

Review Article

# How the SARS coronavirus causes disease: host or organism?

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## Abstract

The previous epidemic of severe acute respiratory syndrome (SARS) has ended. However, many questions concerning how the aetiological agent, the novel SARS coronavirus (CoV), causes illness in humans remain unanswered. The pathology of fatal cases of SARS is dominated by diffuse alveolar damage. Specific histological changes are not detected in other organs. These contrast remarkably with the clinical picture, in which there are apparent manifestations in multiple organs. Both pathogen and host factors are important in the pathogenesis of SARS. The choice of specific receptors and the unique genome of the SARS-CoV are important elements in understanding the biology of the pathogen. For the host cells, the outcome of SARS-CoV infection, whether there are cytopathic effects or not, depends on the cell types that are infected. At the whole-body level, immune-mediated damage, due to activation of cytokines and/or chemokines and, perhaps, autoimmunity, may play key roles in the clinical and pathological features of SARS. Continued research is still required to determine the pathogenetic mechanisms involved and to combat this new emerging human infectious disease.

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Keywords: SARS; coronavirus; pathogenesis; cytokines; chemokines

## Introduction

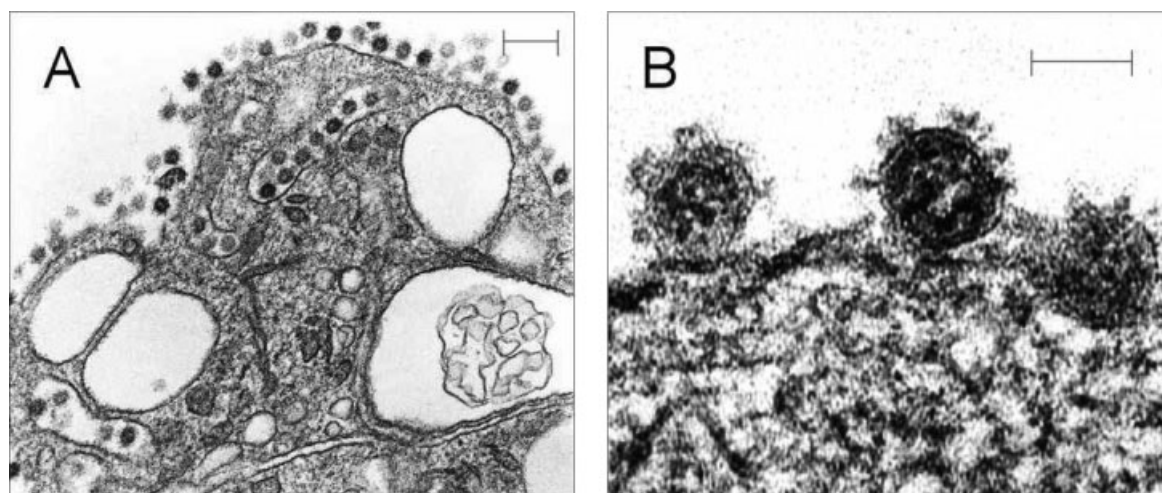
Severe acute respiratory syndrome (SARS) is a new viral disease caused by a novel coronavirus, SARS-CoV (Figure 1) [1,2]. The saga of SARS has officially come to an end, as no more new cases have been reported since 2004. Many questions, particularly those related to how SARS-CoV causes disease, however, remain unanswered.

The disease caused by SARS-CoV differs from the diseases caused by the previously known human coronaviruses, 229E and OC43. SARS-CoV infection results in severe and potentially fatal lung disease [1,2]. Although the majority of patients recovered after 1–2 weeks of debilitating febrile illness, a substantial proportion (up to one-third) developed severe inflammation of the lung, requiring ventilator support and intensive care. Many patients in this group deteriorated into acute respiratory distress syndrome (ARDS). The mortality of this group of patients is high [3]. Manifestations in other organ systems are characteristic. Lymphopenia [4], gastrointestinal symptoms [5], impaired liver function [6,7], and impaired renal function [8] are common. The possibility of viral infection

in multiple organs has been raised and viral replication in the lung, kidney, and gastrointestinal tract was reported [9,10]. In addition, prolonged shedding of virus was found in some convalescent patients [11]. However, chronic infection by SARS-CoV has not, to date, been documented in humans. Moreover, asymptomatic carriage of SARS-CoV is rare [12].

