Molecular Targets for Diagnostics and Therapeutics of Severe Acute Respiratory Syndrome (SARS-CoV)

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Abstract – **PURPOSE.** The large number of deaths in a short period of time due to the spread of severe acute respiratory syndrome (SARS) infection led to the unparalleled collaborative efforts world wide to determine and characterize the new coronavirus (SARS-CoV). The full genome sequence was determined within weeks of the first outbreak by the Canadian group with international collaboration. As per the World Health Organization (WHO), the continual lack of a rapid laboratory test to aid the early diagnosis of suspected cases of SARS makes this area a priority for future research. To prevent deaths in the future, early diagnosis and therapy of this infectious disease is of paramount importance. **METHODS.** This review describes the specific molecular targets for diagnostics and therapeutics of viral infection. **RESULTS.** The three major diagnostic methods available for SARS includes viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR), virus induced antibodies by immunofluorescence assay (IFA) or by enzyme linked immunosorbant assay (ELISA) of nucleocapsid protein (NP). The spike glycoprotein of SARS-CoV is the major inducer of neutralizing antibodies. The receptor binding domain (RBD) in the S1 region of the spike glycoprotein contains multiple conformational epitopes that induces highly potent neutralizing antibodies. The genetically engineered attenuated form of the virus or viral vector vaccine encoding for the SARS-CoV spike glycoprotein has been shown to elicit protective immunity in vaccinated animals. **CONCLUSION.** NP is the preferred target for routine detection of SARS-CoV infection by ELISA which is an economical method compared to other methods. The RBD of the spike glycoprotein is both a functional domain for cell receptor binding and also a major neutralizing determinant of SARS-CoV. The progress in evaluating a therapeutic or vaccine would depend on the availability of clinically relevant animal model.

INTRODUCTION

The outbreak of severe acute respiratory syndrome (SARS) epidemic in 2002-2003 infected over 8000 people globally (in more than 30 countries across 5 continents) and led to the death of over 900 people with a fatality rate of 9.6%. This new emerging disease represented the most recent threat to human health as it has been reported to be highly contagious (1-3). The large number of deaths in a short period of time due to SARS led to the collaborative efforts world wide to determine and characterize the new Coronavirus, the etiological agent of SARS (SARS-CoV). Indirect evidences have suggested that SARS-CoV may have originated from wild animals such as civet cats in southern China, with the isolation of SARS-CoV-like virus from Himalayan palm in a live animal market in Guangdong Province of China (4). There were also reports that horseshoe bats may be a natural reservoir of SARS-CoV (5). The nucleotide sequence homology between the SARS-CoV-like virus isolated from animals and SARS-CoV isolated from human has been found to be 99% (4). As per the WHO, the continual lack of a rapid laboratory test to aid the diagnosis of suspected cases of SARS makes this area a priority for future research. Thus the development of simple inexpensive screening and diagnostic tests for specific and early detection of SARS-CoV will contribute to the risk management of a future disease outbreak.

Coronaviruses are a group of positive sense, single stranded RNA viruses that infect humans and animals. In a short period of time the SARS-CoV was identified and initial laboratory protocols for diagnosing SARS were disseminated. The need for the early diagnosis of SARS is vital due to the difficulty in clinically diagnosing this infection and its rapid nosocomial transmission.

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The current WHO criteria for laboratory confirmation of SARS-CoV is based on either detection of SARS-CoV RNA by PCR, increase in SARS-CoV antibodies in body fluids, or isolation of SARS-CoV from clinical samples. PCR and antibody detection have been the most widely used diagnostic tests for SARS due to the fact that viral culture is time consuming and insensitive. It has also been reported that antibody detection is the most important method during the convalescent phase (6).

