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SARS CORONAVIRUS INFECTIONS OF THE LOWER RESPIRATORY TRACT AND THEIR PREVENTION

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1 INTRODUCTION

The severe acute respiratory syndrome (SARS) coronavirus is a positive-stranded RNA virus, 29.7 kb in length with approximately 14 open reading frames.¹ It was identified as a human pathogen for the first time in 2003 as part of an intensive investigation into the cause of a series of fatal pneumonia cases that started in Hong Kong but then rapidly spread to over 30 different countries.^{2,3} The outbreak was eventually brought under control by quarantine measures but not before more than 8000 people worldwide were infected and there were over 800 confirmed deaths. This translated into an overall case fatality rate of ~10% but with mortality rates approaching 50% in the elderly.⁴ Those most likely to get serious complications or die from SARS virus infection were individuals over 65 years of age or who had a chronic illness, such as, diabetes or hepatitis.

SARS virus is spread by aerosol in the form of respiratory droplets or through close personal contact, being absorbed through mucous membranes. It has a typical incubation period between initial infection and development of symptoms of 3–7 days. Symptoms include a high fever, dry cough, shortness of breath, headache, muscle aches, sore throat, fatigue, and diarrhea. Lung histology in fatal cases revealed a marked diffuse alveolar damage, inflammatory cell infiltrate, bronchial epithelial denudation, loss of cilia, and squamous metaplasia.⁵ Deaths resulted from respiratory, heart and/or liver failure. Infected individuals recovering from coronavirus infections may become susceptible to reinfection due to rapidly waning immunity.^{6,7} In fact, those with waning immunity may be at risk of even more severe disease upon coronavirus reinfection.⁸ SARS homologous reinfection studies showed that although immune animals cleared lung virus much faster than naive animals, the incidence and severity of lung inflammation was not reduced.⁹ This suggests that illness severity is not just dictated by SARS viral load but is also influenced by host factors. Given the possibility of future human outbreaks, development of a safe and effective coronavirus vaccine platform would be beneficial. In particular, lessons learned from development of SARS vaccines may also be applicable to other coronavirus infections, such as, the recently emerged Middle East Respiratory Syndrome (MERS) coronavirus.¹⁰

2 INACTIVATED WHOLE VIRUS VACCINES

Initial attempts to produce SARS vaccine candidates were based on traditional methods where SARS virus was grown in cell culture under biosafety level (BSL)-3 conditions and then inactivated with formaldehyde, beta-propiolactone, ultraviolet irradiation, or a combination of these. These initial inactivated whole virus vaccines provided modest protection in animal models, inducing low titers of neutralizing antibody and earlier lung virus clearance but did not completely prevent infection.¹¹ One such inactivated vaccine was administered to a small number of human subjects in a phase 1 clinical trial and was shown to be able to induce neutralizing antibodies.¹² Although no immediate safety issues were identified, the subjects were not exposed to SARS virus and hence any risk of vaccine exacerbation of lung immune-pathology by this vaccine remains unknown.

Of note, animals immunized with similar inactivated vaccines developed a severe lung eosinophilic immunopathology when challenged with SARS virus.¹³⁻¹⁶ This lung pathology was exacerbated further when SARS vaccines were formulated with alum adjuvant in an attempt to increase their immunogenicity.⁹ Despite inactivated vaccines combined with alum adjuvant being able to reduce virus replication in young animals, they failed to reduce virus replication in older animals in which their use was associated with severe lung eosinophilic pathology postchallenge.¹³ This finding is particularly concerning, as the elderly are a major target population for SARS vaccines, being most at risk of complications and mortality. If the animal data translates to humans, this suggests that inactivated vaccines might not only fail to protect the elderly but may even increase their risk of serious illness if exposed to SARS virus.

The problem of lung eosinophilic pathology was also seen in mice immunized with Venezuelan equine encephalitis virus replication particles expressing the SARS nucleocapsid protein.¹⁷ Similarly, mice immunized with vaccinia virus encoding the SARS nucleocapsid protein developed a severe pneumonia characterized by increased Th2 and Th1 cytokines, reduced IL-10 and TGF-beta, thickening of the alveolar epithelium and lung infiltration by eosinophils, lymphocytes, and neutrophils.¹⁵ Hence, given these problems of poor immunogenicity, difficulty of BSL-3 manufacture, poor efficacy in the elderly, and risk of exacerbated disease due to eosinophilic lung immunopathology, inactivated whole virus formulations are poor candidates for safe and effective SARS vaccines.

