

Mini Review

Immunological Responses against SARS-Coronavirus Infection in Humans

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Since the outbreak of a SARS epidemic last year, significant advances have been made on our understanding of the mechanisms of interaction between the SARS coronavirus (CoV) and the immune system. Strong humoral responses have been found in most patients following SARS-CoV infection, with high titers of neutralizing Abs present in their convalescent sera. The nucleocapsid (N) and spike (S) proteins of SARS-CoV appear to be the dominant antigens recognized by serum Abs. CD4⁺ T cell responses against the N protein have been observed in SARS patients and an HLA-A2-restricted cytotoxic T lymphocyte epitope in the S protein has been identified. It is likely that the immune responses induced by SARS-CoV infection could also cause pathological damage to the host, especially in the case of proinflammatory cytokines. There is also evidence suggesting that SARS-CoV might be able to directly invade cells of the immune system. Our understanding on the interaction between SARS-CoV, the immune system and local tissues is essential to future diagnosis, control and treatment of this very contagious disease. *Cellular & Molecular Immunology*. 2004;1(2):119-122.

Key Words: SARS, coronavirus, immune responses, infection

Introduction

Severe acute respiratory syndrome (SARS), the first infectious disease caused global impact in this century, emerged initially in Guangdong province, China (1, 2) and then spread to Vietnam, Hong Kong, Singapore and far beyond (3-6), infecting more than eight thousand people with a nearly 10% mortality (7). A novel coronavirus (CoV) was soon identified as the causative agent of this highly contagious disease (8-13). Thanks to the close international cooperation and rigorous quarantine measures adopted by many countries, SARS is now well contained. Several new cases have recently been reported in Singapore, Taiwan and mainland China (14-20), however major outbreaks of SARS seem unlikely to occur in the near future.

The successful fight back against SARS was attributed mainly to traditional pavement-pounding epidemiological control measures rather than advances of modern medicine. Identification of SARS-CoV relied on classic tissue-culture isolation procedures followed by electron-microscopic studies, although the latest DNA sequencing technology

played a major part in determination of the viral genome sequence in such a short period of time. It must also be emphasized that the immune system played a vital role in defense against SARS-CoV infection, since none of the drugs employed to treat SARS is able to inhibit viral replication *in vivo*. Our knowledge of the immunological defense mechanisms against SARS-CoV is indispensable for future diagnosis, surveillance, control and treatment of SARS. We herein summarize the main findings of recent studies on the immunological responses induced by SARS-CoV infection in humans and also speculate on the possible future development in this exciting area of research.

Humoral responses against SARS-CoV infection

Antibodies (Abs) are one of the two arms of adaptive immunity against viral infection. In some patients, SARS-CoV-specific serum Abs become detectable in the late first week after the onset of symptoms (21, 22). More than 95% of the patients seroconverted for specific IgG Abs 25 days after the onset of the infection (23, 24). Abs to SARS-CoV were not found in serum samples obtained before the SARS outbreak of last year, suggesting that SARS-CoV had not previously circulated in human populations (25).

Several patients with severe cases of SARS were saved after *i.v.* injections of convalescent sera from recovered SARS patients (26), confirming the protective nature of the anti-SARS-CoV serum Abs. The protection effect of humoral immunity attributes mainly to the neutralizing Abs which are able to block the viral entry into host cells. Laboratory tests have confirmed that convalescent sera from most SARS patients were able to neutralize SARS-CoV infectivity at a titer of 1/40 or higher. The genome of

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Abbreviations: SARS, severe acute respiratory syndrome; CTL, cytotoxic T lymphocyte; CoV, coronavirus; ACTH, adrenocorticotrophic hormone.