SARS-CoV: Three laboratories within the WHO network independently reported the isolation of a novel coronavirus from clinical specimens of patients with SARS (1, 2, 7). The cell lines like MRC5, Hep-2, RDE and HeLaB95-8 that were used by different laboratories to culture the virus proved unproductive. The virus was however successfully isolated in Vero-E6 cells in two laboratories and from FRhK-4 cells in the third laboratory (8). The identification of the causative agent as a coronavirus was made by electron microscopy of virus infected cells. Much later, the Dutch group confirmed the Koch's postulates by infecting primates with the virus isolates (9). Within weeks of the first isolation of the novel coronavirus associated with SARS, a Canadian group from Michael Smith Genome Science Centre in Vancouver, British Columbia, and National Microbiology Laboratory in Winnipeg, Manitoba, completely sequenced the genome of the virus (10) closely followed by another group from the Center for Disease Control (CDC), Atlanta, USA (11). Like other known coronaviruses, SARS-CoV is an enveloped positive stranded RNA virus, featuring a large viral genome of approximately 30 kb in length with 11 Open Reading Frames (ORF's). Coronaviruses have five major ORF's that encodes the replicase, spike, envelope, membrane and NP (12, 13). The replicase gene alone is composed of approximately 21 kb which is larger than the whole genome of picornavirus (14). Similar to other known coronaviruses, SARS-CoV is an enveloped virus containing four structural proteins, namely the small membrane (M), envelope (E) glycoprotein, spike (S) glycoprotein and the NP (15). The virus was detected by RT-PCR and by culture in respiratory tract, gastrointestinal tract, cerebrospinal fluid, faeces and urine (16, 17) indicating that SARS-CoV was not confined only to respiratory tract but spread to different sites. In one study, the highest percentage of positive RT-PCR was found in the stool sample (18) whereas another study reported that stool samples may not be useful for early diagnosis due to the absence of viral RNA in the initial days of the illness (16). Other approaches like real time nested RT-PCR strategies were also used to increase the detection rate of SARS (19, 20). The sensitivity could be increased by testing serial samples collected from the same patients on different days. Initial diagnostic approaches did not focus on serum samples for detection of antigens or antibodies for the diagnosis of SARS. Subsequently it was shown that the viral RNA was found in 78% of the serum samples during the initial days of illness (21). Serodiagnosis has been reported to be the gold standard for confirmation of a diagnosis of SARS by indirect IFA using virus infected cells or extracts of virus infected cells. Employing recombinant viral nucleoprotein and spike protein antigen serological tests have also been explored (6, 22, 23). Three major diagnostic methods are currently developed.

(i) Viral RNA detection using quantitative reverse transcription (RT)-PCR (20, 24, 25) (ii) Antibody detection using indirect fluorescence assay (26) or (iii) using both recombinant NP and culture extract of SARS-CoV-based ELISA (7, 27, 28) . ELISA based antibody detection tests with recombinant antigens are well known to offer higher specificities and reproducibilities. Such tests are easy to standardize and less labour intensive than antibody detection by indirect IFA and thus avoids the requirement of growing SARS-CoV (28-32).

NUCLEOCAPSID AND MEMBRANE PROTEINS: ROLE IN DIAGNOSTICS

Reverse Transcription –Polymerase Chain Reaction (RT-PCR):

RT-PCR has been used widely for the rapid detection of the viral genome in different clinical specimens. Identification of a CoV genome by electron microscopy led to the search for conserved regions of the CoV genome and development of RT-PCR assays for SARS-CoV RNA assays. Early diagnosis of SARS-CoV infection, which involves viral RNA detection by RT-PCR first targeted the polymerase (pol) 1b region of the 5' replicase gene using different formats including one step or two step RT-PCR or real time-PCR assays (2, 7, 33-35). Other report also described an enhanced RT-PCR that involved target preamplification by using two separate amplifications of a region of the pol 1b gene (19). Another group targeted the Nucleocapsid

(nuc) gene which involves one step and two step real-time assays (36). A two step real time TaqMan assay targeting the nuc gene has also been tested for diagnostics (37).

