Minireview

Immunotherapy approaches on innate immunity for SARS-Cov-2

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Summary. – Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused fatal outbreaks of pneumonia. The similarity of S protein of SARS-CoV-2 with SARS-CoV and RaTG13 is about 76% and 97%, respectively. Also its potential receptor-binding domain (RBD) shows similarity with approximately 74% and 90.1% for SARS-CoV and RaTG13, respectively. SARS-CoV-2 has been shown to use the SARS-CoV receptor ACE2 for entry and serine protease TMPRSS2 for S protein priming. Sialic acids are primarily expressed by vertebrates and some microbial pathogens improving the ability to avoid immune system of vertebrate host. Interactions of sialic acid-binding Ig-like lectins (Siglecs) with their ligands play an important role in modulating immune cell function activities as their regulators. Therefore, while Siglecs help immune cells to distinguish between self and non-self, non-self ligands of some sialylated pathogens can recognize Siglecs and reduce immune cell responses or escape from immune surveillance. In this review, innate immunity in SARS-Cov-2 infection was discussed through Siglecs, especially Siglec-7, Siglec-3, NKG2A and neuraminidases.

Keywords: SARS-Cov-2; siglecs; NK cells; NKG2A; neuraminidases

Introduction

Coronaviruses (CoVs) are common human and animal pathogens that can be clasified as zoonoses and cause serious respiratory disease syndromes (Qing *et al*., 2020). Coronaviruses are enveloped RNA viruses divided into 4 types as α, β, γ and δ (Tortorici *et al*., 2019). Serious acute respiratory syndrome coronavirus (SARS-CoV), SARS-Cov-2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) have caused fatal outbreaks of pneumonia. Other members of the Coronaviridae family of zoonotic origin are endemic in the human population and account for up to 30% of mild respiratory infections. Among these viruses, the ones from α-coronaviruses are HCoV-NL63 and HCoV-229E, and HCoV-OC43 and HCoV-HKU1 are members of β-coronaviruses. Currently, there is no specific antiviral therapy or vaccine to combat especially fatal coronaviruses (Hoffmann *et al*., 2020; Ou *et al*., 2020; Tortorici *et al*., 2019).

CoVs can be transmitted from animals to humans and cause serious illness. For this zoonotic conduction, CoVs use spike (S) proteins that bind the virus to host cell receptors. S protein is the main antigen in the virus and induces the production of neutralizing antibodies during infection (Ou *et al*., 2020; Qing *et al*., 2020; Tortorici *et al*., 2019). S proteins also provide virus-cell membrane fusion.

It has two receptor binding domains, S1 and S2. While S1 binds to sialic acid of the host cell, the other domain S2 recognizes host transmembrane proteins (Ou *et al.*, 2020; Qing *et al.*, 2020; Tortorici *et al.*, 2019; Bertram *et al.*, 2011; Shulla *et al.*, 2011).

Two receptors in host cells bound by S proteins have been identified for human coronaviruses: CD13 for hCoV-229E and Angiotensin converting enzyme 2 (ACE2) for SARS-CoV, SARS-Cov-2 and hCoV-NL63 (Ou *et al.*, 2020; Gierer *et al.*, 2013; Kawase *et al.*, 2012). Binding of the S protein to host cell receptors is an important determinant of viral cell and organ tropism (Gierer *et al.*, 2013). S proteins accumulate on the plasma membrane of the infected cell. Sialic acids have been shown to play a role in facilitating CoV infection and CoV cell propagation (Qing *et al.*, 2020).

ACE2 is widely distributed especially in alveolar type II cells (AT2) and capillary endothelium. Although ACE2 expression in lungs has been demonstrated for both type I and type II pneumocytes, SARS-CoV cell tropism is not fully associated with ACE2 expression. It is suggested that other factors are needed to explain the pathogenesis of this disease. S proteins are synthesized as inactive precursors and become active on proteolytic cleavage by proteases (Gierer *et al.*, 2013; Matsuyama *et al.*, 2010).

