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Review

Mechanism of Anosmia Caused by Symptoms of COVID-19 and Emerging Treatments

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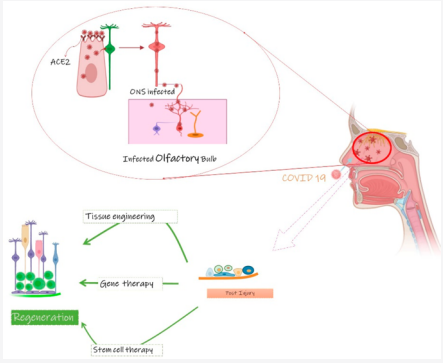
ABSTRACT: The occurrence of anosmia, the loss or change in sense of smell, is one of the most common symptoms of COVID-19 experienced by almost 53% of those affected. Several hypotheses explain the mechanism of anosmia in patients suffering from COVID-19. This study aims to review the related mechanisms and answer the questions regarding COVID-19-related anosmia as well as propose a new strategy for treatment of long-term anosmia as a result of COVID-19 infection. This paper covers all of the studies investigating olfactory disorders following COVID-19 infection and explains the possible reasons for the correlated anosmia, including olfactory cleft syndrome, local inflammation in the nasal epithelium, early apoptosis of olfactory cells, changes in olfactory cilia and odor transmission, damage to microglial cells, effect on olfactory bulbs, epithelial olfactory injury, and impairment of olfactory neurons and stem cells. The key questions that arise in this field have been discussed, such as why prevalent anosmia is varied among the age categories and among sexes and the correlation of anosmia with mild or severe COVID-19 infection. The angiotensin-converting enzyme 2 receptor is a significant player in the mechanism of anosmia in COVID-19 patients. Based on current studies, a novel approach to treat long-COVID-19 with ongoing anosmia has been proposed. The fields of smart drug delivery, tissue engineering, and cell therapy provide a hypothesized strategy that can minimize the side effects of current treatments and support efficient recovery of the olfactory system.



KEYWORDS: *COVID-19, anosmia, COVID-19 symptoms, ACE2 receptor, biomedical strategies, treatment*

1. INTRODUCTION

In December 2019, the COVID-19 outbreak originated in Wuhan, China and rapidly spread across the world, causing a global pandemic.[1] To date, there have been more than 210 million confirmed cases of COVID-19 and 3.9 million reported deaths as a result of the virus.[2] Although the source of the virus is still being researched, the majority of cases have been established to be caused by human-to-human transmission.[3,4] The common symptoms reported by patients with COVID-19 infection include fever, dry cough, shortness of breath (dyspnoea), myalgia, malaise, chills, confusion, headache, sore throat, rhinorrhea, chest pain, diarrhea, nausea/vomiting, conjunctival congestion, nasal congestion, sputum production, and hemoptysis.[5−7] Alongside this, several studies have reported olfactory dysfunction and hypogeusia as frequent symptoms of COVID-19 (Table 1).[8,9] However, some patients with COVID-19 have not exhibited typical respiratory symptoms, such as fever and coughing, at the time of diagnosis; rather, some infected patients have shown only neurological symptoms as the initial symptoms.[5] According to a meta analysis, the prevalence of olfactory dysfunction in COVID-19 patients is estimated to be 52.73%.[10] Kaye et al. reported anosmia in nearly 73% of patients and showed it began before their diagnosis of COVID-19 and, in addition, anosmia was the initial symptom in over 26.6% of these patients.[11] It has been shown that 63−78% of the patients with COVID-19-related anosmia had partial or full recovery of their olfactory senses within the first 30 days of disease. Although the majority of COVID-19-related olfactory dysfunctions seem to recover within a short time period, some patients reported long-term anosmia of more than 30 days.[12] To control the pandemic and stop its spread, it is important to consider and encourage early diagnosis using alarm symptoms so that affected individuals can self-isolate before becoming contagious. In the absence of other respiratory diseases, an unexpected olfactory dysfunction should alert physicians to the differential of infection with COVID-19. Understanding the mechanism of olfactory dysfunction in COVID-19 is the key to finding the right treatment. This Review aims to update

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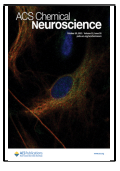

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Table 1. Prevalence of Olfactory Dysfunction in COVID-19 Patients[a]

			sex		age			time taken recovery of olfactory sensation	
year	no. of pts	total	male	female	<30	30−50	>50	<15 day	>15 day
2021[13]	95	60							
2021[14]	183	87						36	50
2021[15]	1072	75							
2021[16]	3448	368							
2020[17]	2428	2097	628	1468	620	1188	287	1921	165
2020[11]	237	176						107	69
2020[18]	1788	118	31	87					
2020[19]	45	18	25	20					
2020[20]	6	5	2	3	3	2	0		
2020[21]	138	117						∼85	∼32
2020[22]	151	75	22	52				64	11
2020[23]	121	50							

[a]Key: no., number; pts, patients.

