Review

SARS Vaccine: Progress and Challenge

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Severe acute respiratory syndrome (SARS) emerged in 2002 as a severe and highly contagious infectious disease that rapidly spread to a number of different countries. The collaborative efforts of the global scientific community have provided, within a short period of time, substantial insights into the molecular biology and immunology of SARS-CoV. Although the outbreak has been contained, there is continuous concern that the virus may resurface into the human population through seasonal changes, animal reservoirs or laboratory accidents. The severe morbidity and mortality associated with SARS make it imperative that an effective vaccine be developed to prevent reemergence and epidemics in the future. *Cellular & Molecular Immunology*. 2005;2(2):101-105.

Key Words: SARS, vaccine, animal model, immune response, coronavirus, emerging infection

Introduction

SARS appears to have originated in Guangdong Province, China, in late 2002. It spread to locations in Asia including Hong Kong, Vietnam, Singapore, and Taiwan and in North America, most notably to Toronto (1-4). By June of 2003, the CDC and WHO reported more than 8,000 cases, with more than 800 deaths in 26 countries spread over 5 continents. The case fatality rate was as high as 15% for patients younger than 60 years old and higher than 50% for patients 60 years or older. The clinical syndrome is characterized by fever, cough, shortness of breath and the development of pneumonia that can progress to severe bilateral pulmonary disease and respiratory failure. Nearly 40% of patients developed respiratory failure causing them to require assisted ventilation. The pathology observed in the lungs of patients was mostly from post mortem tissue, making it difficult to determine the sequence of events that lead up to severe disease and death. The data from several studies of 24 human cases demonstrated diffuse alveolar damage with edema, pneumocyte necrosis and hyaline membrane formation or organization, depending on the duration of illness before death (5-7). The epidemic in 2003 was controlled essentially by isolation. However, in 2004 there were a few cases in China believed to have resulted from a laboratory exposure to the virus in Beijing. At present, there is no effective treatment for SARS.

Virology

A newly identified coronavirus was isolated from SARS patients (1, 3). The proof that this virus is the etiologic agent for SARS was provided by results of infections carried out in non-human primates, in which Koch's postulates were fulfilled (8). The genome of this new coronavirus was quickly sequenced and the sequence information demonstrated that this is a previously unrecognized coronavirus, which was then named SARS-CoV (9-11).

The coronaviruses are a diverse family of large, enveloped, plus-stranded RNA viruses that cause respiratory and enteric diseases in humans and other animals. There are three groups of coronaviruses; groups 1 and 2 contain mammalian viruses, whereas group 3 contains only avian viruses. Within each group, coronaviruses are classified into distinct species by host range, antigenic relationships, and genomic organization. The viruses can cause severe disease in many animals and several of them, including infectious bronchitis virus, feline infectious peritonitis virus, and transmissible gastroenteritis virus, are important veterinary pathogens. Human coronaviruses (HCoVs) are found in both group 1 (HCoV-229E) and group 2 (HCoV-OC43) and are responsible for ~30% of mild upper respiratory tract illnesses (12, 13). The newly identified SARS-CoV has been suggested to define a new, fourth group of viruses; however, one report suggests that it most closely relates to group 2 coronaviruses (11). Like other CoVs, SARS-CoV is an enveloped plus-stranded RNA virus with a ~30 kb genome

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encoding replicase (Rep) and the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) (9, 10). E protein plays a role in viral assembly, M is important for virus budding, N protein is associated with viral RNA packaging (14), and S protein is responsible for binding to a specific cellular receptor, angiotensin-converting enzyme 2 (ACE2) (15).

