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IMMUNOLOGY OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 (SARS COV-2): A REVIEW
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IMMUNOLOGY OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 (SARS COV-2): A REVIEW

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ABSTRACT

***Objectives*: A mysterious respiratory disease named Severe Acute Respiratory Syndrome Coronavirus -2 (SARS CoV-2) broke out in Wuhan, China in December 2019, and has stimulated a rapid and intense public health response. As at 7th of July, 2020, about 11,516,782 confirmed cases and about 535,453 deaths have been reported across over 187 countries/regions of the world as reported by Johns Hopkins University, Maryland USA.**

We need to understand the immunologic responses to this infection, so as to be able to properly manage patients, and also hopefully to be able to produce effective vaccines against SARS CoV-2.

***Data sources and synthesis:* SARS-CoV-2 via virus Spike (S)-protein primarily attaches to the epithelium of the respiratory tract because of its high affinity for the ACE 2 receptor on the epithelium. It gets into the respiratory tract endothelium by means of endocytosis.**

The mast cells in the sub-mucosa of the respiratory tract form a barrier of protection against the virus. Mast cells get primed when they make contact with the virus, and cytokines are then released. The cytokines, however, play an important role both in its virulence and in the outcome of the viral infection.

***Conclusion*: Human convalescent serum can be useful for preventing infection and for treating COVID-19 disease. Antibodies against SARS-CoV-2 are also being developed, but mainly aimed at developing ELISA and other rapid sero-diagnostic reagents for COVID-19. Protein snippets of the Spike (S) glycoprotein that are immunogenic are being produced in *E.coli* to be used in the vaccine-production process.**

INTRODUCTION

Coronaviruses (CoVs) are so called due to their striking crown of surface projections, reminiscent of the solar corona, which can be seen by electron microscopy.[1] Severe Acute Respiratory Syndrome Coronavirus - 2 (SARS CoV-2), the aetiology of Coronavirus disease 2019 (COVID-19) is the seventh specie of coronavirus to infect humans and one of the three (including SARS CoV and MERS CoV) with severe presentations. They belong to the genus *Betacoronavirus* of the Family *Coronaviridae* and display a tropism for epithelial cells of the respiratory or gastrointestinal tract.[1]

The outbreak of SARS CoV-2, which originated from Wuhan, China in December 2019, has stimulated a rapid and intense public health response coordinated by the World Health Organisation (WHO) in collaboration with all health departments of countries and regions across the globe. As at 7th of July 2020, about 11,516782 confirmed cases and 535,453 deaths have been reported across over 187 countries/regions of the world.[2]

CoVs have very large, linear, enveloped, 20- to 160-nm particles that contain an unsegmented genome of single-stranded positive sense RNA (27–32 kb); with polyadenylated genomes at the 3′ end. It utilizes the host receptor Angiotensin-Converting Enzyme-2 (ACE-2) on the epithelial cells located in human nasopharynx and oropharynx. Cell entry is by endocytosis. [1,3]

The pathological manifestation of this viral disease results from cytopathic effect on the epithelium of the respiratory tract and immune responses to the virus. Cytokine production is responsible for the common manifestations of viral diseases such as fevers, chills, and myalgia, and the cytokine response has been associated with complications secondary to some viral infections, such as immunosuppression and dementia.[4] The primary function of these cytokines is either to inhibit viral replication or to activate the cells mediating the cellular and humoral immune responses. The production of inflammatory cytokines during the immune response is not always beneficial to the host. They sometimes contribute to the pathogenicity and side-effects of viral infections. It is also now clear that cytokines produced either directly in infected cells or indirectly by lymphocytes and macrophages activated by the immune response to viral proteins play an important role both in the outcome of the viral infection and in its virulence.[5]

Epitopes Responsible for Binding to the Epithelial Cells of the Respiratory Tract

SARS-CoV-2 predominantly affects the lungs through epithelium of the respiratory tract. The virus enters lung cells by binding to ACE-2 receptor. The virus uses defined receptor-binding domain (RBD) on the glycoprotein spikes (S or HE) that specifically recognizes the host Angiotensin-Converting Enzyme-2 (ACE-2) receptors on the epithelial cells of the nasopharynx and oropharynx. Endocytosis leads to internalization of viruses by host cells. Mast cells contribute to SARS-CoV2 (2019-nCoV) induced inflammation of the submucosa of the respiratory tract and the nasal cavity.[3] Knowledge of the epitopes responsible for binding of SARS-CoV-2 to its receptor is critical to development of monoclonal antibodies against this novel virus

