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SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection

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ABSTRACT: The novel SARS-CoV-2 virus has very high infectivity, which allows it to spread rapidly around the world. Attempts at slowing the pandemic at this stage depend on the number and quality of diagnostic tests performed. We propose that the olfactory epithelium from the nasal cavity may be a more appropriate tissue for detection of SARS-CoV-2 virus at the earliest stages, prior to onset of symptoms or even in asymptomatic people, as compared to commonly used sputum or nasopharyngeal swabs. Here we emphasize that the nasal cavity olfactory epithelium is the likely site of enhanced binding of SARS-CoV-2. Multiple non-neuronal cell types present in the olfactory epithelium express two host receptors, ACE2 and TMPRSS2 proteases, that facilitate SARS-CoV-2 binding, replication, and accumulation. This may be the underlying mechanism for the recently reported cases of smell dysfunction in patients with COVID-19. Moreover, the possibility of subsequent brain infection should be considered which begins in olfactory neurons. In addition, we discuss the possibility that olfactory receptor neurons may initiate rapid immune responses at early stages of the disease. We emphasize the need to undertake research focused on additional aspects of SARS-CoV-2 actions in the nervous system, especially in the olfactory pathway.

KEYWORDS: *SARS-CoV-2, ACE2 expression, TMPRSS2 expression, COVID-19, respiratory epithelium, olfactory epithelium, viral brain infection*

1. FAST, SENSITIVE, AND RELIABLE TESTS ARE CRITICAL TO SLOW DOWN A PANDEMIC

An important factor accelerating the spread of COVID-19 is the high infectivity of this virus. It is related to extraordinarily ability of spike glycoprotein to bind to host receptor with much higher affinity as compared to related SARS-CoV virus. Lower thermostability of SARS-CoV-2 spike protein has also been suggested as a factor contributing to its high infectivity.[1] Although SARS-CoV-2 testing is currently very efficient, public health care systems do not have the capacity to test all the citizens. Thus, the identification of infected but asymptomatic people should be one of the priorities and this should be done as early as possible. Currently, assays that are based on real-time RT-PCR technique are recommended for early detection of the virus. Theoretically, procedures based on RT-PCR are able to detect even a small number of viral RNA particles in biological samples. However, in practice, due to several technical factors, there must be much more viral load in the biological material collected to achieve a reliable diagnosis. Typically nasal and pharyngeal swabs as well as sputum are used as the starting biological material for SARS-CoV-2 testing. It is assumed that this strategy is very efficient in diagnosing infected individuals 5−7 days after onset of symptoms. Unfortunately, it is less efficient in detecting SARS-CoV-2 within 1−4 days after symptoms and in asymptomatic individuals. Therefore, other types of biological samples should be identified to detect SARS-CoV-2 more efficiently about the time of infection.

2. SARS-CoV-2 AFFINITY TO THE RESPIRATORY EPITHELIUM IN THE NASAL CAVITY IS LIKELY MODERATE

Because the nasal cavity is the main gate for SARS-CoV-2 entrance, epithelial cells located within this area can be considered as appropriate clinical sample for early virus detection. The nasal cavity contains three main types of mucosa: squamous, respiratory, and olfactory epithelium (Figure 1). Importantly, all these cells are easily accessible for collection by medical staff. According to some gene expression data deposited in databases such as GEO and MGI, respiratory epithelial cells (RECs) express both of the SARS-CoV-2 human proteins required for host cell entry, namely, ACE2 and TRMPSS2 transmembrane proteases[1,2] (Table 1). On the other hand, recent single cell RNaseq studies in humans showed only TMPRSS2 expression in RECs without detecting ACE2.[3] Other RNaseq studies showed rather low levels of ACE2 in RECs.[4] However, according to the mouse atlas, in embryonic RECs, ACE2 expression was clearly shown by in situ hybridization but TMPRSS2 expression was not examined by this approach. Taken together, current data suggest that RECs present in the nasal cavity express rather low