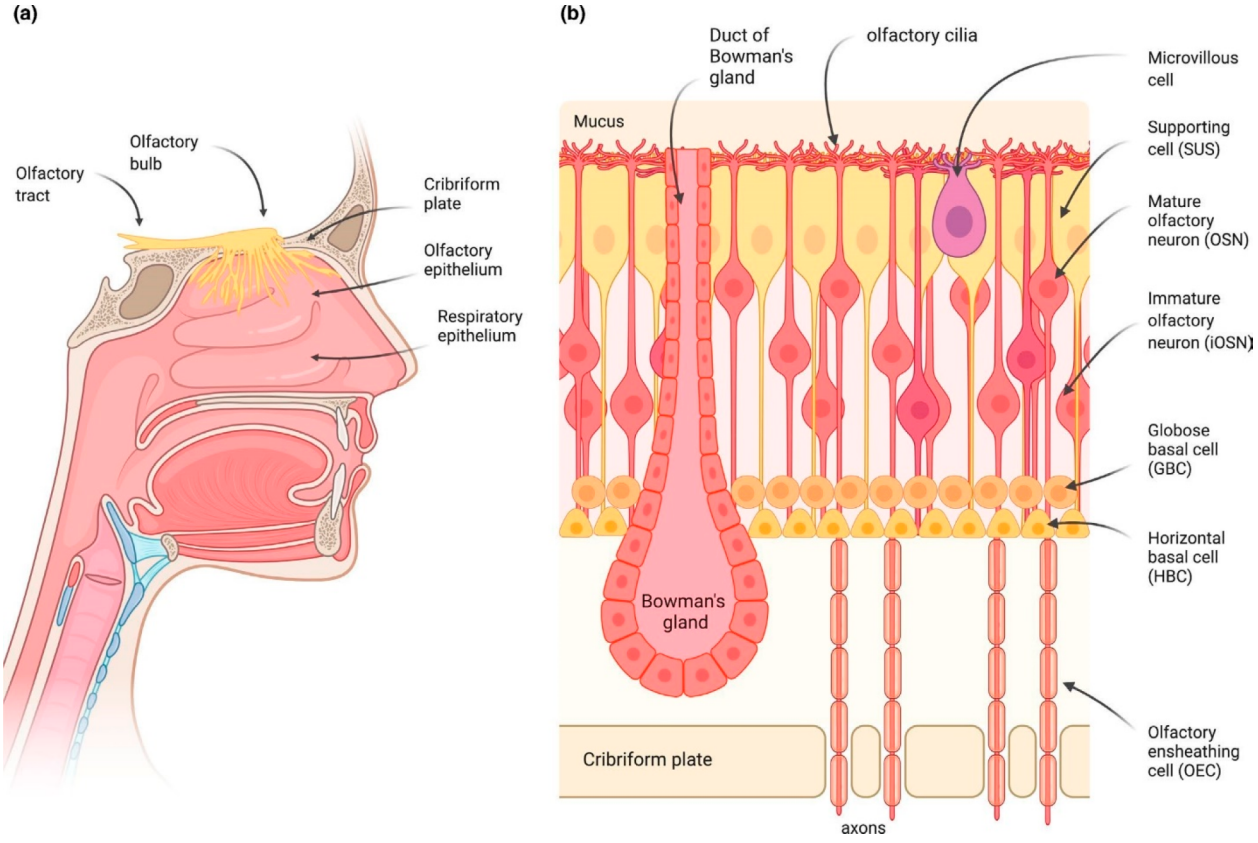

Figure 1. Schematic of the olfactory system. (a) Odor sensing initiates in the OE. The OSN cilia have related receptors (G-protein-coupled) for detection of odorants; OSN synapses with the olfactory bulb project to the glomeruli, and the bulb's cells have axons assigned to various olfactory areas in the CNS. (b) Different cell types of OE, including sustentacular cells, OSNs, microvillar cells, basal cells, and olfactory gland cells. Reproduced with permission from ref 24. Copyright 2020 International Society for Neurochemistry.

the current literature on olfactory physiology and pathways by investigating the mechanisms of virus pathology that result in anosmia and olfactory dysfunction.

2. OLFACTORY PHYSIOLOGY

The olfactory system (Figure 1) provides essential information from the surrounding environment; therefore, substantial neural circuitry is committed for olfaction processing. The nasal cavity is coated with two different types of epithelia: respiratory and olfactory epithelium (OE). The respiratory epithelium covers most of the nasal cavity area, a pseudostratified columnar epithelium made of ciliated cells, secreting (goblet) cells, and basal cells. The goblet cells secrete mucus for epithelium moistening; the ciliated cells propel mucus toward the distal opening to expel mucus from the body, and the basal cells are progenitor cells that differentiate into the necessary respiratory epithelium cell types. The OE initiates the sensation of smell and consists of at least five types of cells, including sustentacular cells, olfactory sensory neurons (OSNs), microvillar cells, basal cells, and olfactory gland cells.

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The cilia of the OSNs have related receptors (G-protein-coupled) for the detection of odors and cause Golf activation. This activation results in adenylyl cyclase stimulation and cyclic adenosine monophosphate formation, leading to chloride channels opening and chloride ions efflux, creating an action potential. OSNs as bipolar neurons form synapses with the olfactory bulb through their axons that project out into the nasal cavity via their dendrites and are covered by sustentacular cells. Each OSN has a unique odor receptor that projects up to the glomeruli, a synapse with the olfactory bulb cells, including mitral and tufted cells. These cell axons are assigned to various olfactory areas in the central nervous system (CNS).[24,25]