The key structural proteins of SARS-CoV-2 include the spike protein (S), the membrane (M) envelope (E) glycoprotein, and nucleocapsid (N) proteins.[6] Framework and

practical analysis of the SARS-CoV-2 reveals that there is a binding between viral S-protein and the ACE-2 receptor of the human alveolar epithelial cells. This gives the impression that SARS-CoV-2 uses the same receptor, ACE-2 as SARS-CoV, though it attaches with a higher affinity. [7, 8]

SARS-CoV-2 shares nearly 79.6% genomic sequence density with SARS-CoV genealogically; thus monoclonal antibodies being developed for SARS-CoV may be useful in lowering the severity of covid-19 disease.[9] For viral attachment, fusion and entry into host cells, the wholly glycosylated spike protein of SARS-COV-2 is essential. SARS-CoV-2 binds to ACE-2 receptor using its N-terminal S_1 subunit, while the C-terminal S_2 subunit of the virus is involved in virus cell membrane fusion.[10]

The ACE-2 receptors of the human respiratory epithelia are the binding sites of CoV during infection and this occurs via the interaction between its S_{1-} receptor binding domin (S1-RBD) and the cell membrane receptors. Subsequently, conformational changes in the virus occurs enabling fusion and entry of the virus into target cells. [9,10]

S protein priming by cellular proteases ($TMPRSS_2$) is essential for entry of the virus, and this involves a protein cleavage at the S_1/S_2 and S_2 sites; thus, allowing fusion of viral and cellular membranes, a process driven by the S_2 subunit. .[10, 11]

The length of the SARS-CoV spike protein has 1,255 amino acids (aa) and it consist of two domains -S_1 (aa residues 17 to 680) responsible for receptor binding and S_2 (aa residues 681-1255) responsible for membrane fusion.[12] The major neutralization determinant that has the ability to induce potent neutralizing antibodies in mice is embedded in the receptor binding domain (aa residues 328 to 510) of the S protein content.[13]

Activation of Mast Cells

Mast cells (MC) are granule containing immune cells that reside in the tissues and are derived from hematopoietic precursor cells.[13] They are found, among other places, in the mucosa of the lungs and the gut. They are also found in the sub-mucosa of blood vessels, lymphatics, and nerve endings. Stem cell factor (SCF) is involved in regulation of mast cell proliferation.[14] Effect of mast cells on SARS-CoV results in production of inflammatory mediators including proteases and pro-inflammatory cytokines.[15,16] Mast cells are involved in the innate and adaptive immune systems.[15] Activation of mast cells are involved in restricting viral replication in the local tissue as well as preventing viral dissemination, which if left uncurbed may cause substantial tissue damage and vascular leakage thereby causing tissue edema.[17]

Toll-like receptors (TLR) expressed by MCs are swiftly identified by the virus in the respiratory tract. Mast cells participate in inflammatory processes; defend the body against microbial infections, takes part in the body defence against SARS-CoV. [17] Mast cell plays a significant role in allergic reactions that follow SARS-CoV infection. Receptors through which MCs recognize and respond to viral infections include TLR signaling, such as TLR3, detection of ds RNA, Spingosin-1-phosphate (S1P) binding to its receptor S1PR and RIG-1-recognition of uncapped VRNA.[17]

Mast cell activation leads to immediate degranulation with subsequent synthesis of eicosanoids within minutes of activation and synthesis of numerous chemokines and growth factors within hours of activation.[17] The large quantity of cytokines, chemokines and growth factors released by mast cells includes synthesis of TNF-α, IL-4, IL-5, IL.6, IL-13, IL-17 and VEGF. [17,18]

Virus-activated MCs may provoke the release, after seconds, of stored chemical mediators such as histamine, tryptases and chymase. After hours of incubation, activated MCs secrete synthesized inflammatory cytokines including IL-6, IL-1, IL-31, IL-33, TNF and chemokines CC5, CCL2, MCP-1 and CXCL8 which attracts white blood cells to the inflammatory sites.[18] Virus activating MCs cause the release of some specific chemokines, such as the ligand 5 (CCL5) which attracts the CD_8T immune cells that defend the lung tissue and fight viral infection.[19]

