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SARS: clinical virology and pathogenesis

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SARS: clinical virology and pathogenesis
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Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus, called the SARS coronavirus (SARS-CoV). Over 95% of well characterized cohorts of SARS have evidence of recent SARS-CoV infection. The genome of SARS-CoV has been sequenced and it is not related to any of the previously known human or animal coronaviruses. It is probable that SARS-CoV was an animal virus that adapted to human-human transmission in the recent past. The virus can be found in nasopharyngeal aspirate, urine and stools of SARS patients. Second generation reverse transcriptase polymerase chain reaction assays are able to detect SARS-CoV in nasopharyngeal aspirates of approximately 80% of patients with SARS within the first 3 days of illness. Seroconversion for SARS-CoV using immunofluorescence on infected cells is an excellent method of confirming the diagnosis, but antibody responses only appear around day 10 of the illness. Within the first 10 days the histological picture is that of acute phase diffuse alveolar damage (DAD) with a mixture of inflammatory infiltrate, oedema and hyaline membrane formation. Desquamation of pneumocytes is prominent and consistent. After 10 days of illness the picture changes to one of organizing DAD with increased fibrosis, squamous metaplasia and multinucleated giant cells. The role of cytokines in the pathogenesis of SARS is still unclear.

Key words: histology, pathology, SARS coronavirus, severe acute respiratory syndrome.

Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus, now called the SARS coronavirus (SARS-CoV).[1–3] Over 95% of well characterised cohorts of SARS have evidence of recent SARS-CoV infection[1,4–6] and experimental infection of cynomolgous macaques with SARS-CoV leads to a SARS-like disease associated with lung pathology and giant cells reminiscent of the human disease.[6,7] Investigations during some outbreaks of SARS have also revealed coinfection with human metapneumovirus or other pathogens in a proportion of patients.[7,8] Whether such coinfections contribute to enhancing the pathogenesis or transmission of the disease is still uncertain.

The genome of SARS-CoV indicates that it is a novel virus within the family coronaviridae, not closely related to any of the human or animal coronaviruses known to date. Healthy humans have no serological evidence of past SARS-CoV infection. Closely related viruses have recently been isolated from animals such as civet cats.[9] It is probable that SARS-CoV was an animal virus that adapted to human-human transmission in the recent past. The presence of this animal reservoir implies that it is possible for this virus to again cross into humans and initiate outbreaks of disease in the future. Many respiratory viruses, including coronaviruses have a seasonality and that of SARS-CoV is yet unknown. It is therefore possible that, similar to other coronaviruses, winter may be more conducive to the transmission of SARS-CoV.

SARS-CoV can be detected by culture in vero-E6 or FRhK-4 cells or by reverse transcriptase polymerase chain reaction (RT-PCR) in respiratory secretions, faeces and urine, and also less frequently in blood. The infection is thus not restricted to the respiratory tract alone and the presence of diarrhoea in a proportion of patients is one indication of this fact. In contrast to most other respiratory viruses, SARS-CoV viral load in the respiratory tract and faeces is low in the first few days of the illness but peaks around day 11 of the disease.[4,5] This accounts for the low sensitivity of the first generation diagnostic tests in the first few days of illness. It may also explain in part the unusual predilection of this virus to spread among health care

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workers, as patients are usually most infectious later in the illness by which time many of them are already hospitalized.

Respiratory specimens and faeces are useful clinical specimens for RT-PCR diagnosis, but faeces is a less satisfactory specimen in the first 5 days of illness. Nasopharyngeal aspirates are respiratory specimens of choice, but throat swabs can also be used, though with lower diagnostic yield. When patients are producing sputum, it is also an excellent clinical specimen, however, in most patients the cough is non-productive. Second generation RT-PCR assays are able to detect SARS-CoV in nasopharyngeal aspirates of approximately 80% of patients with SARS within the first 3 days of illness.[10] Seroconversion for SARS-CoV using immunofluorescence on infected cells is an excellent method of confirming the diagnosis, although antibody responses appear only around day 10 of the illness and such diagnosis is retrospective in nature.[3,5] Since some patients have a delayed antibody response it is advisable to obtain a convalescent serum at least 21 days, but ideally 28 days, after the onset of the disease, especially if patients have been on high dose steroid therapy in the acute stage of the illness.[4]