There are significant age differences in the prognosis of SARS. Children have a good prognosis [13], while elderly patients with chronic illnesses fare badly. SARS is predominantly a lower respiratory tract disease, yet the most consistent and powerful prognostic indicator reported so far is blood lactate dehydrogenase (LDH) concentration [1], which is most likely a surrogate indicator and may reflect the extent of ongoing tissue damage.

Both pathogen and host factors are important for the progression of an infection. Here, we review the pathology of SARS infection. Specific features of the pathogen SARS-CoV itself are then addressed. Finally, host factors, particularly an emerging understanding of immunological and inflammatory responses to SARS-CoV infection, are discussed.



**Figure 1.** SARS-CoV replicates in cultured Vero E6 cells and is produced in large numbers inside cytoplasmic vesicles (A). Virus particles can also be seen budding through the cytoplasmic membrane (B). Each virion particle is 60–90 nm in size by transmission electron microscopy and is characterized by the numerous club-shaped projections on the outside, a ring beneath the envelope, and an electron-lucent centre. Scale bars = 200 nm (A) and 50 nm (B)

### Pathology of SARS in human and animal models

#### Diffuse alveolar damage is the most characteristic pathology in SARS

Most data on the human pathology of SARS come from autopsy studies of fatal cases [14–21]. These reports thus reflect the terminal stages and are likely to represent only the more severe end of the spectrum of SARS. Treatment and co-morbid conditions might also modify the pathological changes. Diffuse alveolar damage at different stages of organization is the most consistent finding in the lungs of SARS patients in the terminal stage (Figures 2A–2F). Multi-nucleated syncytial cells (Figures 2G and 2H) are characteristic, although these cells are rare. Apart from when secondary infection occurs, the lack of a prominent inflammatory response is also distinctive. SARS-CoV is explicitly detected in the alveolar lining cells (Figures 2I and 2J) [10,22–27]. No specific pathology is identified in the gastrointestinal tract (Figure 3) [5], urinary system [8], or other organ systems [28], apart from that related to end-stage multi-organ failure or those changes secondary to treatment. It is important to note that in some organs such as the liver, while definitive and distinct morphological and functional changes are observed, SARS-CoV may not be unequivocally demonstrable [29].

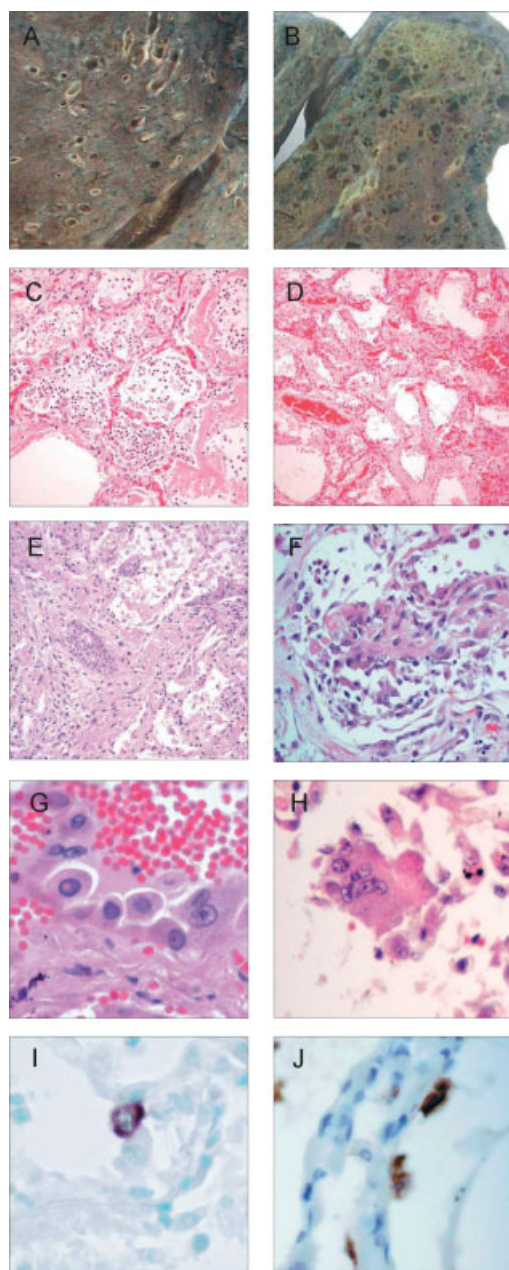
It is clear that our understanding of the pathology of SARS is incomplete. An obvious large gap is the lack of information on the early pathological changes of SARS. During the epidemic, very few biopsies were obtained from patients with clinically active SARS.