Viruses develop particular mechanisms to invade the body and immune cells including MCs. RNA viruses stimulate MCs to produce type 1 interferons (IFNs) which are antiviral cytokines. Type 1 IFNs enhance the cytotoxic activity of natural killer cells against virus infected cells.[20] On the other hand, virus stimulates the mucosa MCs to release pro-inflammatory cytokines such as TNF, IL-1, IL-6 and proteases which aggravate the inflammatory states.[21]

POSSIBLE IMMUNOTHERAPY FOR COVID-19

Antibodies for passive immunotherapy

Vaccines for prevention and treatment of COVID-19 are not presently available, however global effort are being made to produce vaccines. Patients classified as high risk such as those with severe manifestation of COVID-19, those with co-morbidities such as those with diabetes, cardiovascular disease, cancer patients and elderly patients who are sufferers of COVID-19 may benefit from passive immunotherapy. Passive antibody therapy involves the administration of antibodies to a susceptible individual for the purpose of preventing or treating an infectious disease due to a given agent. Thus, passive antibody administration is the only means of providing immediate immunity to susceptible persons.

Researches done with other coronaviruses, such as SARS-CoV-1, showed that such convalescent sera contain neutralizing antibodies to the relevant virus.[22] Viral neutralization is the expected specific means by which passive antibody therapy could mediate its protection against SARS-CoV-2 using convalescent sera. However, other mechanisms include antibody-dependent cellular cytotoxicity and/or phagocytosis.[23] Human convalescent sera from individuals who have recovered from COVID-19, monoclonal antibodies (mAbs), or preparations generated in certain animal hosts, such as genetically engineered cows that produce human antibody are potential sources of antibody for SARS-CoV-2.[23]

The potency of passive antibody therapy is high when used for prophylaxis than for treatment of disease. Antibody administered shortly after the first appearance of symptoms is usually more effective whenever it is used for therapy. Passive antibody functions by neutralizing the initial inoculum, which is probably much smaller than that of deep rooted disease.[24] Passive antibody therapy also works by making alterations to the inflammatory response and this is certainly achieved during the initial immune response, a stage that may be asymptomatic.[25] There are reports that convalescent serum was used for treatment of patients with COVID-19 in China during the current outbreak. Although few details are available from the epidemic in China and published studies involved small numbers of patients, the available information suggests that convalescent serum administration reduced viral load and was safe.[26] COVID-19 convalescent sera can be used for either prophylaxis of infection or

treatment of disease. Convalescent serum administration when used prophylactically can prevent infection and subsequent disease in those who are at high risk for the disease, such as vulnerable individuals with underlying medical conditions, health care providers, and those with exposure to confirmed cases of COVID-19. Therapeutically, convalescent serum would be administered to those with clinical disease in an effort to reduce their symptoms and mortality. Antibody administration would be more effective in preventing disease than in the treatment of established disease.[27]

Administration of convalescent sera as passive immunotherapy has its own hazard. There is a risk of transfer of unpremeditated infections and immune serum sickness. Transfusion-related acute lung injury (TRALI) is another dreadful risk particularly in those with chronic obstructive airway disease as well as other pulmonary disease [28]. Balance of risk and benefit evaluation of convalescent sera therapy should be considered before administration. With modern blood banking techniques that screen for blood-borne pathogens and match the blood type of donors and recipients, the risks of inadvertently transferring known infectious agents or triggering transfusion reactions are low. There could also be a risk of antibody-dependent enhancement of infection (ADE). Although, this is a theoretical risk, it can occur in many viral diseases and entails an amplification of disease in the presence of definite antibodies. Several mechanisms for ADE have been described for coronaviruses, and there is the apprehension that antibodies to one type of coronavirus could amplify infection to another viral strain.[29]

Production of antibody against SARS2-CoV-2 from COVID-19 convalescent sera is being geared up by one pharmaceutical company, Takeda [30] It is important to note that producing highly purified preparations containing a high titer of neutralizing antibodies against SARS2-CoV-2 is preferable to convalescent sera given that these are safer and have higher activity. *Monoclonal Antibodies*

The development of monoclonal antibodies could go a long way in abating SARS-CoV-2 epidemic. A number of effective monoclonal antibodies that target the SARS-CoV spike protein to prevent the virus from entering host cells have been identified by prior researches.[31,32,33] The prime target of neutralizing monoclonal antibodies is the 193-amino acid (residues 318–510) receptor-binding domain (RBD) of the spike protein.[34] The SARS-CoV neutralizing monoclonal antibodies CR3014 and CR3022 were found to attach non-competitively to the SARS-CoV RBD with subsequent neutralization of the virus in a concerted manner.[32] Recent survey showed that CR3022 could combine effectively with SARS-CoV-2 RBD (K_D of 6.3 nM).[35] Moreover, the epitope of CR3022 does not overlap with the binding site of ACE-2 in the SARS-CoV-2 RBD.[5] Thus, CR3022 could be a promising therapeutic candidate, alone or in combination with other neutralizing monoclonal antibodies, for the treatment of COVID-19 infection.

