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EDITORIAL

Potential role of glycoprotein 340 in milder SARS-CoV-2 infection in children

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1. Introduction

It is well reported that the frequency and severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is significantly lower in children compared to the total infected population [1]. This is similar to the lower mortality rate in children during 1918 'Spanish flu' as the most deadly influenza pandemic, SARS-CoV (2002), 2009 'H1N1 flu' outbreak, and MERS-CoV (2012) [2,3]. Less outdoor activities, as well as minimal exposure to air pollution and cigarette smoke, are among possible reasons for lower infection of children to coronavirus disease 2019 (COVID-19) [3,4]. Although the exact cause of lower frequency and mortality of COVID-19 in pediatric patients remains obscure, the difference in the composition and functional responsiveness of the immune system to COVID-19 in children and adults can be of critical importance. It has been suggested that the milder COVID-19 disease presentation in children might be due to [3–5]: (1) the different response of children vs. adults in terms of immunosenescence and inflammaging. Children appear to have protective T helper 2 immune response and eosinophilia, and also lower levels of inflammatory cytokines. (2) The resistance to SARS-CoV-2 in children due to maturational changes in the axonal transport system which can restrict the viral transmission. A similar phenomenon has been observed in immature mice resistant to poliovirus-induced paralysis. (3) Virus–virus interactions in children vs. adults that might mitigate replication of the SARS-CoV-2. Specifically, the simultaneous presence of different viruses in the mucosa of children leads to a cross-reactive humoral immunity, T cell immunity, and growth competitions that limit the severity of COVID-19; (4) lower gene expression of angiotensin-converting enzyme-2 (ACE-2) receptor in children vs. adults. Investigations on ACE-2 gene expression in the nasal epithelium have shown a strong linear trend of ACE-2 expression which increases with the advancing age group [6]. ACE-2 is known to be the receptor for SARS-CoV, SARS-CoV-2, and human coronavirus-NL63 (HCoV-NL63). Studies have indicated that patients under 12 years old with SARS-CoV disease had milder symptoms and were less likely for admission to an intensive care unit. Similarly, HCoV-NL63 infection in patients aged 16–25 years was more severe than those aged 5–16 years; and (6) influence of *Bacillus* Calmette-Guvaérin (BCG) vaccine in children that can lead to protection against the severity of COVID-19, most probably through trained immunity phenomenon. Trained immunity is a memory-like response in the innate immune system, which enhances responsiveness to subsequent triggers. In this article, we discuss the possibility of GP-340 abundance as a potential cause for less severe SARS-CoV-2 infection in children. We also elaborate on the potential role of this glycoprotein as a novel therapeutic target.

2. Glycoprotein 340 (GP-340)/DMBT1

Scavenger receptor-rich glycoprotein 340 (GP-340), the tumor suppressor DMBT1 (deleted in malignant brain tumors), is encoded by the *dmbt1* gene. It belongs to the scavenger receptor cysteine-rich (SRCR) family of proteins that is mainly involved in tumor suppression or innate immune defense. As a part of the innate immune response, GP-340 binds to surfactant A (SP-A) and surfactant D (SP-D) that has been suggested to contribute to the clearance of microorganisms from the lung. Lung GP-340 binds less avidly to SP-D than salivary GP-340. Salivary GP-340 was identified as a key element involved in the innate host defense on mucosal surfaces against a range of oral bacteria as well as HIV-1 and influenza A virus (IAV) [7]. The antiviral activity of GP-340 was noted only against these two viruses suggesting a common mechanism of action. Both of these viruses have an enveloped RNA, with a glycoprotein target on their surfaces, *i.e.* GP-120 in HIV and hemagglutinin in IAV.

