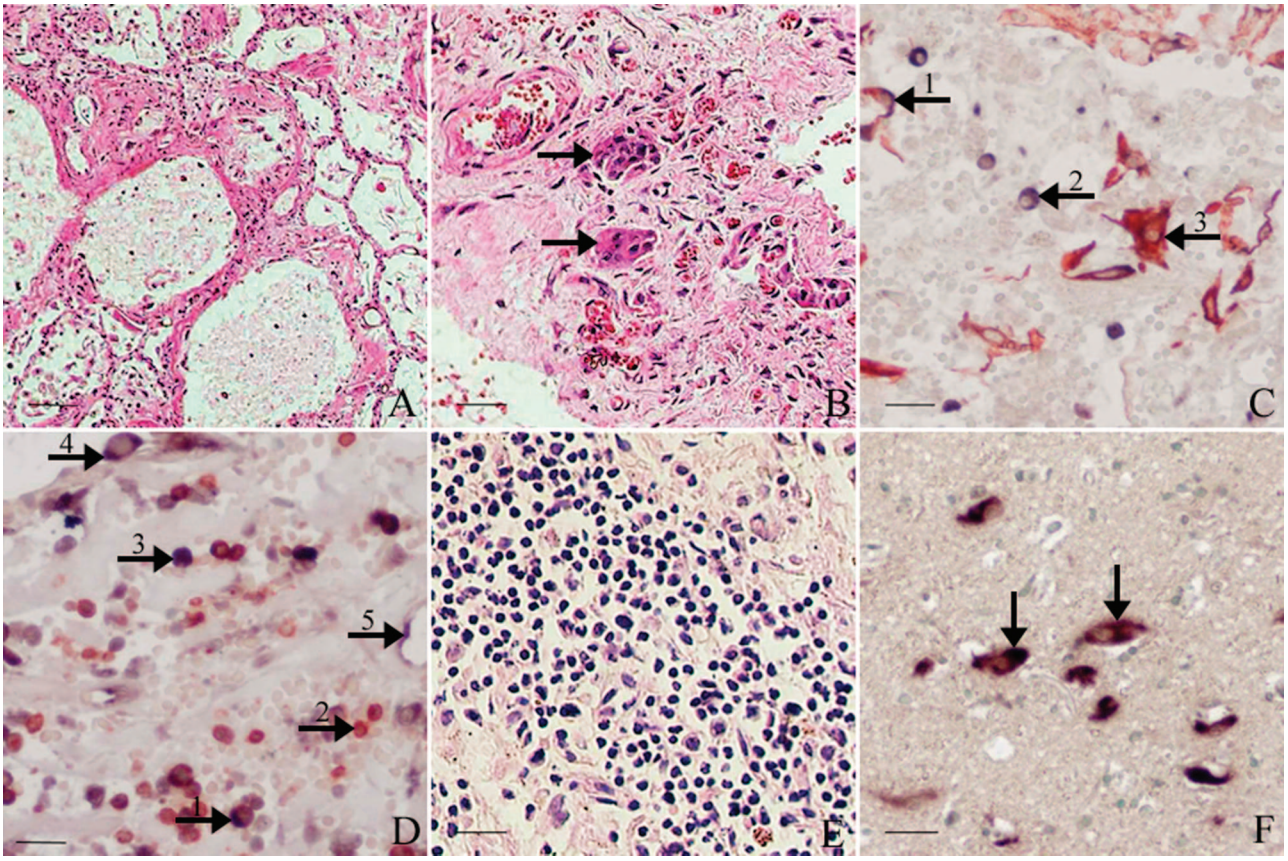

Figure 1. Pathology in the lungs, brain, and spleen. **A:** Lung tissue of a SARS autopsy showing severe damage, hyaline membrane formation, edema, fibrin exudation, and some inflammatory cells (H&E staining). Sample from a 50-year-old male SARS patient who died 33 days after disease onset. **B:** Multinucleated cells (**arrows**) in the lungs of a SARS patient (H&E staining). Sample from a 51-year-old male SARS patient who died on day 45. **C:** Double labeling combining *in situ* hybridization (ISH) of SARS viral genomic sequence and IHC with antibodies to cytokeratin (AE1/AE3) showing both brownish red (cytokeratin) and purplish blue signals for viral genome in the same cells, identifying the infected cells as pneumocytes (**arrow 1**). **Arrow 2** points to an ISH-positive and cytokeratin-negative cell (purplish blue signal only), representing an inflammatory cell that is infected by SARS virus. **Arrow 3** points to an *in situ* hybridization-negative pneumocyte (cytokeratin-positive, brownish red signal only) that is not infected by SARS virus. Sample from a 58-year-old male patient with SARS who died 58 days after disease onset. **D:** SARS-CoV genomic sequence in various cells in the lungs. Both a dark blue *in situ* hybridization signal and a brownish red IHC (CD3) signal are present in the same cell (**arrow 1**), suggesting the infection of T lymphocytes. There are also some uninfected CD3-positive cells (**arrow 2**, brownish red signal only). **Arrow 3** points to *in situ* hybridization-positive mononuclear cell (purplish blue signal only). A spindle-shaped pneumocyte with a positive *in situ* hybridization signal is also shown (**arrow 4**, purplish blue signal only). **Arrow 5** points to an *in situ* hybridization-positive cell morphologically resembling a vascular endothelial cell (purplish blue signal only). Sample from a 24-year-old male SARS patient who died on day 21. **E:** Spleen tissue showing depletion of lymphocytes. Sample from same patient as in **C**. **F:** Positive *in situ* hybridization signals in the cytoplasm of many neurons (**arrows**) in brain tissue of a SARS patient. Sample from a 49-year-old female SARS patient who died on day 32. In **C**, **D**, and **F**, *in situ* hybridization was performed with a 154-nucleotide cRNA probe directed against fragments of the polymerase gene (R1ab) of SARS-CoV. The probe was labeled with digoxigenin, and a NBT/BCIP substrate chromogen kit (Promega Corp., Madison, WI) was used to visualize *in situ* hybridization signals, resulting in a purplish blue color. In **C** and **D**, IHC with antibodies to cytokeratin (AE1/AE3) and CD3 was performed. IHC signals were detected with the HRP reaction kit AEC, which gives a brownish red color. Scale bars: 50 μm (**A**); 25 μm (**B**, **C**, **E**, **F**); 20 μm (**D**).