#### Animal models in the understanding of SARS

The study of animal models is important in a number of ways. It has allowed the establishment of SARS-CoV as the aetiological agent [30]. It also provides controlled conditions for the study of early changes in the disease. Initial studies of macaque models were promising. The histology of infected lung tissue is similar to that in humans [31–33]. Both acute and organized stages of diffuse alveolar damage were seen when the macaques were sacrificed on the sixth day after a heavy dose of the virus. SARS-CoV was detected in the alveolar epithelial cells and in the intra-alveolar syncytial cells. However, detailed morphological studies and viral distribution in other organs in these animal studies are lacking. In studies involving longer observation times, the disease in macaque models appears self-limiting and different from the genuine human disease. The usefulness of the macaque as a model of the disease remains to be established [32,33].

Civet cats, domestic cats, and ferrets are thought to have been potential reservoirs of the virus during the epidemics and subsequent smaller outbreaks in mainland China [34]. The animal coronavirus identified in civet cats shows high sequence identity with, but is distinct from, SARS-CoV [2,35]. Recent evidence also suggests that wide Chinese horseshoe bats harbour a closely related bat-SARS-CoV which might also act as the animal reservoir [36]. Again, details concerning the distribution of virus in different organs in these animals and the information on the pathology in the diseased or carrier animals are, surprisingly, sparse [32,35].

Other common small laboratory animal models, such as the mouse, are not particularly useful. SARS-CoV has a low virulence in ordinary laboratory mice and very high levels of inoculation are required to produce self-limiting diseases. These features may be



**Figure 2.** Diffuse alveolar damage is the most consistent finding in the terminal stages of SARS. The lung may appear grossly consolidated (A) or have a honeycomb appearance (B). Although the latter finding may be related to pre-morbid lung pathology, a correlation with interstitial fibrosis and disease duration has been demonstrated [21]. Diffuse alveolar damage at different stages of organization, from fibrin deposition (C, H&E, original magnification  $\times 200$ ), to interstitial fibrosis (D, H&E, original magnification  $\times 100$ ) and cellular organization (E and F, H&E, original magnification  $\times 400$ ), can be detected. Atypical pneumocytes with enlarged nuclei and prominent nucleoli are often seen and some pneumocytes coalesce into syncytial multi-nucleated cells (G, H&E, original magnification  $\times 600$ ). Multi-nucleated histiocytes may also be found (H, H&E, original magnification  $\times 600$ ). SARS-CoV can be detected in pneumocytes by *in situ* hybridization (I, using a DNA probe against the M gene, original magnification  $\times 600$  [22]). A large array of antibodies against the viral proteins including nucleocapsid N, spike S, membrane M, and SARS-3a [23], has been developed for the detection of SARS-CoV in formalin-fixed, paraffin-embedded tissue sections (J, showing immunohistochemical staining with an anti-peptide antibody against N, original magnification  $\times 600$ )

related to differences in the affinities of SARS-CoV for human receptors and their murine homologues [37].