HIV-1 infects host cells by binding to a cluster of differentiation 4 (CD4) through GP-120 in the presence of coreceptors, such as CXCR4 or CCR5. Specifically, GP-120 undergoes conformational changes and binds to chemokine receptors and facilitates the virus entry. It has been suggested that GP-340 can exhibit an inhibitory effect on HIV-1 infection by binding to viral GP-120 in

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a region different from CD4-binding site. Interestingly, the pre-binding of sCD4 (CD4i epitope) to GP-120 enhances the interaction of GP-340 with GP-120 and can hinder virus entry. Thus, it can be concluded that the inhibitory effect of GP-340 might be due to the blocking access of GP-120 to chemokine receptor that can prevent GP-120 conformational changes for viral and cell membrane fusion [8]. On the contrary, Stoddard et al. reported that GP-340 may promote heterosexual transmission of HIV-1 through transcytosis of the virus in genital tract-derived cell lines and primary endocervical tissues [9].

3. Does GP-340 have an antiviral activity against SARS-CoV-2?

SARS-CoV-2 is a single-stranded and positive-sense RNA virus, which is phylogenetically related to group 2 coronaviruses, but it does not encode the hemagglutinin-esterase. One may argue that there is a lack of glycoprotein target (unlike hemagglutinin in IAV and hemagglutinin-esterase in coronaviruses) on the surface of SARS-CoV-1 and SARS-CoV-2. Moreover, the SARS-CoV-1 spike glycoprotein is selectively recognized by lung SP-D and activates macrophages [10]. GP-340 binds to SP-D and it has been reported that the lung GP-340 has cooperative interactions with SP-D in viral neutralization and aggregation assays. In a study performed by Hartshorn et al., a donor presented noticeably higher salivary GP-340 activity against avian-live flu IAV strains. Furthermore, the density of α(2,3)-linked sialic acids linkage on GP-340 was much higher in this donor. Since IAV strain prefers to bind sialic acid in the α(2,3) linkage, the specificity of sialic acid linkage on GP-340 can be used as a factor of anti-IAV activity. Lung GP-340 is reported to co-operate with SP-D in viral neutralization; however, salivary GP-340 has been reported to antagonize the anti-viral activity of SP-D against IAV strains. The reason for this contrary activity is still unknown [11]. This effect was associated with greater binding of salivary GP-340 to the carbohydrate recognition domain of SP-D as compared with the binding of lung GP-340. Interestingly, there is a direct association between elevated plasma SP-D levels and SARS-CoV-1-specific IgG antibody titers in infected patients, and monitoring systemic SP-D is useful for keeping track of the alveolar integrity in SARS-CoV-1 pneumonia [12].

Recently, the role of GP-340 as a plausible phenotypic target was discussed in a transcriptomic study of a diverse repertoire of airway cell lineages. It was observed that GP-340 is highly expressed in AT2 lung cells compared to the other lung epithelial subset. This expression was also positively correlated with ACE-2 levels. Furthermore, a population of ACE-2-positive AT2 cells was identified to co-express viral pathogen and GP-340 receptor that is crucial for the host defense, thus suggesting a possible phenotypic target for the treatment of COVID-19 [13].

Proteomics analysis of saliva showed that Mucin-7 (MUC7), Mucin-5B (MUC5B), DMBT1/GP-340, and neutrophil defensins can bind to spike protein of SARS-CoV-2. This provides evidence that the saliva glycoproteins bind to spike protein of SARS-CoV-2. Moreover, saliva from healthy donors prevented the binding of spike-protein-specific polyclonal antibodies to spike antigen, suggesting that the spike protein's glycoprotein-binding domains (GBD) may be targeted to obstruct SARS-CoV-2 adherence or internalization [14]. However, further investigations are crucial to prove this hypothesis *in vitro* and *in vivo*. Taking together, the available evidence supports the role of GP-340 as a potential therapeutic target for COVID-19 (Figure.1).