S protein of CoVs can be activated by cathepsins, furin, trypsin, elastase, hypoxanthine-aminopterin-thymidine (HAT), transmembrane protease serine protease-2 (TMPRSS2), TMPRSS11a, and TMPRSS-4 proteases. TMPRSS2 is expressed in epithelial cells of human lungs (Ou *et al.*, 2020; Gierer *et al.*, 2013; Kawase *et al.*, 2012; Bertram *et al.*, 2011). Entering of the virus into the cell through cell membrane depends on the presence of these proteases in the cell (Ou *et al.*, 2020). Endosomal cathepsin B and especially cathepsin L are required for entering of SARS-CoV and hCoV-229E into the cell by endocytosis (Gierer *et al.*, 2013; Bertram *et al.*, 2012; Kawase *et al.*, 2012; Glowacka *et al.*, 2011; Matsuyama *et al.*, 2010). There are several proteases that can work as SARS-CoV entry cofactors, including cathepsin L, elastase, trypsin, factor Xa, and plasmin. Without ACE2 binding, these soluble proteases excessively separate and neutralize virus spikes (Shulla *et al.*, 2011). Understanding of functions of these proteases in SARS-Cov-2 can contribute to elucidation of possible functional differences between SARS-Cov and SARS-Cov-2.

TMPRSS2 activates SARS-CoV S protein on the cell surface following receptor binding during viral entry into cells (Kawase *et al.*, 2012). TMPRSS2 affects virus entry, but not other stages of virus replication (Matsuyama *et al.*, 2010). Neutralizing of TMPRSS2 with antibodies can reduce viral recognition. ACE2 and TMPRSS2 are co-expressed in type II pneumocytes. TMPRSS2 distribution correlates with SARS-CoV infection in lungs (Bertram *et al.*, 2012; Glowacka *et al.*, 2011; Matsuyama *et al.*, 2010).

SARS-CoV-2

The similarity of S protein of SARS-CoV-2 with SARS-CoV and RaTG13 is about 76% and 97%, respectively. Also its potential receptor-binding domain (RBD) shows similarity with approximately 74% and 90.1% for SARS-CoV and RaTG13, respectively. SARS-CoV-2 has been shown to use the SARS-CoV receptor ACE2 for entry and serine protease TMPRSS2 for S protein priming (Hoffmann *et al.*, 2020; Leng *et al.*, 2020; Ou *et al.*, 2020). SARS-CoV-2 has been identified as the genus β-coronavirus and shares 79.5% sequence homology with SARS-CoV (Zheng *et al.*, 2020a).

Lymphocytes play an important role in maintaining immune system function. Therefore, there is a relationship between changes in lymphocyte numbers and subgroups and viral pathogenic mechanisms. In patients infected with SARS-CoV-2, CD4^{+} T cells, CD8^{+} T cells, B cells, and natural killer (NK) cells have been shown to decrease in severe cases to a lower level than mild cases (Wang *et al.*, 2020).

Another study found that the total number of NK and CD8^{+} T cells decreased significantly in patients with SARS-CoV-2 infection and increased expression of NKG2A, an inhibitory receptor in these cells. It is thought that downregulation of NKG2A expression may be associated with disease control in SARS-CoV-2 patients (Zheng *et al.*, 2020a). The virus is able to induce cytokine storm in the lungs, overproducing cytokines such as IL-2, IL-6, IL-7, GSCF. Therefore, avoiding cytokine storm may be the key to treating SARS-CoV-2 infected patients (Leng *et al.*, 2020). The fact that NKG2A is expressed in both NK cells and CD8^{+} T cells makes it an important treatment target against virus infections. Also, can the HLA-E expression levels and polymorphism studies provide information regarding sensitivity to SARS-Cov-2 and progression of the disease?

