Upper Airway Stem Cells: Understanding the Nose and Role for Future Cell Therapy

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Abstract The nose together with the paranasal sinuses has an approximate surface area of 100 to 200 cm² in adults, which is lined with pseudostratified columnar ciliated epithelium. It serves several important physiological functions such as conditioning and filtration of the inspired air and the provision of end organ for the sense of smell. It is also a physical and immunological barrier as it is the first site of interaction between the host tissue and foreign invaders (viruses, bacteria, and allergens). Our understanding of the complex cellular events occurring in response to inhaled agents during the development of common airway diseases has been significantly enhanced by the current status of in vivo and in vitro nasal experimental models. This will allow the development of novel therapeutic strategies designed to improve the physiological and immune defense functions of the nasal epithelium, as well as novel therapies for other common nasal diseases.

Keywords Common nasal disease · Nasal epithelium · Host defense · Human nasal epithelial stem/progenitor cells (hNESPCs) · In vitro nasal experimental models

Introduction

The diseases of the upper airway, such as rhinitis (allergic and infectious) and rhinosinusitis are the most common health problems worldwide, affecting millions of people of all ages. Although it has now been well defined that mucosal inflammation is the principal condition in these diseases, the etiology and pathogenic mechanisms underlying the development and progress of these nasal diseases are still incompletely understood. They can be influenced by multiple risk factors including gene-gene and gene-environment interactions. For example, chronic rhinosinusitis (CRS) with and without nasal polyps (CRSwNP and CRSsNP) can be described as a dysfunctional host-environment interaction that occurs in the nose and paranasal sinuses [1••].

The nasal cavity is lined with pseudostratified columnar ciliated epithelium, which is also found in the lining of the trachea and upper respiratory tract. In recent years, we have seen several advances in our understanding of the host defense mechanisms of the nasal epithelium. The combined function of ciliated epithelial and secretory cells maintaining efficient mucociliary clearance and a variety of other host defense mechanisms can be considered a soldier in the fight against airborne pathogens [2••]. In addition, a new concept on the “immune barrier hypothesis” proposes that defects in the coordinated mechanical barrier and/or the innate immune response of the sinonasal epithelium manifest as CRS [3]. This concept has been confirmed based on respiratory epithelial cells which are the primary targets of the common viruses (influenza virus, respiratory syncytial virus [RSV], adenovirus, rhinovirus, coronavirus, Coxsackie virus, and
ly laminar flow at the vestibule to a highly turbulent flow. Aerodynamics of airflow changes significantly from a relative-interior structure and typical shape of the human nose, the airflow rates may be as large as 150 L/min [5]. Due to the increase to 40 L/min with physical exercise, but extreme adult can range from 5 to 12 L/min for calm breathing and cavities to the lungs. Normal inspiratory nasal airflow for an approximately 10 to 20,000 L of air move daily through the nasal cavities. The nose is known to be important in ventilation as approximately 10 to 20,000 L of air move daily through the nasal cavities to the lungs. Normal inspiratory nasal airflow for an adult can range from 5 to 12 L/min for calm breathing and increase to 40 L/min with physical exercise, but extreme airflow rates may be as large as 150 L/min [5]. Due to the interior structure and typical shape of the human nose, the aerodynamics of airflow changes significantly from a relatively laminar flow at the vestibule to a highly turbulent flow anterior to the head of the inferior turbinate [6]. This is critical to facilitating mucosal contact for heating/cooling, humidification, and filtration of inspired air, which is necessary in conjunction with the functions of the nervous system, vasculature, secretory tissue, and mucociliary clearance of the nasal mucosa.

The mucosal lining of the nasal cavity covers an area of 100–200 cm², extends into the sinuses, and is coated by a layer of mucus 10- to 15-μm thick, which is supplied by goblet cells in the epithelium and submucosal seromucous glands. This will produce 100–200 mL of mucus over 24 h in a resting rate [7]. Epithelial cells, composed of basal cells, goblet cells, and ciliated or non-ciliated columnar cells, are attached to their neighbors by cell-cell junctions, including tight junctions (TJs), adherens junctions (AJs), gap junctions, and desmosomes, which are central components of the physical barrier [2••, 8]. Mucociliary clearance or sometimes referred to as the mucociliary apparatus is the process by which cilia of the nasal epithelial cells transport the viscous mucus blanket of the upper airway to the gastrointestinal tract. In the healthy nose, over 80–90 % of small particles (e.g., 10 μm) in the inhalant air are trapped on the surface of the nasal mucosa [9] and are transported by the mucociliary apparatus to the pharynx where they are either swallowed or coughed up. This phenomenon supports the claim that the nasal epithelium is exposed first, and to a greater extent than that of the bronchial epithelium, to all environmental agents, including infectious agents (e.g., viruses, bacteria, and fungi), allergens, and air pollutants, thus protecting the lower airways [10•].