Immune responses during SARS-CoV infection in human

The immune-pathogenesis of SARS is not well understood. SARS-coronavirus poorly infects human peripheral blood mononuclear cells (PBMCs). Viral replication in PBMCs seems subject to self-limitation, partly due to the production of IFN-α by these cells (16, 17). Yang and colleagues (18) demonstrated that myeloid dendritic cells bind to virus *via* interaction with SARS-CoV S protein. Although these dendritic cells could not be infected by SARS-CoV, the authors suggest that they could serve as vehicles for dissemination of the virus. The potential of monocytes and dendritic cells to take up SARS-CoV may be relevant to SARS pathogenesis *in vivo*. In addition, the levels of serum IL-2, IL-10, and IL-12 were evidently increased while peripheral blood $CD3^+$, $CD4^+$, and $CD8^+$ counts were decreased in the SARS patients, compared to normal individuals (19). To study the differential immune-gene expression patterns induced by SARS-CoV infection, PBMCs derived from SARS patients and normal subjects were examined using DNA microarray technology (20). Surprisingly, there was no significant up-regulation of MHC-I genes and cytokines that were examined including IFNs, nor for genes involved in complement mediated cytolysis.

So far, no conclusive information is available on the immune correlates of protection to SARS in patients. Antibodies against SARS-CoV were detected in patients infected with SARS (1). Recently, using pseudotyped lentiviral particles bearing the SARS-CoV spike protein, it has been shown that spike-mediated infection could be inhibited by sera from SARS patients, demonstrating that spike is a target for neutralizing antibodies (21). Anti-nucleocaspid antibodies were also detected 7-30 days in patients after the onset of SARS symptoms (22). In addition, two identified CD8 T-cell epitopes in SARS-CoV spike protein have been shown to elicit specific T-cell responses in $HLA\text{-}A2^+$ SARS-CoV infected patients (23). 293T cells transfected with a functional receptor for SARS-CoV, ACE2, formed multinucleated syncytia with cells expressing spike protein (15). More significantly, in the post-mortem lung tissue samples from patients who died from SARS, multinucleate giant cells of macrophage and epithelial origins have been observed within the damaged alveoli (5). This suggests that cell-to-cell transmission *via* syncitia formation may occur in SARS-CoV. Thus, eradication of SARS-CoV may not be achieved by humoral response alone and T cell-mediated immunity may also be required to clear infection. However, immune responses may also exacerbate the symptoms and pathology of SARS. During the outbreak, young children appear to be the least susceptible of SARS-CoV infection and the only accepted clinical intervention in SARS has been aggressive suppression of local immune responses using high dose and inhaled steroids.

Animal models for SARS

Critical to an understanding of SARS pathogenesis and the development and evaluation of therapies is the availability of animal models that recapitulate the human disease when infected with clinical or species-adopted isolates of SARS-CoV. A number of animal models have been tested for studying SARS pathogenesis and evaluating therapies. These include macaques, ferrets, hamsters, immune competent mice, and Stat1 deficient mice.

In studies by Kuiken et al. (24), macaques intratracheally infected with SARS-CoV excreted the virus from the nose, mouth, and pharynx 2 days after infection. Three of four macaques developed diffuse alveolar damage, similar to that in SARS patients, that was characterized by epithelial necrosis, serosanguineous exudate, formation of hyaline membranes, type 2 pneumocyte hyperplasia, and the presence of syncytia. SARS-CoV was detected in pneumonic areas by virus isolation and RT-PCR, and was localised to alveolar epithelial cells and syncytia by immunohisto-chemistry and transmission electron microscopy. However, the data from Rowe et al. (25) indicated that high challenge doses of the Tor2 strain of SARS-CoV administered into the lung caused a mild clinical syndrome in macaques that differed from human SARS in a number of important ways. The animals remained afebrile, and the clinical and histological consequences of intrapulmonary administration of virus were mild and self-limited. Lung pathology and recovery of virus was more evident when animals were necropsied at earlier time points than at later time points after the infection resolved (i.e., days 12 to 14), consistent with the findings by Kuiken et al. (i.e., days 4 to 6) (24). Furthermore, the lack of apparent clinical illness and rapid clearance of virus in macaques were also reported by McAuliffe et al. (26). Therefore, it is questionable whether the clinical manifestations of SARS-CoV infected macaques are sufficiently robust to be useful in evaluating pathogenesis of the disease or assessing therapeutic efficacy.

