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Review

Vaccine Design for Severe Acute Respiratory Syndrome Coronavirus

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ABSTRACT

Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a new coronavirus (SARS-CoV). Recent studies suggest that SARS-CoV is zoonotic and may have a broad host range besides humans. Although the global outbreak of SARS has been contained, there are serious concerns over its re-emergence and bioterrorism potential. As a part of preparedness, development of a safe and effective vaccine is one of the highest priorities in fighting SARS. A number of candidate vaccines, using a variety of approaches, are under development. The first vaccine tested in clinical trial is made from the inactivated form of SARS-CoV. Several live attenuated, genetically engineered or vector vaccines encoding the SARS-CoV spike (S) protein have been in pre-clinical studies. These vaccine candidates are effective in terms of eliciting protective immunity in the vaccinated animals. However, caution should be taken with the safety of whole virus or full-length S protein–based immunogens in humans because they may induce harmful immune or inflammatory responses. We propose to use the receptor-binding domain (RBD) of SARS-CoV S protein (residues 318–510) for developing a safe and effective subunit SARS vaccine, as it is not only a functional domain that mediates virus-receptor binding but also a major neutralization determinant of SARS-CoV. It has been demonstrated that the RBD of SARS-CoV S protein contains multiple conformational epitopes capable of inducing highly potent neutralizing antibody responses and protective immunity.

INTRODUCTION

In November 2002, a new infectious pneumonia, known as severe acute respiratory syndrome (SARS), emerged in the Guangdong province of China, rapidly spread to 29 countries, and resulted in more than 8,000 cases with 774 deaths (www.who.int/crs/sars/country/table2004_04_21/en/). The global outbreak of SARS quickly garnered international attention and elicited a strong healthcare response worldwide. Under the coordinated efforts by a network of laboratories created by the World Health Organization (WHO), the causative pathogen was quickly characterized as a novel coronavirus (SARS-CoV) (12,25,31,37,39). With aggressive quarantine measures, the SARS spread was brought under control in July 2003. However, several isolated infections occurred in early 2004 caused by either accidental releases of the SARS-CoV from laboratories or new SARS-CoV–like

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isolates from animal reservoirs (14,29). No one knows whether, when, or where SARS-CoV will re-emerge. Development of a safe and effective vaccine is one of the best control measures for preventing new SARS outbreaks.

SARS-CoV: A NOVEL CORONAVIRUS POSSIBLY FROM ANIMAL RESERVOIRS

There exist three known antigenic groups (I, II, and III) of coronaviruses (CoVs) associated with diseases in animals and humans (27). Major members of group I include the following: the enteric and respiratory porcine CoVs (transmissible gastroenteritis virus [TGEV]; porcine epidemic diarrhea virus [PEDV]; porcine respiratory CoV [PRCV]) the canine and feline enteric CoVs, the feline infectious peritonitis virus (FIPV), and the human CoV 229E. The main members of group II are bovine (BCoV), porcine (HEV), rodent (Rat CoV and murine hepatitis virus, MHV), and human CoV OC43. Group III CoVs consist of avian strains, including infectious bronchitis virus (IBV), which causes respiratory disease in chickens and turkeys and CoV (TCoV), which causes enteritis in young turkeys. Of the previously known human CoVs, 229E (group I) and OC43 (group II), cause ~30% common colds in humans. Similar to other CoVs, the SARS-CoV is an enveloped, positive-stranded RNA viruses featuring the largest viral RNA genome (31,39). However, sequence comparisons indicate that it does not closely resemble any of the previously known CoVs. Recent studies suggest that SARS-CoV is zoonotic and may have a broad host range besides humans (10,18). How this pathogen crossed the species barrier to humans is still a mystery. To this end, the unknown animal reservoirs provide additional difficulties for the prevention of this emerging virus. A number of wildlife species, such as the Himalayan masked palm civet (*Paguma larvata*), the Chinese ferret badger (*Melogale moschata*), and the raccoon dog (*Nyctereutes procyonoides*) have shown laboratory evidence of infection with related CoVs (10,18). Macaques, ferrets, and mice are experimentally susceptible to SARS-CoV (15,33). Domestic cats were also found to be infected with SARS-CoV, suggesting a mechanical vector (34). The masked palm civet is the wildlife species most often associated with animal-to-human transmission, and genetically similar SARS-CoV has been isolated from civet cats found in live wild-game animal markets (18). However, whether the civet is the natural reservoir of SARS-CoV remains to be proven. Therefore, the modes and routes of inter-species transmission from animals to humans or to other animal species need further investigation to track animal reservoirs. Phylogenetically, the SARS-CoV is most closely related to group II CoVs (42), although an evolutionary origin of SARS-CoV through recombination events between mammalian (group I) and avian (group III) CoVs was suggested by recent sequence analysis (44). Therefore, SARS may recur from an uncharacterized animal reservoir.

