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REVIEW

Current Understanding of Nasal Epithelial Cell Mis-Differentiation

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Abstract: The functional role of the respiratory epithelium is to generate a physical barrier. In addition, the epithelium supports the innate and acquired immune system through various cytokines and chemokines. However, epithelial cells are also involved in the pathogenesis of various respiratory diseases, some of which are mediated by increased permeability of the mucosal membrane or disturbed mucociliary transport. In addition, it has been shown that epithelial cells are involved in the development of inflammatory respiratory diseases. The following review article focuses on the aspects of epithelial mis-differentiation, in particular with respect to nasal mucosal barrier function, epithelial immunogenicity, nasal epithelial–mesenchymal transition and nasal microbiome.

Keywords: nasal mucosal barrier function, tight junction, epithelial–mesenchymal transition, microbiome

Anatomy of Nasal Mucosa

Macroscopically, the nose consists of the external part and the internal part, the so-called nasal cavity, which is further divided by the nasal septum into two almost symmetrical halves. The nasal cavity includes various types of epithelium. At the atrium, it is lined with multilayered keratinized squamous epithelium. This area contains sebaceous and sweat glands, apocrine glands and vibrissae, which have a filter function. In the area of the inner nasal valve, the multi-layered squamous epithelium passes into a multi-row cylindrical epithelium. The main nasal cavity, an area of 140–172 cm^2, is completely covered by mucosa, which is divided into two distinct areas, the regio respiratoria 140–170 cm^2 and the regio olfactoria 2–2.5 cm^2. The regio olfactoria is located at the upper nasal concha and at the upper nasal septum, which is covered by olfactory epithelium.[1] The mucosa of the regio respiratoria has a double-row highly prismatic epithelium. The cells contain kinocilia, which beat in a coordinated manner. By this, mucus is transported towards the pharynx expressing the mucociliary clearance to clear the nasal cavity and the paranasal sinuses. Furthermore, respiratory mucosa contains mucus-producing goblet cells, a conspicuously thick basal lamina and an underlying, strongly vascularized lamina propria.[2] Additionally, this layer contains a special venous plexus, which contributes to temperature modification of inhaled air and to the regulation of the nasal cavity cross-sectional area.[3]

The functional role of the nasal epithelium is complex. Most important, it serves as a physical barrier. Furthermore, nasal mucosa produces various cytokines and chemokines and plays an important part in the control of the innate and acquired immune

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response.[4] In addition to the protective functions mentioned above, epithelial cells are also involved in the pathogenesis of various inflammatory respiratory diseases, which are partly mediated by an increased permeability of the mucosa. Decreased integrity of tight junctions, an impaired mucociliary transport and reduced production of antimicrobial peptides are relevant pathophysiological mechanisms.[5–7] Furthermore, it has been shown that epithelial cells with a disorder of innate immune receptors also contribute in the genesis of inflammatory respiratory diseases.[8] The following review article focuses on the aspects of epithelial misdifferentiation, especially with respect to nasal mucosal barrier function, epithelial immunogenicity, nasal epithelial–mesenchymal transition, and nasal microbiome.

Nasal Mucosal Barrier Function

The nasal mucosa represents an interface between the environment and the inside of the human organism. It is the first barrier against continuously inhaled substances such as pathogens and allergens. An important intrinsic defense system is the mucociliary clearance of the nasal cavity. Ciliary beat in a well-orchestrated and coordinated manner, which results in a wave motion leading to a successful elimination of foreign bodies.[9] The respiratory epithelium contains about 200 cilia per cell. These have nine peripheral microtubule pairs that surround a central microtubule pair, which leads to the well-known "9+2" arrangement of microtubules.[10] Chronic inflammation or locally applied medication can have negative effects on epithelium functions, which are associated with the disturbed or missing ciliary activity, epithelial metaplasia leading to an impaired mucociliary clearance. Thus, the integrity of the nasal protective mechanisms may be further compromised.[11] Other possible etiologic factors for nasal epithelia metaplasia are cigarette smoke, ozone, and heavy metals.[12] Chronic inflammation such as chronic rhinosinusitis (CRS) or asthma leads to epithelial damage resulting in increased paracellular permeability, impaired epithelial repair mechanisms and inflammation. Histologically, the respiratory epithelium changes into a hypersecretory mucus state with increased proliferation rates of goblet cells, hypertrophy of submucosal glands, basement membrane thickening, hypertrophy of smooth muscles, and a thick layer of mucus on the apical surface.[13,14]