The reservoir of the coronavirus isolated from patients with severe acute respiratory syndrome (SARS) is still unknown, but is suspected to be a wild animal species. Interestingly, it has been showed that ferrets are susceptible to SARS-CoV infection and that virus can be efficiently transmitted from infected ferrets to uninfected ones by housing them together. In ferrets, SARS-CoV replicates to high levels in the lung and causes a severe and progressively worsening pneumonitis that begins in the small airways and progresses to consolidation in the airspaces (27). While ferrets best recapitulate the human disease, there are essentially no reagents available to study host immune

response in ferrets. Additionally, these animals are difficult and expensive to use.

At the early stage of evaluation of vaccines and antiviral therapeutics, small animal models, such as hamsters and mice, would be very useful. Roberts et al. (28) investigated the ability of SARS-CoV to infect hamsters. When administered intranasally, virus replicated to high titers in the lungs and nasal turbinates. Peak replication in the lower respiratory tract was noted on day 2 post-infection (p.i.) and was cleared by day 7 p.i. Viral replication in epithelial cells of the respiratory tract was accompanied by cellular necrosis early in infection, followed by an inflammatory response with viral clearance, focal consolidation in pulmonary tissue, and eventual pulmonary tissue repair. Despite high levels of virus replication and associated pathology in the respiratory tract, the hamsters showed no evidence of disease. But viremia and extrapulmonary spread of SARS-CoV to livers and spleens were observed in infected hamsters.

Following intranasal administration of BALB/c mice, SARS-CoV replicated to high titers in the respiratory tracts. Peak viral replication was seen on day 1 or 2 after injection. However, BALB/c mice cleared the virus within a week and showed no severe illness (29). Similarly, intranasal inhalation of SARS-CoV in the mouse strain 129SvEv resulted in infection of conducting airway epithelial cells followed by rapid clearance of virus from the lungs and the development of self-limited bronchiolitis (30). In contrast, mice deficient in Stat1 demonstrated a markedly different course following intranasal inhalation of SARS-CoV. In Stat1 deficient mice which are defective in IFNα/β signaling, the severity of infection was substantially worse and bronchiolitis progressed to interstitial pneumonia and mediastinitis, a characteristic commonly described in the histopathology of humans who died from SARS. Systemic spread of virus to livers and spleens were also seen (30). Overall, viral replication and the host response in Stat1 deficient mice are similar in magnitude to what is observed in ferrets. The Stat1 deficient mouse model has enormous advantages over ferret model, in term of affordability, simplicity, and the plethora of reagents available for immunological studies.

Development of vaccines against SARS

A phase I clinical trial of SARS vaccine based on heat-inactivated virus was initiated in September, 2004 in China. Inactivated SARS vaccine appeared to be safe in the volunteers. After the preliminary 56-day observation period, 11 out of 12 (91.6%) of the volunteers who received high-dosage vaccine and 12 out of 12 (100%) of the volunteers who received low-dosage vaccine showed blood serum conversion, with no obvious side-effects (Press release by Sinovac Biotech Ltd, Beijing, China). However, the efficacy and long-term safety of this vaccine remain to be determined.

With the advance of molecular biology techniques, recombinant subunit vaccines and genetic vaccines have been rapidly developed and tested in animals. The most straightforward approach for recombinant subunit vaccine is to use purified protein in combination with adjuvants. Pei et al. (31) reported that orally delivered recombinant N protein of SARS-CoV induced significant N-specific IgG in the mice sera. With the identification of SARS-CoV functional receptor (15) and the mapping of the receptor-binding domain on the S protein (32, 33), it is now possible to develop subunit vaccines targeting the receptor-binding domain that might be more effective. Huang et al. (34) have successfully generated SARS-CoV pseudoparticles by co-transfecting human 293 renal epithelial cells with plasmids encoding S, M, and N proteins. The pseudoparticles, which are safe and conformational mimetics of live virus, may also be served as a novel subunit vaccine against SARS.

