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What is the significance of the conjunctiva as a potential transmission route for SARS-CoV-2 infections?

The WHO declared the outbreak of COVID-19 a health emergency of international concern. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is associated with symptoms such as fever, cough, impaired taste, fatigue, and severe pneumonia [19]. SARS-CoV-2 is highly infectious and primarily transmitted via inhalation of aerosol droplets released by an infected individual, as well as possibly via the feco-oral route [6]. Potential conjunctival transmission of SARS-CoV-2 has not been conclusively elucidated and would have a significant

impact on public health. For example, a handful of studies postulate that SARS-CoV-2 can be transmitted via the mucous membranes, including the conjunctiva [2, 3], and that all ophthalmologists are at increased risk and should therefore wear protective eyewear when examining suspected cases [14].

Route of infection and replication of SARS-CoV-2

Like SARS-CoV, SARS-CoV-2 uses the membrane-bound angiotensin-converting enzyme 2 (ACE2) and the membrane-

bound serine protease TMPRSS2 to enter the host cell [7]. During this process, SARS-CoV-2 binds to ACE2 with its spike (S) glycoprotein and is able to fuse with the host membrane via TMPRSS2-mediated proteolytic activation (■ Fig. 1; [7, 16]). Following membrane fusion, the RNA of the virus is released, replicated, and transcribed into virus-specific proteins on the ribosomes of the host cell. It is assumed that SARS-CoV-2, like SARS-CoV, is absorbed into the endoplasmic reticulum and can leave the host cell via exocytosis. Since the outbreak of COVID-19, a number of stud-

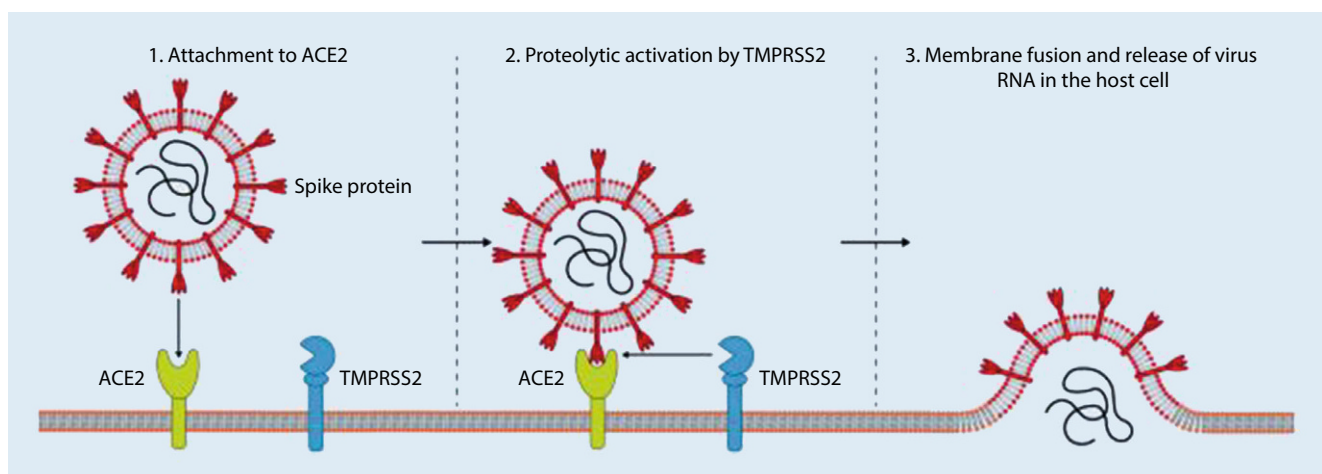


Fig. 1 ▲ The molecular mechanisms and receptors of SARS-CoV-2 cell entry. (Based on [8, 9]).