The mechanical barrier of nasal mucosa results from the formation of tightly bounded cell-cell connections, which are mainly composed of tight junctions (TJ).[15] Further components are desmosomes, adhesion connections and gap junctions.[16] TJ were visualized at the ultrastructural level in 1963 by Farquhar and Palade.[17] TJ separate the apical from the basolateral surface and in addition, they close the intercellular space. In this manner, they form a paracellular barrier, which controls the flow of molecules, ions and dissolved substances.[18] Zonula occludens proteins (ZO) connect the transmembrane proteins of the TJ with the cytoskeleton of the cell. The main components of TJ are claudin, tight junction-associated marvel domain-containing proteins (TAMPs) with its three family members occluding, tricellulin and MarvelD3, junctional adhesion molecules (JAM) and in a broader sense membrane-associated scaffold proteins.[19,20]

The claudin family, comprising 27 members, are transmembrane proteins that form the structural basis for a close TJ connection. Typically, claudins have a unique secondary protein structure: four transmembrane (TM) domains, N- and C-terminal domains aligned to the cytosol, two extracellular domains, and a short intracellular loop.[21–23] Functionally, the main task of the claudins is the formation of the paracellular TJ barrier and therefore the key position with regard to the permeability of individual epithelia. In addition, claudins are categorized according to their abilities, ie, formation of paracellular channels (pore-forming) and restriction of paracellular permeability (sealing claudins).[24,25] This emphasizes the different characteristics of the individual claudin family members with regard to their barrier properties. Some claudin subtypes, such as claudin-3, −4, −5, and −8, are mainly detectable in impermeable epithelial cells.[26–28] Other claudin species, such as claudin-2, are found in permeable epithelia like the surface epithelium of duodenum, ileum and jejunum.[29] This shows the different role of claudins in the epithelial barrier functionality.

The MAL (myelin and lymphocytes protein) and related proteins for vesicle trafficking and membrane link (Marvel) 1, 2 and 3 form the tight junction-associated Marvel protein (TAMP) family, which has in common a conversed four-transmembrane Marvel domain. Members of this family, which are characterized by close connections, are occludin (MarvelD1), tricellulin (MarvelD2) and MarvelD3.[20,30–32]

The function of occludin, which was discovered as the first transmembrane TJ protein in 1993, is still controversially discussed.[30,33] This discussion is further reinforced by diverging results from in vitro and in vivo studies. Occludin-deficient mice, for example, show, on the one hand, an unchanged TJ structure combined with an undisturbed epithelial barrier function. On the other hand, the same defect was related to significant phenotypic changes regarding hyperplasia and inflammation of the

gastrointestinal epithelium as well as cerebral calcifications.[34,35] Saitou and colleagues could even show that occludin-disturbed embryonic stem cells were able to generate polarized epithelial cells with an intact functional barrier.[36] Other studies, in turn, demonstrated that the expression level of occluding is closely associated with the barrier properties of epithelial cells. This is achieved via an interaction between occluding and ZO-1.[37]

Various diseases such as inflammatory bowel disease or celiac disease can destroy the epithelial barrier function. In addition, allergy-driven processes can induce alterations of the TJ as well, which in turn lead to a compromised epithelial barrier function. Probst and colleagues investigated the epithelial barrier restriction as a function of house dust mite (HDM) extract exposure. They were able to show an increase in the permeability of the epithelium induced by HDM extract.[38] Furthermore, genetic factors might influence the weakness of TJ as well, which leads to an impaired barrier function.[39] Regarding experimental settings, in vitro models for the analysis of barrier function are of particular interest, as they can be used to investigate the interaction between functional deficits related to pathogens. Transepithelial electrical resistance (TEER) is a sensitive and reliable method for the evaluation of epithelial integrity and permeability. In addition, the TEER method has the advantage that vital cells can be studied non-invasively in vitro at different phases of cell differentiation and growth phase.[40] Furthermore, this method can be used in order to study the effects of therapeutics on the barrier function in vitro as well.

Nasal Mucosal Epithelial–Mesenchymal Transition (EMT)