weight.[12–17] In most cases, they showed extensive consolidation.[12,14–17]

Histopathologically, the lungs in SARS characteristically show DAD. During the first phase of the disease (7 to 10 days), SARS lungs display the following features of acute exudative DAD[12,13,16,18–23] (Figure 1A): 1) extensive edema, 2) hyaline membrane formation, 3) collapse of alveoli, 4) desquamation of alveolar epithelial cells, and 5) fibrous tissue in alveolar spaces. In cases of longer disease duration, features of fibrous organization of DAD appear after ~10 to 14 days, such as interstitial and airspace fibrosis and pneumocytic hyperplasia.[18,20,21,23,24] The longer the disease, the more extensive becomes the fibrous organization of the lung tissue.[19,20] In SARS cases lasting more than 2 to 3 weeks, dense septal and alveolar fibrosis were demonstrated, in addition to organizing features.[18,21,23] A direct correlation has been found between the extent of fibrosis and the duration of the illness.[14,19] Pathological changes suggesting active pulmonary injury have been observed up to 108 days after the onset of disease.[19] Hwang and colleagues[19] have established a specific pathological pattern in SARS autopsies, characterized by a combination of fibrin balls within airspaces and features of an organizing pneumonia.

In many cases, cellular infiltration has been observed. Immunohistochemical staining has shown that these inflammatory cells predominantly consist of macrophages[13,17,22,24,25] or a combination of macrophages and lymphocytes with or without neutrophils.[12,14–16,18,19,23,26] In other cases, however, a disproportionate scarcity of inflammatory cells has been noted.[14,15]

Large multinucleated cells have frequently been observed in the lungs of SARS patients (Figure 1B).[12,14–16,18–20,23] IHC has identified these cells as macrophages and pneumocytes.[14,19,20] In addition, atypical enlarged pneumocytes with large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were observed in the majority of SARS patients.[14,16,18–20] It should be noted, however, that multinucleated cells in the lungs may be the result of many viral or bacterial infections, whereas atypical pneumocytes often appear as a reaction to alveolar damage. Therefore, neither the presence of multinucleated cells or atypical enlarged pneumocytes can be regarded as a unique characteristic of SARS-related pathology.

Additional pathological features include: 1) squamous metaplasia of bronchial and alveolar epithelial cells[16,18–20]; 2) subpleural proliferation of fibrogranulative tissue in small airways and airspaces[14]; 3) loss of cilia of bronchiolar epithelial cells[16]; 4) hemophagocytosis in mononuclear cells residing in pulmonary tissue[16]; 5) apoptosis in epithelial cells, monocytes/macrophages, lymphocytes, and pneumocytes[23]; and 6) vascular injury. Vascular injury consists of edema of the walls of pulmonary vessels and fibrous thrombi with or without pulmonary infarction.[12,13,16,18–20]

In a number of SARS cases, co-infections have been reported.[17,19] These include infections by *Aspergillus* species, *Mucor* species, *Pseudomonas aeruginosa*, *Klebsiella* species, methicillin-resistant *Staphylococcus aureus*, α-*hemolytic Streptococcus* species, and *cytomegolavirus*. These co-infections are probably related to longer disease durations and/or treatment with high doses of corticosteroids.[19]

Certain studies have compared the pulmonary pathology of SARS cases with non-SARS cases showing SARS-like symptoms.[15,17,20] Thirty-six of 36 SARS autopsies showed DAD, contrasting with only 19 of 40 of such non-SARS cases. Apart from prominent vascular injury, which was more frequently observed in the SARS cases than in the non-SARS cases, no significant differences in terms of morphology and extent of alveolar damage were established. It is therefore of noticeable interest that SARS-related pathology lacks specific characteristics. It seems to be impossible to distinguish DAD caused by SARS from DAD caused by, for instance, trauma, aspiration, oxygen toxicity, or infectious microorganisms. Therefore, additional tests such as *in situ* hybridization, IHC, viral isolation, or RT-PCR are necessary to confirm the diagnosis.