MAJOR STRATEGIES FOR DEVELOPING SARS VACCINES

Prior experiences in infectious disease control suggest that vaccination will be one of the most effective measures to prevent new SARS epidemics. SARS-CoV has a large, positive-stranded RNA genome that harbors open-reading frames (ORFs) encoding a large polyprotein required for virus replication; four structural proteins (spike, S; envelop, E; membrane, M; and nucleocapsid, N); and eight additional polypeptides of unknown function (31,39). Infection of SARS-CoV can trigger humoral and cellular immune responses against viral proteins in humans. The facts of the high rate of recovery from acute illness of SARS in the absence of effective medical therapy and the low rate of re-infection by SARS-CoV suggest that protective immunity is achievable. Recent studies have demonstrated that sera from convalescent-phase SARS patients contain high titers of neutralizing antibodies against SARS-CoV (35,41) and that the convalescent sera could be used for treatment of SARS, suggesting that induction of neutralizing antibody would be an effective strategy for developing SARS vaccine. Taken together, these features suggest that a safe and effective anti-SARS vaccine can be developed.

Since the emergence of SARS, many research groups worldwide have joined the SARS vaccine race. A number of candidate vaccines, using a variety of approaches, are under development (32). Generally, the classic approach using killed or inactivated cell-cultured virus has been considered as a first-generation vaccine to prevent viral infectious disease because it is the fastest and easiest means of vaccine development. Therefore, the inactivated form of the SARS-CoV has been developed as one of the major SARS vaccine candidates (32). Several reports have shown that the SARS-CoV inactivated with formaldehyde, ultraviolet light, or β-propiolactone can induce virus-neutralizing antibodies in immunized animals (21,46,47,55). In the lead, an inactivated SARS vaccine that has been developed by Sinovac Biotech in Beijing, China, is the only one being tested in a clinical trial (1,32). Although the whole viral vaccine is effective in terms of eliciting protective immunity in the vaccinated animals, there is serious concern over its safety in humans since antigenic properties of the SARS-CoV has not been well characterized. SARS-CoV expresses a

number of viral proteins with known or unknown functions; some viral proteins may induce harmful immune or inflammatory responses and may even cause SARS-like disease (32,49,52). In addition, caution should be taken in using the inactivated SARS-CoV as a vaccine, because handling of the highly concentrated live virus may pose a high risk to production workers and incomplete virus inactivation may cause SARS cases in vaccinated populations (24,24,32).