### Pathogen factors: specific features of SARS-CoV

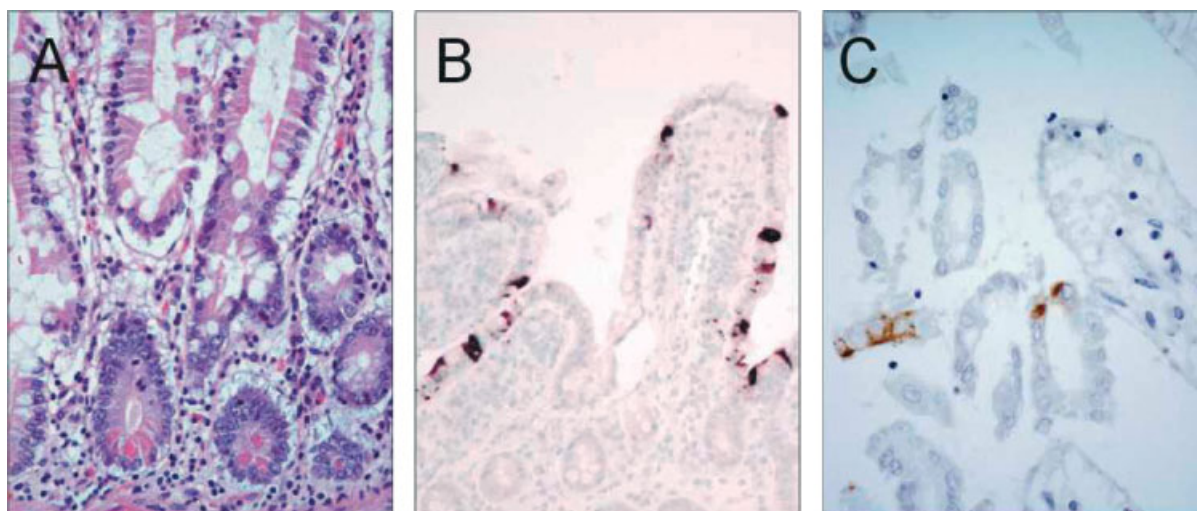
#### SARS-CoV uses a protector of lung damage, angiotensin-converting enzyme 2, as a receptor

Characterization of the functional cellular receptor of SARS-CoV provides important clues to the pathogenesis of SARS. Angiotensin-converting enzyme 2 (ACE2) interacts directly with the Spike (S) proteins of the SARS-CoV [38–42]. The level of expression of ACE2 correlates with the efficiency of SARS-CoV infection in cell culture models [42–44]. ACE2 proteins are expressed by alveolar epithelial cells and by surface enterocytes of the small intestine [45], which are the primary target cells of SARS-CoV. Studies in the intestine cell culture model, however, suggested that, in addition to ACE2, unknown co-factors or co-receptors are required to convey infectivity [46].

In addition to being a cellular receptor, ACE2 may contribute to the pathogenesis of DAD in SARS through its role in the tissue renin–angiotensin system. In a mouse model of alveolar damage induced by acid aspiration, the balance of the renin–angiotensin system appears to affect the development of DAD. ACE2, which acts as a negative regulator of the local renin–angiotensin system, protects the mouse lung against experimental damage [47,48]. SARS-CoV co-infection in these damaged animals down-regulates ACE2 in the lungs of infected mice and the severity of lung damage can be alleviated by blocking the system [49]. Exciting as these findings appear, the case of a new coronavirus, NL63, immediately provides an example that other factors are acting in the overall mechanism of lung damage. NL63 utilizes the same ACE2 protein as its receptor in the lung. However, infection with NL63 results in only minor cold symptoms and alveolar damage is rare [50]. The insert/deletion genotype of the *ACE* gene was associated with DAD after SARS-CoV infection in a small cohort of 44 patients [51]. This association was, however, not replicated subsequently in a larger series [52]. We also could not detect any association between the *ACE2* genotype and disease severity in SARS-CoV infection [53].

#### SARS-CoV may also use the C-type lectins as receptors for infecting immune cells

C-type lectins, including CD209 and CD209L, are also SARS-CoV receptors: these were identified through the study of proteins that interact with the S (Spike) protein. CD209, also known as dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN), was shown to mediate viral entry in a lentiviral pseudo-type experimental model [54]. In



**Figure 3.** The small intestine shows no gross or microscopic pathology in terminal cases of SARS. Apart from autolytic changes, light microscopy reveals no specific abnormalities in the small bowel mucosa (A, original magnification  $\times 400$ ). However, SARS-CoV can be detected on the surface enterocytes using *in situ* hybridization (B, with a DNA probe against the M gene, original magnification  $\times 600$  [22]) or immunohistochemical staining (C, using anti-peptide antibody against SARS-3a, original magnification  $\times 600$  [23])

