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COVID-19

Conjunctivitis as a Sentinel of SARS-CoV-2 Infection: a Need of Revision for Mild Symptoms

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Abstract

COVID-19 has been declared a pandemic by the World Health Organization on March 11, and since then, more than 3 million cases and a quarter million deaths have occurred due to it. Lately, there is a growing evidence for an ophthalmologic symptom (conjunctivitis) to be connected with the disease. This seems to happen in early stages of the infection by SARS-CoV-2, and thus, it is of major importance to understand the mechanism through which the virus can facilitate such a symptom. Here, we are proposing a molecular mechanism through which the novel coronavirus could act in order to affect the eye and use it as another, secondary but alternative, point of entry to the host organism.

Keywords COVID-19 · SARS-CoV-2 · Pandemic · Conjunctivitis · Pink eye · Ophthalmology · ECM proteins · ACE2 · HSPG · Picornavirus · Molecular ophthalmology

Introduction

The SARS-CoV-2 virus, a novel coronavirus, emerged in December 2019 in China, and then Japan, South Korea, Europe, and North America. On March 11, 2020, the World Health Organization declared the spreading novel coronavirus outbreak as a pandemic, thus showing the possibility that the virus spread to all countries worldwide [1]. As of May 13, 2020, about 4.5 million confirmed cases of coronavirus disease 2019 (COVID-19) and almost 300,000 deaths have been reported, with one third of the cases and more than 25% of the deaths to have occurred in the USA (John Hopkins Coronavirus Resource Center statistics). In response to the most serious global health threat in a century, researchers from all biomedical fields worldwide have participated in an unprecedented response to the COVID-19 pandemic, with rapidly increasing resources aimed at finding safe and effective treatments for the disease (comprehensively reviewed in [2]).

Research for treatments has emerged from different backgrounds, pharmacologically with the use of well-known drugs for other diseases [3–7], with corticosteroids [8], immunologically from the serum of antibodies against former coronaviruses or from patients that have recovered from COVID-19 [9–11] or even with the use of revolutionary ideas such as CRIPR-Cas13 [12–14]. Another tremendous effort from NIH (ClinicalTrials.gov Identifier: NCT04283461) and all countries around the globe focuses on the successful development of a vaccine that would prevent the emergence of COVID-19 through the years and create a repeating cycle of spreading, like the influenza virus [15, 16].

While up to mid-April 2020, the only symptoms that were officially recognized as linked with COVID-19 were fever, cough, shortness of breath, or difficulty breathing, the CDC (Centers for Disease Control and Prevention) have lately updated the symptom list based on changes in the disease's definition adopted by the Council of State and Territorial Epidemiologists (CSTE). Chills, rigors, myalgia, headache, sore throat, and new olfactory and taste disorder(s) have been officially added in CDC's website as symptoms connected with SARS-CoV-2 infection. Moreover, gastrointestinal symptoms like nausea, vomiting, and diarrhea are stated as

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reported symptoms for the same disease in CDC's official website (CDC.gov). Importantly, it is already known that a substantial percentage of patients do not exhibit any symptom while infected with SARS-CoV-2 [17, 18].

In this review, we will focus on another symptom that has not been officially recognized, yet is arguably found in a small percentage of COVID-19 patients [19], and is of a major concern for ophthalmologists, i.e., conjunctivitis or pink eye. We will summarize all the cases reported in other publications, and through basic molecular biology mechanisms, we will propose a possible explanation of the etiology of this symptom.

Background

The Molecular Biology of Coronaviruses and SARS-CoV-2

Coronaviruses (CoVs) are RNA viruses with the largest RNA in base length identified so far and belong to the Coronaviridae family. They are divided into 4 groups: α-, β-, γ-, and δ-CoV [20]. SARS-CoV and SARS-CoV-2 have 89.8% sequence identity in their spike (S) protein S2 subunits, which mediate the membrane fusion process, and both of their S1 subunits utilize human angiotensin-converting enzyme 2 (hACE2) as the receptor to infect human cells [21]. Most importantly, the ACE2-binding affinity of the S protein of SARS-CoV-2 is 10- to 20-fold higher than that of SARS-CoV [15], which contributes to the higher infectivity of SARS-CoV-2 as compared with SARS-CoV [22].

