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Antigenicity and Immunogenicity of SARS-CoV

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Abstract

A new coronavirus, severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV), has been identified as the causal agent of SARS. Phylogenetic analyses indicate that it is distinct from the three known antigenic groups of coronaviruses. Similar to other coronaviruses, SARS-CoV contains four structural proteins, including spike (S), nucleocapsid (N), membrane (M) and envelope (E) proteins. All these proteins may induce humoral and cellular immune responses during viral infection. Characterization on the antigenicity and immunogenicity of SARS-CoV is important for developing effective diagnostics, therapeutics and vaccines.

Keywords: SARS-CoV, antigenicity, immunogenicity, vaccine

The global outbreak of severe acute respiratory syndrome [SARS] was caused by a new coronavirus [SARS-CoV] within the family Coronaviridae [1-4]. With aggressive quarantine measures, the epidemic of SARS has been successfully controlled. However, SARS-CoV may re-emerge from an animal reservoir, or from a laboratory source due to accidental release. Also, there is serious concern over the possibility of SARS-CoV being utilized as a bioterrorism agent. Therefore, post-genomic characterization of SARS-CoV is important for exploring the mysteries of SARS and for developing effective diagnostics, therapeutics and vaccines. Similar to other coronaviruses [CoVs], SARS-CoV features a large positive-stranded RNA genome 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 [3, 4]. All these proteins may serve as antigens to trigger immune responses in the infected humans and immunized animals. Here we will mainly discuss the antigenicity and immunogenicity of SARS-CoV S, N and M proteins as well as their cross-reactivity with other CoVs.

1. SARS-CoV S Protein

The S protein of SARS-CoV is a type I transmembrane glycoprotein responsible for receptor binding and membrane fusion. Recent studies indicate that the S protein is highly immunogenic to induce antibody responses in SARS patients and is a potent inducer of neutralizing antibodies against SARS-CoV in the immunized animals [5-7]. Therefore, it has been used as a major antigen for developing diagnostics and as a major immunogen for developing SARS vaccines. Several genetically engineered vaccines encoding the SARS-CoV S protein have been evaluated in pre-clinical studies. A DNA vaccine and a recombinant modified vaccinia virus Ankara [MVA] expressing full-length S protein of SARS-CoV has been shown to induce protective neutralizing antibody responses [8, 9].

The S protein of SARS-CoV can be divided into S1 and S2 domains by sequence alignment with other CoV S proteins [4, 10]. A 193-amino acid fragment [residues 318-510] in the S1 domain has been characterized as the minimal receptor-binding domain [RBD] of SARS-CoV to mediate the S protein binding to the cell receptor angiotensin-converting enzyme 2 [ACE2] [11-17]. We have recently found that the RBD of SARS S protein contains multiple conformational epitopes capable of inducing highly potent neutralizing antibody responses [18-21]. The protective neutralizing antibodies induced by MVA that expresses SARS-CoV S protein primarily target the RBD [8]. Therefore, the RBD of S protein may serve as a major neutralization determinant of SARS-CoV. Using Pepscan analysis, we have shown that the full-length S protein contains several linear immunodominant domains that do not induce neutralizing antibodies [5]. It was reported that vaccination of ferrets with vaccinia virus-based SARS vaccine expressing the full-length S protein enhanced liver damage caused by SARS-CoV infection [22, 23]. Yang et al [24] found that polyclonal and monoclonal antibodies specific for the S protein of SARS-CoV is effective to neutralize homologous virus [Urbani strain]. However, these antibodies could not neutralize, but rather enhanced infections by the early human SARS-CoV isolate [GD03T0013] and palm civet SARS-CoV-like

viruses [24]. The S2 domain of SARS-CoV S protein containing a putative fusion peptide and two heptad repeat [HR1 and HR2] regions is responsible for fusion between viral and target cell membranes [25]. Recent data suggest that the S2 also contains epitopes for neutralizing antibodies [26-28] and several immunodominant T-cell epitopes [29-31].

2. SARS-CoV N Protein

The N proteins of CoVs play important roles in viral pathogenesis, replication and RNA package [32, 33]. They are also immunodominant antigens in the CoV family [34-38]. Antigenic studies have demonstrated that the N proteins are capable of inducing protective immune responses against CoV infection [39-41]. These features make them suitable candidates for developing diagnostic agents and subunit vaccines. Recent studies have shown that the antibodies to SARS-CoV N protein are highly detectable in the sera of SARS patients [42-44], suggesting its potential application for SARS diagnosis. The SARS-CoV N protein has also been considered as a vaccine candidate. A number of reports indicate that DNA vaccines encoding N protein can induce potent humoral and cellular immune responses against SARS-CoV [45-47]. However, it is unclear whether the antibody responses induced by these vaccines are effective in neutralizing infectivity of SARS-CoV. We and others have found that SARS-CoV N protein contains several immunodominant epitopes with a major antigenic site located at the C-terminal region [48, 49], suggesting that truncated proteins containing the immunodominant epitopes may serve as antigens for developing SARS diagnostics and vaccines [50].