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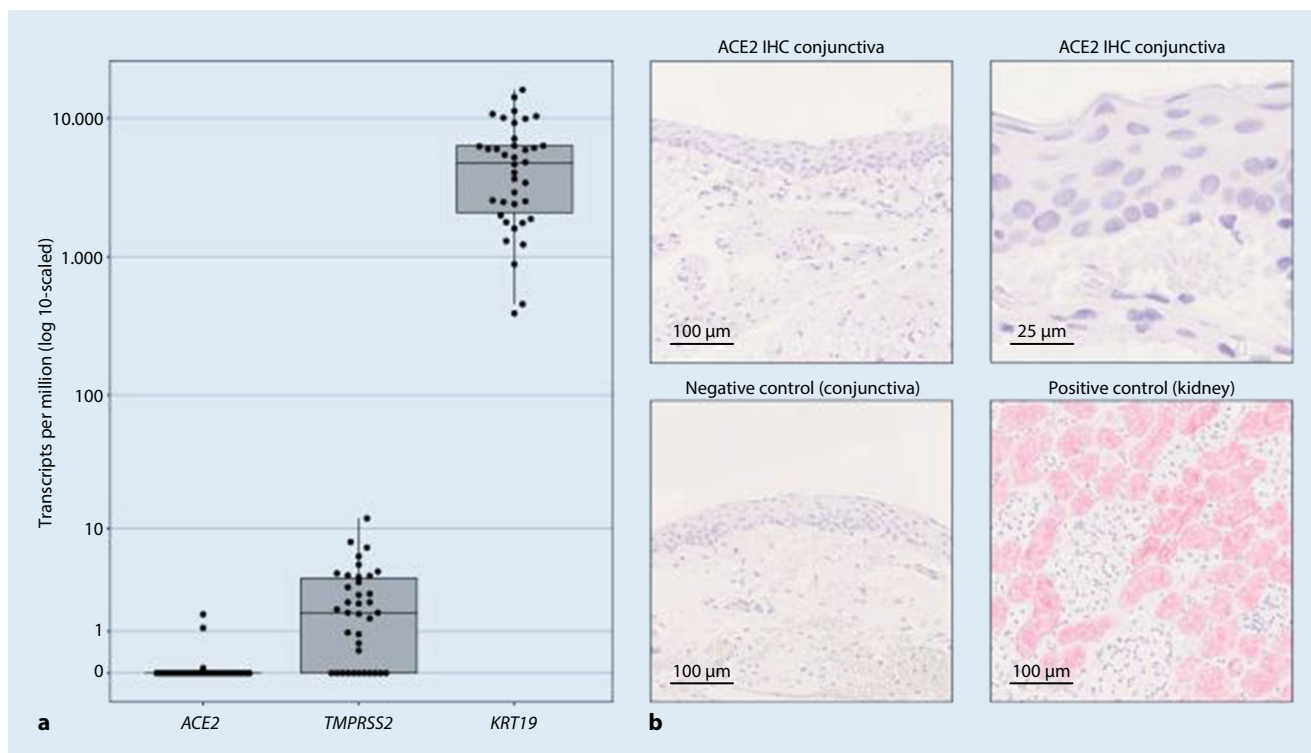


Fig. 2 ▲ Expression of the SARS-CoV-2 receptor *ACE2* and the proteinase *TMPRSS2* in the human conjunctiva. **a** The box plot shows low mRNA expression levels for *ACE2* and *TMPRSS2* compared to the conjunctival marker keratin 19 in 38 analyzed conjunctival samples. Each dot represents one sample. **b** Representative immunohistochemical images of *ACE2* staining of conjunctival and kidney tissue. While kidney tissue shows strong *ACE2* staining, healthy conjunctival samples ($n=8$) show negligible immunoreactivity. For the negative control, the primary antibody was omitted. (Figure modified from [12])

ies have investigated the expression of *ACE2* in a variety of human tissues and, in addition to expression in lung tissue, demonstrated marked expression of the receptor in stomach, colon, liver, and kidney tissue [15, 23]. This highlights the susceptibility of a wide variety of tissues to SARS-CoV-2 infection and explains the clinical observation of possible multiple organ involvement in SARS-CoV-2 infection [19]. It has not yet been definitively elucidated whether ocular surface cells express *ACE2* or *TMPRSS2* and are therefore susceptible to SARS-CoV-2 infection.

Possibilities of SARS-CoV-2 infection via the ocular surface

There are three fundamental questions related to the possibility of virus transmission via the ocular surface.

Can SARS-CoV-2 infect the conjunctiva and replicate there?