However, the possible need for T cell immunity in SARS vaccination and the evasion of antibody neutralization in emerging SARS-CoV (35) suggest that vaccines inducing cellular immunity should also be considered. Jin et al. (36) employed DNA vaccine technology and the newly discovered levamisole as a chemical adjuvant. High levels of specific antibodies, T cell proliferations, IFN-γ, DTH responses, and *in vivo* cytotoxic T cell activities specifically against SARS-CoV antigens were observed in mice after intramuscular immunization. It has also been shown that a DNA vaccine encoding SARS-CoV S protein can induce T cell and neutralizing antibody responses, as well as protective immunity, in a mouse model (37). However, DNA vaccines have performed poorly in human clinical trials so far (38, 39), despite the efficiency shown in experimental animal studies.

Several vaccines based on recombinant viral vectors have achieved promising results in either human trials or nonhuman primate challenge studies. Modified vaccinia virus Ankara (MVA) has been used in large vaccine trials and in clinical practice for primary vaccination of more than 100,000 humans against smallpox (40). No side effects have been associated with its use, even when high-risk patients or experimentally immune-suppressed monkeys received primary vaccination (41-43). Intranasal or intramuscular inoculations of MVA expressing SARS-CoV S protein can elicit protective immunity in BALB/c mice (44). In addition, Chen et al. (45) reported that MVA expressing SARS-CoV S protein protected Chinese rhesus monkeys from pathogenic SARS-CoV challenge. However, the experiments done by Weingartl et al. (46) suggested that vaccination with MVA expressing SARS-CoV S protein in ferrets was associated with enhanced hepatitis. Replication-defective adenoviral vectors based on human serotype 5 (AdHu5) are capable of delivering high level gene transfer and inducing activation of T and B cells specific to the transgene product (47, 48). These properties have been exploited in the development of genetic subunit vaccines against infectious diseases (49, 50). Vaccination of C57BL/6 mice with AdHu5 expressing SARS-CoV N protein generated potent SARS-CoV-specific humoral and T cell- mediated immune responses (51). Gao et al. (52) reported that broad virus-specific immunity was induced in rhesus macaques intramuscularly immunized with a mixture of three AdHu5 vectors that express SARS-CoV S protein S1 fragment, M protein, or N protein. An important limitation of AdHu5 vector based genetic vaccine is the

existence of neutralizing antibodies (NABs) against AdHu5 vector in a large proportion of the human population due to natural infections (53). These NABs substantially diminish its vaccine efficacy (54, 55). To circumvent interference by pre-existing immunity to AdHu5 vector, molecular clones from adenoviruses based on three serologically distinct simian adenoviruses were developed and further used to create stocks of replication defective simian adenoviral vectors (56). These simian adenoviral vectors have shown substantial utilities as vaccine carriers for rabies and HIV (54, 55). Importantly, sera from humans have little if any NABs against the simian adenoviruses (56). Recently, Zhi et al. (57) has shown that immunization of mice with simian adenoviral vector C7 expressing codon optimized SARS-CoV S protein could induce robust S-specific $CD8^+$ T-cell response and this has allowed identification of several murine T cell epitopes in SARS-CoV S protein. The DNA prime and adenovirus or MVA boost approach, which is currently being explored for HIV vaccine development, might be an avenue worth exploring for SARS vaccine. Ultimately, a successful SARS-CoV vaccine is likely the one that induces strong, broad spectrum, and long-lasting neutralizing antibody and protective T cell responses.

Conclusion

As a novel virus, SARS-CoV poses a threat with many unknowns and challenges. While much remains to be discovered about this novel coronavirus, the collaborative efforts of the global scientific community have provided, within a short period of time, substantial insights into the molecular biology and immunology of SARS-CoV. Based on this knowledge, the successful development of SARS vaccine appears feasible and worthwhile.

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