Understanding the pathogenesis of SARS is bedeviled by the fact that most available autopsy tissues are from patients dying later in the illness, at a time when the initial viral pathology is obscured by secondary infections or changes due to ventilator therapy, steroids and other immune modulators. Despite these limitations on autopsy material, it has been possible to determine that there are two phases of SARS pneumonia.[11,12] Within the first 10 days the histological picture is that of acute phase diffuse alveolar damage (DAD) with a mixture of inflammatory infiltrate, oedema and hyaline membrane formation (Fig. 1). Desquamation of pneumocytes is prominent and consistent. One report initially published in Chinese[13] but later in English in another journal[14] mentioned viral intracytoplasmic inclusion bodies but these were not identified in the Hong Kong or Singapore cases.[11,12] After 10 days of illness the picture changes to one of organizing DAD with increased fibrosis, squamous metaplasia and multinucleated giant cells (Fig. 2).[11,12,15] The giant cells that have also been seen in the experimentally induced SARS pneumonia in primates and coronaviruses are known to give rise to syncitia.[16] Histologically, the appearance is not distinctive enough to make a diagnosis without viral detection. In humans, as well as in primates, SARS-CoV was identified in the lung.[1,6,11,12]

The majority of post-mortem examinations performed on patients with SARS have involved patients with a disease duration of 10 days or more, that is, patients with the organizing stage of DAD. In the few cases of patients succumbing before then there were increased numbers of interstitial macrophages (INT) and alveolar macrophage (AM) present with focal haemophagocytosis seen in the former.[11] An experimental study on primates[6] found tracheobronchial lymph node and spleen enlargement but low numbers of lymphocytes. This lymphopenia is a consistent finding in the majority of SARS cases and may be similar in aetiology to influenza-induced leukopenia.[17] The primate study also found increased numbers of alveolar macrophages and this was also present in our single early case of SARS. In influenza this has been explained by chemokines stimulated by influenza preferentially recruiting blood mononuclear cells to the site of infection (reviewed in [18]). In the normal lung AM are supposed to be a developmental end-point stage of monocytes while INT macrophages represent a precursor stage. The two also differ in their function—AM are effective as non-specific first-line defence against infectious agents whereas INT macrophages cooperate with interstitial lymphocytes in inducing a specific immune response.

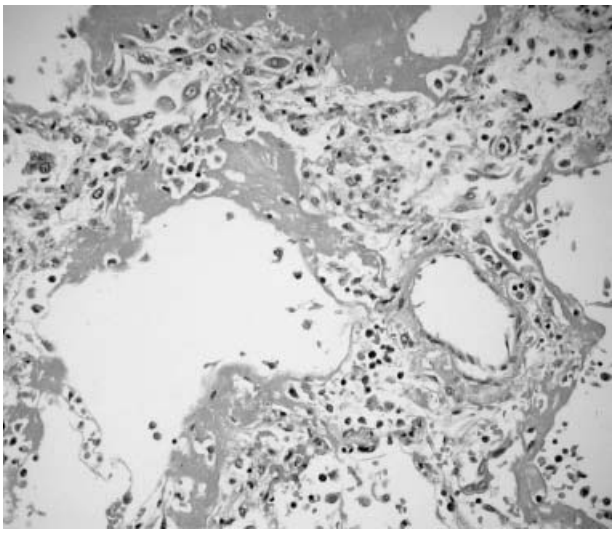

Figure 1 Exudative phase DAD in a patient with SARS pneumonia showing exudation and hyaline membrane formation. H and E × 200.

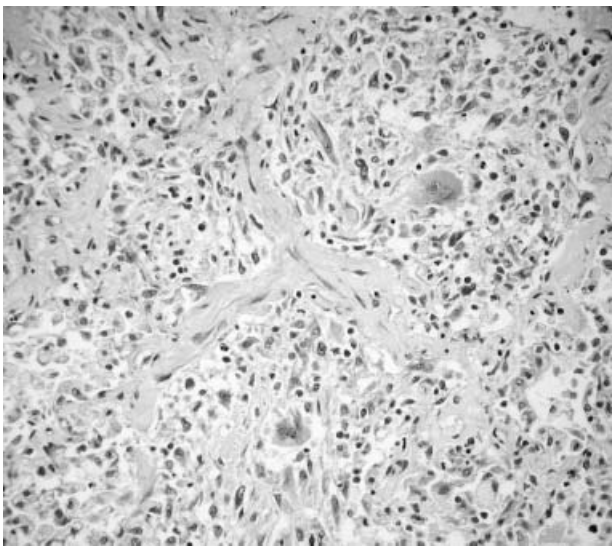

Figure 2 Organizing phase DAD showing fibrosis and giant cell formation. Toludine Blue × 200.