The spike proteins of SARS-CoV-2 (SARS2-S; 1273 residues, strain Wuhan-Hu-1) and SARS-CoV (SARS-S, 1255 residues, strain Urbani) are 77.5% identical by primary amino acid sequence, are structurally very similar [36,37,38] and commonly bind the human angiotensin converting enzyme 2 (ACE-2) protein as a host receptor. However, a recent study showed that a SARS-CoV antibody, CR3022, binds to SARS-CoV-2 RBD [35], but its neutralization capability is uncertain.

SARS-CoV-2 S protein binds ACE-2 with higher affinity than SARS-CoV (10- to 20-fold) [10], suggesting its recognition to ACE-2 could be different with SARS-CoV. Cross-reactivity is limited between the two virus S proteins. Several published SARS-CoV NAbs do not have appreciable binding to SARS-CoV-2 S protein [35,36]. Generating NAbs targeting different epitopes on SARS-CoV-2 will be meaningful.

A research work on Mab for COVID -19, using a trypsin-triggered cell-cell fusion assay, Mab 47D11 had shown to impair SARS-S and SARS2-S mediated syncytia. Data from the study showed that 47D11 neutralizes SARS-CoV and SARS-CoV-2 through a yet unknown mechanism that is different from receptor-binding interference. Alternative mechanisms of coronavirus neutralization by RBD-targeting antibodies have been reported including spike inactivation through antibody-induced destabilization of its perfusion structure, which may also apply for 47D11.[39]

HOPE OF VACCINES FOR COVID-19

Vaccines against COVID-19 are in the production process: a. Scientists convert the virus's RNA into DNA and select pieces of the virus that computer simulations have suggested are immunogenic. Those selected bits of DNA are then inserted into bacteria, which produce large quantities of protein snippets to be used in the vaccine-production process;[39] b. Others have mapped the molecular structure of the spike glycoprotein, in an attempt to use them to produce vaccines that can act specifically on the S glycoprotein.[40] Antibodies against COVID-19 are also being developed, but mainly aimed at developing ELISA and other rapid sero-diagnostic reagents for COVID-19.[41]

DISCUSSION

SARS-CoV uses defined receptor-binding domain (RBD) on the glycoprotein spikes (S or HE) that specifically recognizes and binds to the host Angiotensin-Converting Enzyme-2 (ACE-2) receptors on the epithelial cells of the nasopharynx and oropharynx. Endocytosis leads to internalization of viruses by host cells. [6]

Mast cells constitute a barrier of protection against microorganism.[3,18] The virus activates MCs which release early inflammatory chemical compounds including histamine and protease, while late activation provokes the generation of proinflammatory IL-1 family members including IL-1 and IL-33. Virus-activated MCs immediately release chemical mediators and or late production of pro-inflammatory proteins such as cytokines and chemokines.[18] The cytokines, chemokines and growth factors released by mast cells includes synthesis of TNF-α, IL-4, IL-5, IL.6, IL -13, IL-17 and VEGF are believed to be important causes of cytokine storm in SARS-CoV infection. [17]

IL-37, a member of the IL-1 family of cytokines, is activated by Caspase1. It acts by suppressing immune responses and transcription of pro-inflammatory genes. IL-37 acts by inhibiting IL-1, which is a potent pro-inflammatory cytokine.[21] This anti-inflammatory cytokine may suppress fever and the inflammation provoked by coronavirus.

Vaccine production initiatives are taking place globally. Human convalescent serum could be useful for prevention and treatment of COVID-19 disease. [22,30,31] People who have recovered from the infection should be persuaded to donate their immunoglobulin-containing serum. Effective monoclonal antibodies that target the SARS-CoV spike

protein to prevent the virus from entering host cells are also under development.[31,32,33,41] Efforts are also directed at cloning the S protein spikes of SARS-CoV with the aim of using them as antigen in vaccine production.

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