In addition to the physical barrier, nasal epithelial cells are known to play an active role in both the innate and acquired immune responses, which have been summarized in the European Position paper on rhinosinusitis and nasal polyps 2012 (EPOS 2012) [1••] as (1) expressing membrane-bound and cytoplasmic pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPS), which are conserved molecular patterns found in parasites, viruses, yeasts, bacteria, and mycobacteria; (2) secreting a vast arsenal of host defense molecules, such as antimicrobial molecules in several classes of enzymes (lysozyme, chitinases, and peroxidases), opsonins (complement and pentraxin-3), permeabilizing proteins (A defensins, B defensins, and cathelicidins such as LL-37), collectins (surfactant protein-A, surfactant protein-D, and mannose-binding lectin), and binding proteins (lactoferrin and mucins); (3) producing a variety of inflammatory cytokines, such as IL-1, TNF-α, IFNα/β, GM-CSF, eotaxins, RANTES, IP-10, IL-6, IL-8, GRO-α, MDC, SCF, TARC, MCP-4, BAFF, osteopontin, IL-25, IL-32, IL-33, and thymic stromal lymphopoietin (TSLP), in response to stimulation of the antigens. Many of these cytokines have chemokine properties that attract various leukocytes including eosinophils, mast cells, neutrophils, dendritic cells, and lymphocytes. Some cytokines are also believed to play a key role in dendritic cell polarization, shaping the nature of the T cell response to antigens; and (4) other molecules and enzymes in response to antigenic stimuli.

### Function of the Nose

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### Role of Nasal Epithelium in Common Upper Airway Diseases

It has been reported that in lower airway diseases (e.g., asthma, bronchiolitis obliterans, chronic obstructive pulmonary disease, and cystic fibrosis), the pseudostratified airway surface epithelium is severely damaged and must regenerate to restore its defense functions [11]. The same results have also been found in the sinonasal epithelial cells from CRSwNP, such as up-regulation of MUC1, MUC4, and MUC8 [12, 13]; down-regulation of MUC5AC [12, 13] and TLR9 [14]; up-expression of VPF/VEGF [15, 16] and IL-37 [17, 18]; increased production of GM-CFS [19]; and staph invasion of sinonasal epithelial cells [20]. It is interesting that during the recovery or resolution phase of viral infections in the lower airways, the immune system must help to orchestrate tissue repair to restore normal lung architecture and function and prevent permanent defects in respiratory function [4••].

### Viral Infection

Infection of the respiratory tract by viruses is one of the most common health problems seen, affecting millions of people annually. Respiratory viruses include rhinoviruses,
coronaviruses, RSV, influenza viruses, parainfluenza viruses, and adenoviruses [1••]. Among these, rhinoviruses, influenza viruses, and RSV have been intensively investigated, and their mode of epithelial injury is well understood [21–24]. It has been reported in our previous review [25•] that respiratory viruses infect epithelial cells via cell surface receptors (e.g., ICAM-1, TLR3, α-2,3-SA/α-2,6-SA, RIG-I, and MDA5), up-regulate receptor expression of ICAM-1 via IL-1β and nuclear factor (NF)-κB-dependent mechanisms, and enhance release of inflammatory mediators (e.g., IL-6, IL-8, IL-1α, IP-10, TNFα, RANTES, IRF7, TGF-β1, etc.) with subsequent transcription, replication, virus assembly, and release. After infection of adjacent epithelial cells, damage to tight junctions, membrane disruption, and cell death occur.

The role and mechanisms dominated by the epithelium may have an important role in the host response to a viral infection as it occurs in the initial stages of viral infections in susceptible hosts. Respiratory epithelial cells are able to recognize viruses through PRRs, such as Toll-like receptors (TLRs), which play a crucial role in the initiation of immune responses in the respiratory epithelium and which lead to the induction of type I IFNs [2••, 4••, 27]. Type I IFNs (known as IFN-α and IFN-β) were among the first antiviral agents to be discovered and are classified as type III IFNs, such as IFN-α1/IL-29, IFN-α2/IL-28A, and IFN-α3/IL-28B, which possess antiviral properties similar to those of type I IFNs but appear to be expressed especially by epithelial cells and consequently exert host protection primarily at epithelial surfaces [2••, 28, 29]. It has been reported that the pandemic influenza virus (pH1N1) in 2009 was found to induce type I and type III IFNs and was extremely sensitive to the antiviral actions of type I and type III IFNs [30]. Therefore, an understanding of the mechanisms by which viruses interfere with epithelial innate and adaptive immune responses might contribute to the design of new therapeutic tools to treat or prevent respiratory disorders caused by viral infections [2••].