Both sense and anti-sense probes with specificity for several viral proteins have been used for *in situ* hybridization.[13,15,22–24,27–33] *In situ* hybridization has been performed on lung tissue of 67 SARS cases, of which 31 showed positive staining of epithelial cells. After double-labeling with cytokeratin/anti-epithelial membrane antigen (Figure 1C) and surfactant protein A, these cells were identified as type II pneumocytes.[15,22,24,27,28,30–33] Some studies also found positive *in situ* hybridization signals in epithelial cells of bronchi, bronchioles, trachea, and multinucleated cells.[15,22,23,28,30] In addition, infection of alveolar macrophages[15,22–24,27,28,33] and lymphocytes (Figure 1D)[15,33] has also been confirmed by double labeling. We found positive *in situ* hybridization signals in both fibroblasts and vascular endothelial cells (Figure 1D).[33] Up to 62 days after onset of disease, *in situ* hybridization has detected viral sequences in lung tissue.[15] Three research groups have used immunofluorescence and fluorescence *in situ* hybridization with several cell markers and have found infected pneumocytes, bronchiolar epithelial cells, and macrophages.[22,29,31]

IHC with antibodies against SARS-CoV nucleocapsid (N) protein, spike (S) protein, and nonstructural protein 3a has been performed in 47 SARS cases.[23,24,26,28–30] Positive staining of alveolar epithelial cells and macrophages was observed in 12 of 47 cases.[24,26,28–30] Limited staining of bronchiolar epithelium has also been reported.[28] Positive IHC has not been established in cases with a disease duration exceeding 20 days.[28,30]

Specific immunohistochemical staining with antibodies to P-selectin, dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), and interferon-inducible protein-10 (IP-10) has been performed in a number of cases.[26,34,35] Increased expression of DC-SIGN, P-selectin, and IP-10 in both pneumocytes and macrophages was demonstrated.[24,34,35] The results of immunostaining with antibodies directed against monocyte chemoattractant protein 1, transforming growth factor-β1, tumor necrosis factor-α, interleukin-1, and interleukin-6 in SARS patients have recently been reported by He and colleagues[23] Strong expression of such proinflammatory cytokines was found in angiotensin-converting enzyme 2 (ACE2)-positive cells infected with SARS-CoV.[23]

Ultrastructurally, SARS-CoV infection of cultured cells has shown features similar to those of previously described coronaviruses.[36] SARS-specific characteristics include large granular cytoplasmic areas, nucleocapsid inclusions, and typical double-membrane vesicles.[36] In some SARS autopsies, EM examination has revealed cytoplasmic viral particles in pneumocytes.[12,14,22,24,28,30] The majority of these viral particles were within membrane-bound vesicles. Viral particles have also been observed in macrophages in lung tissue.[22] In addition, the presence of viral inclusion bodies has been reported.[24,30] In some studies the viral origin of the identified particles and inclusion bodies has been confirmed by immunogold labeling.[15,24]

SARS-CoV was successfully isolated from lung tissue in 10 of 23 cases, including cases with a duration of illness of up to 20 days.[14,17,31] By RT-PCR, genomic sequences were found in the lungs of 47 of 55 SARS autopsies.[16,18,22,28,30] Quantitative RT-PCR has detected viral sequences in lung tissues up to 51 days after onset of symptoms.[37]

Similar to SARS, avian influenza A (H5N1) is an emerging viral infectious disease that targets the lungs. Both diseases often result in respiratory distress, with a high fatality rate. Certain pathological similarities and differences between the two diseases have been described in a comparative review.[38] DAD in H5N1 influenza cases shows a more fulminant progression, compared with that in SARS, with marked hemorrhage and necrosis.[38] Multinucleated cells were readily noticeable in SARS

cases, whereas the presence of such cells has thus far not been reported in H5N1 influenza cases. The organizing phase of H5N1 influenza seems to be characterized by paucicellular fibrosis, without the BOOP-like pattern as found in SARS autopsies. With respect to extrapulmonary manifestations, SARS is less often associated with a reactive hemophagocytic syndrome.

Immune System

In most SARS autopsies, both extensive necrosis of the spleen and atrophy of the white pulp with severe lymphocyte depletion have been found.[11–14,17,27] Zhan and colleagues[27] have demonstrated a sharp decrease in the number of periarterial sheaths in the spleen (Figure 1E).[27] Quantification of the various immune cells residing in the spleen including $CD4^+$ lymphocytes, $CD8^+$ lymphocytes, $CD20^+$ lymphocytes, dendritic cells, macrophages, and natural killer cells showed a decrease of 78, 83, 90, 80, 39, and 48%, respectively. The average size of macrophages was found to be increased by more than 100%.[27] Some studies have failed to detect any positive viral signal in splenic cells[30–32] or to isolate virus from cultures of splenic tissue.[11,14,31] In contrast, others have detected infection of T lymphocytes and macrophages in the spleen[15,27] and reported high viral loads in this organ.[37]

Lymph nodes usually show atrophy and reduction of lymphocytes with loss of germinal centers.[12,13,15,17] Focal necrotic inflammation of hilar lymph nodes has been found in some cases.[13] Evidence of hemophagocytosis in lymph nodes was observed in a limited number of cases.[17,38] High viral loads have been detected in lymph nodes, whereas viral isolation was negative.[11,31,37] Both *in situ* hybridization and EM have confirmed SARS-CoV infection of immune cells residing in lymph nodes, and by double labeling these cells were identified as macrophages and T lymphocytes.[15]

In several cases, severe depletion of mucosal lymphoid tissue in the small intestines and appendix has been described. Decrease of lymphocytes, depletion of follicles, and loss of germinal centers were noted.[39] EM and *in situ* hybridization have, respectively, revealed viral particles and genetic sequences in the remaining lymphocytes.[15,39]