Real time PCR assays have been introduced for rapid detection of SARS-CoV infection that targeted both *pol 1b* and *nuc* gene. It was expected that the detection of nucleocapsid transcripts by RT-PCR should enhance the sensitivity of PCR for SARS diagnosis because its template mRNA is the most abundant subgenomic RNA during infection. However, different studies showed that RT-PCR assays targeting the *nuc* gene have not been more sensitive than *pol* gene assays because the bulk of the viral RNA in a clinical specimen is total genomic RNA and not the pure mRNA. In another approach a two step real time assay that targeted the 3' non-coding region of the SARS-CoV genome was tested with excellent sensitivity but has not been evaluated with SARS patients samples (38).

Culturing of SARS-CoV is a less sensitive and difficult method requiring BSL-3 facility. The RT-PCR and quantitative RT-PCR has been the working standard in this routine diagnosis. Although the main clinical symptom of SARS-CoV infection is severe respiratory tract symptoms, it has been found that the virus also infects other organs. It was reported that SARS patients had diarrhea and virus can be cultured from the feces and urine in addition to the respiratory tract (39). A comprehensive monitoring of the time periods of RT-PCR diagnosis after disease onset in different types of specimens such as tracheal and nasopharyngeal aspirates, throat swabs, nasal swabs and rectal swabs has been studied (40). Their study showed that the peak detection rate for SARS-CoV occurred at 2 weeks after the onset of illness for respiratory specimens, at weeks 2-3 for stool or rectal swab specimens and at week 4 for urine specimens. It is likely that the current RT-PCR is not quite sensitive enough to detect the low concentration of SARS-CoV RNA, although RT-PCR is a reliable alternative approach for the early diagnosis of SARS. However, the detection rate for probable SARS was only 37.5-50%.

Detection of Anti-viral antibodies

The presence of specific antibodies against various viral components has been a classical diagnostic method. However, the limitations of this approach are two fold. Firstly, there is a time lag before antibodies appear in body fluids. Secondly the persistence of the antibodies in serum may not distinguish between the current and past infective states. It has been found that anti-NP antibodies are detected early and with high specificity during infection (41). These authors used three different assays namely Western blot, ELISA, and IFA, using both native and bacterially produced antigens of the virus to evaluate serum samples obtained from 46 patients of SARS, 40 patients with non-SARS pneumonia and 38 healthy individuals. It was reported that 89% of the SARS patient's sera were found to be positive to NP antigen. All the subjects without SARS had no anti-NP antibodies. In another study, peptide induced polyclonal antibodies against NP were produced in rabbits using 6x His recombinant NP antigen. Western blot analysis and immunofluorescent staining of affinity purified antibodies showed that these antibodies specifically recognized SARS-CoV (42). In an earlier report, a full length and six truncated fragments of the nucleocapsid gene were cloned, expressed and purified as glutathione S-transferase (GST) tagged recombinant proteins. These recombinant proteins were reacted to a panel of SARS-CoV positive and negative sera. A major immunodomain of recombinant SARS-CoV nucleocapsid, a truncated 195-amino acid fragment from the C terminus of the NP (N195) was identified by Western blot that had a strong ability to detect antibodies against SARS. The sensitivity and specificity was reported to be 98.5 and 100%, respectively (43). No cross reactivity was reported between the N195 protein and antibodies against chicken, pig and canine coronaviruses. The Western blot assay could distinguish patients with fever caused by other diseases from that of SARS patients, thus reducing the possibility of false positives.

Detection of Viral antigens

The presence of viral antigens in different body fluids is suggestive of current infection. Like most viruses, the NP of SARS-CoV is the most conserved and an ideal target for diagnostics. The NP is the most predominant virus derived protein throughout the infection, likely because its template mRNA is the most abundant subgenomic RNA. This feature makes it a suitable candidate for raising antibodies for antigen diagnostics. The amino acid homology of SARS-CoV NP with other members of coronaviruses have been reported to vary from 27 to

