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Structural, Functional, and Immunological Properties of SARS-CoV Spike Glycoprotein

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During the 2002-2003 epidemic, close to 8,100 people were infected worldwide, among which 774 people died. The etiological agent of this atypical respiratory disease has been identified as a novel coronavirus (designated as SARS-CoV). With a mortality rate of over 9%, SARS-CoV had a major health and socioeconomic impact. Due to multiple modes of virus transmission and a wide range of potential non-human reservoirs, including wild animals commonly found in markets, bats and domestic cats, a virus of this nature will likely resurface in the future. Currently, there are no anti-viral drugs, immuno-therapeutic agents or licensed vaccines available against the virus.

Spike (S) protein is a major target of interest for antiviral drug development efforts as well as for developing vaccines. It is a type I membrane glycoprotein, which functions as a trimer. It is responsible for binding to cellular receptors and inducing membrane fusion for entry into target cells. The primary receptor for SARS-CoV has been identified as angiotensin-converting enzyme-related carboxypeptidase (ACE2). Molecular interactions between SARS-CoV S protein and ACE2 are beginning to be understood. The receptor-binding domain (RBD) has been narrowed down to amino acid residues 318-510. Recently solved co-crystal structure of ACE2 bound to the RBD revealed that residues 424-494 form the receptor-binding motif (RBM) that directly contacts ACE2.

Our goal is to characterize structural, functional, biochemical and immunological properties of SARS-CoV S glycoprotein, with long-term goals of understanding viral pathogenesis, discovering antiviral agents, and developing antigens for a vaccine. Towards this goal, we generated SARS pseudoviruses, which are specifically neutralized by convalescent sera from SARS-CoV-infected patients. These pseudoviruses facilitate analyses of S protein function because they can be used outside of BSL3 containment facility. Using them, we have characterized molecular interactions between S protein and its receptors, identified neutralization epitopes, and developed a potent entry inhibitor.

To identify determinants on ACE2 critical for mediating SARS-CoV infection, we performed alanine scanning mutagenesis analyses on charged amino acid residues on the first two α-helices. The results

indicated that eleven charged amino acids between residues 22 and 57 were important for mediating virus infection. Having identified determinants on ACE2 that interact with S protein, we next evaluated whether we could use ACE2-derived peptides as inhibitors of SARS-CoV infection. Out of six peptides we analyzed, one exhibited potent antiviral activity with IC50 of 100 nM. The antiviral activity was specific towards ACE2-mediated SARS-CoV infection. We are presently characterizing biochemical interactions between the peptide inhibitor and S protein to improve antiviral activity. To identify additional peptides that exhibit anti-SARS-CoV activity, phage-displayed peptide libraries are being screened.

Carbohydrate moieties on viral envelope glycoproteins play important functions in the viral life cycle and pathogenesis. While some sugar moieties are critical for protein function, others are important for immune evasion. There are 23 potential N-linked glycosylation sites on SARS-CoV S protein. These sites exist in three distinct clusters on a linear map: Cluster I at the N-terminus, Cluster II in the middle of the protein near the border between S1 and S2 domains; and Cluster III at the C-terminus. Considering the fact that SARS-CoV uses DC-SIGN and L-SIGN as alternative receptors, glycan residues on S protein likely play an important role in S protein function. To demonstrate this more directly, effects of removing carbohydrate moieties on pseudovirus infectivity were examined. Pseudoviruses were treated with endoglycosidase H (Endo H) and their infectivity in cells expressing DC-SIGN, L-SIGN, or ACE2 was determined. As expected, infectivity of viruses treated with Endo H decreased drastically in cells expressing DC-SIGN or L-SIGN. Endo H-treated viruses also lost infectivity in cells expressing ACE2, albeit less pronounced than for SIGN-mediated infections. These results indicate that glycans are involved not only in binding DC- or L-SIGN, but also in maintaining the proper conformation of the protein required for efficient interaction with ACE2. To identify glycosylation sites critical for mediating DC- or L-SIGN-mediated SARS-CoV infections, site-directed mutagenesis was performed to mutate twelve individual glycosylation sites in Cluster I. Infectivity of glycosylation site mutant pseudoviruses showed the importance of glycans on residues N109, N118, N119, N158 and N227 for SIGN-mediated SARS-CoV entry.

To evaluate immunogenic properties of S protein, we have immunized mice with various fragments of S proteins. Potent neutralizing antibodies were elicited. We have thus far identified three neutralization epitopes. The details of these studies will be discussed.