SARS-CoV-2 immunogenicity at the crossroads

To the Editor,

The outbreak of coronavirus disease 2019 (COVID-19) caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a global health emergency and became a worldwide pandemic. We summarize the recent findings with respect to the function, structure, and immunogenicity of the spike (S) protein, arising mutations, and implications on vaccine development and therapeutics.

The novel coronavirus can cause pneumonia leading to severe respiratory failure driven by immune dysregulation. SARS-CoV-2 belongs to the Betacoronavirus genus and is closely related to SARS-CoV, which had caused a previous outbreak in 2002-2003.

Cell entry of coronaviruses requires the concerted action of receptor binding and proteolytic processing of the trimeric surface spike glycoprotein. S protein priming is mediated by cellular proteases into S1 and S2 subunits, harboring the receptor-binding domain (RBD) and the fusion machinery, respectively. Upon receptor binding, conformational changes lead to exposure of a second cleavage site (S2') allowing fusion of viral and cell membrane. SARS-CoV-2 employs the cellular protease TMPRSS2 for S protein priming like SARS-CoV. Strikingly, SARS-CoV-2 S possesses a polybasic furin cleavage site at the boundary of S1/S2 in contrast to SARS-CoV S that harbors only a monobasic site (Figure 1). This suggests that ubiquitously expressed furin-like proteases might contribute in addition to TMPRSS2 to cell entry leading to an expanded tissue tropism or even altered pathogenicity of the novel SARS-CoV-2 relative to SARS-CoV.

Both SARS-CoV-2 and SARS-CoV use angiotensin-converting enzyme 2 (ACE2) as their host entry receptor. Several recent publications resolved the structural basis of the interactions between ACE2 and the RBD of SARS-CoV-2, located in the C-terminal portion of S1 (CTD). Interestingly, most reports indicate that SARS-CoV-2 S binds to human ACE2 with higher affinity than the SARS-CoV S protein, which may impact viral infectivity for SARS-CoV-2.

Based on studies on SARS-CoV and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV), the S protein is the main target for neutralizing antibodies and an ideal candidate target for vaccine studies. Interestingly, S-reactive CD4+ T cells have been reported in more than 80% of COVID-19 patients, targeting both N- and C-terminal epitopes of S. Strikingly, CD4+ T cells in 34% of seronegative healthy donors did react, but only to the C-terminal part of S containing the S2 subunit but not the RBD. This suggests a potential preexisting cross-reactive cellular immunity to SARS-CoV-2 directed to S2. Although the S proteins of SARS-CoV-2 and SARS-CoV share a high degree of sequence similarity and use the same receptor, they seem not to share cross-reactive neutralizing epitopes within S1 or the RBD. Monoclonal and polyclonal antibodies targeting the S1 or RBD of SARS-CoV did not recognize SARS-CoV-2 or poorly neutralized SARS-CoV-2 entry. In line with this observation, Ju et al reported on RBD-specific monoclonal

**Figure 1** Schematic of SARS-CoV and SARS-CoV-2 spike protein. Coronavirus spike protein harbors the S1 and S2 subunits, which are cleaved at the S1/S2 boundary and the S2' cleavage site, as indicated by arrows. SARS-CoV-2 spike protein harbors a polybasic furin cleavage sequence (PRRARS) with an insertion of four amino acid residues distinct from SARS-CoV and other SARS-like viruses. The receptor-binding domain (RBD) is indicated in dark green. Predicted dominant B- and T-cell epitope regions are indicated as red and blue bars, respectively, adapted from Grifoni et al. The two immunodominant B-cell linear epitopes identified by Poh et al are indicated as yellow bars.
antibodies derived from single B cells of eight SARS-CoV-2-infected individuals demonstrating neutralizing activity against SARS-CoV-2. Neither SARS-CoV-2 antibodies nor the infected plasma cross-reacted with RBDS from SARS-CoV or MERS-CoV. However, antibodies elicited by SARS-CoV S protein in sera from convalescent SARS patients revealed some degree of cross-neutralization activity toward SARS-CoV-2. It may be hypothesized that the target of these antibodies is indeed the S2 region. Interestingly, S2 of SARS-CoV and SARS-CoV-2 sequence similarity than the respective S1 subunits (~90%), and importantly, S2 of SARS-CoV-2 might contain neutralizing epitopes.

This is in line with a recent publication that applied several bioinformatic prediction models and identified potential dominant B- and T-cell epitopes in high-homology regions for SARS-CoV and SARS-CoV-2 that are likely to be recognized in humans. Surprisingly, the prediction revealed no B-cell epitope within the RBD of the S protein; however, several candidate epitopes were identified outside the RBD and within S2 (Figure 1). The predictions are corroborated by the identification of two immunodominant linear B-cell epitopes that are highly recognized by neutralizing antibodies in sera of COVID-19 convalescent patients (Figure 1, yellow squares). Importantly, some of the predicted identified epitopes show a high degree of conservation between SARS-CoV and SARS-CoV-2. Moreover, one of the identified immunodominant epitopes encompasses part of the highly conserved fusion peptide suggesting a potential pan-coronavirus epitope. This may infer that immune responses elicited by vaccination strategies designed to target these particular epitopes could be cross-protective to various betacoronaviruses and to emerging virus mutations worldwide.

One major hurdle for therapeutic strategies and vaccine development is in fact the high mutation rate of RNA viruses. The mutation rate drives virus evolution and genome variability, which enables the virus to escape host immunity and to develop drug resistance. To assess the genetic variation, an early study on eighty-six genomes of SARS-CoV-2 detected in infected patients worldwide revealed in particular eight missense mutations in the RBD. Furthermore, Forster et al. classified three central variants A, B, and C based on phylogenetic network analysis of 160 SARS-CoV-2 genomes. These findings suggest that the virus is rapidly evolving. It needs to be seen whether observed mutations will have an influence on immunogenicity or receptor-binding capacity. On the other hand, a report on a 382-nt deletion in ORF8 of SARS-CoV-2 isolated from patients in Singapore implies mutations may arise as result of human adaptation and could be associated with attenuation.
With this in mind, multiple vaccination strategies are currently pursued targeting the S protein as an ideal candidate protein that may elicit cross-protective immunity (Table 1). Additionally, as a therapeutic option, a broadly acting cocktail of repurposing drugs might be the most pragmatic strategy to combat COVID-19. Drugs targeting the replication steps of the virus, and therapeutics alleviating the observed cytokine storm and hyperinflammation reducing acute lung injury would help patients. Interestingly, off-label treatment with the IL-6 blocker tocilizumab has been described in producing acute lung injury would help patients. Interestingly, off-label levitating the observed cytokine storm and hyperinflammation requiring targeted therapy with tocilizumab has been described in COVID-19 patients [1].

The authors declare that they have no conflicts of interest.

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CONFLICTS OF INTEREST

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Is BCG vaccination affecting the spread and severity of COVID-19?

To the Editor,

The influence of infectious stimuli on the development of allergic disorders has been investigated for many years. As allergists and immunologists, we are very familiar with the T-helper (Th)1/Th2 balance in addition to orchestral roles of T regulatory and other effector cells, so we have to consider the possible roles of childhood immunizations within the context of the current coronavirus disease 2019 (COVID-19) outbreak.