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SARS-CoV-2 Cellular Entry and Antigen Recognition: Modulating Immunological Responses

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ABSTRACT

The SARS-CoV-2 coronavirus (COVID-19) pandemic has led to an enormous collaborative international effort within the medical and scientific community. This effort resulted in the development of several vaccines stemming from various platforms within an unprecedented timeframe. The binding of the spike protein, a specific region on the virus surface, to specific regions on certain adhesion molecules is critical at this port of entry. Once infected, the virus begins to replicate, destroys the host cells, and spreads to other cells, leading to disease. This review outlines some of the specific receptors expressed on endothelial and epithelial cells mediating the entry of the virus into the host cells via the virus-binding ligand, the spike protein. This is a key factor to be considered for developing new vaccines and therapeutic strategies against both current and potential future variants.

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Abbreviations: Endothelial Cells (ECs), Angiotensin-converting Enzyme (ACE2), Transmembrane Protease Serine 2 (TMPRSS2)

Introduction

The adhesion of leukocytes to the vascular endothelium is a critical event, taking place prior to their extravasation and migration to inflammatory sites. The process is implicated in the pathogenesis of several diseases including atherosclerosis, myocardial infarction, and gastric ulcers [1]. Given the importance of leukocyte-endothelial cell interactions in impacting tissue injury and organ dysfunction, it has been the focal point of intense investigation to identify and characterize the adhesion molecules and glycoproteins mediating these interactions. Adhesion is a multi-faceted process commencing with the rolling of the leukocyte along the EC surface, followed by firm adhesion, and ending with extravasation through the extravascular space. Some of the main endothelial cell adhesion molecules involved in this process are summarized below.

Selectins

The selectins belong to a family of single transmembrane glycoprotein adhesion molecules that bind to sugar moieties.

P-selectin: Located in the Weibel-Palade bodies of ECs and the α-granules of platelets. Upon stimulation by agents like thrombin or histamine, it is mobilized and translocated to the cell surface where it promotes platelet aggregation and recruits leukocytes to the EC surface [2].

Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1): Inducible EC adhesion molecule following exposure to cytokines such as interleukin-1 (Il-1), endotoxin, or tumor necrosis factor-α (TNF-α) [3].

L-selectin (CD62L): Transmembrane glycoprotein and cell adhesion molecule that is expressed on most circulating leukocytes. It is involved in tethering and rolling on the EC surface and regulating monocyte protrusion during trans-endothelial migration (TEM) [4].

A recently published study highlighting the role of vascular ECs in the lung during COVID-19 reported that despite increased immune cell infiltration in infected patients, both P and E-selectin were down regulated in pulmonary ECs [5].

Immunoglobulins

There are five members of the immunoglobulin superfamily that act as adhesion molecules. Vascular cell adhesion molecule-1 (VCAM-1): Constitutively expressed on EC but significantly increased following several hours of cytokine exposure and binds to integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$ [6].

Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1): Constitutively expressed on platelets, leukocytes, and ECs. It mediates the adhesion of platelets and leukocytes to ECs, and the migration of leukocytes through the endothelial cell junctions [7].

Intracellular Adhesion Molecule-1, 2 (ICAM-1, 2): ICAM-1 is constitutively expressed on the EC surface and can be significantly increased for 5-24 hours following cytokine activation. ICAM-2 is also constitutively expressed on ECs but is not modulated by activation [8].

Mucosal Vascular Addressin Cell Adhesion Molecule 1 (MAdCAM-1): Similar to VCAM-1 and ICAM-1 and interacts with leukocyte $\alpha_4\beta_7$, $\alpha_4\beta_1$, and L-selectin, to direct leukocytes into mucosal and inflamed tissues [9].

It has been reported that serum levels of several adhesion molecules including VCAM-1 and ICAM-1 were elevated in patients with mild COVID, dramatically elevated in severe cases, and decreased in the convalescence phase [10]. The authors concluded the increased expression of EC molecules is related to COVID-19 disease severity and may contribute to coagulation dysfunction.

Integrins

Integrins are heterodimeric glycoproteins expressed on the surface of both leukocytes and ECs. CD11/CD18: Family of adhesion molecules that are expressed on leukocytes and include CD11a, CD11b, CD11c and CD11d. CD11a/CD18 is expressed on most leukocytes and interacts with ICAM-1 and -2 resulting in firm adhesion. CD11b/CD18 and CD11c/CD18 are stored in granules intracellular and released to the cell surface following activation by agents such as platelet-activating factor (PAF) and TNF-a, resulting in a 10-30 fold increase in expression that binds to ICAM-1 [8]. CD11c/CD18 binds ICAM-4 whereas the ligand for CD11d/CD18 is as yet unknown [11].