Recent studies describe subjective ocular symptoms in 7% of all COVID-19 patients [22] and signs of conjunctivitis in approximately 1% [5]. However, these observations were only rarely based on ophthalmological examinations and the inclusion criteria were heterogeneous [10]. In addition, the cited studies did not investigate adequate control cohorts and it is unclear whether the symptoms were caused by SARS-CoV-2 or whether they were an epiphenomenon unrelated to SARS-CoV-2, e.g., resulting from intensive care treatment. There is also controversy as to whether ocular surface cells, e.g., conjunctival epithelial cells, express *ACE2* or *TMPRSS2* and are therefore susceptible to SARS-CoV-2 infection. In light of the lack of data, we recently investigated the expression levels of *ACE2* and *TMPRSS2* in 38 healthy and diseased conjunctival samples [11]. To this end, RNA was isolated from formalin-fixed

paraffin-embedded conjunctival samples as previously described, sequenced [12, 17], and the sequencing data were then bioinformatically analyzed [1]. Whereas the conjunctival samples exhibited significant mRNA expression of the epithelial marker keratin 19, healthy and diseased conjunctival samples showed no relevant expression of the SARS-CoV-2 receptor *ACE2* (■ Fig. 2a). Consistent with the virtually undetectable expression of *ACE2* at the transcriptional level, we also found negligible *ACE2* immunoreactivity in eight healthy conjunctival samples [11], suggesting the absence of relevant *ACE2* protein expression in the conjunctiva (■ Fig. 2b). Furthermore, our data show that the serine protease *TMPRSS2* in conjunctival tissue is also not significantly transcribed (■ Fig. 2a). These results argue against an *ACE2*-mediated conjunctival route of infection for SARS-CoV-2 and are in line with histological investigations conducted on patients having died from COVID-19 that did not detect relevant conjunctivitis [13].

Therefore, SARS-CoV-2 appears to differ from other viruses, such as hepatitis C virus, for which conjunctival infection and transmission are described [8]. Although evidence is lacking to date, it remains to be elucidated whether individual factors such as hypoxia or smoking can trigger *ACE2* expression in the conjunctiva [21]. A recent investigation using an ex-vivo model reported the possibility of cell infection, particularly conjunctival stromal cells, by SARS-CoV-2 [9]. However, it remains unclear to what extent this observation can be extrapolated to the in vivo situation. In addition, more aggressive pretreatment of histological conjunctival specimens appears to induce *ACE2* immunoreactivity of the conjunctival epithelium; however, the clinical relevance of this is unclear [4]. Other investigations, e.g., on autopsy material from COVID-19-deceased patients, are needed in order to gain insight into actual infectivity and possible sites of virus replication.

Can healthy individuals become infected via the tear film?

There are currently no data on SARS-CoV-2 infection of healthy individuals via the tear film. While some data (see above) suggest that SARS-CoV-2 does not infect the conjunctiva, viruses in the tear film could gain access to the nasal mucosa and respiratory tract via the lacrimal drainage system, thereby triggering infection of respiratory epithelial cells. It is currently unclear to what extent rubbing the eyes with contaminated hands can cause infection via the tear film. One can only speculate at present on the importance of virus uptake via the tear ducts compared to direct uptake of virus-containing aerosols via the airways. Due to the protective blinking of the eye and the smaller surfaces, a purely ocular route of SARS-CoV-2 infection likely plays a minor role. However, eye protection appears to be urgently required in the case of close contact with COVID-19 patients or a high risk of exposure, e.g., during intubation or extubation of COVID-19 patients.

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What is the significance of the conjunctiva as a potential transmission route for SARS-CoV-2 infections?

Abstract

Recent studies have described conjunctivitis in approximately 1% of COVID-19 patients and speculated that SARS-CoV-2 can be transmitted via the conjunctiva. In this article we recapitulate the molecular mechanisms of host cell entry of SARS-CoV-2 and discuss the current evidence for a potential conjunctival transmission of SARS-CoV-2. The current body of evidence indicates that SARS-CoV-2 requires the membrane-bound angiotensin-converting enzyme 2 (*ACE2*) and the membrane-bound serine protease *TMPRSS2* to enter cells. Recent studies suggest that COVID-19 patients rarely exhibit viral RNA in tear film and conjunctival smears and that,

ACE2 and *TMPRSS2* are only expressed in small amounts in the conjunctiva, making conjunctival infection with SARS-CoV-2 via these mediators unlikely. Nevertheless, we consider the current evidence to be still too limited to provide a conclusive statement and recommend appropriate protective measures for healthcare personnel who are in close contact with suspected and confirmed COVID-19 patients.

Keywords

SARS-CoV-2 · COVID-19 · *ACE2* · *TMPRSS2* · Conjunctiva

Welche Bedeutung hat die Bindehaut als möglicher Übertragungsweg für eine SARS-CoV-2-Infektion?

