REVIEW

First contact: the role of respiratory cilia in host-pathogen interactions in the airways

Li Eon Kuek¹ and Robert J. Lee¹,²

¹Department of Otorhinolaryngology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; and ²Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania

Submitted 15 June 2020; accepted in final form 28 July 2020

Kuek LE, Lee RJ. First contact: the role of respiratory cilia in host-pathogen interactions in the airways. Am J Physiol Lung Cell Mol Physiol 319: L603–L619, 2020. First published August 12, 2020; doi:10.1152/ajplung.00283.2020.—Respiratory cilia are the driving force of the mucociliary escalator, working in conjunction with secreted airway mucus to clear inhaled debris and pathogens from the conducting airways. Respiratory cilia are also one of the first contact points between host and inhaled pathogens. Impaired ciliary function is a common pathological feature in patients with chronic airway diseases, increasing susceptibility to respiratory infections. Common respiratory pathogens, including viruses, bacteria, and fungi, have been shown to target cilia and/or ciliated airway epithelial cells, resulting in a disruption of mucociliary clearance that may facilitate host infection. Despite being an integral component of airway innate immunity, the role of respiratory cilia and their clinical significance during airway infections are still poorly understood. This review examines the expression, structure, and function of respiratory cilia during pathogenic infection of the airways. This review also discusses specific known points of interaction of bacteria, fungi, and viruses with respiratory cilia function. The emerging biological functions of motile cilia relating to intracellular signaling and their potential immunoregulatory roles during infection will also be discussed.

Aspergillus; chronic rhinosinusitis; coronavirus; cystic fibrosis; Hemophilus influenzae; influenza; lung epithelium; primary ciliary dyskinesia; Pseudomonas aeruginosa; rhinovirus; Streptococcus pneumoniae

INTRODUCTION

During every inspiration, environmental debris and microorganisms are internalized into the conducting airways. To protect the host from infection, the airway epithelium lies strategically at this interface, creating a physiochemical barrier limiting the passage of inhaled foreign matter. To bolster this first-line defense and maintain sterility within the lungs, inhaled particles are continually cleared from the airways by mucociliary clearance (MCC). MCC consists of two primary components: 1) hairlike motile cilia that line the airways and beat continually in a rhythmic waving motion and 2) mucus secreted by goblet cells and submucosal glands that entraps microorganisms and debris (26, 120, 141). Defects in either component impair the lung’s ability to clear inhaled particulates, rendering the airways vulnerable to colonization and infection by pathogenic microbes. Disorders of MCC are linked to chronic airway diseases such as cystic fibrosis (CF), characterized by excessively thickened mucus, and primary ciliary dyskinesia (PCD), characterized by directly impaired ciliary function. Recurrent lung infections are a common symptom in both patients with CF and patients with PCD (20, 115, 120, 187).

Multiple pathogenic microbes have evolved mechanisms to resist MCC to colonize the airway. Common pathogenic bacteria such as Streptococcus pneumoniae, Pseudomonas aeruginosa, and Hemophilus influenzae produce virulence factors that disrupt ciliary motion and coordination (72, 84, 99, 142, 203). Mycotoxins released by the opportunistic fungi such as Aspergillus flavus can reduce ciliary motility (112). Disrupted cilia motility and ultrastructure also occur in infections by respiratory viruses, including coronavirus, influenza, and rhinovirus (24, 53, 60, 117, 128, 175). Although disruption of ciliary functions is a recurrent theme in respiratory infections, the cellular pathways underlying these abnormalities are not fully understood. The relative lack of studies investigating the relationship between motile cilia function and respiratory pathogens is likely due to challenges in procuring primary airway epithelial cells (AECs), culture of AECs under air-liquid interface (ALI) conditions necessary for motile cilia differentiation, and the need for specific equipment to visualize and analyze cilia motility.
Despite cilia being the airway’s first point of contact with pathogens, questions remain regarding how ciliary function and expression are altered during infection. This review will provide greater insight into the dynamic relationship between motile cilia and respiratory pathogens to shed light on mechanisms governing cilia expression, function, and regulation during infection. Various ciliopathies associated with common respiratory viruses, bacteria, and fungi will be compared. Finally, the potential significance of emerging biological functions of motile cilia as immune sensory organelles will be addressed.

**MUCOCILIARY CLEARANCE**

MCC is the primary innate defense mechanism of the airways against the constant threat of inhaled airborne pathogens, pollutants, and allergens. This system comprises two important functional entities: mucus production and transport of that mucus via ciliary beating. The apical airway surface is lined by a complex airway surface liquid (ASL) made up of an upper gel-like mucus layer that entraps inhaled pathogens and debris, and underneath that is a lower-viscosity periciliary liquid (PCL) that lubricates the airway surface and allows the cilia to beat rapidly (Fig. 1A; Refs. 20, 41, 130). The mucus layer is formed by “sticky” carbohydrate side chains of cross-linked MUC5AC and MUC5B mucus, whereas the PCL is kept separate from the mucus layer partly by cilia membrane-tethered MUC1 and MUC4 that take up space surrounding the cilia and create an electrostatic “brush” for the cilia to slide against (21).