Beta-1 Integrin $\alpha_4\beta_1$: Also known as very late antigen-4 [VLA-4], mediates the adhesion of lymphocytes, monocytes, eosinophils and natural killer cells (NK) to activated ECs expressing vascular cell adhesion molecule-1 (VCAM-1) [12]. Integrin α5β1, also known as the fibronectin receptor, binds to matrix macromolecules and proteinases and stimulates angiogenesis [13].

Integrin ($\alpha_v\beta_3$): The vitronectin receptor, has been shown to be involved in mediating monocyte adhesion to, and induction of cyclooxygenase (COX) in, ECs [14, 15]. A recent review reported that blockade of SARS-CoV-2 binding to $\alpha_5\ \beta_1$ and $\alpha_V\beta_3$ integrins using specific small peptides has been shown to reduce viral infectivity in vivo and attenuate vascular inflammation. The authors proposed an urgent examination into the therapeutic potential of integrins as therapeutic targets for SARS-CoV-2 [16].

Angiotensin-Converting Enzyme-2 (ACE2) Receptor

The ACE2 receptor is a transmembrane protein found on endothelial and epithelial and of the upper and lower respiratory tract, kidneys, heart and blood vessels, and portions of the gastrointestinal tract. Its presence on epithelial cells creates protective barriers lining these tissues and also plays an important role in regulating wound healing, inflammation, and blood pressure. Recently it has been the focus of intense investigation as it is the main entry point for SARS-CoV-2 into the host cell [17]. The "spike protein" that is expressed on the surface of the SARS-CoV-2 virus targets, attaches, and enters the host cell by attaching itself to the ACE2 receptor. It is this spike that gives the coronavirus family of viruses their name as they protrude outwards from the spherical virus surface, rendering it a crown-like halo, or "corona".

The specific region on the spike protein that binds ACE2 is the receptor-binding domain (RBD). The spike protein binds to the ACE2 receptor on cells, down regulates their function, and causes damage to the endothelial cells lining the blood vessels. Since ACE2 is also present in the epithelium of the nose, mouth, and lungs, where it is highly abundant in type 2 pneumocystis, an important cell type present in chambers within the lung alveoli [18].

The infection begins when the long spike proteins protruding from the virus surface latch onto the ACE2 protein. From here, the spike transforms by unfolding and refolding itself using coiled spring-like parts that are buried at the core of the spike. The reconfigured spike hooks and docks the virus particle to the host cell. This forms a channel allowing the viral genetic material into the host cell, such as type II lung cells. Most of the damage in COVID-19 results from the immune system going into overdrive to prevent the virus from spreading [19]. The influx of immune cells to the infected tissue causes enormous amounts of damage in the process of cleaning out the virus, infected cells, and bacterial infections, with potentially lethal consequences. It has been reported that the spike protein alone can damage the vascular ECs by down regulating ACE2 and inhibiting mitochondrial function [20]. Moreover, a recent study reported that Severe COVID-19 induces autoantibodies against angiotensin II that correlate with blood pressure dysregulation and disease severity [21].

Trans Membrane Protease Serine 2 (TMPRSS2)

Another key component involved in mediating virus attachment and entry into the cell relates to binding to another cell receptor, transmembrane protease serine 2 (TMPRSS2). TMPRSS2 is a transmembrane protein with serine protease activity that is found in high concentrations on epithelial cells of the liver, heart, and lungs [22].

Recent evidence suggested that SARS-CoV-2 uses the ACE2 receptor for cell entry, in synergy with the host's TMPRSS2. More specifically, the viral S glycoprotein is cleaved by TMPRSS2, thus facilitating viral activation, and representing one of the essential host factors for SARS-CoV-2 pathogenicity [23].

Vaccines

Although several therapeutic modalities have been tried and tested and deployed to treat COVID-19, vaccines ultimately offer the most appealing and robust therapeutic modality as they prevent the disease from taking hold [24]. This resulted in a global vaccine R&D effort unprecedented in terms of scale and delivery, compressing the standard vaccine development timeline from years to months. Vaccines trigger an immune response that generates highly specific antibodies against virus spike protein. Moreover, the immune system is taught to recognize the spike protein specific to the virus. If this spike protein is encountered. While the Pfizer/BioNTech and Moderna vaccines confer over 94% protection rates against symptomatic COVID-19 infection, vaccines have lower efficacy against the variants, but appear to provide protection against severe disease [25].

Viral Variants

It is well established that viruses, such as SARS-CoV-2, evolve continuously due to changes in the genetic code that occurs during replication. These changes lead to variants that have one or more mutations differentiating them from other variants of the same virus. They occur as a result of:

- **Mutations:** During viral replication, its genes undergo random "copying errors" (i.e. genetic mutations). Over time, these mutations may lead to alterations in the virus' surface proteins or antigens. Although mutations occur frequently, they do not always alter the characteristics of the virus.
- **Recombination:** Variants can also occur as a result of recombination, whereby the genomes of two variants combine during replication in an infected person simultaneously, resulting in a variant different from the original variants.

According to the Centers for Disease Control and Prevention the variants are classified into 4 main categories: This new classification was based on the following [26].