Epithelial–mesenchymal transition (EMT) was first described in 1982 by Hay et al.[41] It defines a biological process in which biochemical alterations of epithelial cells lead to mesenchymal characteristics. During gastrulation and organogenesis, epithelial cells have to detach from their united cell structure and migrate through mesenchymal tissue layers in order to form tissues and organs. For this propose, cells must gain the properties to move, penetrate and decompose extracellular-matrix constituents.[42,43] From a biological point of view, EMT can be divided into three subtypes: Type I occurs during embryogenesis; type II takes place during wound healing and type III occurs during metastasis of carcinomas.[44] Expression and activation of EMT-inducing transcription factors occurs in response to various signaling pathways, including those mediated by transforming growth-factor β (TGF-β), bone morphogenetic protein (BMP), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), Wnt, Sonic Hedgehog (SHH), Notch, and integrin signaling.[45–47] Many EMT-inducing signals tend to be cell- and tissue-type-specific. This implies that cells integrate certain signals in different forms depending on their microenvironment and cell state.[42] The following transcription factors can induce EMT: zinc-finger binding transcription factors Snail1 and Snail2, basic helix-loop-helix (bHLH) factors such as zinc-finger E-box–binding homeobox 1 (ZEB1), ZEB2, Twist, and T cell factor (TCF) transcription factor family member called lymphoid enhancer-binding factor-1 (LEF-1).[47] The common feature of all three types of EMT is the loss of epithelial characteristics and the appropriation of mesenchymal characteristics. The loss of epithelial cell markers such as decreased expression of E-cadherin, a protein responsible for maintaining lateral contacts between epithelial cells, is the first step in EMT.[44] Parallel to that, the once epithelial cells gain mesenchymal phenotypic characteristics by expression of markers such as neuronal cadherin (N-cadherin), vimentin, integrin, fibronectin and matrix metalloproteinases (MMPs).[48]

EMT can occur during a chronic inflammation including CRS. Pathological repeated epithelial injuries lead to EMT, which in turn contributes to tissue fibrosis.[49] In a study, Hackett and colleagues were able to show that TGF-β1 stimulates EMT in bronchial epithelial cells through a Smad3-dependent process.[50] Since a number of respiratory diseases tend to involve the lower (bronchial) and upper (sinonasal) respiratory mucosa, this finding suggests that sinonasal remodeling and EMT may have common features at the molecular level.[51] The induction of EMT by TGF-β1 could be inhibited in an in vitro study via the c-Src pathway.[52] As early as 1993, Lyons and colleagues were able to show that after treatment of rat mucosal keratinocytes with EGFR ligands and inflammatory cytokines such as TGF-β or interleukin 1 beta (IL1β), an induction of EMT-associated protein expression MMP-9 and MMP-13 as well as EMT-like changes in cell morphology occur.[44,53] TGF- β and EGF appear to work together synergistically in EMT induction.[54,55] Another research group was able to show an association between CRS and an increased expression of vimentin in the nasal epithelium. In this study, MMP-9 was suspected to be a factor for the increased rate of EMT.[56] Allergy as a widespread disease with inflammation induction also

leads to airway remodeling and fibrosis via EMT. Johnson and colleagues were able to show that exposure of the nasal mucosa to HDM extract caused significant inflammation of the epithelial cells. This resulted in an enhanced expression of TGF-β 1, vimentin, alpha-smooth muscle actin (α-SMA), pro-collagen I combined with a reduction of E-cadherin and occludin synthesis.[57] One possible signaling pathway in these circumstances may be the activation of the Sonic Hedgehog (SHH) pathway. In a publication presented by Zou and colleagues, an activation of the SHH pathway in human bronchial cells after exposure to HDM and TGF-β1 was presented, leading to EMT. The SHH pathway is activated during lung repair as well as in the development of the lung. It results in the nuclear translocation of the transcription factor glioma-associated antigen (Gli). By blocking the SHH signaling pathway with Small-interfering RNA (siRNA) for Gli1, the HDM-induced EMT in human bronchial epithelial cells could be reversed.[58] Thus, for future therapy of chronic inflammatory diseases such as CRS or asthma, molecular manipulation of signaling pathways such as SHH could offer promising approaches. In another publication, it was shown that the expression of snail and MMP-9 was reduced by knockout of Gli1, while the expression of E-cadherin increased.[59]

Hence, EMT acts as an important factor in the remodeling processes of the epithelium of human nasal cells during CRS.[60] Yan and colleagues investigated the differences in EMT formation between eosinophilic CRS with nasal polyps (CRSwNP) and healthy control group with non-eosinophilic CRSwNP. They were able to show that the rate of EMT in the group of eosinophilic CRSwNP was significantly higher than in the control group. There was also a difference in the expression of remodeling-associated molecules such as MMPs, tissue inhibitor of metalloproteinases (TIMPs) and TGF-β family members.[61] BMP as a member of TGF-β superfamily also plays a special role in the regulation of epithelial regeneration after acute damage. This was shown in a study by Masterson and colleagues, in which respiratory cells in vitro were acutely damaged and subsequently exposed to BMP4. BMP4 induced a mesenchymal-like cell morphology associated with a reduction of E-cadherin and an increase in cell motility in terms of EMT induction.[62]