3. COVID-19 VIROLOGY AND ITS ANOSMIA PATHOLOGY

3.1. Molecular Virology of COVID-19. The COVID-19 virus (SARS-CoV-2) has been established to be a positive-sense single-stranded ribonucleotide acid (RNA) virus. These viruses have a glycoprotein spike (S protein) belonging to the class I viral fusion proteins. These include the S1 and S2 proteins (N-terminal and C-terminal), respectively, that play a prominent role in the virus's activity.[26] The N-terminals bind to host cell receptors through its receptor-binding domain (RBD). The C-terminal contains heptad repeat domains (HR1 and HR2) that cause the formation of a six-helix bundle fusion core structure during receptor-spike interaction that then allow viral RNA entry into the cell.[27]

3.2. The Proposed Mechanisms for Viral Anosmia Pathology. Angiotensin-converting enzyme 2 (ACE2) has been detected as the functional receptor for the COVID-19 virus. Furthermore, a priming protease TMPRSS2 facilitates viral uptake.[28,29] This receptor has been seen in several organs, including the lungs, heart, oral mucosa, kidneys, skeletal muscles, respiratory cells, and the CNS. This indicates that the COVID-19 virus can cause multisystem disorders in the human body by affecting some organ systems at once.[27,30] The epithelium of the respiratory system is the primary site of coronavirus attachment and so the viral impacts on the sense of smell and taste have not been surprising.[30] Several probable mechanisms for the anosmia in COVID-19 have been suggested in early studies and hospital observations.

Olfactory cleft syndrome, local inflammation in the nasal epithelium, early apoptosis of olfactory cells, changes in olfactory cilia and odor transmission, damage to microglial cells, effect on olfactory bulbs, epithelial olfactory injury, and damage of olfactory neurons and stem cells have been reported as possible mechanisms.[37] Table 2 presents the currently proposed mechanisms for viral-related anosmia.

3.2.1. Olfactory Cleft Obstruction. Olfactory cleft obstruction is a significant plausible mechanism causing hyposmia or anosmia in viral infection. Olfactory cleft obstruction affects air flow and prevents odors from traveling to the intact OE, hence causing conductive loss. In these cases, nasal mucosal swelling and secretions cause anosmia in viral-related patients. However, a group of patients with COVID-19 has been identified with sudden-onset anosmia without nasal discharge or congestion, indicating an alternative mechanism for COVID-19 anosmia.[38] Studies demonstrated the high intensity of ACE2 receptors in the olfactory cleft region; additionally, a comparison of olfactory cleft size between COVID-19 patients and healthy control showed a significantly greater volume and cleft area in anosmic patients. Therefore, it seems increased

Table 2. Clinical Studies on Anosmia Due to COVID-19 Infection[a]

year	aim of study	methods	outcome	possible effect on olfactory system
2020[31]	determine expression of ACE2 and TMPRSS2 in olfactory sensory tissue	biopsies via nasal endoscopic surgery quantified gene expression	sustentacular cells had a higher amount of ACE2 and TMPRSS2	OE inflammation can depend on the personal immune system and cause anosmia
2020[27]	Cytokine levels assessment in OE	biopsies of the OE and determination of cytokine level	increased in TNF-α and IL-1β levels	OE inflammation can happen and cause short duration of anosmia
2020[32]	microscopic studies of cilia in virus-infected cells	TEM analysis of ciliary ultrastructure	cilia have absorption sites for viruses	change in odor transmission and can cause short duration of anosmia
2020[33]	percentage of cells expressing ACE2 and TMPRSS2	human nasal biopsy samples, quantification of gene expression	ACE2 detected only in supportive cells	OE inflammation can depend on the personal immune system and cause anosmia
2020[34]	correlate interleukin-6 levels with olfactory dysfunctions	IL-6 was evaluated with venous blood samples	levels of IL-6 were significantly related to severe clinical anosmia	OE inflammation and the rapid recovery of smell could be attributed to a decrease in interleukin-6 levels
2020[30]	injury to the olfactory bulbs	brain MRIs of the patients	microbleeding or abnormal enhancement on MR imaging	damage to the olfactory bulb and long duration of anosmia
2020[35]	olfactory cleft measurements	all cases had paranasal sinus CT and MRI OC width and volumes were measured on CT	total OC width was significantly wider in anosmic patients	short duration of anosmia
2020[36]*	investigation of ACE2 expression in different cell types of OE	biopsies of the OE and quantification of gene expression	higher ACE2 and TMPRSS2 in sustentacular cells no detection on olfactory receptor neurons	OE inflammation can depend on the personal immune system and cause anosmia

[a]An asterisk (*) indicates the study was in a mouse model.

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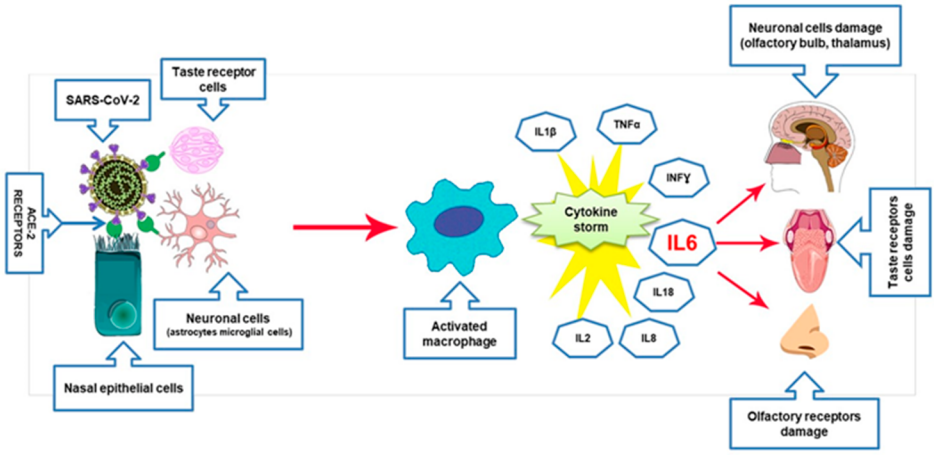