Zusammenfassung

Aktuelle Studien haben bei ca. 1 % aller COVID-19-Patienten eine Bindehautentzündung beschrieben und spekuliert, dass SARS-CoV-2 über die Bindehaut übertragen werden kann. In der vorliegenden Arbeit rekapitulieren wir die molekularen Mechanismen des Eintritts von SARS-CoV-2 in die Wirtszelle und diskutieren die aktuelle Studienlage zu einer möglichen konjunktivalen Transmission. Derzeit geht man davon aus, dass SARS-CoV-2 das membran-gebundene Angiotensin-konvertierende Enzym 2 (*ACE2*) sowie die Membran-gebundene Serinprotease *TMPRSS2* benötigt, um in die Wirtszelle einzudringen. Aktuelle Studien weisen darauf hin, dass COVID-19-Patienten nur selten Virus-RNA im Tränenfilm und

Bindehautabstrichen aufweisen und dass *ACE2* und *TMPRSS2* in der Bindehaut nur in sehr geringen Mengen gebildet werden, was eine konjunktivale Infektion durch SARS-CoV-2 über diese Mediatoren wenig wahrscheinlich macht. Dennoch halten wir die derzeitige Studienlage für zu begrenzt, um eine abschließende Aussage treffen zu können, und empfehlen konsequente und adäquate Schutzmaßnahmen für medizinisches Personal, das in engem Kontakt mit verdächtigen und bestätigten COVID-19-Patienten steht.

Schlüsselwörter

SARS-CoV-2 · COVID-19 · *ACE2* · *TMPRSS2* · Bindehaut

Is the tear film in COVID-19 patients infectious?

Three recently published studies demonstrated SARS-CoV-2 RNA in only a small proportion of conjunctival swabs from COVID-19 patients [18, 20, 22]. Zhou et al. analyzed the conjunctival swabs from 67 confirmed or suspected COVID-19 cases and reported that only one patient had a positive PCR result and two patients had likely positive results. None

of the three patients had conjunctivitis [22]. Similarly, Xia et al. investigated a total of 30 patients with confirmed SARS-CoV-2 detection in sputum samples and reported that SARS-CoV-2 RNA was additionally detected in the conjunctival swab of only one of these patients. This patient also exhibited signs of conjunctivitis [20]. In another study, Seah et al. investigated 17 COVID-19 inpatients who had positive nasopharyngeal swabs. Despite repeated testing, they

could neither isolate the virus nor detect SARS-CoV-2 in tears from any of the patients [18]. These data suggest that even in patients with florid COVID-19 disease, the tear film only rarely contains virus RNA. The detection of viral RNA cannot be equated to the presence of infectious virus particles. Therefore, the risk of SARS-CoV-2 infection via tear fluid from infected patients appears to be low.

Conclusion

Due to the low number of COVID-19 patients investigated, the current evidence does not permit a conclusive statement to be made on possible SARS-CoV-2 infection of the conjunctiva. However, since COVID-19 patients only rarely exhibit clinical signs of conjunctivitis and SARS-CoV-2 RNA has been detected only sporadically in tear fluid, conjunctival SARS-CoV-2 infection appears to be unlikely. These clinical observations are supported by basic scientific research that describes low expression of *ACE2* and *TMPRSS2*. This makes conjunctival SARS-CoV-2 transmission via these mediators unlikely—but does not rule out other routes of infection via hitherto unknown receptors. In addition, inoculation of SARS-CoV-2 could occur via tears transporting the virus through the nasolacrimal drainage system to the nose and throat region where cells get infected. Other investigations, e.g., on autopsy material from COVID-19-deceased patients, are needed in order to gain insight into actual infectiosity and possible sites of virus replication. Until these possibilities are reliably ruled out, effective prophylactic measures that protect the mouth, nose, and, where necessary, the eyes, should be used by physicians coming into close contact with COVID-19. In routine ophthalmological practice, the risk of infection via respiratory aerosols and close contact to patients during certain ophthalmological examinations is likely to be higher than via the tear film and ocular surface of patients.

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Compliance with ethical guidelines

Conflict of interest. C. Lange, J. Wolf, C. Auw-Haedrich, A. Schlecht, S. Boneva, T. Lapp, H. Agostini, G. Martin, T. Reinhard, and G. Schlunck declare that they have no competing interests.

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