EM has also detected viral particles in circulating monocytes and T lymphocytes and to a lesser extent in natural killer cells and B lymphocytes found in blood samples collected in the early phase of the disease.[15] In several SARS autopsies, infection of T lymphocytes and monocytes within blood vessels were confirmed by *in situ* hybridization and EM.[15]

The Central Nervous System

Several observations suggest that SARS-CoV is capable of causing an infection of the central nervous system. RT-PCR has detected SARS-CoV genomic sequences in cerebral spinal fluid and in brain tissue specimens (Figure 1F).[40–42] The virus has been successfully isolated from brain tissue.[42] Edema and focal degeneration of neurons have been observed in the brains of SARS autopsies.[13,15] IHC, *in situ* hybridization, and EM have confirmed viral infection of neurons.[13,15,42] Gliocytes have also been found infected by SARS-CoV.[42]

The Urogenital Tract

Kidneys of autopsied SARS patients have shown focal necrosis and vasculitis of small veins in the renal interstitial tissue.[12] In addition, monocytic infiltration, acute tubular necrosis, and other nonspecific changes, such as glomerular fibrosis and nephrosclerosis, have all been observed.[12,13,17,43,44] Quantitative RT-PCR has detected high viral loads in the renal tissue specimens of several SARS patients.[37] *In situ* hybridization and IHC have identified viral genomic sequences and proteins, respectively, in the epithelial cells of the distal tubules.[15,23,30] Viral particles have been localized to the cytoplasm of these cells through EM.[15] These findings may explain the presence of SARS-CoV sequences in urinary samples of SARS patients.[6,7]

The testes of seven of seven male SARS patients displayed germ cell destruction, showing few or no spermatozoa in the seminiferous epithelium or lumen and a mixed cellular infiltrate. Significantly increased numbers of apoptotic spermatogenetic cells were identified.[45] *In situ* hybridization and EM have failed to demonstrate SARS viral sequences and viral particles in the testes.[15,30,45]

Gastrointestinal Tract

Gastrointestinal manifestations are commonly reported in SARS cases, with more than 20% of the patients presenting with watery diarrhea and up to 67% of patients developing diarrhea during the course of the illness.[6,8,46] Microscopic examination has not detected any evident pathological changes, other than nonspecific changes in tissue specimens of small and large intestines, such as autolysis and mild focal inflammation.[13,14,39,46] The most evident pathological finding was depletion of the mucosal lymphoid tissue in the pharynx, appendix, and small intestines, as described above. The pancreas, stomach, and salivary glands have not shown any obvious pathological changes.[39,46] Positive *in situ* hybridization signals have been observed in the cytoplasm of mucosal epithelial cells,[15,23,29–31,39] as well as in mucosal and submucosal lymphocytes.[39] Viral particles were identified by EM in the mucosal epithelial cells and were localized to the dilated endoplasmic reticulum and the surface of the microvilli.[29,39,46] Viral sequences were not detected in the esophagus.[39] Viral proteins and genomic sequences have been observed in gastric parietal cells but not in gastric chief cells.[23] RT-PCR and viral isolation were positive on intestinal tissue specimens.[14,46,31,32] Infection of the intestinal tract may provide an explanation for the detection of viral RNA in stool samples.[6,7] Nevertheless, SARS-CoV could not readily be isolated from stool samples.[7]

Liver

The majority of SARS patients showed a transient increase in serum alanine aminotransferase levels during the course of their disease.[47] High peak alanine aminotransferase levels have been associated with an adverse outcome.[47] In some autopsy cases fatty degeneration, necrosis of hepatocytes, and cellular infiltration were observed,[12,13,17,39] whereas in other cases no specific pathological changes have been described.[9] RT-PCR was positive on liver tissue in several cases.[9,37,48] However, *in situ* hybridization and EM failed to detect either viral genomic sequences or particles in most cases.[31,32,39,48] Liver tissues obtained through percutaneous biopsies showed mitotic hepatocytes with evidence of apoptosis.[48]

Bone Marrow

In some cases, evidence of reactive hemophagocytosis or bone marrow hypoplasia was present.[13,25,38] In other cases, however, active bone marrow without reactive hemophagocytosis has been demonstrated.[11] *In situ* hybridization and IHC have detected neither viral genomic sequences nor antigens.[30,31] Both viral isolation and RT-PCR performed on bone marrow were negative.[11,25,31]

Thyroid Gland

Destruction of epithelial cells with significant changes in the follicular architecture was present in the thyroid glands of five of five SARS autopsies. No distinct calcitonin-positive cells were identified. Cellular infiltration was not observed.[49] Fibrosis in the interfollicular connective tissue has been described in one case. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay has demonstrated several apoptotic cells.[49] In contrast, however, *in situ* hybridization has not detected any viral sequences in thyroid tissues[15,31] except in leukocytes within blood vessels distributed in the organ.[15] Acidophilic cells of the parathyroid gland showed positive *in situ* hybridization signals.[23,30]