After binding of the S protein of the virion to the ACE2 receptor on the target cell, the heptad repeat 1 (HR1) and 2 (HR2) domains in its S2 subunit of the S protein interact with each other to form a six-helix bundle (6-HB) fusion core [23], bringing viral and cellular membranes into close proximity for fusion and infection [24]. Therefore, the specificity of the virus is determined through the S-protein–receptor interaction to a host cell receptor. Cathepsin protease action is the first step for the virus in order to access the host cell's cytosol by proteolytic cleavage of the S protein, followed by fusion of the virus and the host's membranes. Fusion occurs within the endosomes and the formation of the bundle after fusion mixes viral and cellular membranes. As a result, the viral genome is released into the cytoplasm [24].

Coronavirus lifecycle proceeds with the translation of the replicase gene from their genomic RNA, where the polyproteins pp1a and pp1ab encoded from two large ORFs [25, 26]. Polyproteins of coronaviruses are further cleaved by a group of proteases [27, 28].

Importantly, many non-structural proteins (nsps) are assembling the replicase–transcriptase complex (RTC) needed for RNA synthesis, while specifically nsp12 encodes the RNA-dependent RNA polymerase (RdRp) domain, arguably the most important enzyme for the replication of the virus. This is the enzyme that will elongate new positive sense RNA molecules from the original RNA of the virion [29].

Subgenomic RNAs (sgRNAs) are abundantly produced by the virus. SgRNAs serve as mRNAs mainly for the structure of the virus. Importantly, homologous and non-homologous recombination can happen in the virus genome at this stage [30, 31].

After replication and sgRNA synthesis, S, E, and M structural proteins are translated and transferred into the endoplasmic reticulum (ER). These proteins move to the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) [32, 33] and are encapsulated into membranes to form mature virions [34]. The M protein is responsible for most protein–protein interactions required for assembly of coronaviruses, while the E protein functions as a chaperone to the M protein [35]. Lastly, the S (spike) protein that is not required for assembly is transferred to virions by interacting with the M protein. As already stated, the trimeric S protein is the spike-like protein on the surface of the virus [36, 37] and acts as a class I fusion protein [24] that ensures attachment to the host receptor. Following assembly, newly made viruses transport to the cell surface and are released to the environment by exocytosis [38].

Viral Conjunctivitis

Conjunctivitis, or pink eye, is an irritation or inflammation of the conjunctiva, which covers the white part of the eyeball [39] . It can be caused by bacteria, viruses, or allergies. It can be contagious as it is spread by contact with eye secretions. Symptoms include itching, redness, and tearing of the eyes. It can also lead to discharge or crusting around the eyes [40].

It is important to stop wearing contact lenses while affected by conjunctivitis. While allergic conjunctivitis can be treated with antihistamines and bacterial conjunctivitis can be treated with antibiotic eye drops to speed up the recovery process, the only way to recover from viral conjunctivitis is to let it resolve on its own while taking care of the overall good health of the patient [41].

Adenovirus is the most common cause of viral conjunctivitis. Viruses of the Adenoviridae family consist of non-enveloped, double-stranded DNA. The most frequent infections caused by the adenovirus are eye infections, upper respiratory tract infections, and diarrhea in children [42]. Except for adenovirus derived, herpes conjunctivitis is also common in children [43]. Except for DNA viruses though, RNA viruses are often associated with conjunctivitis. Picornaviruses can cause acute hemorrhagic conjunctivitis and are highly infectious, and HIV can also cause conjunctivitis producing redness, irritation, and tearing [44].