3 RECOMBINANT SPIKE PROTEIN VACCINES

A major advance in vaccine development was the identification that the SARS virus spike (S) protein mediates cell entry via its ability to bind angiotensin-converting enzyme 2 and CD209L, thereby triggering virus endocytosis into target cells.^{18,19} A human monoclonal antibody binding the S protein N-terminal domain was shown to be able to block infection, thereby identifying S protein as a major target of SARS virus neutralizing antibodies.²⁰ Consistent with this, monkeys could be protected against SARS infection by intranasal immunization with a S protein-encoding live parainfluenza vector.²¹ S protein was also shown to be the target of CD4 and CD8 T cell responses suggesting these may also be important to SARS protection.²² A recombinant S protein vaccine was manufactured using an insect cell expression system but was found to be considerably less immunogenic than inactivated whole virus vaccine, requiring ~100 times more antigen to achieve the same level of immunogenicity.²³ Attempts to improve the immunogenicity of S protein vaccine

by formulation with alum adjuvant again resulted in severe lung eosinophilic immunopathology in response to SARS virus infection, marking this as another potentially unsafe approach.^{13,16} This confirmed that the problem of lung eosinophilic immunopathology was not just confined to inactivated or nucleocapsid protein vaccines but was a more general problem of vaccines made from any SARS virus antigen.

4 SARS-ASSOCIATED EOSINOPHILIC LUNG IMMUNOPATHOLOGY

What is the mechanistic basis of lung eosinophilic immunopathology? This question is not just of academic interest as SARS-associated lung immunopathology bears a striking resemblance to the lung eosinophilic pathology previously seen with an alum-adjuvanted formalin-inactivated respiratory syncytial virus (RSV) vaccine. In clinical trials this vaccine caused increased mortality when immunized children became infected with RSV.²⁴ Hence, any SARS vaccine that induces lung eosinophilic immunopathology could be equally unsafe in human subjects.

Lung eosinophilic immunopathology did not arise in naive mice pretreated with anti-S protein antibody before challenge suggesting it is not due to an antibody-dependent enhancement mechanism.¹⁷ Lung immunopathology might instead be due to an aberrant host immune response to SARS virus, exacerbated by vaccine priming. As the ability of inactivated or recombinant vaccines to induce eosinophilic immunopathology is dramatically exacerbated by formulation with alum adjuvant, a known Th2-polarising adjuvant, this implies that the problem might be vaccines that prime an excessive Th-2-response and/or that fail to prime for a sufficient Th1 response. SARS virus itself mediates broad ranging immune modulatory effects that might be expected to lead to strong Th2 immune bias. S protein binds lung surfactant protein D, a collectin found in the lung that activates macrophages but not dendritic cells (DC).²⁵ Autopsy lung samples of patients dying after SARS infection revealed down regulation of type 1 interferon and CXCL10, a chemokine involved in T cell recruitment and inhibition of the STAT1 pathway.²⁶ Conversely, SARS virus enhances production of interleukin-6 (IL-6) and IL-8, inflammatory cytokines that inhibit the ability of DC to prime T cells.²⁷ GU-rich ssRNAs from SARS virus activate TLR7 and TLR8 in mice and thereby induce high levels of pro-inflammatory cytokines including TNF- α , IL-6, and IL-12 leading to lethal acute lung injury.²⁸ Thus SARS virus has the capacity to exacerbate lung inflammation while at the same time inhibiting antiviral interferon responses.

SARS virus uses multiple mechanisms to inhibit the host type 1 interferon response with open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins all playing a major role; nucleocapsid protein inhibits the synthesis of interferon while ORF 3b and ORF 6 proteins inhibit both interferon synthesis and signaling.²⁹ In addition, ORF 6 protein inhibits nuclear translocation of STAT1. The importance of STAT1 to protection is demonstrated by STAT1 knockout (STAT1^{-/-}) mice being unable to clear SARS virus infection, resulting in increased virus lethality.³⁰ Furthermore, STAT1^{-/-} mice infected with SARS virus had evidence of T cell and macrophage dysregulation with increased alternatively activated macrophages and a Th2-biased immune response. As STAT6 is essential for development of alternatively activated macrophage, the importance of alternatively activated macrophages to SARS pathology was able to be demonstrated using STAT1/STAT6 double-knockout mice, which exhibited reduced lung disease in response to SARS virus challenge.³¹ On the host side, CD8 T cells are responsible for virus clearance and adoptive transfer of immune splenocytes or SARS-specific T cells reduced lung

