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Visions & Reflections

The SARS-CoV S glycoprotein

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Abstract. The severe acute respiratory syndrome-coronavirus (SARS-CoV) spike (S) glycoprotein alone can mediate the membrane fusion required for virus entry and cell fusion. It is also a major immunogen and a target for entry inhibitors. Recent rapid advances in our knowledge of the structure and function of this protein have lead to the development of a number of candidate vaccine immunogens and SARS-CoV entry inhibitors.

Key words. SARS; S glycoprotein; S protein; virus; fusion; entry; ACE2.

Viral envelope glycoproteins initiate entry of viruses into cells by binding to cell surface receptors followed by conformational changes leading to membrane fusion and delivery of the genome to the cytoplasm [1]. The spike (S) glycoproteins of coronaviruses are no exception and mediate binding to host cells followed by membrane fusion; they are major targets for neutralizing antibodies and form the characteristic corona of large, distinctive spikes in the viral envelopes [2, 3]. Such 20-nm complex surface projections also surround the periphery of the severe acute respiratory syndrome-coranavirus (SARS-CoV) particles [4]. The level of overall sequence similarity between the predicted amino acid sequence of the SARS-CoV S glycoprotein and the S glycoproteins of other coronaviruses is low (20–27% pairwise amino acid identity) except for some conserved sequences in the S2 subunit [5]. The low level of sequence similarity precludes definite conclusions about functional and structural similarity.

The full-length SARS-CoV S glycoprotein and various soluble fragments have been recently cloned, expressed and characterized biochemically and biophysically [6–11]. The S glycoprotein runs at about 180–200 kDa in SDS gels suggesting posttranslational modifications as predicted by previous computer analysis and observed for other coronaviruses [6, 11]. Cells expressing S fused with receptor-expressing cells at neutral pH suggesting that the recombinant glycoprotein is functional, its membrane fusogenic activity does not require other viral proteins, and that low pH is not required for triggering membrane fusion; fusion was not observed at low receptor concentrations [6]. Although low pH is not required for membrane fusion, entry of pseudoviruses mediated by the S glycoprotein into cells may occur at low pH by an unknown mechanism [9, 12]. However, a very recent study based on pseudotyped murine leukemia virus particles did not confirm these results [13]. Entry was found to occur at concentrations of NH_4Cl (0–50 μM) which are sufficient to inhibit VSV G protein-mediated entry but much lower than those used previously for inhibition of pseudotyped viruses containing S (in the millimolar range [9]). Further studies are needed to clarify these differences. S and its soluble ectodomain, S_e, were not cleaved to any significant degree [6]. Because the S protein of coronaviruses is a class I fusion protein [14], this observation classifies the SARS-CoV S protein as an exception to the rule that class I fusion proteins are cleaved exposing an N-terminal fusogenic sequence (fusion peptide), although cleavage of S could enhance fusion [9].

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Because S is not cleaved, defining the exact location of the boundary between S1 and S2 is difficult; presumably it is somewhere between residues around 672 and 758 [6, 7]. Computer analysis suggested the existence of two heptad repeats; peptides from the N- and C-terminal regions of S2 (NP and CP, respectively) can form stable complexes (six-helix bundle structures) indicating that as for other class I fusion proteins, such structures are an important intermediate in the fusion process [15–18]. The formation of these structures could be disrupted by NP and CP. Indeed, several recent studies demonstrated that SARS-CoV infection can be inhibited by CP although in most cases the inhibitory peptide concentrations were in the micromolar range [15–18], except for one isolate which appears to be inhibited at nanomolar concentrations [17]. In contrast, T20, which is the first virus entry inhibitor approved by the FDA for clinical use, can inhibit virus entry much more efficiently in HIV-1 infections. The underlying mechanism of these differences is not known but could be related to the different pathways of entry of these two viruses – endocytosis (SARS-CoV) and entry at the cytoplasmic membrane (HIV).

Fragments containing the N-terminal amino acid residues 17–537 and 272–537 but not 17–276 bound specifically to Vero E6 cells and purified soluble receptor molecules. Together with data for inhibition of binding by antibodies, developed against peptides from S, these findings suggest that the receptor-binding domain is located between amino acid residues 303 and 537 [6]. Two other groups obtained similar results and found that independently folded fragments as short as 193 residues can specifically bind receptor molecules [8, 10]. Currently, these fragments are being further characterized to better understand the interactions of the virus with its receptor. They also have potential as inhibitors of the virus entry by blocking these interactions.

The functional receptor for SARS-CoV is angiotensin-converting enzyme 2 (ACE2) [7], finding which has been confirmed [6, 19]. Its crystal structure was recently reported [20] in general agreement with two previously developed homology models [21, 22]. The S-binding domain on ACE2 was proposed to involve residues on the ridges surrounding the enzymatic site [22]; the exact location and properties of this domain are now being elucidated. ACE2 binds with high (nanomolar) affinity to S and is likely to induce conformational changes required for membrane fusion [6–8, 23]. The structure of the S-ACE2 complex is currently being elucidated. Soluble ACE2 (sACE2), and various fusion constructs or fragments, can serve as potent inhibitors of the virus infection in vivo.

The entry of SARS-CoV into cells can also be inhibited by antibodies that bind the S glycoprotein. Such a human monoclonal antibody that potently inhibits membrane fusion at nanomolar concentrations was recently identified by screening phage display libraries [24]. This antibody competed with ACE2 for binding to the S glycoprotein, suggesting that its mechanism of neutralization involves inhibition of the virus-receptor interaction. Sera from convalescent SARS patients also neutralized the virus [25]. Antibodies to the S glycoprotein appear to play an important role in virus neutralization in vivo. Such antibodies can be elicited in vivo by using the full-length S glycoprotein or soluble fragments including the whole ectodomain and fragments containing the receptor-binding domain. Immunization of macaques [26] and mice [11, 27] elicited neutralizing antibodies that protected mice from infection. Careful examination of candidate vaccines is required because of the possibility for enhancing effects and animal model-dependent effects.

In conclusion, the S glycoprotein has been characterized as an entry mediator, vaccine immunogen, inhibitor and a target for inhibitors in an amazingly short period of time. Future studies will elucidate detailed mechanisms of its function that may help in the further development of clinically useful inhibitors and vaccines.

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