The S proteins of CoVs are the major antigenic determinants that induce neutralizing antibodies (7,40). Thus, it is reasonable to use S proteins as antigens for developing vaccines against CoVs (40). Like other CoVs, the SARS-CoV S protein is also a type I transmembrane glycoprotein that contains a leader (residues 1–12), an ectodomain (residues 13–1193), a transmembrane domain (residues 1194–1215), and a short intracellular tail (residues 1216–1255) (39). Recent studies have shown that the S protein of SARS-CoV is a major inducer of protective immunity among structural proteins (5), and a number of live attenuated, genetically engineered or vector vaccines encoding the full-length SARS-CoV S protein have been studied. Yang *et al.* (56) demonstrated that a DNA vaccine candidate encoding the S-protein induced T-cell and neutralizing-antibody responses (Table 1), and that the humoral immunity, rather than the cellular immune response, protected mice from SARS-CoV challenge. Wang *et al.* (51) used DNA vaccines encoding the full-length and segments of S protein to immunize rabbits and generated higher titers (1:>2,000) of neutralizing antibodies against SARS-CoV. Bisht *et al.* (3) showed that intranasal or intramuscular administration of the highly attenuated, modified vaccinia virus vectors virus Ankara (MVA), containing the gene encoding full-length SARS-CoV S protein into mice, elicited SARS-CoV–neutralizing antibodies (Table 1) and protected mice from SARS-CoV infection after transfer of serum from immunized mice. Using similar approaches, Chen *et al.* (8) were able to induce much higher titers of neutralizing antibodies in mice and rabbits (Table 1). Recently, Bukreyev *et al.* (6) demonstrated that African green monkeys immunized with an attenuated parainfluenza virus expressing S protein produced low titers of neutralizing of antibodies but that the animals were protected from SARS-CoV challenge. These data indicate that the S protein of SARS-CoV is a protective antigen capable of inducing neutralizing antibodies, although its antigenic determinants remain to be defined. However, the full-length S protein-based immunogen has been compromised because of its potential risk associated with antibody-dependent enhancement of disease (52,57). A clear precedent is that several vaccines designed against feline CoVs predispose some cats to accelerated disease and death. For instance, the S protein of feline infectious peritonitis virus (FIPV) expressed by recombinant vaccinia can cause antibody-dependent enhancement of disease if the vaccinated animals are subsequently infected with wild-type virus (9,36,48). We have shown that the full-length S protein contains several linear immunodominant domains inducing non-neutralizing antibodies (22), although we do not know whether these non-neutralizing antibodies may enhance SARS-CoV infection or mediate harmful immune responses. Cao *et al.* reported that vaccination of ferrets with MVA expressing the full-length S protein aggravated liver damage caused by SARS-CoV challenge (13,52). Yang *et al.* (57) demonstrated that antibodies against the S protein of SARS-CoV (Urbani strain) could neutralize homologous SARS-CoV strains, but enhanced infections by an early human SARS-CoV isolate (GD03T0013) and two palm-civet, SARS-CoV–like virus strains. Therefore, the full-length S-protein–based vaccines may contain some epitopes that induce infection-enhancing antibodies or other harmful immune and inflammatory responses. To design and develop a safe and effective SARS vaccine, it is necessary to identify the neutralizing epitopes in the S protein of SARS-CoV.

Table 1. Comparison of Efficacy of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Antigens in Eliciting Neutralizing Antibodies

Antigen	*Animals*	*Virus tested*	*Neutralizing titer*	*Reference*
Inactivated SARS-CoV	Mice	SARS-CoV	1:2,200–7,400	Tang *et al.* (47)
Inactivated SARS-CoV	Mice/rabbits	Pseudovirus	1:7,393/1:2,060	He *et al.* (21)
DNA expressing S protein	Mice	SARS-CoV	1:50–1:150	Yang *et al.* (56)
DNA expressing S protein & segments	Rabbits	SARS-CoV	~1:>2,000	Wang *et al.* (51)
MVA expressing S protein	Mice	SARS-CoV	1:284	Bisht *et al.* (3)
MVA expressing S protein	Mice/rabbits	Pseudovirus	1:1,000–10,000	Chen *et al.* (8)
Parainfluenza virus expressing S protein	Monkeys	SARS-CoV	~1:16	Bukreyev (6)
RBD-Fc	Mice/rabbits	SARS-CoV	1:>10,000	He *et al.* (19, 20)