Figure 2. Cytokine release in the OE following interaction between the SARS-CoV-2 virus and ACE2 receptors. Reproduced from ref 34. Copyright 2020 American Chemical Society.

olfactory cleft volume and area in anosmic patients can result from the overall increased ACE2 receptor number, which results in inflammation of more olfactory mucosal surface area that could affect air flow and prevents odors from traveling to the intact OE. However, if olfactory cleft obstruction causes smell loss, it appears that cleft obstruction may occur in certain patients who have a rapid olfactory recovery.[38,39]

3.2.2. Local Inflammation in the Olfactory Epithelium. OE inflammation is one possible mechanism for COVID-19-related anosmia. A high level of ACE2 receptor expression was reported on OE cells when exposed to strains of COVID-19. The binding between the virus and these cells causes cytokine release and promotes OE inflammation (Figure 2). Torabi et al. investigated levels of local pro-inflammatory cytokines in the OE and found that COVID-19 infection significantly increased tumor necrosis factor α (TNF-α) levels.[27] Inflammation in the OE is a probable mechanism of the quick renewal and recovery of COVID-19-related anosmia. High ACE2 expression in non-neuronal OE cells may result in COVID-19 targeting the peripheral nasal system cells rather than directly entering olfactory neurons. Therefore, in the cases where the virus affects the OE, the olfactory can quickly regenerate and recover after the viral infection.[33,40] The cause of the prolonged recovery of the olfactory system in some patients can be related to damage to the olfactory receptor neurons and nasal stem cells or olfactory bulbs. This is further explained in the following section.

3.2.2.1. Interleukin Level. Interleukin-6 (IL-6) plays a significant role in anosmia; IL-6 can activate apoptotic pathways through TNF-α or neuropilin and directly inhibit the sense of smell.[34] One study investigated the relationship between IL-6 levels and olfactory disorders in COVID-19 patients. Significant correlations were found between the decreased levels of IL-6 and time taken for recovery from anosmia secondary to COVID-19.[34] Furthermore, studies have shown a rise in IL-6 levels in the serum, olfactory bulb, and CNS of patients with hyposmia during various human influenza virus infections.[41−43] Also, some other cytokines, including IL-6, IL-12, IL-15, and TNF-α, were increased in viral-infected cells.[44]

3.2.3. Early Apoptosis of Olfactory Cells. Early apoptosis of olfactory cells during viral infections has been studied in animal models. Intranasal inoculation using some viral strains in mice revealed evidence of apoptosis in anosmic mice. Intranasal inoculation with Sendai virus 52, a mouse counterpart to human parainfluenza virus, showed apoptosis and decreased proliferation of OE cells. A similar study on Influenza R404BP strains demonstrated apoptosis of the olfactory neurons.[45] There is evidence that apoptosis in olfactory cells can inhibit anterograde propagation of the virus to the olfactory bulb and CNS and prevent prolonged olfactory disturbance. This may be an inherent reaction to prevent severe infection secondary to the regenerative capacity of the olfactory neurons. Viruses that block or delay OSN apoptosis are more likely to enter the olfactory nerve and the brain.[46]

3.2.4. Changes in Olfactory Cilia and Odor Transmission. Numerous sensory receptors accumulate on cilia in the OE that perceive related odors and transduce the signal to the cortex of the olfactory in the brain.[47] Viral infections may disrupt the cilia structure by destroying olfactory receptors' ciliary localization, thus inhibiting the perception of odor molecules. Pathological studies have shown the expression of COVID-19 antigens in ciliated nasal epithelial cells. Transmission electron microscopy (TEM) demonstrated an absorption site on cilia for viral infection entry. Investigations of the connection between the COVID-19 virus and nasal cilia found that the Nsp13 protein, a highly conserved protein in coronaviruses, binds with the centrosome of cilia through the pericentrosomal region. Nsp13 competes with endogenous binding partners of the centrosome proteins and damages physiological interactions in the structure of cilia. Therefore, this interaction disrupts the centriolar structure and interaction between the centriole and cilia, leading to deciliation.[32]

3.2.5. Effect on Olfactory Bulbs. A number of studies investigated the effect of COVID-19 infection on the olfactory bulb and the damage caused as a potential mechanism for anosmia. Aragão et al. used the detection of bleeding or abnormal enhancement on MRI to identify five cases of olfactory bulb injury secondary to COVID-19 infection. The mechanism is thought to involve the virus entering the CNS via OSNs in the OE. The virus crosses the cribriform plate to reach the olfactory bulbs, which contain the second olfactory neurons.[30,47,48]

3.2.6. Olfactory Epithelium Injury. The regeneration ability of OE has been researched in studies investigating damage to the olfactory system and has demonstrated the regeneration

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potential for basal cells to grow new axons and revert to the olfactory bulb. Histological changes were shown in the postviral human OE, including disorganization, scarring, atrophy, and marked reduction of olfactory epithelial density and receptors. These amplified and supported the possibility of anosmia secondary to direct OE damage.[49] Scar tissue formation because of severe inflammation may inhibit regrowth and regeneration and cause a delay in OE recovery.[50]

