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Implications of the SARS-CoV-2 spike protein interaction with type-1 macrophages via α7-nAChR.

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Abstract

The human immune system has a generic response strategy to viral infections that begins with local inflammation at the infected site along with the initiation of a primary innate immune response. Beyond a certain threshold, innate immune cells cannot handle the infection, and they call upon adaptive immunity. The innate cells then take up their role in “The Resolution of Inflammation”. The recognition of the SARS-CoV-2 antigen triggers an eicosanoid storm and initiates a robust inflammatory response, leading to a sustained cytokine storm that interferes with the activation of adaptive immune cells, specifically BCL6+ B cells. The mechanism of this interaction, and hence the pathogenesis of the virus with the immune system, is yet to be determined. In silico studies predict a direct viral interaction with type 1 resident macrophages, which could initiate the cascade of events described above. The macrophage population being the target would also explain why older age groups are relatively more susceptible to the virus. Here, we review the interaction of the SARS-CoV-2 spike protein via a cryptic epitope with the α7-nAChR in Type-1 macrophages, discuss the implications it might have on our approach to the generic treatment of COVID-19 patients, and present better prospects for the design and dissemination of more effective vaccines and their importance.

The high expression levels of the ACE2 receptor on lung epithelial cells [1][2] explains why the lung is severely affected by COVID-19, but the infection of resident alveolar macrophages seems counterintuitive, as their expression of the ACE2 receptor is fairly limited [3][4]. Macrophages are an attractive target due to their ability to cause type 1 IFN disruption via the activation of various types of PRRs they express [5]. In theory, the phagocytosis of infected cells by resident macrophages exposes them to the virus [5][6]. The activation of PRRs in macrophages and other innate cells triggers the release of CD14+ monocytes from the bone marrow into the bloodstream via CCL-2 signaling. They are then induced as resident macrophages at the infection site [5][7][8][9]. Monocytes, on the other hand, express the ACE2 receptor and are highly susceptible to SARS-CoV-2 [10][11][12][13]. They play a fundamental role in generating an adaptive immune response, as tissue-resident macrophages are poor APCs and fail to migrate to regional lymph nodes [14].

The local concentration of proinflammatory cytokines is proportional to the rate of signaling to the bone marrow for the release of monocytes, which further contributes to the production of local proinflammatory cytokines, thereby establishing a positive feedback system. This is naturally regulated by polarization of resident M1 macrophages to the M2 phenotype,

which mediate the anti-inflammatory effects and initiate the recovery process [15][16][17][18]. By keeping the macrophage population balanced towards the M1 phenotype in the microenvironment, the virus ensures an increase in the concentration of proinflammatory cytokines while avoiding the hindrance caused by anti-inflammatory cytokines [19]. This initiates an eicosanoid storm and then a subsequent cytokine storm with increased expression of TNF-α, as is seen in COVID-19 cases [20][21][22][23][24][25][26][27]. The direct interaction of the virus with macrophages indicates the involvement of α7-nAChR in the pathogenesis of COVID-19, hinting at a dysregulation of the polarization mechanism, which is an explanation for the dysfunction observed in the innate immune response to this virus [28].

Upon the initiation of the adaptive immune response, neural pathways play a major role in transmitting early signals from one part of the body to another. The inflammatory reflex is an example; it is responsible for priming the spleen for the adaptive immune response [29][30]. Indirect activation of α7-nAChR via acetylcholine produced by splenic T cells inhibits splenic macrophages from expressing proinflammatory cytokines. This is one of the functions associated with M2 macrophages. Whether polarization is triggered by the activation of this receptor or is only a small part of the grand polarization scheme awaits investigation, but it plays a role in the dysfunction of innate immune cells.

SARS-CoV-2 has one of the largest known viral genomes (approximately 2-3 times the average viral length) and features exonuclease activity with proofreading capabilities via nsp14 (ExoN) [31][32]. The term “error catastrophe” has not been defined f1or this viral candidate. It has adapted well, and a good example here would be the mutations seen in the S1 subunit of the spike protein enabling it to have better binding affinities for the ACE2 receptor while maintaining ~76% similarity with the S1 subunit of SARS-CoV [33]. The S2 subunit is even more highly conserved with one striking feature, the biosynthesis of a furin cleavage site that aids the transmissibility of the virus. This is particularly intriguing because the cleavage of S1/S2 subunits is not even necessary for its biosynthesis [33]. So why did that happen? Why increase the chances for cleavage of a site that is not necessary in the first place? This suggests that the S1 residue still has a few tricks up its sleeve and these come into effect post binding with the transmembrane pentameric glycoprotein receptor ACE2.