The mucus layer also possesses potent antimicrobial activity. Secretory cells secrete a cocktail of antimicrobial molecules, including defensins, lysozyme, and immunoglobulins A and G (IgA/IgG; Refs. 10, 54, 70, 200). When coupled with the synchronous and continual beating of motile cilia, airway mucus is constantly moved from the lower airways up to the oropharynx, where it is either expectorated or swallowed. Thus the mucociliary escalator provides an effective means to quickly neutralize and remove most inhaled pathogens from the lungs.

**RESPIRATORY CILIA: STRUCTURE, FUNCTION, AND EXPRESSION**

Respiratory cilia are microtubule-based hairlike projections emanating from basal bodies found on the apical membranes of AECs (Fig. 1, B and C). These organelles are 6 – 7 μm long and 0.2 – 0.3 μm in diameter (17, 160). Ciliated AECs possess about 200–300 cilia per cell. The architecture of the motile cilium includes a microtubule scaffold termed the axoneme, made up of a ring of 9 doublet microtubule protofilaments that surround a central pair of singlet protofilaments, termed a “9 + 2” arrangement. Each outer microtubule pair possesses inner and outer dynein arms that generate the required energy for motility through ATP hydrolysis (Fig. 2A; Refs. 160, 187).

Airway cilia beat continuously in a coordinated metachronous pattern to move mucus up the airway tree. To generate the required force to move the mucus gel layer, the cilium moves forward rapidly and forcefully during the power stroke, followed by a slower recovery stroke in which the cilium bends backward 90° along the same plane of motion to return to its original starting position (Fig. 2B; Refs. 25, 71). Cilia beat frequency (CBF) normally ranges between 10 and 15 Hz and is regulated by several mechanisms in a Ca2+-dependent manner, both via direct Ca2+ binding as well as calmodulin binding (160). Physiologically relevant signaling molecules such as ATP (35, 67, 218), acetylcholine (103, 161, 215), cyclic AMP (cAMP) and cGMP (167, 168, 219), nitric oxide (NO; Refs. 86, 114, 191), and inositol trisphosphate (12) can modulate CBF. Interestingly, pathogenic bacteria and fungi that colonize the respiratory tract secrete virulence factors that interfere with these mechanisms to bypass mucociliary defense (72, 84, 99, 112, 142, 143, 203, 222).

Ciliogenesis in AECs is tightly regulated by multiple transcription factors. FOXJ1, CBE1, GemC1, TAp73, and TAp79 are key drivers of multimotile ciliogenesis in differentiating AECs by upregulating the expression of downstream genes...
involved in cilia assembly, motility, and structure (6, 62, 76, 81, 144, 214). In vivo studies of knockout mice showed a pronounced loss of cilia and an absence of airway MCC (6, 28, 144, 202). Interestingly, infection by common respiratory viruses such as respiratory syncytial virus (RSV), rhinovirus, and influenza can downregulate the expression of critical genes involved in development of motile cilia, although the pathophysiology remains undefined (40, 117, 145a).

The dogma whereby motile cilia simply clear the airways has now been expanded by studies demonstrating that they also possess mechanosensory traits. It is now understood that respiratory cilia share chemosensory abilities once thought unique to primary cilia, long known to be involved in chemosensation, signal transduction, and regulation of cellular growth (51, 87, 122, 146, 170). Studies have also shown possible involvement of cilia in lung repair processes (148, 155) and in regulating cytokine and antimicrobial production (11, 56, 131), thus suggesting an even greater involvement of these organelles in lung function than initially thought. Studies by our group and others have shown that airway cilia express members of the bitter taste family of receptors (T2Rs) that may hold functional importance in airway innate immunity against bacterial infections by driving innate immune defenses in response to bacterial antigens (52, 65, 113, 170).

CONSEQUENCES OF CILIARY DYSFUNCTION

Defects in cilia function will severely impede MCC and greatly increase the risk of respiratory infections due to inability to remove inhaled pathogens. CF and PCD are classic examples of respiratory disorders resulting from defective MCC, with patients often presenting with recurrent lung infections (33, 38, 78, 115, 164, 179a). The pathophysiology of CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein that leads to impaired secretion of chloride and bicarbonate into the ASL. This results in a characteristic buildup of thick, sticky mucus in affected patients that impedes cilia beating (38, 119). PCD is a genetic disorder caused by mutations in genes that encode for ciliary components involved in the formation of axonemal microtubules and/or associated motor proteins (55, 78). Affected patients often present with dyskinetic or static cilia that also results in recurrent lung infections.