3.2.7. Damage of Olfactory Neurons. Different algorithms of ACE2 expression in OE cells have been shown: the olfactory neurons (ON) do not have ACE2 receptors, while other types of OE cells, including sustentacular cells (SUS), progenitor/stem cells, and Bowman's glands, indicated a high level of ACE2 and TMPRSS2 expression (Figure 3).[33,36] Therefore,

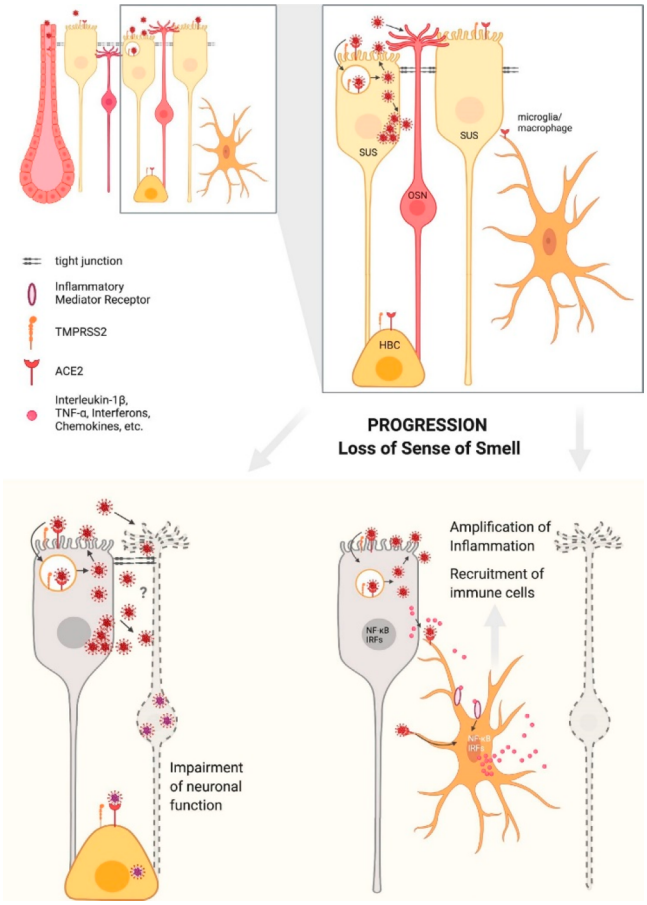

Figure 3. COVID-19 virus and olfactory neuron function. SUS and other supporting cells express a high level of ACE2 receptors. So, SARS-CoV-2 primarily infects the supporting cells. Supporting cell damage indirectly disrupts the ONS. Reproduced with permission from ref 24. Copyright 2020 International Society for Neurochemistry.

ONs might not be infected directly, although the virus can inter to the ONs through other cells, for example, SUS cells functionally and anatomically tightly link with ONs; these cells support ON with detoxifying volatile chemicals by expressing the cytochrome P450 family enzymes and play an essential role in the olfactory transduction cascade.[37] Hence, ON viral infection could be more expected; briefly, SUS cells are infected first and affect olfactory receptor neurons (ORNs), so the odor perception cascade (double lines) is inhibited. Simultaneously, the rapid immune response is induced in a subset of ORNs and microvillar cells (MVCs), and this inflammation in ORNs may cause ON destruction. In this regard, olfactory ensheathing glial cells (surrounding the axons of ORNs) cause olfactory fila formation, which are other candidates by which ACE2-independent virus transfer and can occur into olfactory receptor neurons by way of exosomes. Thus, olfactory receptor neurons may initiate a rapid immune response in the host and cause olfactory dysfunction.[36,51]

3.2.8. Damage of Olfactory Stem Cell Neurons. There have been reports of persistent anosmia ongoing for over two months in a small group of COVID-19 patients.[52] In these cases, it is likely that stem cell damage is perpetuating persistent anosmia by delaying the regeneration capacity of OE. There is evidence that stem cell apoptosis at sites of inflammation is caused secondary to complement factors, complement-activated neutrophils, and cytotoxic cells.[53] As aforementioned, inflammation in the olfactory systems is one of the critical factors for anosmia. Therefore, damage to olfactory stem cell neurons may be a crucial cause for persistent COVID-19-related anosmia.

4. WHY IS OLFACTORY LOSS DIFFERENT IN EACH AGE CATEGORY?

Studies have demonstrated that COVID-19-related anosmia prevalence varies among each age category; it rarely occurs in either extreme age and is most common in the 40−50-year-old age bracket. There is correlation between age category and volume of expression of ACE2 receptors as well as other entry proteins or sustentacular cells. Age-related loss of sustentacular cell nuclei as well as olfactory receptor neuron nuclei in animal models have been demonstrated in previous studies.[54] Because the sustentacular cells express the highest volume of ACE2 receptors in the OE, degenerative injury secondary to age for these cells could explain the less major incidence of anosmia in old patients. For the middle-age bracket, the greatest volume of ACE2 expression was shown, and nasal gene expression of ACE2 was found to increase with age (between 4 and 60 years old).[55] Evidently, this hypothesis needs further research, although it does provide potential mechanisms for the age-related difference in prevalence.