When we separate treatment processes of the infection as early and late phase, the first part can be effective solution for low NK activity and immunotherapy approaches that will increase innate immunity, and in the late phase, inhibitory cytokine treatment approaches that will suppress cytokine storm. Can the factor lowering NK activity be explained by innate immunity-escape mechanism of SARS-Cov-2? In this review, this possibility is discussed through the role of sialic acids in innate immunity. To determine the main regulatory factors, increasing detailed proteomics studies and biomarkers showing progression of the disease will contribute to the identification of possible drug targets.

Sialic acids

Sialic acids are made up of over 50 carbohydrate families that share a nine-carbon backbone. Sialic acid

(Neu5Ac) can attach to proteins and lipids as terminal sugars of oligosaccharides (C1-9) (Mikulak *et al.*, 2017).

They are primarily expressed by vertebrates and some microbial pathogens improving the ability to avoid immune system of vertebrate host. These negatively charged sugars are found at the terminal of a number of glyco-conjugates, including N-linked glycan chains, O-linked mucin chains, and glycosphingolipids on proteins (Pillai *et al.*, 2012).

In humans there is Neu5Ac instead of Neu5Gc which is commonly found in other mammals. However, the dietary consumption of Neu5Gc, primarily from red meat, can be metabolically incorporated into cellular glycoproteins and glycolipids, following the same route used by the endogenous sialic acid Neu5Ac after absorption by the gastrointestinal tract. The exogenous Neu5Gc consumed in this way is presented in the cell surface glycocalyx where it can act as a foreign "xeno-autoantigen". Although the amounts included are small, most people have circulating anti-Neu5Gc "xeno-autoantibodies" and therefore Neu5Gc can develop local chronic inflammation in the accumulation sites (Kawanishi *et al.*, 2019).

Sialic acids are host recognition elements binding hemagglutinins or spike proteins of type A and B influenza viruses. These hemagglutinins represent viral sialic acid-binding lectins. Hemagglutinin or hemagglutinin esterases of type C influenza viruses and some nidoviruses may recognize the 9-O-acetylated form of sialic acid. This form of sialic acid is necessary for the virus to enter the human cell. Sialic acid glyco-epitope is sensitive to hemagglutinin esterase sialic acid O-acetyl esterase activity of bovine coronavirus (Mahajan *et al.*, 2019; Pillai *et al.*, 2012).

Sialic acid can regulate the alternative way of complement activation. Major serum protein complement factor H binds to the exocyclic side chain of sialic acids on host cells and prevents the interaction of complement factor B with C3b and C3 convertase formation. Many microbial pathogens can escape natural immunity in vertebrates by binding to factor H or by triggering sialic acid-binding receptors in innate immune cells. Selectins, C type or calcium dependent lectins have sialic acid containing ligands (Kim *et al.*, 2017; Pillai *et al.*, 2012).

Siglecs

Siglecs (sialic acid-binding Ig-like lectins) are characterized by the Ig domain, which mediates binding of sialic acid (Crocker *et al.*, 2001).

Siglecs can be divided into 2 groups: first group; Siglec-1 (Sialoadhesin), Siglec-2 (CD22), Siglec-4 (MAG) and Siglec-15. The second group is CD33-related Siglecs. Members of this group have two to five Ig-like domains

that share 50% to 80% sequence identity (Lübbers *et al.*, 2018; Macauley *et al.*, 2014; Nicoll *et al.*, 2003). Siglec-12 has lost the ability to bind sialic acids (Mikulak *et al.*, 2017).

Interactions of Siglecs with their ligands play an important role in modulating immune cell functions activities as their regulators. Therefore, while Siglecs help immune cells to distinguish between self and non-self, non-self ligands of some sialylated pathogens can recognize Siglecs and reduce immune cell responses or escape from immune surveillance (Macauley *et al.*, 2014).