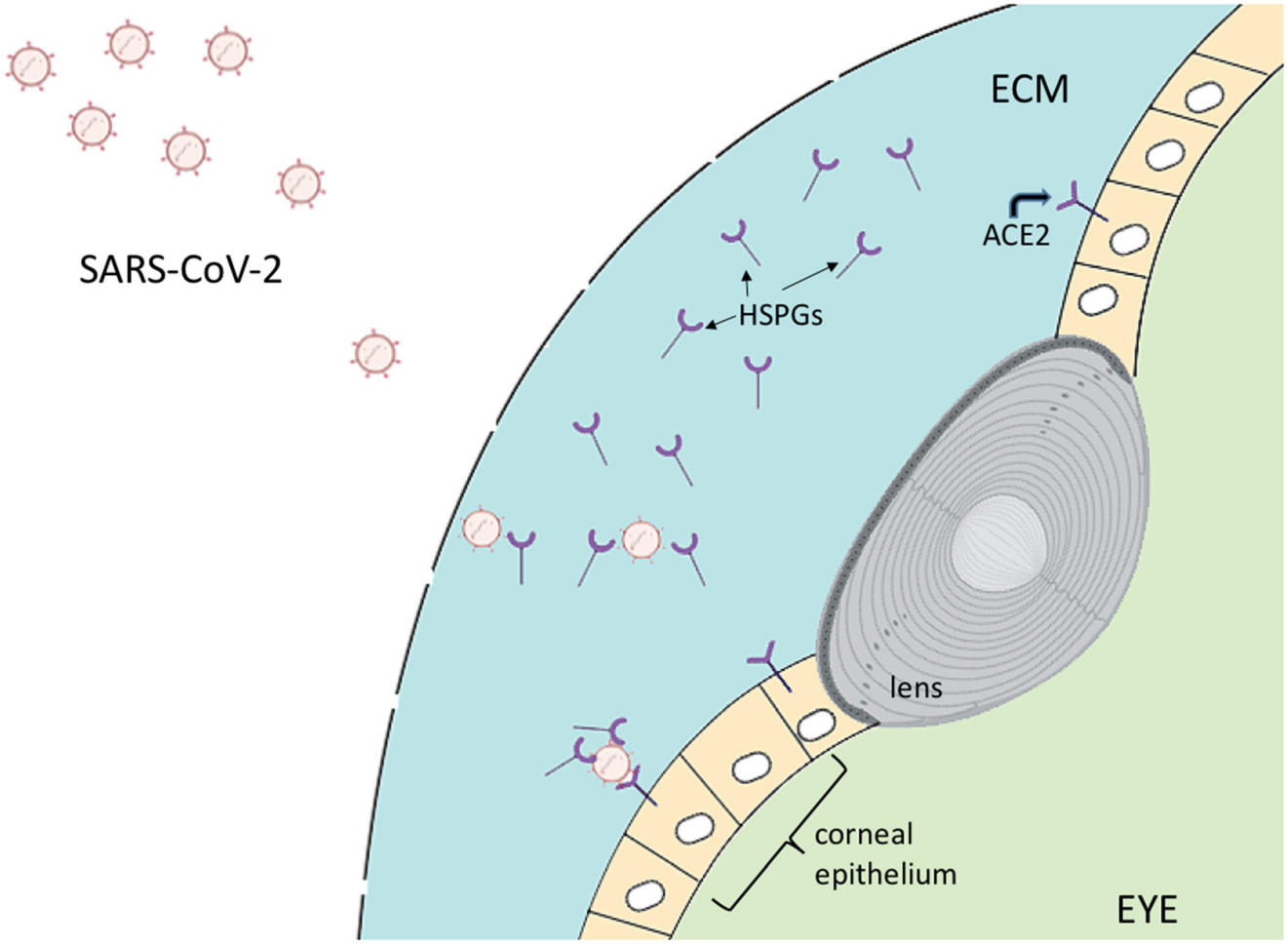

Fig. 1 SARS-CoV-2 viruses find the ACE2 receptor on corneal epithelium cells via the help of HSPG receptors in the eye's ECM

Cell infection of picornaviruses starts with its attachment to cell receptors. These receptors are subdivided into two major groups, i.e., canyon and non-canyon binders, which refers to different structures of the virus' surface [45, 46]. Canyon receptors like ICAM-1, PV receptor, or $\alpha_v\beta_3$ and $\alpha_v\beta_6$ integrin receptors bind into the canyon of the viral surface, triggering conformational changes of the virus essential for infection, while non-canyon binders such as the LDL receptor, P-selectin glycoprotein ligand-1 (PSGL-1), and heparan sulfate proteoglycan (HSPG) receptors attach to the virus surface elsewhere except for the canyon, guiding the virus to the host cell surface and as a result signal for virus endocytosis [47–50]. The HSPGs are continuously reported as providers of an increased efficiency of viral attachment to host cells, thus allowing the binding of the virus to another receptor [51–53].