5. CORRELATION OF OLFACTORY LOSS WITH SEVERE OR MILD CORONA TYPE

Epidemiological studies related to COVID-19 anosmia demonstrated less olfactory involvement in patients with a moderate to severe infection.[56] Ear, nose, and throat complaints were less reported in patients with severe infection compared to mild infection.[9,10] A recent study proposed that patients suffering from a mild form of COVID-19 might have stronger local immunity where the virus replicates in the nasal and olfactory mucosa. The infection causes an inflammatory reaction in the OE and bulb regions; therefore, an otolaryngological pattern of disease takes place.[57] Also, immunohistological analysis has found ACE2 receptor expression in the airways with higher ACE2 expression in the upper airway compared to the lower airways; hence, the upper airway is the initial site of COVID-19 infection.[58]

6. WHY IS OLFACTORY LOSS DIFFERENT BETWEEN MEN AND WOMEN?

Epidemiological data reports that females are more likely to suffer COVID-19-related olfactory dysfunction although less

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Table 3. Applied Treatment of COVID-19-Related Anosmia in Various Studies[a]

year	treatment	no. of pts plus related details	outcome
2021[62]	nasal betamethasone drops	276, PCR-confirmed COVID-19 patients with anosmia	nasal application of betamethasone drops has no significant effect on the recovery time of anosmia
2021[63]	fluticasone nasal sprays	120, mean age for all cases is 51 ± 16 years	statistically significant improvement in recognizing all the odors
2021[64]	corticosteroid nasal spray	100, received mometasone furoate nasal spray	no statistically significant differences between both groups
2020[65]	coffee	doses of 15−20 mg for nonunderlying patients and 25−30 mg for underlying patients	caffeine in coffee reduced the reversibility of the sense of smell of people with COVID-19
2020[66]	intranasal plateletrich plasma (PRP)	7, olfactory loss >6 months, no evidence of sinonasal inflammation and no improvement with olfactory training	all patients reported a subjective improvement of their smell shortly after injection but then stabilized
		pts received a single intranasal injection of PRP into the mucosa of the olfactory cleft	
2020[67]	oral corticosteroid	A 35-year-old woman presented with anosmia after recovery from COVID-19	after 6 days, her anosmia reversed
		rhinocort spray, one puff BID for 10 days	
2020[68]	prescribed systemic prednisone and nasal irrigation with betamethasone	18 (with COVID-19-related anosmia)	a mix of drugs including steroids could represent a useful specific therapy to reduce the prevalence of this long-term morbidity
		nasal irrigation with betamethasone, ambroxol and rinazine for 15 days	

[a]Key: no., number; pts, patients.

likely to suffer severe forms of COVID-19 infection than men, which might result from differences in ACE2 expression.[59] It can be related to different innate immunity, steroid hormones, and factors related to sex chromosomes.[60] The ACE2 receptor gene and other immunological systems genes are on the X chromosome, where women are heterozygous, and men are homozygous.[61] Also, there is evidence that estrogen reduces the expression of ACE2 receptors in females, while in males, the expression of ACE2 receptors is much higher due to a lower amount of estrogen. Therefore, women have a smaller volume of ACE2 receptors resulting in less virus loading, and as a result, a smaller risk of severe COVID-19. Besides, women have been found to have stronger immunity due to the activation of immune cells compared to men that may cause a stronger immune response in OE as an initial target of virus in the body and lead to more olfactory dysfunction.[59]

7. POTENTIAL THERAPEUTIC OPTIONS: FROM APPLIED TREATMENTS TO TISSUE ENGINEERING

COVID-19-related anosmia that is persistent for over two weeks is significant and warrants consideration for medical treatment. Currently, the efficacy of available therapeutic options for COVID-19-related anosmia is unknown, but certain treatments show potential in curing the dysfunction.[69] Table 3 demonstrates the studies investigating available therapies for COVID-19-related persistent anosmia. Olfactory exercises consisting of the repeat and deliberate sniffing of odors including lemon, rose, cloves, and eucalyptus have been shown to symptomatically improve the dysfunction.[70]

Oral and intranasal corticosteroids have been proposed for the treatment of postinfection anosmia. However, in the absence of demonstrable inflammatory disease observed with endoscopy or imaging, it is unlikely that treatment with corticosteroids would benefit. In infected cases, corticosteroid treatment can manage the initial step of disease and prevent inflammation in the olfactory system. Nanotechnology provides a safe and controlled drug delivery system for the treatment of COVID-19-related anosmia. The fabrication of smart drug carriers can provide drug release compatible with stages of disease that can minimize side effects to the olfactory systems; as an instance, one study was able to prepare pH-sensitive liposomes to identify areas of inflammation.[71]

This technique can be used to detect inflammation in the olfactory systems and release anti-inflammatory drugs such as corticosteroids in these defective sites to prevent further inflammation and treat long-term anosmia. Insulin therapy has been considered for the treatment of anosmia and hyposmia, and several studies have investigated the intranasal effects of insulin with positive results.[72] Consequently, we can use intranasal insulin for the treatment of COVID-19-related anosmia.[73] Research has shown formulated intranasal insulin fast-dissolving films can be used for the treatment of anosmia in patients following COVID-19 infection. Current clinical studies have exhibited a significant increase in olfactory discrimination values and olfactory detection scores in patients treated with the aforementioned formulated intranasal insulin fat-dissolving films.[74]