Skeletal Muscle

Both myofiber necrosis and atrophy were observed in the limited number of skeletal muscle tissue specimens of SARS autopsies examined in this respect.[50] Such necrosis was characterized as coagulative and karyorrhexic, with cellular debris in some cells.[50] Regenerative myofibers and infiltrating macrophages were scarce. These changes may possibly be ascribed to a combination of the use of corticosteroids during treatment, critical illness neuropathy, and SARS-CoV-related myopathy.[50] Edema of the walls of small veins and arteries has also been reported.[12] *In situ* hybridization and EM examinations have not detected any viral genetic sequences or particles.[30,32,50] Although RT-PCR on skeletal muscle tissue was positive in a few cases, SARS-CoV could not be isolated from skeletal muscle tissue.[37,50]

Other Organs

The few available studies on adrenal glands described the presence of necrosis and vasculitis of the medulla with monocytic and lymphocytic infiltration.[12,13] Viral antigens and genomic sequences have been identified in adrenal glands.[23,30] Edema of both myocardial stroma, as well as vascular walls, and atrophy of cardiac muscle fibers have all been demonstrated.[12,13] In addition, in one SARS autopsy vegetations on the mitral, tricuspid, and aortic valve were observed.[17] Viral isolation, *in situ* hybridization, and IHC were negative in most cases, whereas RT-PCR on cardiac tissue was positive in some cases.[14,17,30–32,37] SARS-CoV genomic sequences and antigens have also been detected in sweat glands and pancreatic islet cells.[23,30] Because SARS-CoV genomic sequences may be carried by immune cells circulating in a particular organ, positive RT-PCR results do not imply that the parenchymal cells of that organ are also infected.

Pathogenesis

Although reports describing cell and organ pathology and viral distribution have contributed to a better understanding of the pathogenesis of SARS, research regarding receptor interaction, immune system response, and genetic factors will provide additional insights. Below, we summarize the important discoveries in these regards and the major etiological mechanisms are discussed. Figure 2 contains a schematic representation of these mechanisms.

ACE2 and Other SARS-CoV Receptors

ACE2, a metallopeptidase, was identified as the functional receptor for SARS-CoV.[51] Subsequent studies have described the tissue distribution of ACE2 through immunohistochemical staining.[23,52,53] With respect to the respiratory tract, *ex vivo* experiments have detected ACE2 on the luminal surface of tracheobronchial and alveolar epithelium.[52,54] In general, the receptor distribution pattern resembles that of infected organs and cells as demonstrated by *in situ* hybridization and RT-PCR. However, the abundant expression of ACE2 in endothelial cells and smooth muscle cells of several visceral organs is discordant with the absence of the virus in these organs.[53] In addition, only one of seven ACE2-expressing intestinal cell lines seemed to be susceptible to SARS-CoV infection *in vitro*.[55] At the same time, however, the lack of ACE2 expression in immune cells, colonic epithelial cells, and in neuronal cells of the brain contrasts with the confirmed infection of such cells. These contradictions suggest that other receptors, coreceptors, or mechanisms may be involved in the interaction between the virus and its target cells.[15,53] Recently, human autopsy studies have shown that SARS-CoV S protein and its RNA could be detected in ACE2-positive cells and not in ACE2-negative cells, implying that only ACE2-positive cells are susceptible to SARS-CoV infection.[23] However, this contradicts the above re-