Allergic Rhinitis

The development of allergic rhinitis (AR) is known to be due to a complex interaction between environmental and genetic factors. During the past few decades, there has been significant progress in understanding the IgE-mediated immunologic mechanisms which play a key role by triggering the release of mediators (e.g., histamine) which are responsible for allergic symptoms. In addition, transendothelial migration of inflammatory cells and their activation within the reactive tissue are characteristic features, which represent the result of a complex network of interactions between various mediators, cytokines, chemokines, and adhesion molecules. However, our understanding of the disease origin and development of truly curative therapies have proved to be challenging and elusive goals [31].

An intact nasal epithelial barrier is known to be important in protecting against environmental agents, including allergens. Epithelium has emerged as an active and complex organ with mechanical, biochemical, and immunological functions [32]. It has been demonstrated by immune-electron microscopy that an in vivo birch pollen challenge in sensitized AR patients could lead to a very rapid binding of the allergens to at least 16 Bet v 1-binding proteins (e.g., ACTG, PLEC1, STML2, KCNA5, CALM, and ANXA2) in nasal epithelium, which then travel through the nasal epithelium via a lipid raft and caveolar-dependent process before binding to mast cells in the lamina propria [33]. However, the mechanism of how allergens travel through the nasal epithelium remains unknown. Other epithelial barrier markers, such as filaggrin (FLG), a member of the epidermal differentiation complex on chromosome 1q21, were reported to have polymorphisms and loss of functional mutations which were associated with atopic dermatitis and AR [34, 35].

Chronic Rhinosinusitis With and Without Nasal Polyps

CRS is characterized by chronic inflammation of the nose and the paranasal sinus mucosa that persists for at least 12 weeks. It can be divided into CRSwNP and CRSsNP [1••]. Both CRSsNP and CRSwNP are multifactorial diseases and are associated with a wide range of pathogenic risk factors, such as ciliary impairment or malfunction, allergy, asthma, aspirin sensitivity, immunocompromised state, genetic abnormalities, pregnancy and endocrine state, local host factors, biofilm,
environmental factors, iatrogenic factors, *Helicobacter pylori* and laryngopharyngeal reflux, osteitis, etc. [1••].

In the past few decades, large numbers of research data have been able to show infiltration and activation of various inflammatory cells in the sinonasal mucosa and defects in the host and adaptive immune defense functions, which play important roles in the pathogenesis of CRS. Identification of gene susceptibility to CRS as well as expression signatures and molecular pathways in CRS pathogenesis have also contributed significantly to a better understanding of the genetic and molecular alterations underlying CRS development and progression [10•, 36]. The major inflammatory manifestations of CRS include (1) increased infiltration of eosinophils, neutrophils, lymphocytes, and macrophages; (2) abnormal regulation of Th1, Th2, and regulatory T cell (Treg) gene expression; (3) defects in the epithelial barrier and in both innate and adaptive host defense functions; (4) epithelial damage followed by aberrant remodeling; (5) alterations of eicosanoid pathways; and (6) fibrosis or edema [10•]. However, the pathogenic mechanisms causing the initial process and sustaining the abnormal inflammation are incompletely understood. Future studies are needed to identify details in genetic interactions and the interacting pathways underlying CRS and to investigate the interactions with environmental factors that influence the complex pathology of CRS. Only then are we likely to have the understanding necessary for improved prevention, diagnosis, and treatment of CRS and NPs.

In both CRSwNP and CRSsNP, the epithelium is known to be structurally and functionally abnormal, which may be crucial in the development and progression of CRS. However, the underlying mechanisms leading to epithelial damage and formation of abnormal cilia remain unclear. In patients with CRSwNP, the epithelium appears to respond inappropriately to injury, and this can lead to aberrant epithelial damage including hyperplasia or squamous metaplasia [1••, 37, 38•]. Furthermore, goblet cell hyperplasia with excessive mucus production, abnormalities in cilia architecture, and function can be found in hyperplasia or squamous metaplasia of the nasal epithelium [1••, 37, 40, 41••]. These pathological findings are similar to that seen in asthma where the epithelium damage and more mucus-producing cells than normal make the airway epithelial barrier more permeable and more sensitive to oxidants resulting in a deficient innate immune response to respiratory viral infections compared with that seen in normal individuals [42•]. It is important that the potential for a susceptible epithelium and the underlying mesenchyme to create a microenvironment which enables a deviation of the immune and inflammatory responses to external stimuli thus being crucial in the development and progression of asthma [42•].

