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Commentary

Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2)

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Abstract

Severe acute respiratory syndrome (SARS) is an emerging infectious disease associated with a new coronavirus, SARS-CoV. Pulmonary involvement is the dominant clinical feature but extra-pulmonary manifestations are also common. Factors that account for the wide spectrum of organ system involvement and disease severity are poorly understood and the pathogenesis of SARS-CoV infection remains unclear. Angiotensin converting enzyme 2 (ACE2) has recently been identified as the functional cellular receptor for SARS-CoV. Studies of the tissue and cellular distribution of SARS-CoV, and ACE2 protein expression, reveal new insights into the pathogenesis of this deadly disease. ACE2 is expressed at high level in the primary target cells of SARS-CoV, namely pneumocytes and surface enterocytes of the small intestine. Despite the fact that SARS-CoV can infect the lung and intestine, the tissue responses in these two organs are different. All other tissues and cell types expressing ACE2 may be potential targets of SARS-CoV infection. Remarkably, endothelial cells, which express ACE2 to a high level, have not been shown to be infected by SARS-CoV. There is also evidence that cell types without detectable ACE2 expression may also be infected by the virus. Furthermore, studies in a new human cell culture model have indicated that the presence of ACE2 alone is not sufficient for maintaining viral infection. Therefore, other virus receptors or co-receptors may be required in different tissues. Moreover, the interaction between SARS-CoV and the immunological or lymphoid system remains to be defined. It is clear that we are only at the dawn of our understanding of the pathogenesis of SARS. As our knowledge of the pathogenic mechanisms improves, a more rational approach to therapeutic and vaccine development can be designed in order to combat this new and fatal human disease.

Keywords: SARS; coronavirus; ACE2; pathogenesis

Severe acute respiratory syndrome (SARS) haunted the world from November 2002 to July 2003, affecting more than 8000 people in 28 regions with a mortality of 9.6% [1]. Since then, sporadic mini-outbreaks have arisen from laboratory and various other sources, resulting in public panic as well as posting continuous reminders to the medical world of this new disease [2]. The SARS epidemic, on the other hand, clearly demonstrated international co-operation and advances in genomic technology [3]. The causative agent of SARS, a new coronavirus (SARS-CoV), was quickly identified and the genomes of various isolates were determined [4–7]. Patients with SARS-CoV infection have a wide spectrum of disease, varying from a self-limiting illness to a fatal outcome [8,9]. The clinical picture was dominated by respiratory system involvement [8,10] but gastrointestinal symptoms were also common [11,12]. Raised creatine kinase, an increase in lactate dehydrogenase, and a decrease in absolute lymphocyte count are the most common laboratory findings. The best treatment protocol has not yet been defined, although vaccines are currently being developed in major centres around the world [13–16]. The factors that account for the wide spectrum of organ system involvement and disease severity are poorly understood. The pathogenesis of SARS-CoV infection remains uncertain.

As an important step towards better understanding of the pathogenesis, pathologists play a pivotal role in defining the tropism of the SARS-CoV and the body's responses to viral infection at the cellular and tissue levels. Several studies have attempted to characterize the lung pathology of SARS. Most of these studies analysed fatal cases, representing the more severe end of the clinical spectrum [8,17–22]. In the lung, diffuse alveolar damage and syncytial cells are most

consistently seen. Using electron microscopy (EM) directly for viral particles, *in situ* hybridization (ISH) using various regions of the viral genome [23–25], and immunohistochemical (IHC) studies using antibodies against various viral proteins (Figure 1A and ref 26), SARS-CoV infections have been clearly demonstrated in pneumocytes. This cell type is probably the primary target of SARS-CoV.

A substantial number of patients with SARS have diarrhoea [12,27]. In the intestine, little pathology is observed at the light microscopy level, either in biopsies taken during early phases [12] or in autopsy specimens [22,25]. EM [12], ISH [25], and IHC (Figure 1B), however, revealed the presence of SARS-CoV in surface enterocytes. The presence of virus in stool [12,28] and contamination of sewage were implicated as a possible mechanism of transmission in one major outbreak in Hong Kong [29].