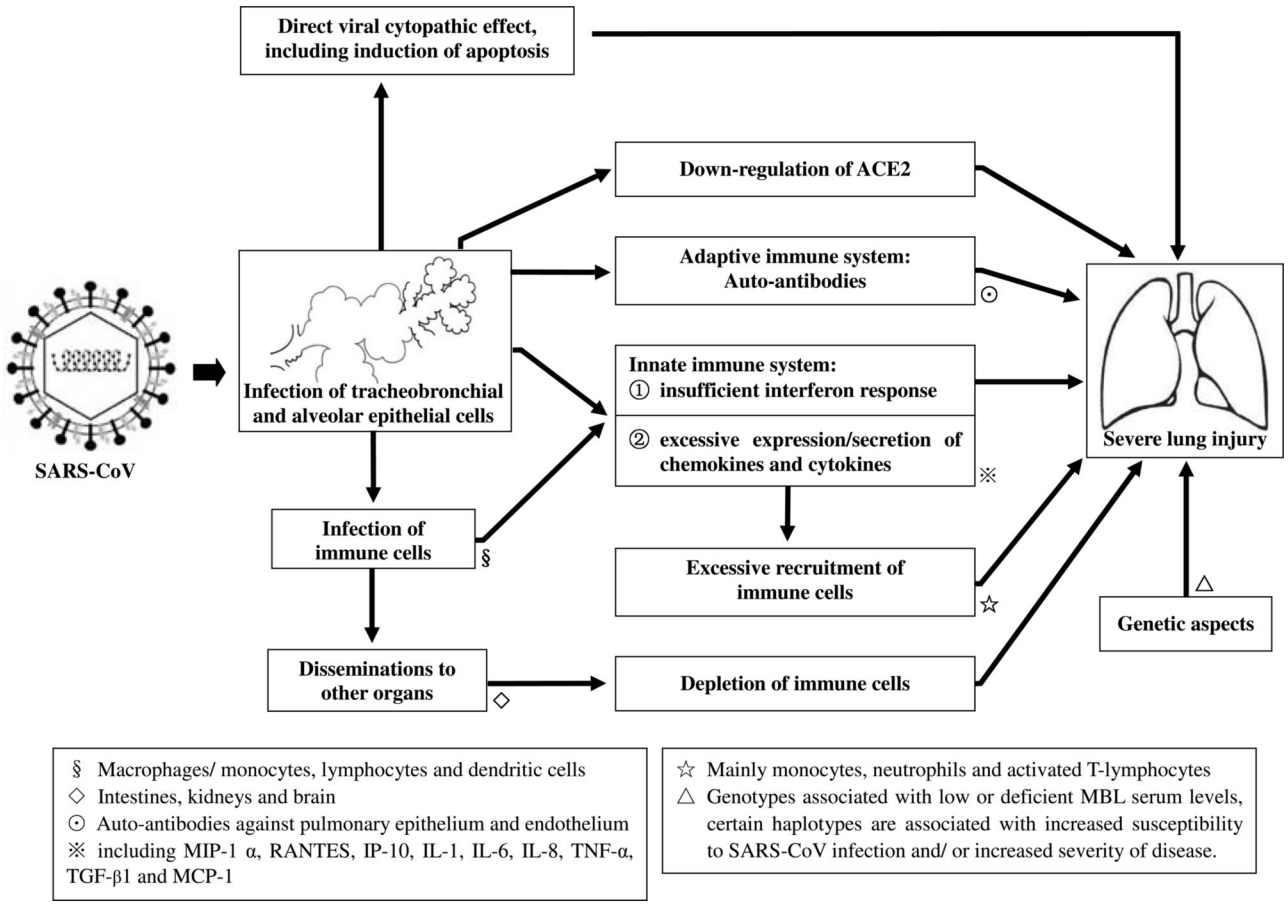

Figure 2. Major mechanisms contributing to the pathogenesis of SARS. These pathological events and cascade of changes form the basis for clinical symptoms and pathological findings at different stages of SARS. Correct understanding of the pathogenesis will provide guidance to prevention, diagnosis, and treatment of this new disease. MIP-1α, macrophage inflammatory protein-1α; RANTES, regulated on activation normal T cell expressed and secreted; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; MCP-1, monocyte chemoattractant protein-1.

ports of the presence of SARS-CoV sequence in ACE2-negative cell types. Further investigations are called for to address this controversy.

At the molecular level, SARS-CoV enters the apical surface of well-differentiated epithelium of the respiratory tract, where ACE2 is expressed more abundantly than basolaterally.[51,56] The apical surface also seems to be the preferential site of viral exit.[56] SARS-CoV infection of ACE2-expressing cells seems to be dependent on the proteolytic enzyme cathepsin L.[57,58] Cathepsin L is poorly expressed in endothelial cells which may explain the low infection rate of these cells despite the high expression of ACE2.[57] SARS-CoV infection seems to be pH-dependent because the activation of cathepsin L is pH sensitive.[58] Differential expression of cathepsin L in various cell types may explain the differences in viral distribution in relation to the ACE2 expression pattern.

Liver/lymph node-specific ICAM3-grabbing nonintegrin (L-SIGN) and dendritic-cell-specific DC-SIGN have both been identified as alternative SARS-CoV receptors.[59,60] L-SIGN expression is generally found in lymph nodes and liver sinusoidal cells.[61] By IHC it has been demonstrated that L-SIGN is also expressed on type II pneumocytes and endothelial cells.[59] In general, DC-SIGN is mainly expressed in certain types of dendritic cells and alveolar macrophages.[62] However, in the lung tissue of SARS autopsies, DC-SIGN has also been localized to pneumocytes, which may indicate that SARS infection is capable of inducing DC-SIGN expression.[26] *In vitro* experiments have demonstrated that cells expressing DC-SIGN or L-SIGN without ACE-2 are not, or are only partially, susceptible to SARS-CoV infection.[59,60,63] This would imply that these molecules are much less efficient receptors than ACE2 as specific receptors and may therefore merely enhance infection of permissive cells.[60,63] Dendritic cells expressing DC-SIGN may transfer SARS-CoV to susceptible cells such as pneumocytes through a synapse-like structure.[63]

Not only does ACE2 function as a SARS-CoV receptor, it also plays an essential role in the pathogenesis of SARS. ACE2 is a key molecule in the renin-angiotensin system. It counteracts the effects of ACE on the renin-angiotensin system and down-regulates the production of angiotensin II.[64] ACE2 and AT2-receptors play protective roles in severe acute lung injury, whereas ACE, angiotensin II, and AT1-receptors probably induce lung failure.[64] Animal experiments have demonstrated that binding of SARS-CoV S protein to ACE2 down-regulates the expression of ACE2, resulting in a diminished protective role of ACE2 and, subsequently, acute respiratory

failure.[65] Likewise, the SARS-CoV-mediated down-regulation of ACE2 in humans provides an explanation for the progression to severe lung injury in some SARS patients.