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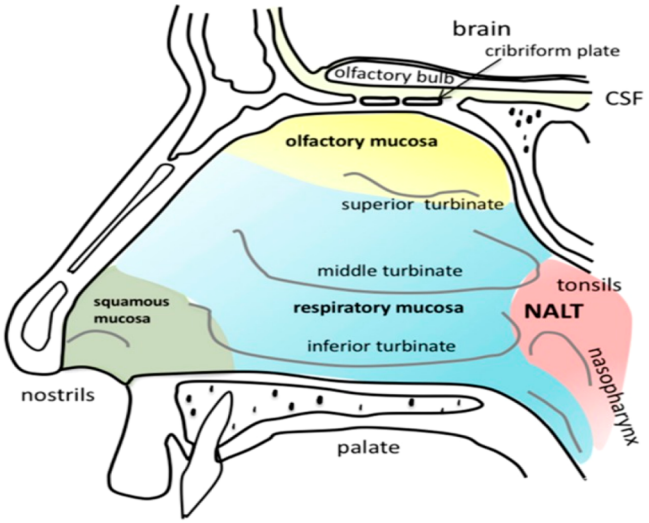

Figure 1. Diagram of human nasal cavity with respiratory and olfactory epithelium areas indicated in blue and yellow, respectively.

levels of ACE2 and TMPRSS2 proteins as compared to epithelial cells located at lower parts of the human respiratory pathway. However, it should be emphasized that the expression data in RECs are clearly incomplete and require further detailed examination. Before drawing a final conclusions about SARS-CoV-2 affinity to these cells, different aspects such as age-dependence and possible effects of pathological conditions on ACE2/TMPRSS2 expression should also be addressed, preferably at cell-type resolution.

3. THE OLFACTORY EPITHELIUM AS A SITE OF SARS-CoV-2 REPLICATION, ACCUMULATION, AND BRAIN ENTRANCE

Another suitable source of biological samples for early SARS-CoV-2 detection is the olfactory epithelium (OE), which is easily accessible within the nasal cavity (Figure 1). Recent reports indicate that total anosmia or partial loss of the sense of smell are early markers of SARS-CoV-2 infection. This phenomenon may be caused by different and yet unidentified factors, e.g., "cytokine storm" initiated in some patients or direct damage of the olfactory receptor neurons (ORNs) located in the olfactory epithelium (Figure 2). The latter possibility is particularly likely due to the fact that cells located in the OE express both protein receptors required for effiecient SARS-CoV-2 infection in humans. Several data sets deposited in gene expression databases show relatively high expression levels of ACE2 and TMPRSS2 in human and murine olfactory mucosa (Table 1). In mammals, OE is a continuously regenerating multilayer structure containing both neuronal and non-neuronal cells (Figure 2). The key question is whether ACE2 and TRMPSS2 expression in the OE is neuronal or non-neuronal or whether it occurs in both cell types. Neuronal expression of host receptors will likely facilitate SARS-CoV-2 brain infection through the uptake into ciliated dendrites/soma and subsequent anterograde axonal transport along the olfactory nerve. Non-neuronal expression of ACE2/TRMPSS2 may possibly establish nasal cavity OE as a virus reservoir. Three major RNaseq transcriptome studies conducted in human and murine OE consistently suggest non-neuronal expression of ACE2.[4−6] Hence, ACE2 expression is not clearly detected in mature ORNs, which are the only OE neurons connected to the brain. Expression of TMPRS2 seems to be higher compared to that of ACE2 and takes place likely in both neuronal and non-neuronal OE cells.[5,6] One state-of-the-art RNaseq study showed intriguingly mosaic TMPRSS2 expression which occurs only in subpopulation of mature ORNs, even though the majority of other genes were more evenly expressed in these neurons.[6] It suggests that some olfactory neurons in the OE may be more vulnerable for viral infection than other morphologically similar ORNs. Moreover, expression of murine ACE2 and TMPRSS2 evaluated by microarrays has a tendency to increase with age (Table 2). If it is true in humans, then in elderly people the OE may be more sensitive to SARS-CoV-2 accumulation. However, it should be remembered that although ACE2 is a mandatory factor for viral entry into the cell, TMPRSS2 can probably be replaced by other proteases from this family such as TMPRSS4, TMPRSS11A, 11D, and 11E1. Of these proteases, only TMPRSS4 is also present in the OE, likely in immature neurons and in non-neuronal cells.[5,6]