THE RECEPTOR-BINDING DOMAIN OF SARS-CoV S PROTEIN IS A MAJOR NEUTRALIZATION DETERMINANT

CoV infection is initiated by attachment of the S protein to the specific host receptor, which triggers a conformational change in the S protein. Unlike many other CoVs, such as mouse hepatitis virus (MHV) (16,30), in which the S protein is post-translationally cleaved into S1 and S2 subunits, no typical cleavage motif has been identified in the SARS-CoV S protein (39). However, the S protein of SARS-CoV can be divided into S1 and S2 domains by sequence alignment with other CoV S proteins (39,43). Its S2 domain (residues 681–1255) contains putative fusion peptide and two heptad repeat (HR1 and HR2) regions responsible for fusion between viral and target cell membranes. Its S1 domain mediates virus binding with angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV on susceptible cells (11,28,38,50). A 193–amino-acid fragment within S1 domain (residues 318–510) has been identified as a minimal receptor-binding domain (RBD), which is sufficient to associate with ACE2 (2,53,54).

Previous studies suggest that the RBDs on the S proteins of other CoVs, such as mouse hepatitis virus (MHV), transmissible gastroenteritis virus (TGEV), and human CoV (HCoV-229E), contain major antigenic determinants that are capable of inducing neutralizing antibodies (4,17,26). We have recently found that the RBD of SARS-CoV S protein is a highly immunogenic site to induce antibody responses in mice and rabbits immunized with inactivated SARS-CoV (21). The antibodies from the immunized animals were able to block the receptor binding and virus entry, highlighting their capacities to neutralize infection of the SARS-CoV (21). The immunogenicity of the RBD has been further demonstrated by its high reactivity with the antisera from SARS patients in the convalescent phase (23). With an immunoabsorption assay, we demonstrated that the anti-RBD antibodies function as a main population of neutralizing antibodies in the convalescent sera of SARS patients or antisera of rabbits immunized with inactivated viral vaccine (23). After depletion of the RBD-specific antibodies from patient or rabbit immune sera with RBD-conjugated beads, a majority of the neutralizing antibodies were removed, whereas the affinity-purified anti-RBD antibodies had potent neutralizing activity (23). This is in consistent with the report by Chen *et al.* (8), who demonstrated that the protective neutralizing antibodies induced by the MVA-based vaccine expressing the SARS-CoV S protein primarily targeted the RBD in S protein. Using a fusion protein containing the RBD linked to human IgG1 Fc fragment (designated RBD-Fc) as an immunogen, we have shown that RBD-Fc can induce highly potent neutralizing antibodies with 50% neutralizing titers at 1:>10,000 in immunized mice and rabbits (19,20). We have also found that the RBD-induced neutralizing antibody response can protect mice from infection by SARS-CoV challenge (unpublished data). These data suggest that the RBD of S protein is a major neutralization determinant of SARS-CoV during viral infection and immunization and may serve as an important target site for developing SARS vaccines.

The mechanism by which the RBD of S protein can induce highly potent neutralizing antibodies against SARS-CoV has been highlighted by monoclonal antibodies (mAbs) isolated from the inactivated virus-immunized mice and from human antibody libraries (23,45). To define further the antigenic determinants responsible for virus neutralization, we have generated a panel of 27 mouse mAbs using the RBD-Fc as an immunogen (19). Six groups of conformation-dependent epitopes (designated as Conf I-VI) have been characterized. The Conf IV and Conf V mAbs significantly blocked RBD-Fc binding to ACE2, suggesting that their epitopes overlap with the receptor-binding sites in the S protein. Most of the mAbs that recognized the conformational epitopes possessed potent neutralizing activities against SARS pseudovirus, suggesting that the RBD of SARS S protein contains multiple conformational epitopes capable of inducing potent neutralizing antibody responses.

In conclusion, the RBD of SARS-CoV S protein is not only a functional domain mediating virus-receptor binding but also a major neutralization determinant of SARS-CoV. We thus believe that proteins containing the RBD sequence or vectors encoding the RBD of S protein can be used for developing safe and effective SARS vaccines.

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