In a number of our recent studies, we were able to show (1) down-regulation of the AP-1 (c-Jun/c-Fos heterodimers) transcription factor and its associated genes (e.g., FOS, EGR1, AREG, HBEGF, IL6, and COX2) in nasal biopsies from CRSwNP were at least partially restored after oral steroid treatment [43]; (2) overexpression of p63 that is associated with the CRSwNP epithelial remodeling that was suppressed following oral steroid treatment [37•]; (3) a reduced protein expression level of epithelial membrane protein 1 (EMP1), which is an integral membrane glycoprotein in nasal epithelium correlated significantly with epithelial remodeling status in CRSwNP [38•]; and (4) motile cilia impairment with abnormal cilia architecture (untidy, overly dense, and lengthened) that is positively associated with increased levels of protein and mRNA and with ciliogenesis-associated markers (CP110, FoxJ1, and TAP73) in CRSwNP [41••]. Our data suggest a new possibility that abnormal mucociliary clearance associated with epithelial hyperplasia in airway diseases is likely due to the impairment of both cilia architecture and function, which could be a likely cause of chronic mucosal inflammation or infection (e.g., biofilm) observed in CRSwNP.
Nasal Epithelium and Epithelial Cells

Airway epithelium is central in respiratory disease, but it is notoriously difficult to distinguish between cause and effect with regard to the epithelium’s role in the context of diseases. It has been shown that airway epithelial cells play a key role in regulation of tissue homeostasis by the modulation of numerous molecules, from antioxidants and lipid mediators to growth factors, cytokines, and chemokines [44]. In addition, the airway epithelium is also able to suppress mechanisms...
involved in inflammation to maintain homeostasis [44]. Therefore, it is important to gain insights into the mechanism by which human nasal epithelial cells (hNECs) respond to various pathogens or antigens, where such an event might take place in nature. Moreover, in the complex interplay between hNECs and environmental pathogens, host factors play an important role in disease severity, progression, and response to pharmacological treatments.

One way to study an intrinsic alteration or defect of nasal epithelial cells in response to environmental risk factors is to isolate and grow nasal epithelial cells from healthy and diseased epithelium and to compare them under conditions that are conducive for normal cell growth and differentiation. Instead of using commercially available respiratory cell lines which have often undergone long-term in vitro cultivation and therefore may not represent the real biological features of hNECs, primary hNEC culture has been used in many studies. Briefly, the epithelial cells are enzymatically dissociated from nasal biopsies and seeded on cell culture flasks. After expanding the epithelial cells in mixed cell clusters (including inflammatory cells, submucosal glands, and fibroblasts) by using a monolayer culture method, the harvested cells are then transferred into an air-liquid interface (ALI) culture to form pseudostratified epithelium. This model has been successfully used in some studies such as a study that demonstrated intrinsic alterations in innate immune gene expression in CRS epithelial cells when grown in a 6-week culture in vitro [45]. There was increased CP110 expression in epithelial cells when grown in a 6-week culture in vitro and showed increased CP110 expression in epithelial cells when grown in a 6-week culture in vitro. In our recent study, we have been successful in isolating adult hNESPCs from nasal biopsies of healthy subjects and patients with CRSwNP [44, 49, 50, 51, 52]. Some key findings from these studies are summarized in Fig. 1a. Briefly, single-cell-derived colonies stain uniformly for basal cell markers such as p63 and keratin 5 (Krt5), and about 80 % of the colonies show potential for long-term self-renewal, because they can be propagated successively for at least an estimated 20–50 additional doublings, while maintaining an immature phenotype [51••]. Lineage potential has been assessed through multiple differentiation assays, in which the pedigree lines developed from single cells can differentiate into stratified mucociliary airway epithelium composed of both ciliated columnar cells and goblet cells. This in vitro culture system provides (1) cells in large numbers (both progenitor and differentiated hNECs), (2) cells which maintain their original biological characteristics, and (3) the in vitro differentiated epithelial cells are viable for more than 1 month in ALI culture. It opens up many research possibilities to understand the molecular mechanisms and pathways underlying both healthy and diseased nasal epithelium and to identify more targeted and cellular therapies for common nasal diseases.