37% (44, 45). This is a phosphoprotein and amino acid residues 397-423 interact with the viral RNA genome to form a helical nucleocapsid (45, 46). The biological function of coronavirus NP is thought to be in the replication and transcription of viral RNA and to interfere with the cell cycle events of host cells (47, 48). In several other coronaviruses, such as murine and turkey coronaviruses, NP has been reported to be highly immunogenic (49, 50). It has been reported that NP accumulates intra-cellularly even before it is packed in the mature virus and is the most abundant virus derived protein throughout the infection. A serum antigen assay could avoid the need for nasal aspirate collection and molecular diagnostic techniques. Detection of circulating antigen has been in use for diagnosis and prognosis of a variety of diseases involving HIV p24, Hepatitis B virus, Hepatitis C virus, viral hemorrhagic fever and Cytomegalovirus (51-57). The detailed analysis in hemorrhagic fever caused by Ebola virus suggested that while RT-PCR assay is extremely useful, it should always be supported in combination with antigen-captured ELISA making the diagnosis more reliable (58).

The SARS-CoV NP antigen capture ELISA was developed using a mixture of three monoclonal antibodies (44) and rabbit polyclonal antibodies for detection. The monoclonal antibodies were of IgG1 type and recognized three different epitopes on NP antigen. The sensitivity of the assay was established to be 50 pg/ml using recombinant NP antigen. The sensitivity of the assay was 84.6% (11 out of 13) of serologically confirmed cases of SARS when samples were obtained between 6 to 10 days of the onset of infection. The specificity of the capture assay was found to be 98.5% in (1253 out of 1,272) healthy individuals. The presence of NP antigen was also detected in the early phase of the disease (3-5 days) in 50% of the patients. Several additional monoclonal antibodies (MAbs) against the NP have also been generated (59). One among these is an IgG1 monoclonal antibody (S-A5D5) that reacts specifically with both recombinant and native NP of SARS-CoV. This MAb was used as detector antibody for detection of NP antigen whereas anti-Guinea Pig polyclonal antibody used as capture antibody in heterosandwich ELISA format. The sensitivity of the capture assay was found to be 37.5 pg of purified NP protein. No false positives results were observed among the sera tested from 60 healthy individuals. Further more, no cross reactivity was observed when infectious bronchitis virus (IBV) and chicken coronavirus were tested. It has also been reported that SARS-CoV NP could be detected using MAb-based antigen capture ELISA in early acute phase serum samples of patients with SARS (60). All of the sera collected from 1 to 10 days after the onset of the symptoms were positive for NP antigen. Nearly all sera were found positive for the NP antigen between days 3 and 5. However, beyond day 11, NP antigen was not detected in group of samples analyzed. The percentage positivity of sera collected between 11 to 15 and 16 to 20 days after the onset of symptoms showed only 36.4 and 21.1% respectively (44, 60). The NP protein was not detected beyond day 19. We have cloned the full length codon optimized NP gene in *E. coli*, expressed and purified as His_6 tagged recombinant protein (61). More recently we have developed a point of care immunoswab assay for detection of SARS-CoV NP antigen using MAb and bispecific MAb (BsMAb). The BsMAb has one arm specific to horseradish peroxidase (HRPO) and the other arm specific to SARS-CoV NP antigen (62). The BsMAb was developed by hybrid-hybridoma technology and purified by a novel dual sequential affinity chromatography method for the detection of NP antigen on immunoswabs (63).

With regard to the other molecular targets, very little information is available on the immune response to the M proteins of SARS-CoV. Some studies have demonstrated that the M proteins of coronaviruses were able to induce antibody response in hosts infected by coronavirus or immunized by attenuated recombinant virus expressing M proteins (64-68) thus making M protein also another target for developing diagnostics and a vaccine candidate. The antigenic site on the M proteins has not been determined as yet in SARS-CoV. Studies using Pepscan analysis identified two immunodominant epitopes on the M proteins at the N-terminus (1-31 amino acids) and C terminal region (residues 132-161) and showed that the N-terminal epitope is more immunogenic in patients infected with SARS but less immunogenic in rabbits than the epitope at the C-terminal (69). Their observations brought out the heterogenicity of SARS-CoV in infection and immunization. It has also been shown that the inactivated SARS-CoV was not only able to induce high titers of antibody response against spike glycoprotein and NP antigen (70-72) but also against

N terminal and C terminal antigenic sites of M protein in immunized mice and rabbits (69).