It is known from a previous SARS-CoV pandemic that, that even though lungs were the major site of infection, the brain was also involved in some patients. In addition, it was shown in transgenic mice expressing human ACE2 that SARS-CoV infected the brain through ORNs.[7] Genetically modified mice express only human ACE2 and not human TMPRSS2. This may additionally suggest that murine ORNs express endogenous TMPRSS2, because both proteins are required for efficient infection. Intriguingly, there was an approximately 60 h delay from the time of nasal infection until SAR-CoV virus detection in the olfactory bulb. During that time the virus likely replicated and accumulated in different OE cells, because its subsequent transport to further parts of the brain required a relatively short time of an additional 12−20 h.[7] The results from transgenic hACE2 mice indicate that SARS-CoV probably uses transneuronal/transsynaptic routes employing axonal transport in the brain and this can also be true for SARS-CoV-2. It is known for other viruses, e.g., rabies virus, that they can hijack existing vesicular axonal transport machineries to spread within the brain. There is very recent evidence that SARS-CoV-2 enters early and late endosomal compartments in non-neuronal cells; thus, it may possibly be directed to the vesicular axonal pathway in neurons.[1] However,

Table 1. ACE2 and TMPRSS2 Expression in Human and Mouse Nasal Cavity Epithelia[a]

nasal cavity	hACE2	hTMPRSS2	mACE2	mTMPRSS2	database
respiratory epithelium	+	+	+	ND	Bgee, GEO
olfactory epithelium	+	ND	+	+	Bgee, GEO
olfactory receptor neurons	ND	ND	− or low	+	Bgee, GEO

[a]Data based on Affymetrix and RNAseq. hACE2, human ACE2; hTMPRSS2, human TMPRSS2; mACE2, mouse ACE2; mTMPRSS2, mouse TMPRSS2. +, positive expression; ND, no data available. Note that olfactory receptor neurons are major part of OE; however, OE also contains several types of non-neuronal cells.

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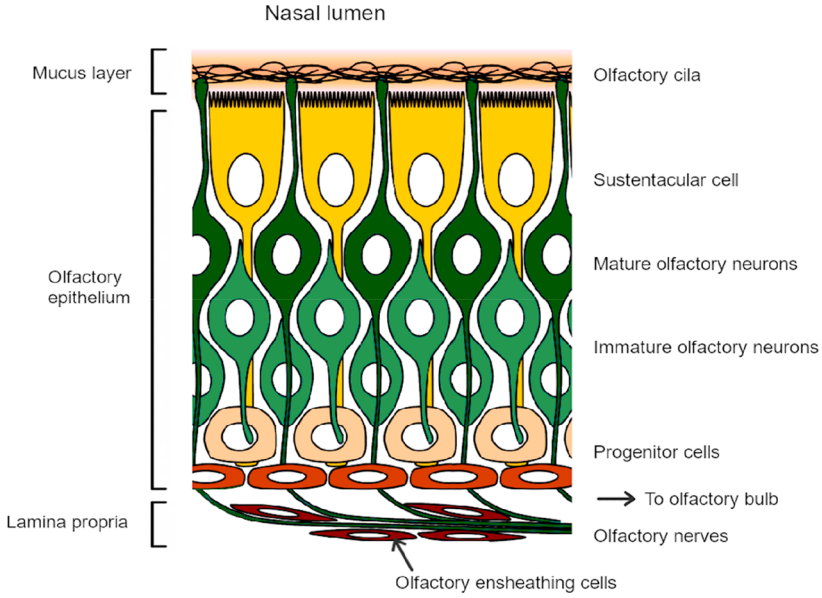

Figure 2. Basic organization of the olfactory epithelium (OE). Olfactory neurons continuously regenerate through human life and therefore are at different stages of differentiation. Some non-neuronal cells are shown, e.g., progenitors, sustentacular cells, and olfactory ensheathing cells.