Chinese hamster ovary (CHO) cells expressing a human lung cDNA library, S protein and its fragments interacted directly with a second related cell surface glycoprotein, CD209L, also known as L-SIGN or DC-SIGNR [55]. CD209L acts in conjunction with LSEctin (liver and lymph node sinusoidal endothelial cell C-type lectin) and enhances viral infection [56]. Tissue cultures expressing CD209 or CD209L were also susceptible to SARS-CoV infection [54,55,57].

The possible involvement of dendritic cells is particularly interesting. Although SARS-CoV does not replicate in dendritic cells, these cells may act as a reservoir and distribute the virus to other cell types [54,58]. This is an attractive concept and similar biological behaviours have been proposed for human immunodeficiency virus I (HIV I) [59]. No SARS-CoV has been detected in dendritic cells in autopsy and biopsy studies reported so far.

#### The unique 3' end of the SARS-CoV may hold the key to specific viral behaviours

The genome of SARS-CoV consists of a single 27.69 kb positive-strand RNA. The genomic sequences derived from different phases of the SARS epidemic revealed no association with sequence variation and virulence [60,61].

There are two large open reading frames (ORFs) and 12 potential ORFs in the SARS-CoV genome. The two large ORFs encode non-structural proteins involved in replication. These proteins have relatively higher homologies to known coronaviruses. The remaining 12 ORFs are squeezed into the 3' end of the genome. These ORFs include four genes encoding known structural proteins (envelope, membrane, nucleocapsid, and spike proteins, respectively). The remaining potential ORFs encode hypothetical SARS-CoV-specific proteins which lack obvious sequence similarity to known

proteins [62,63]. The functions of these hypothetical proteins and their roles in SARS pathogenesis remain obscure [64,65]. Antibodies against some of these putative proteins, notably SARS3a and SARS6, can be detected in the serum of SARS patients [66]. There is also evidence suggesting that a number of these proteins, including SARS3a, 3b, 7a, and 9b, were expressed in pneumocytes and enterocytes in deceased patients [23]. However, differential expression patterns of these proteins in cell types showing different responses to SARS-CoV infection have not been confirmed.

By expressing the hypothetical proteins individually in tissue culture, we are beginning to see data on the cellular functions of these proteins. SARS3a appears to be important in mediating apoptosis in some cell types [67]. The SARS3a protein is incorporated into the viron particle and may also act as one of the structural proteins [68–70]. Through an unknown mechanism, host cells overexpressing SARS3a have increased expression of *fibrinogen* mRNA [71]. SARS7a has been implied in mediating apoptosis through the caspase-dependent pathways [72].

#### Host responses are important in SARS-CoV infection

The effect of SARS-CoV infection varies in different cell types. Apoptosis and syncytial formation are seen in infected monkey renal epithelial cells (Vero E6) [67]. Persistent infection with no change in cellular morphology or doubling time was detected in the colon cancer cell line LoVo [46]. In clinical specimens, SARS-CoV was detected in the lungs and small intestine. Severe cellular damage is characteristically detected in the lungs of SARS patients, while no morphological changes are observed in the small

intestine. The basis of these differences in cellular responses is not clear. The tissue/cellular tropism may be partly related to differential expression of membrane receptors for the SARS-CoV [22]. These observations highlight the importance of host cell responses in SARS-CoV infection. It is also clear from these observations that cytopathic damage alone cannot explain the pathogenesis of SARS.

The marked heterogeneity of the disease course and outcome after SARS infection suggests that host responses may play an important role in pathogenesis. DAD or ARDS appears to be a common pathway of lung parenchyma damage initiated by a variety of aetiologies, including SARS-CoV infection itself, systemic sepsis, shock, and direct lung contusion. Once an inflammatory process reaches a certain intensity, it may self-perpetuate. The cellular inflammatory infiltrate releases toxic metabolites and proteolytic enzymes, which may cause further damage to the lung parenchyma. The surrounding inflamed capillaries launch the coagulation cascade and recruit more immune cells [73,74].