SPIKE GLYCOPROTEIN: ROLE IN THERAPEUTICS

The SARS-CoV spike gene encodes a glycoprotein of approximate 150 kDa containing 1255 amino acids. Spike protein of animal coronaviruses have been reported to be immunogenic and immunization of animals using spike protein based vaccine produced neutralizing antibodies that were able to prevent infection caused by the corresponding pathogen (73, 74). Spike glycoprotein of SARS-CoV is made up of two domains, S1 and S2. The S1 is near the N-terminus and the S2 near the C- terminus. The S1 and S2 domains are located at the region of amino acid residues 17-680 and 681-1255, respectively (75). The S1 and S2 domains form the globular head and the stalk of the spike protein (76) and play an important role in specific receptor recognition and subsequent cell fusion. Hence this protein is a major target for neutralizing antibodies. It has also been demonstrated that RBD of the S protein contains a key neutralization determinant which can induce potent neutralizing antibodies that block the replication of SARS-CoV in monkeys (69). The receptor for SARS-CoV has been identified as the ACE-2 (77, 78). Many neutralizing antibodies have been reported which are capable of blocking the interaction between the spike glycoprotein and the cellular ACE-2 receptor (79). Hence the ACE-2 binding site of the spike glycoprotein provides an attractive target for the development of a SARS therapeutics as well as a vaccine. The spike glycoprotein may mediate membrane fusion and induce neutralizing antibodies in host, raising the possibility that antibodies against the SARS-CoV spike glycoprotein may also be a good tool for early detection and neutralization of the virus. A 193 amino acid fragment (residues 318-510) in the S1 region was identified as the receptor binding domain of SARS-CoV (74, 80, 81). In an other report, it has been shown that the recombinant RBD of SARS-CoV spike glycoprotein induced neutralizing antibodies (82). These findings were further supported by the fact that depletion of RBD specific antibodies reduced serum mediated neutralizing activity. Furthermore, a MAb (2C5) was able to block the binding of the RBD to the functional receptor (ACE-2) on the target cells, suggesting that the RBD of spike proteins is an important neutralization determinant of SARS-CoV (72). In another study S1 domain of the spike protein and three truncated fragments expressed by fusion of GST in a pGEX-6p-1 vector were evaluated for antigenicity and immunogenicity. Western blot results showed the 510-672 fragment of the S1 domain is a linear immunogenic dominant epitope.

Immunization of mice with four overlapping fragments of spike glycoprotein revealed that all four proteins could elicit spike protein specific antisera and showed reactivity to the whole spike glycoprotein expressed by recombinant baculovirus in insect cells (83). The computer based analysis also suggested that amino acid residues 450-650 of spike glycoprotein (S450-650) of SARS is solvent accessible (84). It was also supported by the findings (85) that residues 485-625 of spike glycoprotein of SARS-CoV generated neutralizing antibodies against the virus. This is further supported by the work of another group who identified a major immunodominant domain of SARS-CoV spike protein from the S1 region (441-700 amino acids), designated as protein C (86). This recombinant baculovirus expressed protein reacted with all the SARS patients sera. The spike glycoprotein based IFA based on the protein C showed 100% specificity and sensitivity (87). The anti-spike protein antibodies were detected in 63% of the patients in a study conducted on 46 patients with SARS infection (41). Two chimeric immunodominant genes coding for the proteins of SARS-CoV based on bio-informatics were identified and cloned into plasmid pGEX-6p-1 vector and expressed in *E. coli* (88). The proteins expressed by the two chimeric genes were recognized by the SARS patient sera. MAbs produced against these two peptides specifically recognized expression product of S2 and S5 domains, respectively. The S2 and S5 domains correspond to 447-458 aa and 789-799 aa of SARS-CoV spike glycoprotein respectively. A 15 amino acid segment in the C-terminal (1143-1157) of spike glycoprotein has been shown to induce neutralizing antibodies preventing the SARS-CoV infection in VERO-E6 cells (13).

