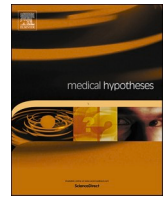




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More than ACE2? NRP1 may play a central role in the underlying pathophysiological mechanism of olfactory dysfunction in COVID-19 and its association with enhanced survival

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ABSTRACT

Three mechanisms have been proposed to account for COVID-19 associated olfactory dysfunction; obstruction of the olfactory cleft; epithelial injury and infection of the sustentacular supporting cells, which are known to express ACE2, or injury to the olfactory bulb due to axonal transport through olfactory sensory neurones. The absence of ACE2 expression by olfactory sensory neurones has led to the neurotropic potential of COVID-19 to be discounted.

While an accumulating body of evidence supports olfactory epithelial injury as an important mechanism, this does not account for all the features of olfactory dysfunction seen in COVID-19; for example the duration of loss in some patients, evidence of changes within the olfactory bulb on MRI imaging, identification of viral particles within the olfactory bulb in post-mortem specimens and the inverse association between severity of COVID-19 and the prevalence of olfactory loss.

The recent identification of a second route of viral entry mediated by NRP1 addresses many of these inconsistencies. Expression by the olfactory sensory neurones and their progenitor cells may facilitate direct injury and axonal transport to the olfactory bulb as well as a mechanism for delayed or absent recovery. Expression by regulatory T cells may play a central role in the cytokine storm. Variability in expression by age, race or gender may explain differing morbidity of infection and inverse association between anosmia and severity; in the case of higher expression there may be a higher risk of olfactory function but greater activation of regulatory T cells that may suppress the cytokine storm.

Introduction

Olfactory dysfunction (OD) has now been shown to be one of the most prevalent symptoms of COVID-19 [1], and the best predictor of COVID-19 status of all the associated symptoms [2]. However the underlying mechanism has yet to be fully elucidated.

In terms of the underlying pathophysiology, several hypotheses were proposed at the outset of the pandemic; that viral induced injury to the olfactory epithelium leads to olfactory cleft inflammation and

obstruction causing a localized conductive loss, that injury to the olfactory epithelium caused a sensorineural deficit directly or, given the known neurotropic potential of coronavirus, that the virus could invade and damage the olfactory bulb [3]. Current thinking is that olfactory epithelial injury is the predominant underlying mechanism, mediated by infection of the sustentacular cells which express ACE2.

Our hypothesis is that all three mechanisms likely play a part; early olfactory cleft obstruction occurs secondarily to olfactory epithelial injury mediated by ACE2 related cell infection. However, in those

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patients with more persistent olfactory deficits, there is direct injury to olfactory sensory neurones and consequently the olfactory bulb, facilitated by NRP1 mediated cell entry and infection. Loss of the progenitor cells may lead to permanent loss in some patients

Is olfactory dysfunction due to obstruction of the nasal cavity and olfactory cleft?

We performed sinus computed tomography in patients with persistent anosmia on psychophysical olfactory evaluation but found that few had complete bilateral obstruction of the olfactory cleft [4], suggesting that while olfactory cleft obstruction may contribute to the olfactory dysfunction in some patients, perhaps most likely in those that show very early resolution, it cannot account for the loss in all patients.

Is olfactory dysfunction secondary to ACE2 mediated entry and injury to olfactory epithelial cells?

CoV-2, like SARS-CoV, has been shown to infect cells through binding of its spike protein to ACE2 proteins on target cells [5]. Chen and colleagues showed that ACE2 immunohistochemical expression was 200 to 700 times greater in the sustentacular cells of the olfactory neuroepithelium than nasal or tracheal epithelia [6]. Brann et al. confirmed high levels of expression of both ACE2 and TMPRSS2, a protease required to cleave the spike protein to allow viral entry, on the sustentacular cells of the olfactory epithelium, but also, importantly, demonstrated the absence of ACE2 expression on olfactory sensory neurones (OSNs) [7]. A post-mortem study of 2 patients reporting anosmia showed focal atrophy of the olfactory epithelium, leukocytic infiltration of the lamina propria and evidence of axonal damage in the olfactory nerve fibres [8]. Animal models demonstrated large increases in macrophages in the OE and lamina propria after SARS-CoV-2 infection [9]. The first reported in vivo biopsy of a patient with anosmia persisting 3 months after diagnosis showed extensive destruction of the olfactory epithelium consistent with mucosal biopsies harvested early in the course of infection in animal models [10].

These findings have been taken as support for injury to the olfactory epithelium as the predominant mechanism, through direct disruption of the normal epithelial architecture and function and indirect actions on the olfactory bulb through loss of the support cells. Attention thus shifted away from viral entry into and along the olfactory sensory neurones into the olfactory bulb, as extension of viral infection from the sustentacular cells to the OSNs seemed less plausible.

What about the neurotropic potential of coronaviruses?