Table 2. ACE2 and TMPRSS2 Expression Scores in Mouse Olfactory Epithelium According to the Bgee Database[a]

age of mice	ACE2	TMPRSS2
6 weeks old	49.4	78.7
6 months old	61.4	89.5

[a]www.bgee.org, Affymetrix microarrays, score range 0−10).

it should be remembered that the hACE2 mouse is an overexpressor model with expression of human ACE2 controlled by human keratin K18 promoter. For this reason, alternative and more physiological knock-in models for SARS-CoV-2 studies in the nervous system would be desirable. Alternatively to the olfactory axonal route, SARS-CoV-2 may pass from non-neuronal OE cells directly to cerebrospinal fluid surrounding olfactory nerve bundles, located near the cribriform plate. Once in cerebrospinal fluid, the virus could reach most of the brain areas including *medulla oblongata* where cardiorespiratory controlling nuclei are located.[8]

Brain infection in COVID-19 patients is currently being seriously considered because of many reports of neurological impairments such as stroke, epilepsy ,and encephalitis. The ACE2 expression in glia and in neurons in the brain is low but also well documented. But the specific sites where SARS-CoV-2 enters the brain are not clearly identified.[8] Mature olfactory neurons present in the OE are probably one such place. However, SARS-CoV-2 virus must first invade high ACE2-expressing yet unidentified non-neuronal OE cells and then pass to low-ACE2-expressing mature ORNs to be finally transported along olfactory axons to the brain. A good candidate for such cells is specialized glia known as olfactory ensheathing cells (OECs). OECs were previously shown to enhance human herpesvirus-6 replication and accumulation in the OE before virus infected the brain.[9] Many studies have already shown that this type of glia cells can supply axons with macromolecules by way of exosomes and this could be a mechanism of ACE2-independent virus transfer from OEC to ORN axons.

4. OLFACTORY NEURONS IN OE MAY MEDIATE ANTIVIRAL RESPONSES

It is known that the nervous system can shape responses of the innate immunity system. ORNs which are located with direct contact with the external environment are ideally suited for that role. Recetly it was shown in fish that ORNs initiate ultrarapid immune responses after binding rhabdovirus surface glycoprotein.[10] The virus binding results in neuronal activation and proinflammatory effects in OE but inhibits inflammation in the brain. As a consequence, some neurons undergo apoptosis, which may inhibit the reception of olfactory stimuli for some time. This data reveals the possible universal role of ORNs as first line viral sensors and initiators of antiviral protective immune responses. Based on the above conclusion, there is an exciting possibility that SARS-CoV-2 binding to ORNs initiates that kind of rapid immune response. Induction of the innate immune system through ORNs does not necessarily have to be mediated by ACE2 and TMPRSS2, but it may require additional yet unidentified host protein(s) with the ability to transmit intracellular signaling. From this point of view, infected people who show signs of olfactory dysfunctions may actually represents those individuals with faster and/or stronger immune response and better body mobilization against the SARS-CoV-2 infection. Therefore, it will be interesting to examine groups of patients with and without olfactory dysfunction and correlate it with the severity of their symptoms and percentage of recovery. Intriguingly, older patients which are known to be much more sensitive to SARS-CoV-2 infection are also those who have their sense of smell compromised simply because of their age. Reduced numbers of ORNs in older people can potentially slow down their early immune response and, consequently, lead to more severe COVID-19 symptoms.