Secondary, acquired nongenetic defects affecting ciliary function can also be caused by factors such as cigarette smoke exposure and inflammation during asthma and chronic obstructive pulmonary disease (COPD), with studies showing that affected individuals are at higher risk of lung infections (17, 61, 183, 209). Multiple ciliary defects have been reported in the asthmatic airways, ranging from decreased motility, disorientation in beat direction, and ultrastructural damage (136, 137, 185). Likewise in COPD, the airway epithelium exhibits significant morphological alterations, with the pseudostratified epithelium being replaced with squamous cells and a significant reduction in ciliated cuboidal cell numbers (57, 68, 210). Respiratory cilia have also been noted to be shorter in length in individuals with COPD as a result of dysregulated intraflagellar transport (IFT) gene expression (69). These impairments in mucociliary function translate to increased risk of respiratory infections, and it is perhaps unsurprising that many respiratory pathogens exploit such defects in MCC to colonize the airway epithelium. However, as previously mentioned, the specific processes leading to various ciliary defects seen in respiratory infections are not fully understood. A better understanding of ciliopathies during infections will provide a greater appreciation of the important roles of respiratory cilia in lung health and disease and may reveal novel therapeutic targets to enhance respiratory defense. The following sections will, therefore, discuss in greater detail the specific known ciliary defects during infection by common respiratory pathogens.

VIRAL INFECTIONS

Respiratory viral infections are major public health and economic burdens worldwide, with annual costs in the United States alone estimated to be $40 billion and $87 billion for the common cold and influenza, respectively (47, 135). Highly pathogenic respiratory viruses such as avian influenza (H5N1), severe acute respiratory syndrome coronavirus (SARS-CoV or SARS-CoV1), and Middle East respiratory syndrome coronavirus (MERS-CoV) are a constant threat to human health, with the more recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) resulting in a global pandemic (39a, 93, 176). Studies dedicated to the interactions
between respiratory viruses and AECs have reported detrimental defects related to cilia function and expression. In almost all cases, viral infection inadvertently leads to reduced or absent MCC of the airways that likely explains why primary viral infections substantially increase the risk for a secondary bacterial infection (96, 124, 140). The following sections will detail various acquired cilia defects associated with specific respiratory viruses. Virus-induced abnormalities of cilia structure and function are summarized in Table 1.

Influenza

Unlike the common cold, influenza symptoms are comparatively more severe, and complications often lead to hospitalizations. Influenza viruses can be classified into three distinct types, A, B, and C. Influenzas A and B cause seasonal epidemics and can bring significant morbidity and mortality, whereas most influenza C infections are generally subclinical (105, 184).

Influenza are enveloped, negative-sense RNA viruses that gain entry into host cells by binding to sialic acids on glycosylated cell surface molecules via the viral hemagglutinin protein. The viral surface protein neuraminidase catalyzes the removal of host sialic acid residues to permit viral escape from the host cell (184). Human influenza preferentially binds to α-2,6-linked sialic acid, found in both ciliated and nonciliated secretory cells in the airways. Avian influenza, on the other hand, has a preference for α-2,3-linked sialic acid, found more specifically on the apical surface of ciliated cells (82, 129, 186, 208). In ciliated AECs, both α-2,6- and α-2,3-linked sialic acids localize to the base of the cilia, with Thompson et al. (186) suggesting that this corresponds to microvilli. More recently, Smith and colleagues (174) demonstrated that ciliated nasal epithelial cells are targeted by human influenza A (H1N1). Interestingly, immunofluorescence (IF) experiments showed viral antigen binding to the ciliary tips, suggesting a novel route of infection where influenza attaches to the distal portions of cilia, which then travels down along the cilium to infect the host. Studies suggest that influenza infections of the airway epithelium are particularly aggressive, with in vitro inoculation of ALI-differentiated AECs quickly resulting in apoptosis and loss of ciliated AECs within 1–2 days (40, 174, 208). An in vivo study conducted in murine tracheal explants demonstrated that influenza inoculation caused significant loss of MCC within 3 days (152). Surprisingly, Smith and colleagues (174) showed that influenza infection of nasal AECs did not reduce CBF nor cause ciliary dyskinesia despite a loss in the number of ciliated cells after 24 h. Taken together, these findings suggest that MCC dysfunction resulting from influenza is primarily due to a direct loss of ciliated AECs rather than dysfunctional cilia motility. Transcriptomic analysis of ALI-differentiated nasal primary AECs revealed significant downregulation of multiple ciliogenesis, IFT, and cilia motor genes independently of cell death (145a). Further studies are needed to determine the pathways and promoters that regulate these gene expression changes.