However, coronaviruses have long been identified as a family of viruses that may be neurotropic. Netland et al. demonstrated that SARS-CoV enter the central nervous system of transgenic mice through the olfactory bulb, leading to rapid transneuronal spread [11]. Interestingly, authors demonstrated that the virus antigen was first detected 60 to 66 h post-infection and was most abundant in the olfactory bulb. Regions of the cortex connected to the olfactory bulb were also strongly infected after the virus had spread. Emerging autopsy reports have shown SARS-CoV-2 tracking along the olfactory bulb, gyrus rectus and medulla of several patients with COVID related anosmia who subsequently died [12,13]. More recent imaging reports in the literature show evidence of hyperintense signal and oedema of the olfactory bulb which subsequently resolved [14,15], giving further support to a central mechanism of anosmia in at least some patients. The current ACE2 mediated route of viral entry does not support a neurotropic mechanism; perhaps the virus could reach the bulb independently of axonal transport, for example via the vascular supply?

Are there other flaws in the current hypothesis?

The current proposed model of epithelial injury secondary to ACE2 mediated entry also does not fully explain the apparent inverse association between OD and severity of disease. Yan et al. found that self-reported olfactory loss associates with milder forms of disease in patients who did not require hospitalization [16]. There is a risk that this represents recall bias due to the over-riding severity of respiratory symptoms and delays in olfactory testing of such patients compared to those with mild disease. Older patients are at higher risk of more severe disease; of course olfactory function diminishes with increasing age [17] and, therefore, hospitalized patients may simply have higher rates of pre-existing loss and may be less able to detect further decline.

However, if it is indeed true that OD is less prevalent in more severe forms, what mechanism may account for this? Nasal gene expression of ACE2 was studied on biobank samples taken from individuals aged between 4 and 60 years old, and was found to increase with age; unfortunately, samples from older patients were not available for comparison [18]. As COVID related anosmia appears rare in childhood, and most prevalent in the 40–50 year old age group, perhaps this is related to peak expression in ACE2?

Ablation of the olfactory bulb may prevent anterograde propagation of the virus to the olfactory bulb in murine models of viral infection [19,20]. Thus, given the capacity to of the olfactory neurones to regenerate, it has been proposed that apoptosis of infected olfactory receptor neurones may be a programmed protective response to neurotropic viruses that lessens the severity of infection [21]. The lower prevalence of anosmia in more severe disease may reflect a failure of this line of defence – but this again does not sit well with a ACE2 mediated route of entry. Furthermore, COVID-19 mortality seems to relate more to the associated cytokine storm rather than central nervous system complications [22].

Is there another route of viral entry?

Perhaps the missing piece of the jigsaw has been discovered. Two teams have independently discovered an alternative pathway of SARS-CoV-2 entry mediated by the neuropilin-1 receptor (NRP1), which can also bind with the Spike protein [23,24]. Cantuti-Castelvetri and colleagues also demonstrated abundant expression of NRP1 in almost all olfactory cells, including neuronal progenitor cells and OSNs in post-mortem olfactory epithelial specimens [23].

Binding to NRP1 could facilitate direct entry and damage to OSNs causing loss of smell, loss of the progenitor cells leading to delayed recovery, and permit axonal transport to the olfactory bulb; this NRP1 mediated mechanism may be responsible for the more persistent olfactory dysfunction seen in some COVID-19 patients.

Implications

Could this also explain the inverse relationship between olfactory dysfunction and disease severity? Neuropilins are involved in many physiological processes, including neuronal development, angiogenesis but also immune regulation. Amongst other immune cells, NRP1 is expressed by regulatory T cells and appears to exert an immunosuppressive effect – perhaps those most susceptible to NRP1 mediated olfactory loss due to higher expression are at the same time better equipped to suppress the cytotoxic storm that is seen in COVID-19, through activation of regulatory T cells? Production of regulatory T cells declines with age [25], and are down-regulated in obesity [26]. Racial variation in T-cell function has also been described; while higher rates of malignancy described in African-Americans is multifactorial [27], they have been shown to have higher levels of T helpers cells and lower levels of regulatory T cells – the same variation could contribute to higher mortality from a cytokine storm in COVID-19.

Finally, regulatory T cells are often associated with chronic viral

infection, while some acute forms are characterised by hyperinflammation but viral clearance – perhaps this is why many patients with olfactory disturbance and a relatively mild initial course report long-lasting fluctuation symptoms associated with Long-COVID.

If NRP1 is the link between olfactory loss and enhanced survival, and given that the majority of patients recover from their olfactory dysfunction, perhaps this is a reasonable price to pay. However, better understanding of the pathophysiology may allow uncoupling of olfactory loss and severity of disease so that better outcomes with regards to both short and long term olfactory dysfunction can be achieved while reducing the risk of mortality. Further study into variation in the expression on NRP1 is clearly required, along with enhanced understanding of the role in both olfactory dysfunction and disease severity. However, this finding highlights the importance of remaining open-minded to the possibility of alternative mechanisms to those that emerged as early front-runners. Perhaps we have become too blinkered by the allure of ACE2 and this has been detrimental to progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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