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SARS-CoV encodes at least 4 major structural proteins: spike (S), nucleocapsid (N), membrane (M) and envelope (E) proteins (11-13). The neutralizing Abs are almost certainly directed against the S glycoprotein, as membrane fusion between SARS-CoV and the host cell is mediated by the S glycoprotein that binds to angiotensin-converting enzyme 2, a metalloproteinase, expressed by Vero E6 and certain other epithelial cells (27-29). Our recent study has found S protein-specific IgG Abs in convalescent sera from most of the 30 SARS patients tested. Yang et al. recently showed that a DNA vaccine encoding the S glycoprotein of SARS-CoV induced T cell and neutralizing antibody responses, as well as protective immunity, in a mouse model (30). They also demonstrated that the protection was mainly mediated by a humoral but not a T-cell-dependent immune mechanism.

Virus-specific serum Abs are also valuable for diagnostic purposes, irrespective of their neutralization potential. Positive detection of specific Abs in patient sera can be used as a confirmative diagnosis criterion for SARS-CoV infection (31). In a recent report by the American CDC, nearly half of probable cases reported in the United States in 2003 were excluded because of negative results of serological tests (23, 32). However, the attempt to establish Ab detection methods as early surveillance and diagnosis tools has enjoyed little success (12).

Specificity of anti-SARS-CoV serum Abs

It is obviously of great importance to dissect the fine specificity of the serum Abs of SARS patients. N protein is the first expressed and most abundant viral protein in cells infected by SARS-CoV. SDS-PAGE gel analysis of purified SARS-CoV particles revealed that N protein was significantly more abundant compared to S and M proteins. Recombinant N protein of SARS-CoV is more easily recognizable than recombinant S protein by convalescent sera from SARS patients as determined in ELISA and Western blot assays, possibly because SARS-CoV-specific Abs recognize conformational epitopes. By using ELISA kits with N protein or a fragment (amino acid residues 450-650) of S protein as coating antigens, specific IgG Abs were detected in convalescent serum samples from most of SARS patients tested in our study. Crossreactive Abs against these proteins were detected in less than 5% of serum samples from healthy subjects collected a year before the SARS epidemic (manuscript in preparation). So far Ab responses against the M protein of SARS-CoV following an infection in humans have not been clearly defined. 3CL is one of the nonstructural proteins of SARS-CoV and was poorly recognized by convalescent sera from SARS patients. On the contrary, X4, another nonstructural protein encoded by SARS-CoV genome, has been shown to be able to induce strong Ab responses in patients (YY Chen, personal communication).

Cellular responses against SARS-CoV

T cells are essential for adaptive immunity against viral infections *in vivo*. Anti-viral CD4⁺ helper T cells help the production of virus-specific Abs by B cells, while CD8⁺

CTLs can kill virus-infected host cells. The structural proteins of SARS-CoV should be able to induce strong cellular immune responses *in vivo*, although only limited data are available at the present stage. Peripheral blood T lymphocytes from SARS patients vigorously responded to stimulation with recombinant N protein of SARS-CoV *in vitro* (XM Gao, et al., unpublished observation). An HLA-A2-restricted cytotoxic T lymphocyte (CTL) epitope (amino acid residues 1167-1175) has recently been identified in the S protein of SARS-CoV (33). Synthetic peptides (RLNEVAKL) representing this epitope induced peptide-specific CTLs both *in vivo* (transgenic mice) and *in vitro* (human PBLs). The CTLs also lysed MHC-matched tumor cell lines expressing the S glycoprotein. In a mouse model described by Yang et al., both CD4 and CD8 T cell responses were induced by immunization with expression vectors encoding for the S protein of SARS-CoV (30).