3. SARS-CoV M Protein

The M protein of CoV is the most abundant glycoprotein in the virus particles and is the key player for viral particle formation. The structure of M protein is characterized as having three domains: a short N-terminal ectodomain, a triple-spanning transmembrane domain, and a large interior C-terminal domain. Previous studies demonstrated that the M proteins of CoVs were able to induce antibody responses in the host infected by CoV or immunized by attenuated recombinant virus that express the M protein [41, 51-54]. Computer-aided analyses reveal that the M protein of SARS-CoV has a similar structure with M proteins of other CoVs. The antigenicity of M protein has been shown by its reactivity with the serum samples from SARS patients [55]. Furthermore, Pang et al [56] demonstrated that a recombinant M protein expressed in Pichia Pastoris was able to induce protective humoral responses against SARS-CoV, suggesting its potential application for designing SARS vaccine. We have recently identified two major immunodominant epitopes on the M protein located in the extreme N-terminal region [residues 1-31] and the interior C-terminal region [residues 132-161], respectively, by Pepscan analyses against convalescent sera from SARS patients and antisera from virus-immunized mice and rabbits. Synthetic peptides M1-31 derived from the N-terminal epitope and M132-161 derived from the C-terminal epitope were able to induce high titers of antibody responses in the immunized rabbits; highlighting the antigenic and immunogenic properties of SARS-CoV M protein [He et al. unpublished data].

4. Antigenic Cross-reactions between SARS-CoV and Other CoVs

SARS-CoV may originate from animals and have a broad host range besides humans [57, 58]. How this pathogen crosses the species barrier to humans is still a mystery. There exist three known antigenic groups of CoVs: i.e., Group I, including transmissible gastroenteritis virus [TGEV], porcine epidemic diarrhea virus [PEDV], porcine respiratory CoV [PRCV], feline infectious peritonitis virus [FIPV], canine CoV [CCoV], and human CoV [HCoV-229E], etc.; Group 2, including bovine coronavirus [BCoV], murine hepatitis virus [MHV], and human CoV [HCoV-OC43]; and Group III, consisting of avian CoVs - infectious bronchitis virus [IBV] that causes respiratory disease in chickens and turkey CoV [TCoV] that causes enteritis in young turkeys. Phylogenetically, the SARS-CoV is most closely related to group II CoVs [59]. Sequence analyses by Stavrinides et al [60] suggest an evolutionary origin of SARS-CoV through recombination events between mammalian [group I] and avian [group III] CoVs. Infection with SARS-CoV-related viruses has been detected in a number of wildlife species - the Himalayan masked palm civet [Paguma larvata], the Chinese ferret badger [Melogale moschata], and the raccoon dog [Nyctereutes procyonoides] [57, 58]. The macaques, ferrets and domestic cats are experimentally susceptible to SARS-CoV [61, 62]. Antigenic relationships within group I CoVs have been extensively studied. Four group I CoVs, including TGEV, PRCV, CCoV and FIPV, share antigenic determinants to cross-react with each other in virus neutralization and immunofluorescence tests and with MAbs to the S, N or M proteins of these CoVs. A number of studies have shown the interspecies transmission of several group I CoVs [TGEV, FIPV, and CCoV], supporting their close genetic and antigenic relationships.

Since the emergence of SARS, the serological tests have been widely used since the seroconversion to SARS-CoV is a definitive criterion for laboratory determination of SARS-CoV infection in humans or animals [63, 64]. However, we still lack sensitive and specific laboratory diagnostic tests for differential diagnosis of infection by SARS-CoV and

other CoVs in humans and animals. The major problem with current serologic tests is antigenic cross-reactions between SARS-CoV and other CoVs. The antigenic cross-reactivity between SARS-CoV and group I CoVs has been documented by recent observations [2, 65]. Ksiazek et al showed that polyclonal antibodies to TGEV, FIPV and human CoV 229E cross-reacted with SARS-CoV-infected cells [2]; Sun and Meng showed that the N protein of SARS-CoV cross-reacted with the polyclonal antibodies against group I CoVs including TGEV, canine CoV, and FIPV, but not with the antibodies to group II and III CoVs [65]. Che et al have recently demonstrated that SARS-CoV shares antigenic reactivity with other two human CoVs [229E and OC43] that cause ~30% common colds [66]. Yuen and colleagues found that false-positive results in a recombinant SARS-CoV N protein-based ELISA were due to HCoV OC43 and 229E infections [67]. It was also reported that the recombinant N protein-based ELISA showed approximately 1% positivity among healthy blood donors [6, 68]. Therefore, further characterization of antigenic relationships between SARS-CoV and other CoVs is very important for developing specific and sensitive serologic tests and for investigating the animal reservoirs of SARS-CoV.

5. Conclusion

In conclusion, identification of the immuno-dominant antigenic sites and neutralizing epitopes involved in the immune responses against SARS-CoV is highly important for developing SARS diagnostics, therapeutics and vaccines. We believe that the immunodominant epitopes specific for SARS-CoV can be used as ideal antigens for designing SARS diagnostic kits. We are in process to develop immunoassays using a peptide pool and a set of mosaic fusion proteins bearing the selected immunodominant epitopes on the SARS-CoV proteins without cross-reactivity with antibodies against other CoVs as antigens for specific serologic detection. We also believe that a safe and effective vaccine will be developed using antigens containing the neutralizing epitopes but no sequences to induce antibodies mediating enhancement of SARS-CoV infection. We propose to use the receptor-binding domain of S protein as a major target for developing SARS vaccines and immunotherapeutics since it contains multiple conformational neutralizing epitopes and is a major neutralization determinant of SARS-CoV.

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