Such comprehensive evidence for the presence of all three viral components — viral genome, viral proteins, and virus particles — is lacking in other cell types. In one of two papers on SARS published in the June 2004 issue of *The Journal of Pathology*, Ding *et al* extended the list of organs harbouring the virus in fatal cases using both IHC with antibodies to the nucleocapsid (N) protein and ISH [26]. Among the various organs in which IHC and ISH are positive for SARS-CoV, the presence of virus in the sweat glands suggests that SARS may be spread via contact with the skin. Although such a phenomenon has not been demonstrated clinically or epidemiologically, this finding might have major implications in clinical practice, infection control, and waste handling. Similarly, the presence of virus in the distal convoluted tubules of the kidney is consistent with detection of the viral genome in urine and suggests that urine may be an additional source of sewage contamination [28]. However, specific renal tissue damage was not observed, nor was viral tropism in kidney demonstrated in a previous ISH study [25], and the virus was not explicitly seen by EM [17–22].

Observations in the liver are also interesting. A high proliferative index has been demonstrated in hepatocytes in some cases [30]. Viral particles are again not detected by EM [30]. In both the liver and the kidney, signals for SARS-CoV were detected by both IHC and ISH [26], yet EM failed to reveal recognizable viral particles. If the observations are real, this raises the question of whether the virus exists in a non-packaged form. The discrepancy between these observations remains unexplained. The presence of viral infection in some endocrine organs such as the adrenals and the pituitary is puzzling. This finding might open up new insights into the pathophysiology of SARS. However, clinical presentations of SARS have not been linked to any particular endocrine disturbance.

The discovery and characterization of cellular receptor of SARS-CoV might provide important clues to the pathogenesis of this novel virus. Angiotensin converting enzyme 2 (ACE2), a metalloproteinase that has previously attracted attention as a result of its role in the cardiovascular system [31], was recently identified as the receptor of SARS-CoV in Vero E6 cells by isolation of the receptor protein through its direct interaction with the Spike (S) proteins of the SARS-CoV [32] and by expression cloning [33]. Further biochemical analysis pinpointed specific regions of the S1 domain of the S protein of SARS-CoV that interacted strongly with ACE2 [34,35]. These findings will have important bearings on vaccine development. Transfection of ACE2 into NIH 3T3 cells apparently conveyed infectivity to these non-permissive cells. Syncytium formation in ACE2-transfected cells has