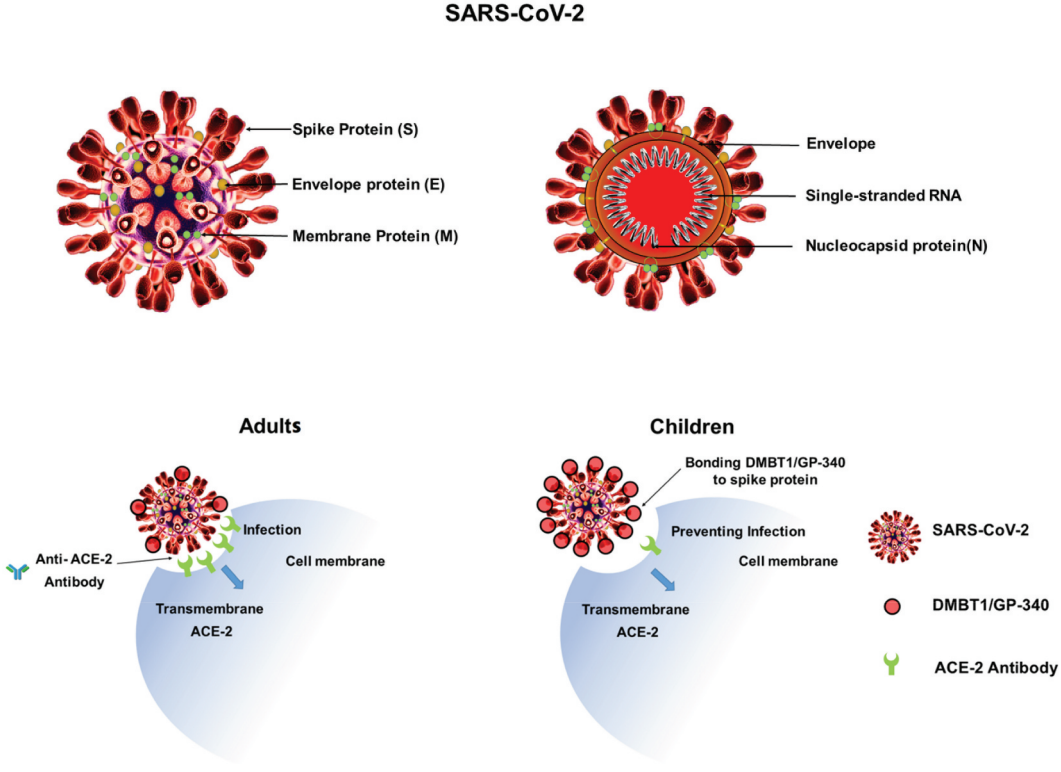

Figure 1. Schematic diagram of the binding of GP-340 to spike protein of SARS-CoV-2 in children and adults. Children have higher GP-340 along with lower ACE-2 receptors. GP-340 targets spike protein's glycoprotein-binding domains (GBD) and blocks virus adherence or entry of SARS-CoV-2.

4. Children have abundance of GP-340 compared to adults: is it the reason for milder SARS-CoV-2 infection?

The study by Sonesson et al. was done on major and minor gland saliva where GP-340 is abundant along with sialic acid. Sialic acid is a common terminal structure of salivary glycoproteins that interacts with microorganisms. The study was done in minor gland and whole saliva of children (3 years old), adolescents (14 years old), and adults (20–25 years old). The results showed a higher content of GP-340 in the whole saliva of the youngest age group compared with the adult group, thereby suggesting a vital innate immunity factor present in children's saliva [15]. One can speculate that since children have higher GP-340 along with lower ACE-2 receptors [13], they present a different response against SARS-CoV-2 infection compared with adults.

5. Expert opinion

Hitherto, there has been no approved therapy available for SARS-CoV-2 infection. However, the differences between children and adults in terms of prevalence and severity of COVID-19 can potentially be harnessed to understand the pathology of the disease and develop effective therapeutics. Among all distinctive features of children and adults in response to viruses, age-related abundance of GP-340 protein in children seems promising as this protein can bind to spike protein of SARS-CoV-2, thereby reducing cell adherence and entry of viral particles. Therefore, proof-of-concept studies on the role of GP-340 as a promising therapeutic target for COVID-19 are greatly recommended. Finally, more studies need to verify this hypothesis *in vitro* and *in vivo* models.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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• **Describes vital innate factors of immunity in children's saliva via age related salivary GP-340.**