Roles of proinflammatory cytokines

Immunological response against viral infection can be a double-edged sword: they could limit virus spreading on one hand and cause pathological damages to the host tissues on the other hand (Figure 1). This is especially a concern in the case of proinflammatory cytokines. Nicholls and colleagues suggested that pro-inflammatory cytokines released by activated macrophages in alveoli could have a prominent role in the pathogenesis of SARS (34). Glass et al. hypothesized that certain chemokines (e.g. CCL3 and CXCL8) could also participate in local inflammatory response in the lungs of SARS patients (35). There is also evidence suggesting that intervention of intra-balance among different cytokines, Th1/Th2 cells, and innate/adaptive immunity may also be an effective way of treating the disease. Beneficial effect of steroids, which are effective cytokine modulators, in treating SARS patients supports this view. In addition, interferon preparations were used as a preventive treatment against SARS during the epidemic period in China (36), although properly

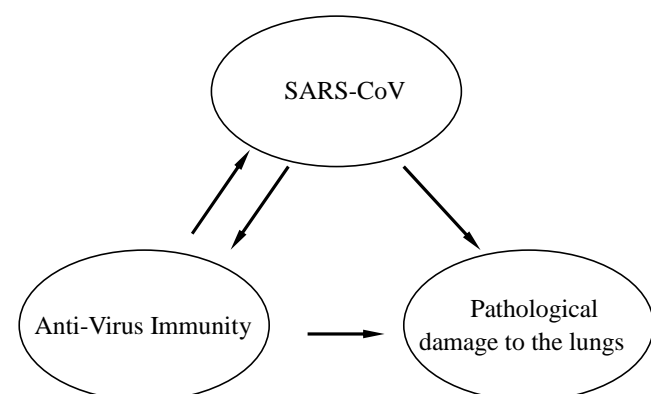


Figure 1. SARS-CoV attacks the respiratory system and also triggers cellular as well as humoral immune responses. The anti-viral immunity controls the spreading of the invading virus *in vivo* but also causes immunopathological damage to the lungs. Meanwhile, cells of the immune system could also be directly infected by SARS-CoV.

controlled studies have not yet been carried out to confirm the speculated effectiveness.

Does SARS-CoV directly attack the immune system *in vivo*?

Lymphopenia and neutropenia were observed in more than half of the SARS patients at the initial phase of their infection and the mechanisms for this phenomenon have been a topic of debate (37-42). Increased adrenocorticotrophic hormone (ACTH) and cortisol production is evident in critical illness leading to activation of the hypothalamic-pituitary-adrenal axis (43). Pituitary ACTH could easily cause the adrenal cortex to release large quantity of cortisol in a person under severe stress and drive T lymphocytes out of the peripheral circulation (44, 45). Therefore the possibility of lymphopenia seen in some SARS patients might be, at least partially, the result of the involvement of hypothalamic-pituitary-adrenal axis. Glucocorticoids were widely used to treat SARS patients, especially in mainland China, which might also have resulted in lymphopenia in some patients (32, 44). Virus induced apoptosis of lymphocytes has also been suggested as a cause for lymphopenia seen in SARS patients (46). It should also be emphasized that leukopenia and lymphopenia are present in other viral diseases such as measles, respiratory syncytial virus disease and sepsis (47), the mechanism for which is also unclear.

There is also evidence suggesting that SARS-CoV could directly infect human immune cells. Histological examination of the postmortem lymphoid tissues from SARS patients revealed significant pathological damages to the organs (48). By using FITC-labeled anti-SARS-CoV human IgG and peroxidase-conjugated anti-N protein monoclonal Abs, we have identified SARS-CoV-infected mononuclear cells collected from SARS patients 10-14 days after the onset of symptoms. Moreover, coronavirus-like particles have also been found inside mononuclear cells of the same specimens under electron-microscope (manuscript in preparation). Furthermore, positive detection of SARS-CoV RNA in mononuclear cells of peripheral blood from SARS patients has also been documented (49).

Concluding remarks

The interplay between SARS-CoV, the immune system and local tissues is clearly at the center of the pathogenesis of SARS (Figure 1). Immune responses against SARS-CoV do not only lead to the clearance of the virus in most cases but also cause pathological damage to the tissues of the host. Direct invasion of the immune cells by SARS-CoV further complicates the situation and increases the severity of the disease. The mechanisms of interaction between SARS-CoV, the immune system and the immunopathological damage to the host merit further investigation.

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