ANTI-SARS-CoV DRUG:

The metallopeptidase, ACE-2, efficiently binds the S1 domain of the SARS-CoV spike protein and has been reported to be a functional receptor for SARS-

CoV. The interruption of virus receptor interaction could be a potential target for therapeutics strategies. The receptor-binding S1 domain of SARS-CoV spike protein is a possible target for new SARS antiviral drugs. It has been suggested that antibodies against ACE-2 and not the inhibitors binding to the active site of ACE-2, may be more useful for the development of therapeutic strategies (89). There are several potential targets for the development of antiviral drugs against SARS-CoV. Strategies include a) the disruption of the binding of the virus to the target cell, b) inhibiting the spike RBD domain or its receptor such as ACE-2, c) cell entry inhibitors or d) inhibitors of replication steps, e) virus assembly disruption and f) targeting viral exit. The main SARS-CoV protease also called the 3C-like protease plays an important role in mediating viral replication and transcription functions through extensive proteolytic processing of two replicase polyproteins , pp1a (486 KDa) and pp1ab (790 KDa) . The crystal structure of the main protease M^{pro} for SARS-CoV (strain 229E) and its inhibitor complex led to the design of anti-SARS drugs (14, 90). An irreversible inhibition of SARS-CoV M^{pro} by aza-peptide epoxide (APE) has been reported (91) suggesting that APE has excellent potential as inhibitors of SARS-CoV M^{pro} and could lead to additional compounds for anti-SARS therapeutics. Furthermore, the aza-peptide component of APE binds to the substrate binding regions of M^{pro} in a substrate like manner with excellent structural and chemical complementarity. The kinetic studies and crystal structure studies have shown that M^{pro} reacts only with the S,S diastereomer of the APE and not it's R,R diastereomer of APE (91).

SARS-CoV VACCINE DEVELOPMENT

It has been reported that SARS patients show clinical improvement if they are treated with serum from previously infected patients suggesting that passive immunotherapy could be developed for the treatment of infectious diseases (92). The development of SARS vaccine can be approached using several techniques such as live attenuated vaccine (93, 94), whole killed vaccine (95), recombinant subunit vaccines (82), recombinant vectored vaccine (96, 97), DNA vaccine (98-101) and epitope based vaccine (102, 103).

For SARS-CoV, it has been demonstrated that prior infection provided protective immunity in a mouse model and the passive transfer of neutralizing antibodies to naïve mice also protected them from infection (104). This suggested that neutralizing antibodies have an important role in reducing the viral load or preventing further viral replication. A DNA vaccine encoding for the spike glycoprotein alone was able to induce T cell responses along with the neutralizing antibodies. Such antibodies were able to protect the mice from SARS-CoV (100). These findings suggested that spike glycoprotein is in fact the target for viral neutralization in SARS-CoV infection. Similar findings were also reported by other group of workers (96, 105). These reports emphasized the potential for developing peptide based vaccine candidates if the neutralizing epitope on spike protein is identified. In a recent study (106) rabbit polyclonal antibodies against five bacterially expressed fragments of spike glycoprotein covering the entire ectodomain (aa 48-1192) were raised. It was demonstrated that all the antibodies were specific and sensitive to both native and denatured forms of full length spike glycoprotein. It was further reported that the anti-SΔ10 antibody which was targeted to amino acid residues 1029-1192 has strong neutralizing activities. This led to the conclusion that this region of spike glycoprotein carries neutralizing epitopes. A high level expression of S1 domain of SARS-CoV in tomato and tobacco has recently been reported (107). This plant derived peptide induces immune responses in mice and showed increased level of SARS-CoV specific IgA and IgG respectively. Other workers (13, 72, 108) have also identified the regions in the spike glycoproteins responsible for inducing the neutralizing antibodies. These observations confirm that there is a major neutralizing determinant exposed *in vivo* for generating high levels of neutralizing antibodies which blocks the interaction between the spike glycoprotein of SARS-CoV and the cellular receptor ACE-2. In a novel study, the neutralization and viral entry determinants were characterized by deleting a positively charged region, SΔ (422-463) in the RBD of spike glycoprotein (79). The deletion resulted in loss of the ability to induce potent neutralizing antibody and reported that a single positively charged amino acid (R441A) in the spike glycoprotein is an indispensable component for determining the immunogenicity of SARS-CoV major neutralizing domain. The substitution of another amino acid (R453A) abolished the viral entry. Their findings provided evidence indicating