Siglecs are mostly derived from hematopoietic lineage and are structurally expressed on cells of natural immunity such as monocytes/macrophages, neutrophils, basophils, eosinophils, dendritic cells (DCs) and natural killer (NK) cells. Siglec-1 and -11 are found structurally only in macrophages. Siglec-5 are expressed on B cells and neutrophils (Mikulak *et al.*, 2017). Siglecs generally lead to a strong inhibitory signal when they bind to sialic acids. Pathogens increase sialic acid expression as a mechanism that modifies the immune system (Lübbers *et al.*, 2018; Kim *et al.*, 2017; Mikulak *et al.*, 2017).

Macrophages localized in the marginal zone of the spleen and in the subcapsular sinuses of the lymph nodes express a high level of CD169. CD169 is also known as a sialoadhesin or Ig-like lectin-1 (Siglec-1) that binds sialic acid. Unlike other Siglecs, sialoadhesin have 17 Ig-like domains. CD169+ macrophages play a major role in inducing both humoral and cellular adaptive immunity. CD169+ macrophages can directly stimulate CD8+ T cells after viral infection (van Dinther *et al.*, 2018; Crocker *et al.*, 2001). Siglec-1 and Siglec-2 mediate cell-to-cell interaction through sialylated glycoconjugates. Masking of Siglecs on the cell surface as a result of cis interaction with sialic acid prevents cell-cell interactions (Crocker *et al.*, 2001).

Treatment of monocytes with Siglec-3 Ab increases their IL-8, TNF-α and IL-1beta proinflammatory cytokine production (Lübbers *et al.*, 2018). Considering these results, can Siglec-3 activation suppress cytokine production in the late phase when cytokine storm is established in SARS-Cov-2-infected patients?

Siglec-7 (also called p75/AIRM1) is a sialic acid-binding receptor expressed on NK cells and monocytes (Nicoll *et al.*, 2003). NK cells express endogenous ligands for Siglec-7, which could bind Siglec-7 and possibly keep these cells in an inhibited state (Pillai *et al.*, 2012). In terms of the amino acid sequence, Siglec-7 and Siglec-9 are approximately in 80% similar to each other (Daly *et al.*, 2019). Siglec-7 are structurally masked on NK cells. However, when it is treated with sialidase, Siglec-7 can interact with GD3 and inhibit killing. Expression of GD3 on target cells has been shown to increase susceptibility to killing independently of Siglec-7. It can modulate NK cell killing activity and has
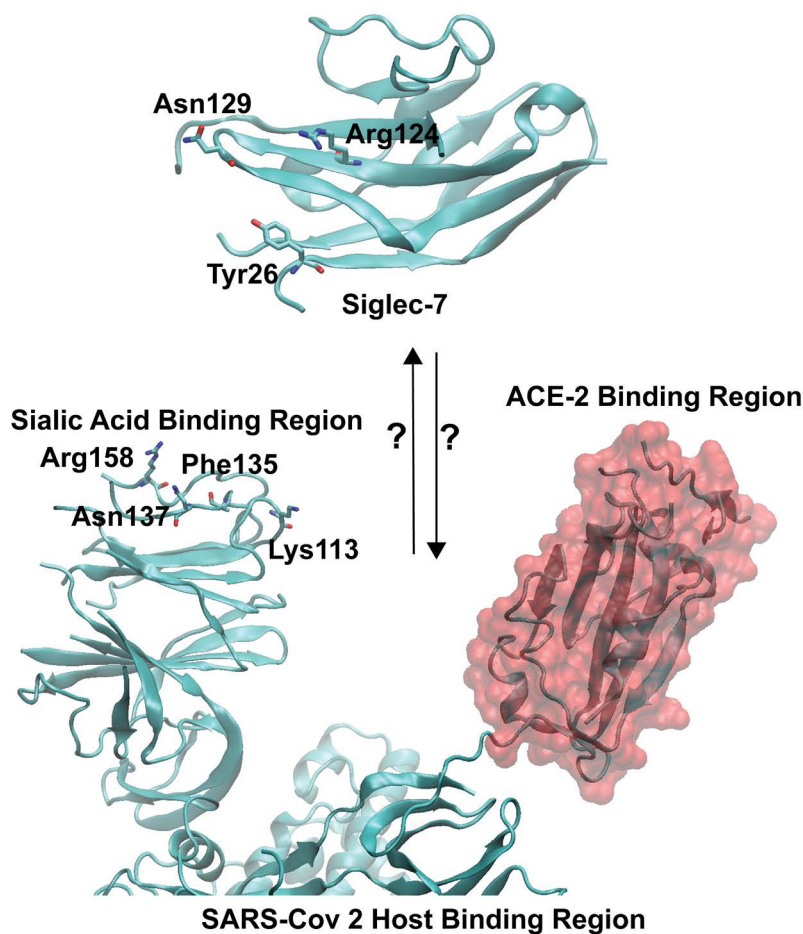
Fig. 1