Viral Cytopathic Effects

Direct viral effects are also likely to contribute to the serious pulmonary injury resulting from SARS-CoV infection. In particular, during the first 10 days of the disease when virus replication is prevalent, viral effects seem to play an important role. The virus is capable of causing cytopathic effects in both Vero E6 cells and ciliated tracheobronchial epithelial cells *in vitro*.[1,52] The presence of multinucleated cells in SARS lungs may also be the result of viral cytopathic effects (Figure 1B).[23] Induction of apoptosis in SARS-CoV-infected Vero E6 cells has been confirmed by morphological and biochemical analyses.[66] SARS-CoV nonstructural proteins may be involved in this process.[67,68] Furthermore, SARS-CoV structural proteins also have the ability to trigger apoptosis *in vitro*.[69] Overexpression of SARS-CoV 7a induces apoptosis through a caspase-dependent pathway in several human cell lines, including cells derived from the liver, kidneys, and lungs.[67] SARS-CoV E proteins may be involved in T-cell apoptosis through inhibition of anti-apoptotic proteins Bcl-xL.[69] *In vivo* studies have reported evidence of apoptosis in cells of thyroid glands, spermatogenetic cells, epithelial cells, pneumocytes, monocytes/macrophages, lymphocytes, and hepatocytes.[23,45,48,49] As mentioned above, increased expression of transforming growth factor-β1 has been detected in infected alveolar and bronchial epithelial cells.[23] Because transforming growth factor-β1 is an enhancer of Fas-mediated cell apoptosis, strong induction of this cytokine may also partially account for apoptosis of such cells.[23]

Infection of Immune Cells

In vitro infected peripheral blood mononuclear cells (PBMCs) have shown viral replication up to 8 days.[70] In other experiments, however, SARS-CoV infection of macrophages, monocytes, and dendritic cells seemed to be abortive.[71,72] In PBMCs obtained from SARS patients, SARS-CoV has also been found to infect and replicate, although replication was self-limiting.[73] We have confirmed infection of various circulating immune cells including monocytes and T lymphocytes in the early phase of the disease.[15] Mean infection rates of lymphocytes and monocytes amounted to 51.5 and 29.7%, respectively. Furthermore, we have provided evidence of infection of both T lymphocytes (Figure 1D) and macrophages/monocytes in the circulating blood, lymph nodes, lungs, and spleens in SARS autopsies.[15,27,33] These findings may provide a partial explanation for the lymphopenia and the widespread destruction of spleen and lymphoid tissue in the majority of SARS patients. However, considering the relatively long half-life of circulating lymphocytes, other factors, such as aberrant homing of lymphocytes, apoptosis, autophagy, and infection of bone marrow precursor cells may also contribute to the severe reduction of lymphocytes. As for SARS, the role of these factors has not yet been clarified. Infected immune cells may cause widespread dissemination to various organs, as has been reported in some studies.[15,27,30,49] Because monocytes and T cells are involved in both the innate and adaptive immune system, the destruction of such cells may result in a compromised immune response. In our view, this may contribute to the occurrence of severe pulmonary injury.[15] This is supported by observations that low CD4 and CD8 T-lymphocyte counts correlate with disease severity and adverse outcome.[10,11] Lymphopenia is frequently observed in other viral infectious viral diseases, such as measles, Ebola, Lassa fever, and respiratory syncytial virus infections.[74–76] However, in these diseases, direct infection and subsequent destruction of lymphocytes are generally regarded unlikely to account for the severe lymphocyte depletion. In measles, for instance, only a small proportion of the patient's PBMCs are infected during acute infection,[74] contrasting with the high infection rates of PBMCs in SARS. In respiratory syncytial virus infections, macrophages/monocytes are the primary immune cells targeted by the virus,[76] whereas viruses causing hemorrhagic fever are not capable of infecting lymphocytes.[76]

Chemokines and Cytokines

Both cytokines and chemokines (chemotactic cytokines) are soluble proteins with a key function in the innate immune system. Dysregulation of these proteins may result in immune-mediated injury. In the early reports after the emergence of SARS, it had already been suggested that the severe SARS-related injury may be attributable to an excessive reaction of the host's immune system, particularly dysregulation of proinflammatory cytokines.[16] This assumption is supported by the clinical deterioration of many patients in the second week of the disease's course, despite decreasing viral loads.[6,16] Increased serum levels of several cytokines were found in the majority of the SARS patients.[60,65] However, no consistent cytokine profile has been reported to date. Later reports have failed to demonstrate significant increases of serum levels of cytokines that normally play an important role in the immune defense against viruses.[34,77] In contrast, an increase of immunosuppressive soluble factors prostaglandin E2 and transforming growth factor-β in serum was detected in certain cases, providing an alternative explanation for the prolonged and severe clinical course.[53]

Recent studies have focused on the role of chemokines rather than cytokines in SARS infection. High serum levels of various types of chemokines were detected in several SARS patients.[34,35,78,79] *In vitro* experiments have demonstrated that SARS-CoV infection of macrophages, dendritic cells, and alveolar epithelial cells induces significant gene overexpression of chemokines, including macrophage inflammatory protein-1α, regulated on activation normal T cell expressed and secreted, IP-10, interleukin-8, and monocyte chemoattractant protein-1.[26,71,72] Increased secretion of these

chemokines has been confirmed in culture supernatants of infected cells.[26,72] In addition, expression of the IP-10 gene seemed to be significantly increased in lung tissue and lymphoid tissue of autopsied SARS patients, which was also confirmed by IHC with anti-IP-10 antibodies.[34,35] Increased IP-10 concentration has been found to be an independent predictor of adverse outcome.[35] He and colleagues[23] have recently demonstrated strong induction of several proinflammatory cytokines and chemokines in cells expressing both ACE2 and SARS-CoV S protein in SARS patients, which may imply that up-regulation of such cytokines in virus-infected cells may contribute to acute lung injury.[23]

Based on the results of the above-mentioned *in vitro* and *in vivo* experiments, SARS-CoV infection of pneumocytic epithelial cells may theoretically induce secretion of chemokines *in vivo*, mediating migration of monocytes and neutrophils to the infection site.[26] Infection of dendritic cells and recruited macrophages may subsequently result in secretion of additional chemokines, thus further enhancing migration of several types of immune cells, including activated T cells.[26,71,72] Excessive induction of proinflammatory cytokines and chemokines and recruitment of immune cells together possibly explain the severity of the injury often observed in the lungs of SARS patients.