5. CONCLUSIONS AND FUTURE DIRECTIONS

There are many reasons to urgently start thorough studies on the role of the OE in SARS-CoV-2 binding, accumulation, and brain infection and of participating in the early response of the immune system. Primarily, the OE may possibly serve as a

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tissue source for early virus detection to minimize false-negative test results. Analyzing viral loads in OE may also improve virus detection in asymptomatic individuals. Second, it should be noted that brain infection may cause delayed and long-lasting neurological impairment even in patients who no longer show respiratory symptoms and are currently considered recovered. Therefore, there is a need to establish which cell types in the OE bind and accumulate virus particles and whether the virus is transferred between OE cells. The large-scale transcriptomic data are only an approximation, and they lack the necessary resolution which can only be achieved by in vivo studies focused on single proteins. The transgenic hACE2 mouse together with epidemiologic COVID-19 data suggest a very dangerous phenomenon that hypothetical coronavirus with more neurotropic properties could be much more deadly to humans. By examining complex interactions of SARS-CoV-2 with the cells present within the OE, we could be better prepared for that type of virus. Future studies should also investigate how age-related differences affect SARS-CoV-2 actions in the OE. Finally, the scenario that the OE/ORN is involved in mediating the rapid response of the immune system is well worth investigating and must be experimentally verified. Epidemiological data should be collected to search for potential correlation between olfactory dysfunction and COVID-19 symptoms as well as severity.

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REFERENCES

(1) Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., and Ren, L. (2020) Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat. Commun. 11*, 1620.

(2) Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, E. S., Herrler, G., Wu, N.-H., Nitsche, A., Muller, M. A., Drosten, C., and Pohlmann, S. (2020) SARS-CoV-2 cell entry depends on ACE2 and TRMPSS2 and is blocked by a clinically proven protease inhibitor. *Cell 181*, 1−10.

(3) Ruiz Garcia, S., Deprez, M., Lebrigand, K., Cavard, A., Paquet, A., Arguel, M.-J., and Zaragosi, L.-E. (2019) Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development 146*, dev.177428.

(4) Olender, T., Keydar, I., Pinto, J. M., Tatarskyy, P., Alkelai, A., Chien, M.-S., Fishilevich, S., Restrepo, D., Matsunami, H., Gilad, Y., and Lancet, D. (2016) The human olfactory transcriptome. *BMC Genomics 17*, 619.

(5) Kangeswaran, N., Demond, M., and Nagel, M. (2015) Deep sequencing of the murine olfactory receptor transcriptome. *PLoS One 10* (1), e0113170.

(6) Saraiva, L. R., Ibarra-Soria, X., Khan, M., Omura, M., Scialdone, A., Mombaerts, P., Marioni, J. C., and Logan, D. W. (2015) Hierarchical deconstruction of mouse olfactory sensory neurons: from whole mucosa to single-cell RNA-seq. *Sci. Rep. 5*, 18178.

(7) Netland, J., Meyerholz, D. K., Moore, S., Cassell, M., and Perlman, S. (2008) Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J. Virol 82* (5), 7264−75.

(8) Harberts, E., Yao, K., Wohler, J. E., Maric, D., Ohayon, J., Henkin, R., and Jacobson, S. (2011) Human herpesvirus-6 entry into CNS through the olfactory pathway. *Proc. Natl. Acad. Sci. U. S. A. 108* (33), 13734.

(9) Baig, A. M., Khaleeq, A., Ali, U., and Syeda, H. (2020) Evidence of the COVID-19 virus targeting the CNS: host-virus interactions and proposed neurotropic mechanisms. *ACS Chem. Neurosci. 11*, 995.

(10) Sepahi, A., Kraus, A., Casadei, E., Johnston, C. A., Galindo-Villegas, J., Kelly, C., Garcia-Moreno, D., Munoz, P., Mulero, V., Huertas, M., and Salinas, I. (2019) Olfactory sensory neurons mediate ultrarapid antiviral immune responses in a TrkA-dependent manner. *Proc. Natl. Acad. Sci. U. S. A. 116* (25), 12428−36.

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