Spike protein of SARS-Cov-2 has its own residues as potential targets for Siglec-7
Spike protein has its own sialic acid recognition region for host cells targeting, together with ACE-2 binding region. Do these two mechanisms interact under certains settings? The answer to this question has yet to be determined. Siglec-7 (PDB ID:2DF3), SARS-Cov-2 spike protein (PDB ID:6VSB). The amino acids, responsible for binding to sialic acid are shown. ACE-2 binding region on spike protein is highlighted with surface representation together with secondary structure representation.

significant effects on tumor escape from NK cell cytotoxicity (Nicoll *et al.*, 2003).

Siglec-7, which is the most abundantly found Siglec on natural killer cells, presents high binding preference towards α(2,8)-linked disialic acids, lower preference toward α(2,6)- and lowest preference for α(2,3)-linked sialic acids (Alphey *et al.*, 2003; Zheng *et al.*, 2020b). Within the frame of this property of Siglec-7, it has also been shown to be involved in viral infections. Low expression levels of Siglec-7 on NK cell populations was shown to be associated with viremia of HIV (Brunetta *et al.*, 2009). Further *in vitro* studies revealed that sialic acid residues on HIV gp120 utilizes Siglec-7 for protection against immune system and systemic increase of soluble Siglec-7 during HIV infections was suggested to be due to NK cell apoptosis (Brunetta

et al., 2009; Mikulak *et al.*, 2017; Varchetta *et al.*, 2013). Similar Siglec-7 dependent events were also observed for HCV and HBV infections (Zheng *et al.*, 2020b). Given that spike protein of SARS-Cov-2 contains glycosylated regions (Walls *et al.*, 2020), here we speculate that Siglec-7, probably together with other Siglecs, may contribute to the escape of SARS-Cov-2 from immunological surveillance, which prompts the need for further studies in this area. On the other hand, a potential drug candidate in COVID-19, chloroquine, has been shown to inhibit the sialic acid-binding of NTD region of the spike protein, potentially leading to decreased binding of the virus to the sialic acid residues found on the host cells (Fantini *et al.*, 2020). If exists, could Siglec-7 or any other Siglec interaction with glycosylated viral amino acid residues enhance or disrupt the capac-

ity of the virus to interact with host sialic acid residues though its NTD region? Discovery of such interactions will pave a path to design and development of new biomimicry based inhibitors and drugs (Fig. 1).

Enveloped viruses, such as HIV and porcine reproductive and respiratory syndrome virus (PRRSV), are covered with sialic acids that can be recognized by sialoadhesin in macrophages and promote uptake and infection. Sialoadhesin has been shown to be the major receptor for GM3 providing HIV trans-infection and spread to endosomal compartments to ensure and spread. HIV can interact with other Siglecs, including Siglec-3, Siglec-5, Siglec-7 and Siglec-9 via glycoprotein 120 (gp120) (Macauley et al., 2014).