virus titers and enhanced survival.³² The importance of Th1-cellular immunity to viral control may help explain the many mechanisms that SARS virus has developed to subvert Th1-cellular responses. By suppressing Th1 responses, the SARS virus may thereby impart an unbalanced Th2 bias to the antiviral lung immune response, which could amplify and exaggerate any preexisting Th2 bias already imparted by immunization with a Th-2 biased SARS vaccine. This thereby provides a plausible explanation for the vaccine-exacerbated lung eosinophilic pathology observed in recipients of alum-adjuvanted or unadjuvanted inactivated whole virus and recombinant S protein vaccines. This is supported by data from a ferret SARS virus reinfection model where reinfected animals or ferrets previously immunized with an alum-adjuvanted inactivated vaccine candidate failed to mount an effective type 1 interferon response.³³ In mice, SARS-associated lung pathology was shown to be prevented by pretreatment of alveolar macrophages with poly(I:C), a TLR3 agonist, which may reflect its ability to prime for a strong type 1 interferon response.³⁴ Hence the aberrant host immune response causing SARS-associated lung immunopathology is complex, with roles played by alternatively activated macrophages, neutrophils, NFkB activation, lack of a type 1 interferon response, and excess IL-1 β , IL-6, and TNF production due to inflammasome activation by the SARS envelope protein-encoded ion channel.^{31,35–39}

5 SARS PATHOLOGY IN THE ELDERLY

Elderly human subjects infected with SARS virus experienced an extremely high mortality rate, approaching 50%.⁴ SARS-infected aged macaques similarly developed more severe pathology than young adult animals, even though viral replication levels were similar. This suggests that increased SARS mortality in the elderly may reflect as much an aberrant host response as toxic effects of the virus. In keeping with this, aged macaques showed greater NFkB activation in response to SARS infection and this was associated with increased inflammatory gene expression but lower expression of type I interferon.⁴⁰ Treatment of aged animals with type 1 interferon reduced expression of inflammatory genes, such as IL-8, and reduced lung pathology, despite not changing lung virus levels.⁴⁰ It has been shown that aged mice exhibit increased lung prostaglandin D2 in response to lung infection that correlates with impairment of DC migration and lower T cell responses.⁴¹ More severe clinical disease in aged animals could be attenuated by blocking prostaglandin D2 with small-molecule antagonists.⁴¹ Thus, multiple factors likely contribute to the increased mortality in elderly subjects suffering from SARS infection, including intrinsic DC defects, increased production of DC-inhibitory factors, such as prostaglandin D2, predisposition to an increased inflammatory response, and reduced Th1 immune responses. An ongoing challenge is to develop a SARS virus vaccine strategy that is able to overcome all of these obstacles and is thereby safe and effective in the elderly.

6 PREVENTION OF VACCINE-EXACERBATED SARS LUNG IMMUNOPATHOLOGY

There is a critical need to identify suitable SARS vaccines that do not run the risk of exacerbating coronavirus-associated lung immunopathology. AdvaxTM is a polysaccharide adjuvant based on microcrystalline particles of β -D-[2-1]poly(fructo-furanosyl) α -D-glucose (delta inulin).^{42–44} It has been shown to enhance vaccine immunity against a wide variety of pathogens, including influenza,^{45,46} Japanese encephalitis,⁴⁷ West Nile virus,⁴⁸ and hepatitis B,⁴⁹ enhancing both humoral and

cellular immunity, and inducing a balanced Th1-Th2 response.^{46,49} It has also been shown to be safe and nonreactogenic in human vaccine trials.^{50,51} When combined with either recombinant S protein or inactivated SARS, Advax™ adjuvant alone or combined with a TLR9 agonist enhanced neutralizing antibody and cellular immune responses, reduced lung viral load and completely protected against SARS lethality.⁵² Notably, animals immunized with Advax™ adjuvant formulations had minimal or no lung eosinophilic pathology postchallenge in stark contrast to the severe immunopathology observed in animals immunized with antigen alone or combined with alum adjuvant (Fig. 3.1) thereby highlighting the critical importance of adjuvant selection for SARS vaccine development. Protection against lung immunopathology correlated with a strong memory Th1 response in the Advax™-immunized animals.⁵²