#### Immune-mediated damage may be the main key to SARS pathology

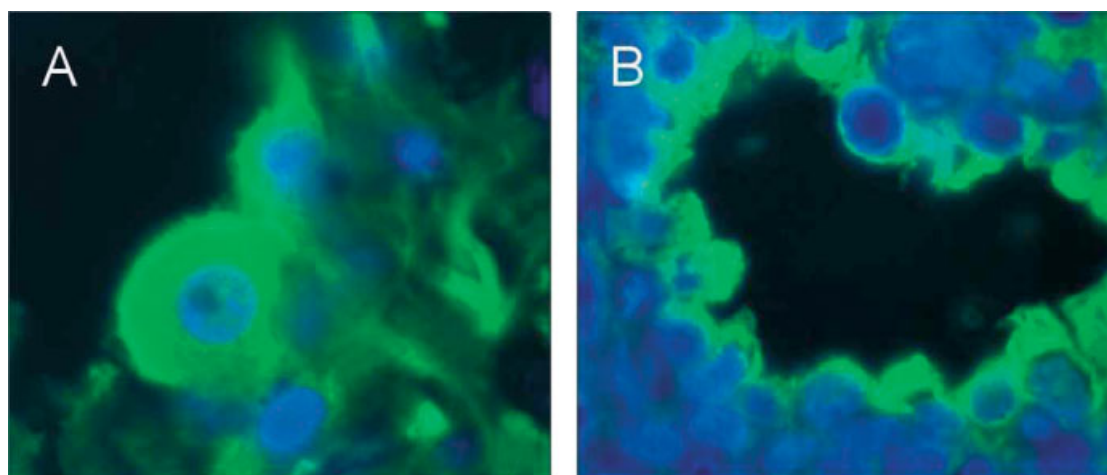
Our previous investigation in the 1997 H5N1 influenza outbreak showed that patients who died of the disease had lymphoid depletion associated with marked elevation of circulating concentrations of cytokines, including interleukin-6 (IL-6), IL-2 receptor, and interferon-gamma [75]. With the observation of characteristic lymphopenia in SARS, it has been postulated that the SARS-CoV may similarly trigger an exaggerated hyper-cytokinemic response in patients with DAD after viral infection [20]. Current understanding indicates that patients with a more intense immune response are those at risk of a poor outcome, as the immune system also mounts a profound reaction to the bystander, the lung parenchyma, and causes DAD [76]. SARS patients have variable humoral

responses to individual epitopes [66]. However, early sero-conversion and high peak total SARS-CoV IgG levels were associated with more severe disease in a cohort of 325 patients [77]. Hence, particularly strong humoral responses to SARS-CoV infection might not be protective but, perhaps, might be harmful to the host. The specific epitopes upon which these 'damaging' antibodies act await further characterization.

There is evidence that disarray of the immune system towards the host's own antigens may play a role in the pathology of SARS. In the early phase, within 1 week of SARS-CoV infection, IgM and IgG autoantibodies against antigens located in the cytoplasm of lung epithelial cells (Figure 4) were detected in the sera of 36 Chinese SARS patients (Lo, unpublished observations). In another cohort of 22 SARS patients, immune activity against antigens from lung epithelial cell lines and endothelial cell lines was found in some patients' sera obtained approximately 1 month after infection [78]. Moreover, high levels of these autoimmune activities in the sera were shown to be cytotoxic to lung epithelial cells and endothelial cells in culture. Autoimmune antibodies may be important in mediating tissue damage at certain stages of the disease. The cause of the autoimmunity is not fully understood. These autoantibodies may be the result of humoral responses to innate antigens exposed accidentally during direct damage of the lung and, perhaps, the endothelium by SARS-CoV. Alternatively, autoimmunity may be due to cross-reactivity of antibodies against some specific epitopes of the SARS-CoV proteins.