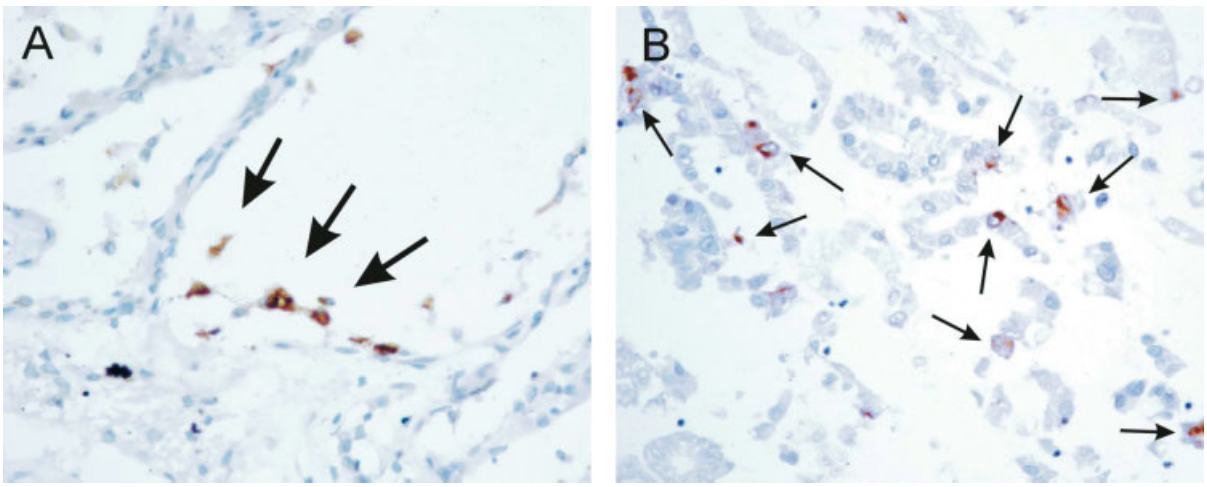

Figure 1. Detection of SARS-CoV membrane (M) protein in tissues from SARS patients by immunohistochemistry (1 : 100) using an anti-peptide antibody with a previously described routine protocol [22]. Note the cytoplasmic expression of this protein in SARS-CoV-infected cells (arrows). (A) Pneumocytes of the lung (original magnification ×400) and (B) surface enterocytes of the small intestine (original magnification ×200). Similar results were obtained using anti-peptide antibodies against N (nucleocapsid) and S (spike) proteins. These antibodies were generated from rabbits immunized with a KLH-conjugated synthetic peptide selected from the N- or C-termini of the respective SARS-CoV proteins. These figures are from autopsy samples, which were used as part of our ongoing project on SARS, approved by the local ethical committee

also been reported [32]. In the second paper on SARS published in the June 2004 issue of *The Journal of Pathology*, Hamming *et al* provided important information on the tissue and cellular distribution of ACE2 protein [36]. The general pattern of ACE2 expression correlates roughly with the tropism of SARS-CoV in fatal cases. In particular, ACE2 proteins were seen in the alveolar epithelial cells and surface enterocytes of the small intestine. The physiological role of ACE2 expression in these epithelial cell types is currently unknown. Interesting discrepancies between the tissue distribution of ACE2 and the tropism of SARS-CoV are, however, immediately apparent. While high levels of ACE2 are seen in endothelial cells, viral infection has not been demonstrated extensively in these cells in any organ, although vasculitis has been reported [18]. Similarly, ACE2 is clearly demonstratable by IHC in glomerular visceral and parietal epithelial cells as well as in the proximal tubules, but SARS-CoV infection has not been observed in these cell types. In contrast, no ACE2 expression was noted in the different cell types in the liver, including hepatocytes, Kupffer cells, and sinusoidal endothelium.

In the search for cell culture models for SARS-CoV infection, it has become clear that ACE2, as a receptor of SARS-CoV, cannot be the only determinant of tissue tropism. Several intestinal cell lines were found to have significant expression of ACE2, but only one human intestinal adenocarcinoma cell line, Lovo, was eventually identified to be permissive for SARS-CoV infection [37]. In contrast to the infected Vero E6 cell line, Lovo cells showed no cytopathic effect upon infection by SARS-CoV. Instead, persistent infection was observed. Virus particles, viral genome, and viral proteins were all demonstrated in SARS-CoV-infected Lovo cells. This cell culture model appears to recapitulate the natural course of intestinal SARS-CoV infection. It is apparent that the effect of SARS-CoV infection is different in different cell types and it is possible that the virus may utilize different receptors, or involve various co-receptors, in these different cells.

Immunological aspects of the study of pathogenesis cannot be overlooked. Lymphopenia is a characteristic feature of SARS [38]. Curiously, lymphoid cells and lymphoid organs, including the spleen, do not harbour SARS-CoV. The viral genome has rarely been demonstrated in macrophages of the lung [39]. The absence of viral proteins and viral particles, however, suggests a passive role for macrophages as scavengers, rather than being the primary target [25,26]. While direct infection of the lymphoid system by SARS-CoV seems unlikely, elevated levels of certain cytokines and chemokines are consistently observed in the serum of SARS patients [40,41]. In addition, the severity of SARS-CoV infection may be related to the HLA haplotypes of different individuals [42,43]. It is likely that there are complex interactions between SARS-CoV-infected cells in various organs and the lymphoid network. Studies of expression and changes in cytokines, chemokines, and their corresponding receptors both at the tissue level and in individual cell types will be necessary to address these issues.

It is clear that we are only at the dawn of our understanding of the pathogenesis of SARS. Among all the cell types being investigated, pneumocytes and small intestinal enterocytes have consistently been shown to be the targets of SARS-CoV infection. However, the cellular and tissue responses in these sites are different. The possibility of another cellular receptor(s) or co-receptor(s) remains open. The interaction between SARS-CoV and the immunological or lymphoid system needs to be defined. Further insights into the pathogenesis of SARS-CoV may be gained from emerging new human cell culture models. As our understanding of the pathogenic mechanisms improves, a more rational approach to therapeutic and vaccine development can be designed in order to combat this new and fatal human disease.

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