A soluble form of Siglec-7 has been shown to act as a biomarker in HIV-1 infected patients. It has been shown that Siglec-7 level is increased in the supernatant of NK cells stimulated in vitro with HCV and there is a decrease in Siglec-7 expression from NK cell surface (Mikulak et al., 2017). It has been hypothesized that soluble Siglec-7 may cause spreading of the infection and increase the susceptibility to infection due to its HIV-1 carrying capacity. (Mikulak et al., 2017).

Due to the very limited expression of the Siglecs in the immune system and the differential expression of the Siglecs in various immune cells, they are increasingly recognized as targets for the development of immuno-therapeutics (Mikulak et al., 2017; Macauley et al., 2014). Siglec binding sites are masked on the same cell surface in cis form by interacting with high and low affinity sialic acids. It can interact in trans form when it encounters a high affinity ligand or pathogen that can compete with this interaction (Mikulak et al., 2017).

Natural killer (NK) cells

The immune response against viral infection is based on effector mechanisms from both a natural and adaptive immunity. Among these mechanisms, cytotoxicity mediated by NK cells and cytotoxic CD8+ T cells (CTL) is responsible for killing infected cells. In human T lymphotropic virus type 1 (HTLV-1) infection, while NK cells try to limit proliferation of virus-infected cells and the proviral load in the early stages of infection, CTLs are responsible for controlling viral delay (Bangham, 2009).

NK cells play a central role in defending against viral infections and eliminating transformed cells. Its activity is regulated by the interaction of activating or inhibitory NK cell surface receptors, effects of cytokines and their interactions with other immune cells. Most inhibitor receptors recognize the major histocompatibility complex (MHC) class I and provide tolerance of NK cells against self-antigens (Jarahian et al., 2009). Activating receptors

on NK cells include CD16; NKG2D; natural cytotoxicity receptors (NCR) NKp30, NKp44 and NKp46 (Atasever-Arslan et al., 2013; Jarahian et al., 2009).

Hemagglutinin (HA) of the influenza virus and hemagglutinin-neuraminidase (HN) proteins of Sendai virus can bind to NKp46 and NKp44 and activate NK cells. Binding of NKp44 and NKp46 to viral HA mediated by specific sialic acid residues found in these receptors leads to the elimination of infected cells (Duev-Cohen et al., 2016). NKp30 do not bind the HA of influenza virus. The human cytomegalovirus pp65 protein has been shown to bind NKp30 and inhibit its function. Also, HIV virus and herpes simplex virus have been shown to upregulate expression of NCR ligands in infected cells (Bar-On et al., 2014; Jarahian et al., 2009). Influenza virus has been shown to use viral neuraminidase (NA) protein to avoid NKp46-mediated elimination, and NA inhibition leads to increased elimination of influenza virus-infected cells in vitro and in vivo (Bar-On et al., 2014).

There are four types of sialidase as neuraminidases (NEU) 1-4. They function by separating sialic acids from glycans by hydrolysis. Increased Siglec-7 and Siglec-9 levels in NK cells of healthy donors treated with sialidase neuraminidase have been observed. Treating only NK cells with sialidase is thought to prevent masking of Siglec-7 with sialic acids in cis form and may increase NK cytotoxicity. Another potential therapeutic approach is using of Siglec-7 inhibitors to prevent Siglec-7-mediated NK activity inhibition (Daly et al., 2019).

Consequently, understanding of importance of Siglec-7 and neuraminidase therapy for SARS-Cov-2 infection may contribute to enhancing innate immunity in the early phase of infection. Increasing NKG2A level in patients infected with SARS-Cov-2 is an important finding related to innate immunity escape mechanisms. Considering this result, investigating HLA-E levels and polymorphisms in patients may contribute to the determination of new drug targets. Could increasing the activity of inhibitory mechanisms in cytokine producing cells such as Siglec-3 for cytokine storm be an effective treatment and approach for the late phase of infection?

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