In fact, various changes occur within the remodeling processes in CRS, which include fibrosis, alteration of the epithelium, thickening of the basement membrane, hyperplasia of the goblet cells, infiltrates of inflammatory cells and angiogenesis.[63–65] This alteration during EMT might appear in patients suffering from chronic airway diseases like asthma, chronic pulmonary disease and bronchiolitis obliterans.[66] Besides TGF-β and MMPs, other proteins such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, epithelial growth factor, and fibroblast growth factor-2 can induce EMT as well.[67,68] Furthermore, clinical factors, such as hypoxia, which is one of the major risk factors for CRS can induce EMT. Shin and colleagues were able to show that hypoxia-inducible factor (HIF)-1α and HIF-2α are expressed in the nasal epithelium. In this study, they also found that HIF-1α, instead of HIF-2α, determined E-cadherin loss in hypoxia.[51]

Nasal Epithelial Innate Immunogenicity

The mechanical barrier function of the nose has been known for a long time, but its immunogenic function has been the subject of new findings in recent decades, which show that the nasal mucosa is an amazingly active participant in the innate immunity of the respiratory tract. However, the detailed function of this immunogenicity is not fully understood. As part of the innate immune defense, the nasal mucosa includes receptors for the identification of pathogenic structures of microorganisms, fungi and viruses. Further mechanisms are chemical components such as antimicrobial peptides and cellular components such as neutrophil granulocytes, macrophages and dendritic cells.[69–71] Although pathogens can be distinguished from non-pathogens, innate immunity is relatively unspecific compared to the adaptive immune response. The recognition of pathogens is achieved by pattern-recognition receptors (PRRs) on the mucosal surface, which were first described by Charles Janeway Jr. PRRs can be divided into three large subunits, namely the Toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs).[72]

TLRs are transmembrane proteins with an extra- and intracellular domain. The extracellular domain is responsible for the registration of pathogen-associated molecular patterns (PAMPs) and the cytoplasmic domain that indicates downstream signaling.[73] PAMPs are uniquely expressed in microbes but not in vertebrates. Thus, after recognition, an immune response is initiated during an infection.[74] To date, 10 different TLRs have been identified, which can be divided

into two main groups based on their localization. It is known that TLRs 1, 2, 4, 5, 6 and 11 are located on the cell surface and recognize PAMPs derived from bacteria, fungi and protozoa, whereas TLRs 3, 7, 8 and 9 are exclusively expressed within endocytic compartments and primarily recognize nucleic acid PAMPs derived from various viruses and certain bacteria.[75,76]

The RLRs family includes RIG-I, MDA5 and LGP2, which recognizes RNA-viruses in the cytoplasm of infected cells and induce inflammatory cytokines and type I interferons. Inflammatory cytokines primarily coordinate innate immune responses by recruiting immune cells such as macrophages and dendritic cells.[75,77] Type I interferons bind directly to infected cells and initiate the transcription of multiple interferon-stimulated genes (ISGs), inducing an antiviral state in all infected cells through altering cellular processes. This inhibits viral replication and induces apoptosis of the infected cells. In addition, the lytic capacity of natural killer cells is increased, expression of MHC class 1 molecules is upregulated and the adaptive immune response is activated.[75]