The Innate Immune Response

Recent studies have investigated the role of the innate immune response in the pathogenesis of SARS. The fact that the virus replicates progressively in the upper respiratory tract during the first 10 days of the disease has raised the suggestion that SARS infection may cause deficiencies in the innate immune response.[7,71,72] The innate immune system constitutes the first line of the immune defense against viruses and involves several cellular components and soluble factors.[56] With respect to the role of the innate immune system in SARS-CoV infection, interferons, mannose-binding lectin (MBL), macrophages, and dendritic cells have all been studied. In viral infections in general, interferons are produced and secreted by infected cells. These cells cause adjacent cells to synthesize antiviral agents that tend to restrict viral dissemination. Contrary to other viruses, SARS-CoV is not capable of inducing significant interferon-α or -β gene expression in infected macrophages, PBMCs, or infected dendritic cells.[71,72] SARS-CoV may evade the effects of interferon induction by blocking a specific molecular step in the activation of interferon-β.[80] In addition, SARS-CoV seems to impair the phagocytic capacity of macrophages, which may render SARS patients prone to secondary pulmonary infections.[81] SARS-CoV also causes phenotypic and functional maturation of dendritic cells *in vitro*, resulting in a moderate production of cytokines and an enhanced T-cell-stimulatory capacity.[81] Activated T cells may exert cytotoxic activity in the lungs, further contributing to pulmonary injury.

MBL deficiency seems to play a key role in the pathogenesis of SARS. MBL is a serum protein that can bind to the ligands of various pathogens, flagging them for immune destruction, independently of a specific antibody response.[82] MBL is capable of binding to SARS-CoV and inhibiting SARS-CoV infectivity *in vitro*.[83] Both low MBL serum levels and haplotypes associated with MBL deficiency have been detected in SARS patients.[83]

Autoimmunity

Autoimmunity may also be involved in the pathogenesis of SARS. Autoantibodies against pulmonary epithelial cells and endothelial cells have been detected in SARS patients.[84,85] These autoantibodies may cause cytotoxic injury to the pulmonary epithelial cells and may induce systemic vasculitis, both frequently observed in SARS autopsies.[84,85] Autoimmunity may be partially attributable to the development of cross-reacting antibodies against specific SARS-CoV epitopes. IgG antibodies against the domain 2 of spike protein have indeed been found to cross-react with pulmonary epithelial cells.[84] Another mechanism that possibly explains autoimmunity is the exposure of autoantigens caused by cytokine-induced organ injury.[85]

Host Factors

Certain host factors have been found to affect the course of disease and outcome of SARS, including age, sex, and pre-existing co-morbid conditions.[8,86–90] Advanced age is independently associated with higher mortality,[8,86] as evidenced by fatality rates ranging from 3% in the youngest age group to 55% in the oldest age group.[86] Survival is also associated with sex, with male cases showing a significantly higher mortality than female cases.[86,87] Patients with co-morbid conditions such as diabetes mellitus, cardiac diseases, pulmonary diseases, and chronic hepatitis furthermore showed a significant increase of mortality.[86–90] In an analysis of 1755 patients, the case fatality rate for patients with co-morbid conditions amounted to 46%, compared with 10% for those without co-morbid conditions.[86] The fact that these co-morbidities are all characterized by a decreased cardiopulmonary status and/or a compromised immune system[88,89] possibly accounts for the increased mortality. Use of glucocorticoids, a key component of SARS treatment, may further aggravate the pre-existing illness, resulting in an even worse outcome.[88]

Genetics

Genetic factors also seem to play a causative role in the pathogenesis of SARS. For a group of Taiwanese patients, the HLA-B*4601 haplotype was associated with severity of SARS infection.[91] This association has not been established for certain Hong Kong Chinese patients.[92] Nevertheless, in the latter group of patients, a strong association was demonstrated between HLA-B*0703 and HLA-DRB1*0301 alleles and an increased susceptibility to SARS infection.[92] In contrast, L-SIGN homozygote individuals seem to have a significantly

lower risk of SARS infection.[93] Furthermore, genotypes associated with low or deficient MBL serum levels have more frequently been detected in SARS patients than in control cases.[83]

Concluding Remarks

In conclusion, extensive reports about the pathology and pathogenesis of SARS present a complex picture of the etiological factors involved, the intricate causes and consequences and their interplay. It is noteworthy that although the major clinical manifestations of the disease involve the respiratory system, the key component of the pathogenesis seems to be related to the immune system. Hyperinduction of chemokines and cytokines, insufficient interferon reaction, and a compromised cellular immune response caused by direct infection or indirect injury of immune cells may all contribute to SARS-related pathological changes.[94] A compromised immune response may lead to aggravation of SARS-CoV-induced lung injury, which otherwise might not have been so widespread and devastating. A more comprehensive understanding of the key features of the pathogenesis is crucial to establish appropriate animal models, which are essential for the production of vaccines. Advances made in the fields of pathology and pathogenesis of SARS will also serve to guide the diagnosis, prevention, and treatment of SARS should it or a similar disease appear in the future.

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