#### Chemokines are important immune mediators for lung pathology in SARS

The chemokines are a family of small proteins that play important roles in intercellular signalling and chemotaxis. Based on their protein sequences, they are broadly divided into  $\alpha$ -chemokines with a common C-X-C (cysteine-other-cysteine) structure of amino



**Figure 4.** Serum taken from SARS patients during the acute phase of the disease contains IgG against cytoplasmic antigens of pneumocytes. Application of acute phase serum as a primary antibody lights up the cytoplasm of pneumocytes of autopsy adult lung sections (A, original magnification  $\times 1000$ ) as well as fetal lung sections (B, original magnification  $\times 1000$ )

acid residues near the amino-terminus which interacts predominantly with neutrophils, and  $\beta$ -chemokines with a C–C (cysteine–cysteine) structure interacting with mononuclear cells. Recently, chemokines have been recognized for their roles in integrating the innate and adaptive immune responses to viral infection through a cytokine-to-chemokine-to-cytokine signalling cascade [79–81].

A global view of the spectrum of expression of the immune mediators was studied in SARS by measuring the circulating concentrations of these mediators at different stages of the disease. Most cytokines showed only transient and short-lived activation in patients after SARS-CoV infection [82]. Even in patients who developed DAD, most cytokine concentrations were not significantly increased [83]. In contrast, circulating concentrations of several chemokines, including CXCL9 (chemokine C–X–C motif ligand 9 or monokine induced by  $\gamma$ -interferon), CXCL10 (chemokine C–X–C motif ligand 10 or interferon-inducible protein-10), and CCL2 (C–C motif ligand 2 or monocyte chemoattractant protein-1), were markedly increased in SARS patients [82,84,85]. Remarkably, the circulating concentration of CXCL10 measured early after infection is an independent prognostic indicator of disease outcome [86]. These chemokines therefore appear to be important elements of the pathogenesis of SARS.

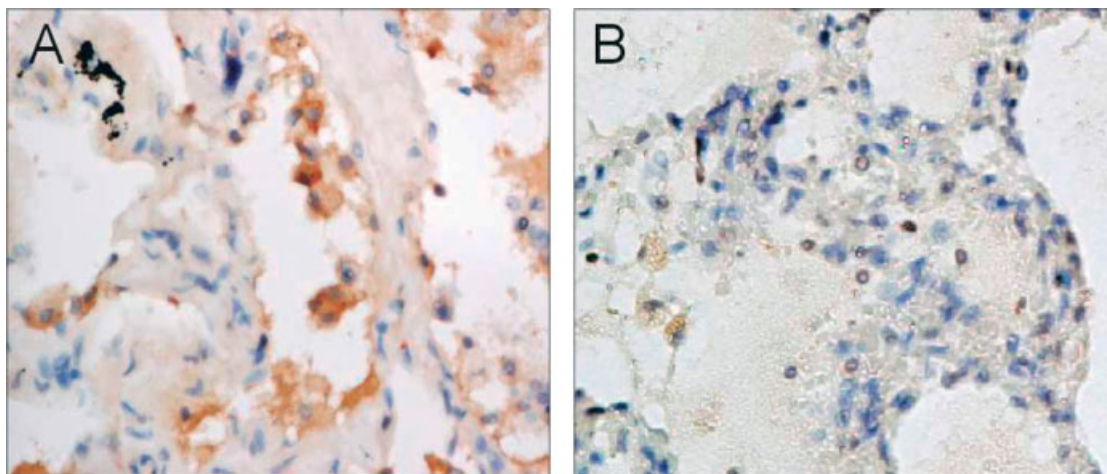
In the lung tissues obtained from seven SARS patients who died [86], chemokines CXCL10 (Figure 5) and IL-18 were markedly activated (25- and 40-fold compared with controls, respectively). The important roles of chemokines are underscored by the findings in an experimental mouse model of SARS-CoV infection in which CXCL10 and a neutrophil chemokine, CXCL8 (chemokine C–X–C motif ligand 8), were also markedly activated [87]. These findings in SARS compare favourably with the specific situation in HIV patients with lung allograft rejection and interstitial alveolitis, in which similar

activation of the chemokine CXCL10 and its receptor CXCR3 (chemokine C–X–C motif receptor 3) was also found [88,89].