NLRs are intracellular PRRs that induce an immune response after the detection of PAMPs or damage-associated molecular patterns (DAMPs). The activation of NLRs shows different functions, which can be divided into four main categories: inflammasome formation, signaling transduction, transcription activation, and autophagy. In humans, 22 NLRs are known, and the association malfunction with human diseases reflect their vital role in host defense.[78] NLRs are able to recognize different ligands of pathogenes. However, some of the NLRs do not act as PRRS but instead react to cytokines such as interferons.[79] The role of PRRs in epithelial dysfunction is crucial. This can be illustrated by various examples. It is known that the CRS can generally be divided into two different major phenotypes: CRS with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP). Although the etiopathology is still unclear, the influence of lymphocytes, especially T-cells, on the maintenance of chronic inflammation in the nasal mucosa is becoming increasingly evident. The CRSwNP is characterized by a T-helper (TH)-2 cells and CRSsNP by a mixed TH1- and TH17-cell answer.[80] Furthermore, allergy-driven rhinosinusitis is characterized by an increased eosinophil-triggered inflammation, which is believed to be secondary to the influx of CD4+ TH-2 cells. Thus, in a murine model for allergic rhinitis, Hussain et al were able to show that treatment of BALB/c mice with TLR9 agonist CpG significantly decreased allergic symptoms during ovalbumin sensitization. These effects were attributed to a decreased eosinophilic inflammatory response associated with reduced IL4 and IL5 levels.[81] Another study by Hammed and colleagues showed the link between TLR4 and HDM-driven allergic airway inflammation. In this study, TLR4 triggering caused the production of the innate pro-allergic cytokines: thymic stromal lymphopoietin, granulocyte-macrophage colony-stimulating factor, IL25, and IL33.[82] These effects were reversed by adding a TLR4 antagonist. NLRs are as well responsible for the maintenance of mucosal inflammation in the presence of PAMPs. A study by Hysi and colleagues showed a correlation between asthma and insertion-deletion polymorphism of the NOD1 gene. In addition, the authors found increased IgE levels.[83] We can, therefore, state that epithelial cells of the upper respiratory tract react to stimulation by PAMP and DAMP with the secretion of inflammatory cytokines. Hammad and colleagues were able to show that some of these cytokines play an important role in the polarization of dendritic cells and thus have an influence on the T-cell response to antigens. These cytokines include IL25 and IL33, which also have the ability to influence T-cells into a TH2 profile.[84] According to Bachert, it can be assumed that epithelial cells are not only significantly involved in mediating reactions of the innate immune defense but also influence the subsequent adaptive immune defense. Furthermore, it cannot be excluded that the etiology and pathogenesis of CRS are based on primary variations of the reaction pattern of epithelial cells.[85]

Mucosal Inflammation and Microbiome

Some studies suggest a correlation between the microbiome and various diseases such as metabolic dysregulation, gastrointestinal diseases and infectious diseases. Trillions of microorganisms such as bacteria, fungi, and viruses populate the body of humans as well as animals. The microbiome consists of these microorganisms and their genes. The study of the microbiome in relation to anatomical localizations combined with diseases has significantly expanded the understanding of the physiological and pathological relationship within these symbiotic communities. Much more, these investigations have brought possible factors in the development of diseases due to dysbiotic changes into the focus of current research.[86] The vast majority of these microorganisms are symbionts. However, also potentially pathogenic microorganism can be part of the microbiome, which may play a role in the development of chronic inflammatory diseases and cancer.[87]

Microorganisms can trigger epithelial reactions like CRS. The relationship between airborne allergens such as pollen, fungus spores, HDM or pets, and respiratory allergic diseases is well understood. In a study, Eidi and colleagues investigated the presence of various fungal species in the nasal cavity and living space of the affected patients. They were able to detect different fungal species and concluded that the detection of fungi in the nasal cavity should be considered as a significant factor for health hazards.[88] Unfortunately, this study did not investigate the rate of chronic respiratory disease in relation to the fungal presence in the nasal cavity, which of course somewhat invalidates their conclusion. Carlson investigated the presence of bacterial population in patients with lung transplantation. The rational of this study was the limited long-term survival of these patients, which was mediated by infectious complications. They could find higher bacterial burden and frequent appearance of dominant organisms in bronchoalveolar lavage in transplanted patients compared to control. Furthermore, a higher rate of fungal population typically dominated by Candida and Aspergillus ssp. were present in transplanted patients as well.[89] There are some studies suggesting that microbial composition might affect the etiology or progression of CRS. In a study by Hoggard et al, the bacterial profile of patients with CRS was investigated. They could find, that dysbiotic bacterial composition was more frequent in subjects with comorbidities such as asthma and cystic fibrosis. The authors concluded that bacterial dysbiosis might play a role in the pathogenesis or influence the severity of CRS.[90] Choi and colleagues could show an altered nasal microbiome and decreased diversity in bacterial compositions as well as an increased S. aureus abundance in patients with CRSwNP.[91]

In summary, epithelial changes in the sense of epithelial mis-differentiation can be either endogenous or exogenously triggered. Under these circumstances, various changes might play an important role. The invalidation of the nasal mucosal barrier by chronic inflammation and associated changes in the histology and expression of proteins such as TJ play a significant role in the epithelial mis-differentiation. In addition, chronic inflammation such as CRS plays a role in EMT induction and is responsible for remodeling processes of the airway mucosa. The nasal epithelial innate immunogenicity, which is ensured by recognition of pathogens by PRRs on the mucosal surface and resulting in a cascade of cytokine release and alteration of the immune response is a pivotal player in the pathology of epithelial mis-differentiation. Important exogenous factors are allergens but also the microbiome. This review only gives an insight into a very complex pathological process that has not yet been fully understood. Further studies are warranted to clarify this fascinating and complex process.

Disclosure

The authors have no conflicts of interest to disclose.

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