- Variants being monitored (VBM)

- Variant of interest (VOI)
- Variant of concern (VOC)
- Variant of high consequence VOHC)

They can be further subdivided as outlined in Table 1:

Table 1

Variant	Lineage
Variant being monitored (VBM	Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Delta (B.1.617.2 and AY lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2)
Variant of interest (VOI)	None
VOC	Omicron (B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4 and BA.5 lineages)
VOHC	None

The Omicron Variant

The Omicron variant has been reported to likely be the fastest-spreading virus in human history. When compared to other viruses, for example, the measles virus may infect 15 people within 12 days, whereas the omicron virus could infect 216 people within the same time frame [27]. This particular variant accounted for almost all new cases of COVID infections in the US by the end of February 2022. One of the main reasons for its greater transmissibility relative to some of the earlier variants, such as the alpha variant, is because of the 50 or more mutations. It possesses twice as many mutations as some of the earlier variants, and its sub variant BA.2 may have even more. Moreover, whereas previous variants required both the ACE2 and TMPRSS2, Omicron required only ACE2 for binding and releasing its genome into the host cell. This alone conferred two main advantages to Omicron transmission:

- There is a wider array of cells to infect as not all cells express TMPRSS2, therefore Omicron can effectively enter cells in a TMPRSS2-independent manner (via the endosomal route) [28].
- Since lung cells are rich in TMPRSS2 expression, the delta variant navigated deep into the airways, and the Omicron variant replicated more rapidly in the upper airway, thus promoting its spread. Moreover, the lower replication ability of Omicron in the human lungs may explain the reduced severity of Omicron that is now being reported in epidemiological studies [29].

Although the Omicron variant was more transmissible, some of the alterations to its structure actually weakened it and rendered it more susceptible to the host's defensive innate immune system. Studies examining the virus's response to interferon's showed that Delta was very efficient at subduing the interferon response, whereas, by contrast, Omicron actually enhanced the same response [30]. Although the reasons for this are as yet unclear 11 of the virus's 26 proteins interacted with the interferon system, many of which were mutated in the Omicron variant.

Another interesting transition of the spike protein was that it became sturdier. The spike contains two subunits, S1 and S2 that are loosely connected, allowing them to split and attach to a human cell. However, if the subunits split prematurely then cell attachment cannot occur. Some of the earlier variants were more loosely connected rendering cell attachment and entry more difficult.

In addition to the aforementioned alterations, another important reason cited for its enhanced transmissibility is its ability to evade immune detection. This is because unlike the original variants which possess 1-3 mutations on the RBD, enough to prevent some but not all antibodies from recognizing it, Omicron possessed at least 15 RBD mutations, many on prime antibody-binding sites, thus camouflaging them from antibody recognition.

Future Treatment Strategies

One analysis that dealt with this dramatic transformation reported that only one out of eight natural antibody treatments, used in hospitals to treat COVID, is still effectively bound to RBDs [31]. Other research has shown that mutations on RBDs and a second site called the N-terminal rapidly enable the virus to evade antibodies generated either by vaccination or infection [32].

Although this camouflage ultimately enabled the virus to spread rapidly the available vaccines, while incapable of preventing transmission, did prevent serious illness, especially among those that received booster shots. As a result of the alterations to the spike of the omicron variant, it more effectively avoided detection from the immune system. Moreover, although it lost some of its ability to bind to ACE2, other mutations compensated [33].

Finally, a recent report from the Scripps Institute reported that a variation of an FDA- approved therapeutic drug has the ability to turn the COVID-19 virus against itself [34]. The team had been researching memantine, used for treating neurological diseases such as Alzheimers, and found that a modified version of the drug could attach itself to the surface of the COVID virus and chemically alter ACE2. The drug, NMT5, which was tested both *in vitro* and *in vivo*, binds tightly to SARS-CoV-2 viral particles as the viruses move through the body. When the virus approaches ACE2 to infect a cell, the drug adds a nitro group to the receptor thus altering its structure for about 12 hours, thus preventing infection. Most anti-viral drugs work by direct inhibition of a portion of the virus such as the spike protein, resulting in mutations leading to drug resistance. NMT5, however, only uses the virus as a carrier, leading the researchers to believe the drug may be effective against many other variants of SARS-CoV-2. The team is currently creating a version for testing in humans.

Conclusion

The SARS-CoV-2 pandemic has been compounded because the virus spreads asymptomatically. The emerging variants have resulted in increased transmission, morbidity, and mortality. Although much is known about the main port of viral entry, the ACE2 receptor, little is known about how viral binding may modulate ACE2 expression and enzymatic activity, and whether this can be affected by known inhibitors or activators of ACE2. These are important questions as future variants, if and when they appear, may have yet other modifications to their structures and abilities. It is likely that neutralizing monoclonal antibodies administered in combination with anti-viral drugs offers an important strategy to curtail infection, transmission, and disease.

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