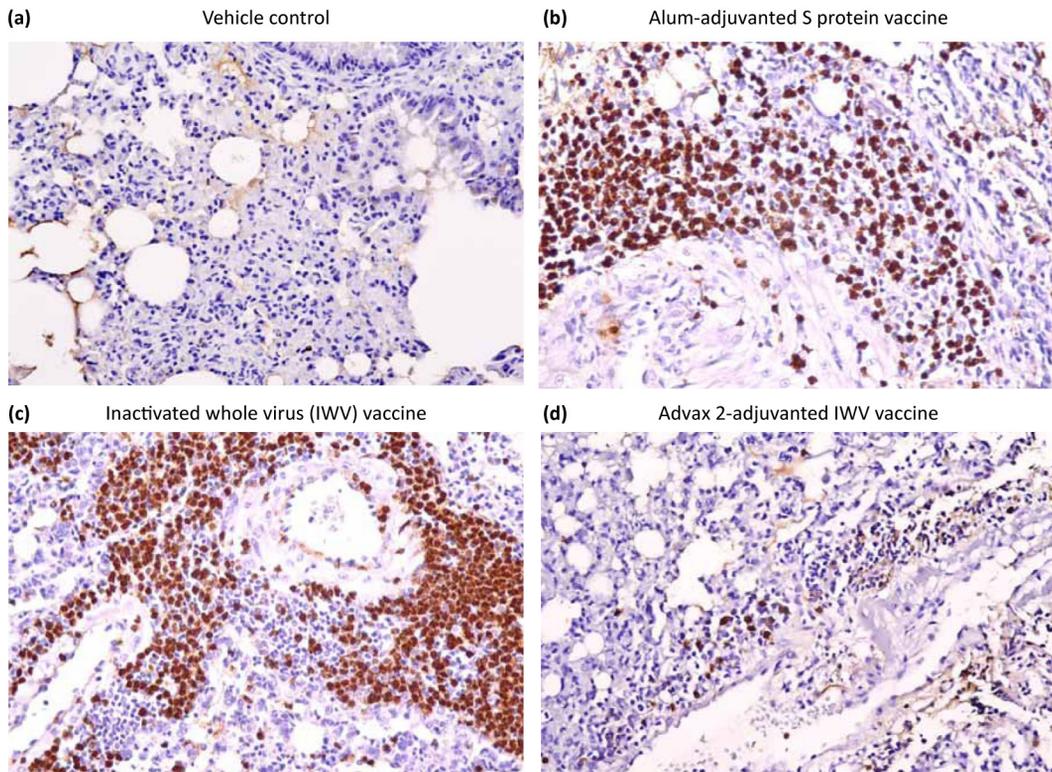


FIGURE 3.1 Lung Eosinophilic Immunopathology in SARS Vaccine Recipients

Mice were immunized with various SARS antigen formulations then challenged with SARS virus. Lungs were harvested day 6 postchallenge for staining with H&E plus a specific eosinophil stain (*brown*).⁵² After SARS challenge, mice that received only vehicle control had marked lung inflammation but minimal eosinophils (a) By contrast, mice immunized with either alum-adjuvanted S protein (b) or unadjuvanted inactivated whole virus (c) exhibited florid lung eosinophil infiltration. However, minimal eosinophils were seen in mice immunized with the same inactivated whole virus vaccine formulated with Advax-2 adjuvant (d).

Pictures courtesy of Bi-Hung Peng, University of Texas Medical Branch, Galveston, Texas.

TLR agonists including lipopolysaccharide, poly(U) or poly(I:C) when formulated with inactivated SARS virus, have similarly been shown to help prevent lung immunopathology.⁵³ What all of these successful strategies have in common is that they prime for a Th1-immune response, suggesting that this is the key to avoiding lung eosinophilic immunopathology.

7 CONCLUSIONS AND FUTURE PROSPECTS

Protection against SARS virus involves coordinated action of memory CD4 and CD8 T cells and neutralizing antibody. Vaccines that fail to induce Th1 immunity against the SARS virus run the risk of exacerbating lung disease.⁵⁴ Hence, the key to any successful SARS vaccine will be the inclusion of an adjuvant able to induce robust cellular immunity together with long-lived neutralizing antibodies. Important questions remain on how best to protect elderly subjects, the most vulnerable population for lethal human coronaviruses. Another important issue is how best to provide protection against heterologous SARS virus strains. Unfortunately, with the passage of time since the SARS epidemic, funding for SARS research has dried up and some of these questions may never be answered. While the SARS epidemic was halted by quarantine measures, there is an ongoing need for a safe and effective vaccine platform that could be used in the event of future human coronavirus threats. Notably, the MERS coronavirus has emerged to become a major human threat. Unlike SARS, MERS has not been halted by quarantine procedures, creating an urgent need for a safe and effective MERS vaccine.⁵⁵ It is not yet known whether MERS vaccines will suffer from the same problems as SARS vaccines, such as, low immunogenicity and lung eosinophilic immunopathology, but this seems likely. Hence, similar strategies as described above will be required to make MERS vaccines safe and effective. MERS will almost certainly not be the last new coronavirus to cause human disease, identifying the need for ongoing research into the unique host-pathogen interactions contributing to coronavirus pathology and infection outcomes, and to develop an effective coronavirus vaccine platform.

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