Tissue engineering is a promising approach for the regeneration of damaged tissue.[75,76] Scaffolds play an essential role in the regeneration of tissues and organs in tissue engineering based-treatments; therefore, the selection of an appropriate material is essential for synthesizing suitable scaffolds.[75,77] Glycosaminoglycans (GAGs) have a role in axon guidance and cell differentiation and are abundant in the native ON of rats. Briefly, GAGs bind to the insulin-like growth factor-binding protein-2 (IGFBP2) that permits focal concentration of IGFBP2 bound insulin-like growth factors (IGFs) in the pericellular environment and modulates the interaction of IGFs with their receptors, regulating IGF biological activity. Several biopolymers, including chitosan, alginate, and hydronic acid, can affect cellular function similarly to GAGs.[78] Studies show chitosan to be a suitable candidate to promote the regeneration of olfactory neuroepithelial cells.[79,80] The effect of chitosan was investigated on a 3-methylindole-induced anosmic rat model, and its ability to regenerate ON and additional results show it regulates ON homeostasis and reduces ORN apoptosis. Electroconductive scaffolds have also been studied in regulating olfactory cells. Fengyu et al. synthesized a biodegradable conductive composite using conductive polypyrrole and biodegradable chitosan to stimulate olfactory ensheathing cells electrically.[81] The scaffold supported cell adhesion, spreading, and proliferation, and the electrical stimulation-induced scaffolds positively influenced neurotrophin secretion in olfactory ensheathing cells.[82] Further

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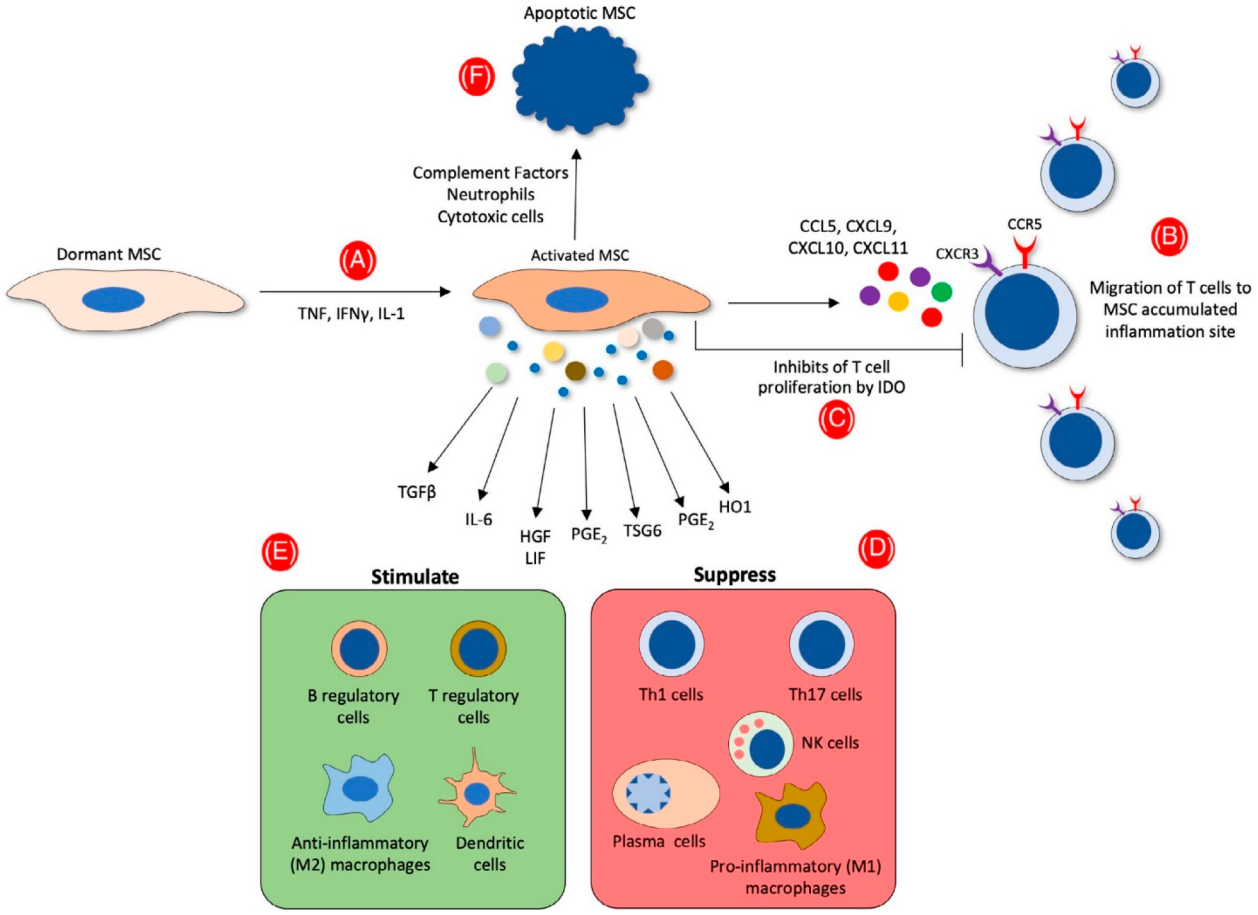

Figure 4. MSCs interact with the inflammation microenvironment and produce TNF, IFN-γ, and IL-1. Two major events are triggered: (B) MSCs produce CCL5, CXCL9, CXCL10, and CXCL11 and cause T cells to be required and (C) with IDO secretion, the proliferation and activity of T cells are suppressed. Reproduced with permission from ref 53. Copyright 2020 The Authors. Stem Cells Translational Medicine published by Wiley Periodicals LLC on behalf of AlphaMed Press.