### Nasal Epithelial Progenitor and Stem Cell Research

During the past decade, there is a great interest in the biology of adult stem cells because of their capacity to self-renew and high plasticity. Different methods for in vitro culture of progenitor/stem cells have been developed, including the use of feeder layer cells, feeder layer cell-free conditions, extracellular matrix (ECM) molecules, and in the presence of diverse growth factors and cytokines. Thus, techniques in isolation and in vitro culture of human nasal epithelial stem/progenitor cells (hNESPCs) become feasible and will provide us with an in-depth understanding of the complex mechanisms of self-renewal, proliferation, differentiation, and epithelial remodeling that occurs during development and homeostasis with common infectious and inflammatory nasal diseases [11, 49, 50].

There are some potential limitations for primary hNECs studies in vitro, such as limited cell numbers and the lifespan of nasal epithelial cells using primary cell culture, high heterogeneity in endogenous gene expression levels, and varied pathogenesis among patients (donors). The choice of experimental methodology is also important as this can greatly influence the results and interpretation when comparing the expression profiles of innate immune genes between hNECs obtained from nasal brushes and from tissue biopsies [45]. It was reported in an early study that hNECs from NPs became squamous and lose their cilia within 2 to 6 weeks in monolayer cultures, while cilia reappeared after changing to suspension cultures [48]. Therefore, it is critical for in vitro studies to establish a hNEC culture model with enough living hNECs and to maintain them in an in vitro culture system for days or weeks without changing their primary biological characteristics.
positively with stem cell marker p63 and KRT5 and presented a consistent high Ki67 expression level over four passages. In contrast, hNESPCs from NPs showed a reduced growth and proliferation rate at each passage by evaluating colony-forming efficiency and doubling time and a lower percentage of Ki67+ cells among p63+ cells in the colonies in late passages. This was also confirmed by immunostaining in the NP specimens. These intrinsic differences in growth and proliferation properties could be an important pathological phenomenon in NPs [52••].

In another study, aberrant epithelial remodeling was found by histo- and immuno-histochemical evaluation to be associated with significant impairments of cilia architecture (untidy, overly dense, and lengthened) and reduced ciliary beating frequency (CBF) in chronically inflamed nasal mucosa of NPs. Further confirmation was performed in vitro with differentiated epithelial cells derived from hNESPCs in the same patients and healthy controls. The results from both in vivo and in vitro measurements are concordant and are associated with increased expression of ciliogenesis-associated markers (e.g., CP110, Foxj1, and TAP73) that confirm the cause of these pathological changes that is likely intrinsic (Fig. 1a) [41••]. These findings are of significant importance to our understanding of the clinical nature of NPs with persistent inflammation and their high recurrence even after maximal anti-inflammatory treatment and surgery. Hopefully, these results will aid in the discovery and development of more specific and directed treatments for NPs and for other inflammatory and hyperplastic airway diseases.

With the pandemic of SARS (in 2003) and H1N1 (in 2009), global concerns for viral airway infections has risen. In addition to continuous efforts in surveillance of naturally occurring viruses in humans, experimental studies elucidating susceptibilities to respiratory viruses and response to infection by human nasal epithelium, which is the primary target site for common cold and influenza viruses, are required. The nature and severity of viral infection observed is dependent on both the direct harmful effects of the virus itself and on the damage caused to host tissues as a consequence of the host immune response to the virus. Thus, hNESPCs and differentiated epithelial cells derived from hNESPCs in an ALI culture may offer possibilities in screening and quantitative assessment of the virulence of common viruses, and the host defense functions including viral fusion and uncoating, transport of viral ribonucleoprotein (RNP) complexes from the nucleus, replication, transcription and translation of the viral genome, export of viral RNP complexes from the nucleus, viral assembly and budding, and potential drug targets. This would provide us with a useful reference for public health risk assessment, interventions, disease control, and prognosis for viral upper respiratory infections. Figure 1b provides a schematic diagram of our current study protocol and some preliminary data elucidating viral infection in specific cell types at 24 h post viral infection. By using this model, we are able to confirm all reported and possible new specific markers (e.g., mediators, cytokines, chemokines, and receptors) from nasal epithelium at the different time points after viral infection (unpublished data) [2••, 4••, 25•, 26].

Conclusion

Nasal epithelium is a physical and immunological barrier as it is the first site of interaction between the host tissue and environmental risk factors. It is also known to play an important role in the innate and acquired immune response of the complex mechanisms which occur during development, homeostasis, and with common infectious and inflammatory nasal diseases. Following the recent advances in molecular and cell biology, nasal epithelial stem/progenitor cells research is now feasible with a significant implication for a better understanding of molecular mechanisms and pathways underlying both healthy and diseased nasal epithelium which will thus aid the preclinical development of novel therapies for common nasal diseases.

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Compliance with Ethics Guidelines

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