The epitope (aa 365-390) belongs to a domain of the S1 subunit and it is highly conserved among the global mutations in this pandemic [34][35][36]. Since this epitope is conserved, the antibody CR3022 developed against SARS-CoV also interacts with high affinity with SARS-CoV-2 and is accessible only in the open configuration of the trimeric spike RBD [37][38]. CR3022 in combination with CR3014 works to neutralize SARS-CoV but failed in this case due to the mutational changes observed between the S1 regions of SARS-CoV and SARS-CoV-2 [39]. Suggesting that this epitope is tasked with a function. Upon further investigation, it is involved in a direct interaction with innate immune cells expressing the ACE2 receptor and α7-nAChR [40][41]. This theory is supported by the rare SARS-CoV-2 antibody COVA1–16 [42].

Implications for clinical therapy

The viral load a COVID-19 host faces is comparable to that of a simultaneous attack by multiple viruses due to the mutations observed in the spike protein. This is why one antibody may work against a few strands but is ineffective in neutralizing the others, thereby reducing the overall efficacy of our vaccines. There is no generic antibody capable of neutralizing all of the mutated spike proteins, yet the only way to develop long-term protective immunity against this virus is with an antibody. The human body is capable of generating a diverse antibody population required to handle immense

viral loads, provided that the immune system is intact. Kaneko et al. observed low but diverse antibody production in COVID-19 cases [27].

With the onset of SARS-CoV-2 infection, the immune system starts to dysregulate, and B-cell lymphopenia is observed. An increase in proinflammatory cytokines is accompanied by an increase in TNF-α, which inhibits the differentiation of active CD4+ T cells into BCL6+ GC-T_{FH} cells, resulting in a loss of germinal centers. Naturally, the antibodies formed are insufficient, leaving us susceptible to reinfection, assuming that the patient has recovered from the first infection [27].

An antibody targeting an epitope that is conserved across all mutations worldwide that interacts with immune cells has major benefits. Neutralizing this epitope would handicap the ability of the virus to cause dysfunction, but this is only a part of the S1 residue. The main virion continues its infection cycle.

To restore the integrity of the immune system, additional stimulation of α7-nAChR would be essential. A combination of α7-nAChR agonists along with the proposed antibody will definitely help regulate the proinflammatory cytokine concentrations, but whether that alone would suffice to restore the ability of the body to generate sufficient quantities of antibodies requires further evaluation [43][44][45][46].

Discussion

COVID-19 shares its clinical features with other diseases, and cholinergic activation in those conditions has shown alleviating effects. Due to these similarities, studies have focused on inactivating certain pathways and cytokines, but the results are inconclusive, mainly because they interfere with a recovery process that is essential at some point during the infection cycle. Dysregulation of this causes downstream effects that eventually benefit the virus or worsen the condition of the patient. Treatment should be designed in such a way that it does not interfere with natural processes while still effectively terminating the viral infection. The spike protein, being the entry point, is extensively surveilled, and the mutations registered have been found to contribute to increased transmission rates and better adhesion capabilities [47][48].

The evolution of SARS-CoV-2 is mediated through variants. The proofreading activity ensures that each variant has a chance to thrive and survive. They adapt to evade the immune response and hence there is a need to upgrade from a cocktail of antibodies to mRNA and adenovirus vectors.

The issue with mRNA and adenoviral vectored vaccines is that they all encode the spike protein, which is subject to mutations. They might work to reduce initial infection rates, but they also allow for recombinant mutations. The spike template used for the vaccine may contribute to increasing the genetic diversity in an area, thereby expanding the scope for local recombinant mutations. This reduces the efficacy of the vaccines, as the body is forced to start again with basic antigen recognition. With respect to the mutation rate, the best plan of action would be to target not the whole protein but only a highly conserved epitope across all variants. From an evolutionary perspective, only the epitopes that are functional for replication would be conserved. This provides us with better options for vaccine development.

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