Though cytokines may not play a major role in the fibrotic stage of DAD[19] the role of undue immunopathology in the lung damage observed in early SARS remains unclear. In animal models, TNFα upregulation by macrophages led to a T-lymphocyte predominant alveolitis that progressed to a histological picture resembling idiopathic pulmonary fibrosis.[20] Immunohistochemical stains on our SARS patients have shown the presence of T-lymphocytes in the alveolar walls and interstitium. If SARS-CoV is similar to other CoV infections where there is early clearance of the virus by day 9, a period similar to the exudative phase of SARS DAD, the findings suggest that the first phase of the disease is cell damage with macrophage and T-lymphocyte infiltration. The second stage is the organizing fibrotic DAD aggravated by mechanical ventilation, in which cytokines play a minor role and only small quantities of virus are still present in lung tissues. Further studies in the primate model may elucidate the pathogenesis of the lung damage and provide a better foundation for patient management.

REFERENCES

1 Peiris JS, Lai ST, Poon LL *et al.* SARS Study Group. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003; **361**: 1319–25.
2 Drosten C, Gunther S, Preiser W *et al.* Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 2003; **348**: 1967–76.
3 Ksiazek TG, Erdman D, Goldsmith CS *et al.* SARS Working Group. A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 2003; **348**: 1953–66.
4 Peiris JS, Chu CM, Cheng VC *et al.* Clinical progression and viral load in a community outbreak of coronavirus associated-SARS pneumonia: a prospective study. *Lancet* 2003; **361**: 1767–72.
5 Chan KH, Poon LLM, Cheng VCC *et al.* Detection of SARS coronavirus (SCoV) by RT-PCR, culture and serology in patients with severe acute respiratory syndrome (SARS). *Emerg. Infect. Dis.* 2003; in press.
6 Kuiken T, Fouchier RA, Schutten M *et al.* Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 2003; **362**: 263–70.
7 Fouchier RA, Kuiken T, Schutten M *et al.* Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* 2003; **423**: 240.
8 Poutanen SM, Low DE, Henry B et al. National Microbiology Laboratory Canada; Canadian Severe Acute Respiratory Syndrome Study Team. Identification of severe acute respiratory syndrome in Canada. *N. Engl. J. Med.* 2003; **348**: 1995–2005.
9 Guan Y, Zheng BJ, He YQ *et al.* Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003; **302**: 276–8.
10 Poon LLM, Chan KH, Wong OK *et al.* Early diagnosis of SARS Coronavirus infection by real-time RT-PCR. *J. Clin. Virol.* 2003; **28**: 233–8.
11 Nicholls JM, Poon LL, Lee KC *et al.* Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003; **361**: 1773–8.
12 Lung pathology of severe acute respiratory syndrome (SARS). A study of 8 autopsy cases from Singapore. *Hum. Pathol.* 2003; **34**: 729.
13 Ding YQ, Wang HJ, Shen H *et al.* [Study on etiology and pathology of severe acute respiratory syndrome]. *Zhonghua Bing Li Xue Za Zhi* 2003; **32**: 195–200.
14 Ding Y, Wang H, Shen H *et al.* The clinical pathology of severe acute respiratory syndrome (SARS): A report from China. *J. Pathol.* 2003; **200**: 282–9.
15 Lee N, Hui D, Wu A *et al.* A major outbreak of severe acute respiratory syndrome in Hong Kong. *N. Engl. J. Med.* 2003; **348**: 1986–94.
16 Cavanagh D. The coronavirus suface glycoprotein. In: Siddell S, ed. *The coronaviridae*. Plenum Press, New York, 1995; 73–113.
17 Nichols JE, Niles JA, Roberts NJ Jr. Human lymphocyte apoptosis after exposure to influenza A virus. *J. Virol.* 2001; **75**: 5921–9.
18 Julkunen I, Sareneva T, Pirhonen J *et al.* Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression. *Cytokine Growth Factor Rev.* 2001; **12**: 171–80.
19 Ricard JD, Dreyfuss D, Saumon G. Production of inflammatory cytokines in ventilator-induced lung injury: a reappraisal. *Am. J. Respir. Crit. Care Med.* 2001; **163**: 1176–80.
20 Miyazaki Y, Araki K, Vesin C *et al.* Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J. Clin. Invest.* 1995; **96**: 250–9.