Conjunctivitis as a COVID-19 Symptom

It is not uncommon for coronaviruses to be found in tears through the years. SARS-CoV, HCoV-NL63, and SARS-CoV-2 coronaviruses have been detected active through RT-PCR in tears in previous coronavirus outbreaks [54–58]. On the other hand, other studies have shown no evidence of live viruses in tears of patients infected with several different strains of coronaviruses [59, 60].

Given the uprising number of publications and case reports of COVID-19 patients showing conjunctivitis [61, 62] and the history of other coronaviruses that are found in tears, we have to consider the possibility of a separate, alternative viral mechanism through which the virus can enter the patient's organism through epithelial cells of the eye [63]. The growing evidence on COVID-19 and its ocular implications and manifestations, in both animals and humans, is covered by many interesting reviews, all published 5 to 6 months after the novel coronavirus' outbreak [64–68], something that reveals the need to understand the virus from different perspectives—which at first may have seemed secondary in priority—in order to be able to reach a treatment.

Discussion

As not much has been yet published about the SARS-CoV-2 pathogenic mechanism, from genomic and structural analyses,

it is known that the SARS-CoV-2 has a similar receptor binding mechanism as SARS-CoV. The angiotensin-converting enzyme-2 (ACE2) receptor is so far the best candidate for the main entry mechanism of SARS-CoV-2 [69].

The ocular surface is comprised by the conjunctival and corneal epithelia which are connected to the upper respiratory system [70]. Liquid from the eye is absorbed by the conjunctiva and cornea epithelium and drained into the nasal cavity through the nasolacrimal duct to the respiratory tract through the trachea [71]. As a result, pathogens from the eye can be transported to the respiratory system.

Interestingly, the human eye has its own intraocular angiotensin system (RAS), which has been popular as a way of antiglaucoma drug development. As secondary evidence, ACE2, the main entry receptor of HCoV-NL63, SARS-CoV, and SARS-CoV-2, has been found in the aqueous humor [72] and at the conjunctival epithelial cells of the ocular surface [73]. However, ACE2 expression in human ocular surface is much lower than in other tissues [74].

Besides the immune conditions of the host, the efficiency of a virus infection depends on the infection rate of the virus and the viral receptors on the host cell membrane. As with picornavirus and other viruses, HSPG receptors are known to create a first attachment of the virus close to an epithelium that consists of cells with a low number of the ACE2 receptors. The entry of the virus inside these cells is facilitated through the ACE2 receptors, but HSPGs provide an environment of enrichment of the virus load close to the host cells through low affinity interactions [45].

While the exact mechanism still remains unclear, many investigations point to the fact that the infection of SARS-CoV and HCoV-NL63 into human cells is mediated by more receptors other than ACE2 on host cell membrane. Among other factors, HSPGs can clearly also serve as first attachment receptors [75]. First, the virus is docked to the host cells with a first link between the S protein on viral surface and the heparan sulfate chains of HSPGs on the host cell membrane [76, 77]. This binding event acts as an anchor for the more stable binding of the S protein to ACE2 receptor of the host cell membrane, followed by endocytosis of the viral particles [78, 79]. As shown in Fig. 1, this is very likely to be the mechanism of the invasion of SARS-CoV-2 in the epithelial cell of the cornea and conjunctiva as well.

By better understanding the mechanism discussed here with more clinical and experimental trials, ophthalmologists can play a major role on tracking early symptoms of COVID-19 and helping in the better treatment of the disease.

Compliance with Ethical Standards There was no research involving human participants or animals for the preparation of this review.

Conflict of Interest The author declares that there are no conflicts of interest.

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