research could prove a promising approach for the treatment of long-term anosmia using tissue engineering.[83,84]

Stem cell therapeutics would provide a novel approach for the treatment of COVID-19-related anosmia. Adipose-derived mesenchymal stem cells (ASCs) have been investigated for olfactory function restoration in rats, and results showed regeneration of the olfactory neuroepithelium.[85] Studies showed the anti-inflammatory effect of stem cells and the promotion of immune cells apoptosis. When inflammatory factors are released from damaged tissues, the mesenchymal stem cells (MSCs) sense and migrate toward inflammatory signals and express receptors for chemokines. Innate immune cells at the site of inflammation, including neutrophils, macrophages, myeloid-derived suppressor cells, mast cells, dendritic cells, and natural killer (NK) cells, can be synchronized by MSCs and chemokine expression variations and can impact MSC function. The MSCs assist the transition of monocyte-to-macrophage, and their microbicidal responses were potentiated. These cells attenuate the already activated pro-inflammatory macrophages and enhance anti-inflammatory activation through delivering immunosuppressive molecules and metabolites, such as TNF, IFN-γ, and IL-1. The MSC cells then secrete CCL5, CXCL9, CXCL10, CXCL11 and cause T cells migration to the site of inflammation and expression of related receptors so that the activity of T cells is blocked (Figure 4).[53] Current research proves promising for stem cell therapy as a potential treatment approach for COVID-19-related anosmia.

Gene therapy might be a promising treatment strategy for application in COVID-19-related anosmia treatment. Chitinase-like protein Ym2 has been shown to support cells that potentiate OE regeneration with expression of this protein rising after OE injury. There is evidence that a drop in Ym2 expression delays in OE regeneration, and so in turn, inducing Ym2 overexpression would counteract this and cause regeneration of OE.[86] Therefore, it is suggested to consider this protein as a possible strategy for gene therapy-related treatment to manage anosmia in COVID-19 patients. Designing small interfering RNAs (siRNAs), a type of double-stranded noncoding RNA molecule that controls gene expression, which can upregulate the expression of Ym2 would encourage OE regeneration.[87] The siRNAs successfully target highly conserved SARS-CoV-2 sequences, including RNA polymerase, helicase, and proteolytic enzymes, resulting in a 95% reduction in viral load. Various delivery systems have been identified for the delivery of siRNAs to the targeted tissue. Nanoparticle-based carriers and viral vectors could be used for siRNA-based drug delivery onto olfactory epithelial cells to prevent SARS-CoV-2 or other viral outbreaks. For the treatment of COVID-19-related anosmia, an inhaled siRNA formulation can be developed as a promising treatment option.[88]

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Intranasal injection of supportive factors to olfactory regeneration provides an alternative approach for the treatment of persistent COVID-19-related anosmia. The results of a study of the intranasal administration in mice of a hydrogel containing fibroblast growth factor-2 and IGF-1 in gelatin found an increase in the number of olfactory marker protein-positive cells, resulting in an enhanced thickness of OE.[89] Furthermore, combined VEGF/PDGF increases olfactory regeneration in mice with bulbectomy through an increment in the number of immature neurons and mature olfactory neurons, hence extending regenerating axons and reducing the astrocytic glial scar.[89]

Among these potential treatment options for OE regeneration, tissue engineering proves to be the most promising treatment due to the appropriate application of polysaccharide-based materials such as chitosan. These biomaterials are inexpensive and easy to process, making them more favorable than gene or cell therapy, which are more expensive and require more research to further analyze safety risks.

8. CONCLUSION

Based on the current literature reviews and our own clinical experience, the concluding outcome is that a significantly high percentage of COVID-19 patients with SARS-CoV-2 infection have symptoms of anosmia, of which the cellular and molecular mechanisms remain unclear. With ACE2 receptor expression levels significantly high in the OE, inflammation in this area can be one of the main reasons for the cause of anosmia. Although the ONs do not have ACE2 receptors, inflammation may propagate to these cells or stem cells through supported cells and cause damage to the olfactory bulb and central brain systems, hence resulting in anosmia. The majority of COVID-19-related anosmia cases recover rapidly, and OE injury and inflammation or changes in olfactory cilia and odor transmission are likely to be the most crucial causes for this anosmia. Currently, therapy is considered only if the anosmia persists for more than two weeks. Treatment options include olfactory exercises, intranasal or oral corticosteroids, and intranasal sodium citrate. There are several promising novel therapeutic options under development, including tissue engineering and stem cell therapy.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ACE2, angiotensin-converting enzyme 2; OE, olfactory epithelium; OSNs, olfactory sensory neurons; CNS, central nervous system; SARS-CoV-2, COVID-19 virus; RNA, ribonucleotide acid; siRNAs, small interfering RNAs; S protein, glycoprotein spike protein; RBD, receptor-binding domain; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; ON, olfactory neurons; SUS, sustentacular cells; ORNs, olfactory receptor neurons; MVCs, microvillar cells; GAGs, glycosaminoglycans; IGFBP2, insulin-like growth factor-binding protein-2; IGFs, insulin-like growth factors; ASCs, adipose-derived mesenchymal stem cells; MSCs, mesenchymal stem cells; NK, natural killer

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