Other than pneumocytes, chemokines are also expressed and secreted by various different cell types. Global gene expression profiles, generated by cDNA microarray analysis of peripheral blood mononuclear cells (PBMCs) after *in vitro* exposure to SARS-CoV, also reveal the importance of chemokine activation. Within 1 day after exposure to the virus, a number of chemokines (including CXCL10, CXCL9, and CCL2) were activated [90]. PBMCs and macrophages do not support productive infection as viral replication is abortive and no infectious virus is produced. The roles of these cell types in the pathogenesis of SARS remain to be clarified. Nonetheless, these easily obtainable cell types provide convenient experimental models and allow some insight into the patterns of host responses to the infection to be studied. Similar findings were also reported in other cell types, such as dendritic cells, where the cytokine expression profiles are predominantly of inflammatory chemokines CCL3 (chemokine C–C motif ligand 3), CCL5 (chemokine C–C motif ligand 5), CXCL10, and CCL2. Unlike the usual response of dendritic cells to viral infection, anti-viral cytokines, including IFN- $\alpha$  (interferon-alpha), IFN- $\beta$ , IFN- $\gamma$ , and IL-12B, were not activated [58].

#### Immunogenetics of the host may affect the severity of SARS

Other than using serum inflammatory mediators to reflect the different degree of host inflammatory reaction during an infection, the intensity of the immune response is also genetically determined. The difference in genetic makeup between individuals is mostly accounted for by single base differences (single nucleotide polymorphisms, SNPs). Many studies have shown an association between SNPs and predisposition to ARDS, and survival after sepsis or other



**Figure 5.** Chemokines are aberrantly expressed in terminal cases of SARS. Immunohistochemical staining using a monoclonal antibody against CXCL10 (IP-10) demonstrated overexpression of CXCL10 in the pneumocytes of SARS patients (A, original magnification  $\times 600$ ) but not in control autopsy lung (B, original magnification  $\times 600$ )

insults [91,92]. In the context of predisposition to ARDS after trauma, among parameters such as circulating concentrations of IL-1, tumour necrosis factor and plasminogen activator inhibitor-1 (PAI-1), and the genotype of *PAI-1*, insertion alleles at the promoter of *PAI-1* were associated with high concentrations of PAI-1 in the plasma and a poor survival rate [93]. In addition to PAI-1, other genetic polymorphisms, such as angiotensin-converting enzyme (ACE) [94], CD14 [95], surfactant protein [96], and HLA genotypes [97], are also associated with predisposition to, severity, and outcome of ARDS. Although SARS-CoV utilizes ACE2 as its receptor and ACE2 is known to be an important protector of lung damage in experimental ARDS, we and other groups found no solid association between alleles of the two *ACE* genes (*ACE* and *ACE2*) and the severity of ARDS after SARS infection [52,53,98].

Several immunogenetic studies have been reported in association with SARS infection. Among 37 Taiwanese SARS patients, HLA-B\*4601 was associated with both predisposition to infection and severity of infection [99]. However, the association of this allele was replicated in another Chinese community of Hong Kong involving 90 SARS patients [100]. HLA-B\*0703 was found to be a predisposition allele in the latter study. It should be noted that this latter allele is rare and is found in ~3% of the general population. Hence, this allele cannot be considered a major predisposition factor for SARS infection [100]. Immunogenotype may play a role in determining the severity of host responses. There is considerable variability in the prevalence of immunogenotypes among different populations and the significance of detecting so-called 'predisposing' alleles in clinical practice is questionable. More studies are needed to uncover fully the real genetic determinants for both predisposition to infection and the host-pathogen interaction after infection with the virus.

## Conclusion

A considerable amount of knowledge of SARS infection has accumulated as a result of almost 3 years of research since the emergence of SARS. Some key issues about the pathogen, SARS-CoV, have been addressed. These include the rapid discovery of SARS-CoV receptors and the actions of some of the specific viral proteins in different host cells. Understanding the molecular basis of differences in host cell responses to SARS-CoV infection will be crucial in delineating its pathogenesis. It is also clear from clinical and experimental data that host immune responses may be the key determinant in disease progression after initial SARS-CoV infection. Future studies aimed at characterization of the variability of host immune and inflammatory responses will be important in understanding this new emerging infectious disease.

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