that determinants for immunogenicity and viral entry might not be the same. In summary, spike glycoprotein of SARS-CoV is the major inducer of neutralizing antibodies and the RBD in the S1 domain of spike glycoprotein contains multiple conformational neutralizing epitopes that induces highly potent neutralizing antibodies against SARS-CoV (70, 72, 109, 110). The recombinant proteins containing the RBD and vector encoding the RBD sequence can be used to develop a safe vaccine (111). Human MAb to the spike glycoprotein of the SARS-CoV have been developed by various groups. These antibodies were able to neutralize the virus in tissue culture and an animal model and thus may have potential for prevention and treatment (112). The first vaccine tested in clinical trials was made from inactivated form of SARS-CoV. However, this traditional approach to generate polyclonal antibodies to SARS-CoV has presented difficulties for several reasons including safety concerns in handling SARS particles (3, 95). The attenuated form of virus, genetically engineered or vector vaccine encoding for the SARS-CoV spike glycoprotein have shown promising effectiveness in eliciting protective immunity in vaccinated animals. A candidate vaccine has been attempted using three adenoviral-based vectors that expressed SARS-CoV structural antigens including the S1 fragment of spike glycoprotein, the membrane protein and the NP (113). Immunization of rhesus monkeys induced antibody responses against the S1 spike glycoprotein and T cell responses against the NP. In another study, immunization of mice by DNA vaccination with NP, M and E gene induced SARS specific antibodies, T cell proliferation, CTL responses with a pronounced Th1 type of response against NP (114). Two different SARS vaccines, a whole killed SARS-CoV (WKV) and a combination of two adenovirus-based vectors-one expressing the NP and the other expressing the spike (S) protein (Ad S/N), were evaluated for the induction of neutralizing antibodies and their ability to protect against SARS-CoV challenge in a murine model of infection (115). The WKV vaccine given subcutaneously to 129S6/SvEv mice was more effective than Ad S/N given intranasally or intramuscularly in inhibiting SARS-CoV replication in murine respiratory tract. Among the vaccine groups, SARS-CoV specific IgA was found only in sera of mice immunized intranasally with Ad S/N, suggesting that mucosal immunity may play a role in protection. The sera of vaccinated mice contained antibodies to S protein, suggesting a role of this protein in conferring protective immunity against SARS-CoV infection. Current knowledge of the potency of inactivated SARS-CoV vaccine is limited. This SARS-CoV vaccine induced high levels of antibodies against NP, S and M antigen with neutralizing activity (95, 116). Intranasal immunization of inactivated SARS-CoV failed to induce detectable levels of specific IgG and IgA antibodies in sera, IgA antibodies in saliva, lungs and intestine unless given with adjuvants (117). Intranasal vaccination of recombinant adeno-associated virus based RBD (RBD-rAAV) could induce highly potent neutralizing mucosal and systemic antibodies specific to SARS-CoV (118, 119).

In conclusion, the RBD of spike glycoprotein is both a functional domain for receptor binding and a major neutralizing determinant of SARS-CoV. All vaccines based on spike glycoprotein seem to induce neutralizing antibody responses and those carrying NP can induce NP specific cell mediated immunity. The success however in evaluating a SARS vaccine would depend on the availability of clinically relevant SARS disease animal model to test the efficacy of the vaccines.

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