Respiratory Syncytial Virus

RSV belongs to the paramyxoviridae family that also includes parainfluenza, mumps, and measles viruses. RSV is a major cause of lower respiratory tract infections and hospitalizations in infants, with most children infected by 2 yr of age (16, 193). Although adult infections are generally mild and limited to the upper respiratory tract, adults with underlying conditions and the elderly may develop severe lower respiratory disease (42, 90, 196). RSV infections also increase the risk of bacterial coinfection with H. influenzae, S. pneumoniae, or Staphylococcus aureus (63, 74, 110, 182). Importantly, RSV infection during childhood is strongly correlated with development of asthma in later life (16).

RSV is an enveloped virus containing single-stranded, negative-sense RNA. Viral entry into the host cell is facilitated by two key viral surface glycoproteins, the fusion (F) and attachment (G) proteins. The G protein mediates attachment of the virus to the host fractalkine receptor CX3CR1, followed by interaction of the RSV fusion protein with nucleolin that enables the virus to enter the host cell (27, 92). Ciliated AECs are the primary target of RSV, with CX3CR1 expressed in the cilium itself under normal physiological conditions (92). Interestingly, CX3CR1 is redistributed from motile cilia to punctate vesicles close to the cell nucleus after RSV infection. This redistribution of CX3CR1 occurs as soon as 1 day after infection in ALI-differentiated AECs. Like most respiratory viruses, RSV does not lead to cell death, independent of cell death (145a).

Table 1. Ciliary defects resulting from respiratory viral infections and their associated host receptors

| Virus               | Host Cell Entry Receptor | Associated Ciliary Defects | References                                                                 |
|---------------------|--------------------------|---------------------------|---------------------------------------------------------------------------|
| Common coronavirus  | hAPN (CD13)              | Dyskinetic cilia, loss of ciliated AECs | Chilvers et al. (24)                                                      |
| HCoV-E229           |                          |                           |                                                                           |
| Common coronavirus  | HLA class I or sialic acid | None reported             | Collins (31), Dijkman et al. (34), Essaidi-Laziosi et al. (40)            |
| HCoV-OC43           |                          |                           |                                                                           |
| SARS-CoV            | ACE2                     | Shedding of ciliated AECs | Sims et al. (173)                                                         |
| SARS-CoV2           | ACE2                     | Shedding of ciliated AECs | Fang et al. (43)                                                          |
| Influenza (A/B)     | Sialic acid              | Reduced MCC, static cilia, shedding of ciliated AECs | Thompson et al. (186), Pittet et al. (152), Wu et al. (208), Essaidi-Laziosi et al. (40), Nicolas de Lamballe et al. (145a) |
| RSV                 | CX3CR1                   | Reduced MCC, dyskinetic cilia, ultrastructural abnormalities, cilia loss from ciliated AECs | Look et al. (117), Mata et al. (128), Smith et al. (175), Jeong et al. (92), Jumat et al. (98), Essaidi-Laziosi et al. (40), Nicolas de Lamballe et al. (145a) |
| Rhinovirus          | ICAM-1/LDL               | Reduced MCC, shedding of ciliated AECs | Sakakura et al. (159), Turner et al. (189), Griggs et al. (60), Essaidi-Laziosi et al. (40) |

ACE2, angiotensin-converting enzyme 2; AECs, airway epithelial cells; hAPN, human aminopeptidase N; HCoV, human coronavirus; HLA, human leukocyte antigen; MCC, mucociliary clearance; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus.

AJP-Lung Cell Mol Physiol • doi:10.1152/ajplung.00283.2020 • www.ajplung.org
infection (27, 92). This suggests that RSV infection begins by attachment to the cilia, followed by viral internalization before transportation to the cell nucleus along the ciliary shaft via IFT. It remains to be investigated whether inhibitors of IFT on ciliated AECs disrupt trafficking of RSV between the cilia and nucleus. A study by Feng and colleagues (48) demonstrated that application of histone deacetylase (HDAC) inhibitors was able to limit RSV replication in inoculated BEAS-2B cultures. The HDAC family of proteins are pleiotropic regulators of cellular function, which includes transcriptional repression, epigenetic modification, and signal transduction (133, 150). Importantly, HDACs regulate microtubule disassembly and IFT processes in cilia and are critical in control of cilia length (58, 149, 213). Building on from this information, it would be valuable to assess whether the use of HDAC inhibitors on ciliated airway epithelial cultures may similarly inhibit RSV replication and maintain cilia expression and function.

The cytopathology of RSV infections of the airway epithelium is noted to be milder compared with influenza. Although cilia expression and dyskinesia are disrupted by RSV infections, in vitro studies show that the integrity of the epithelium remains mostly intact, at least in the time points observed (40, 98, 175). Thus most of the pathogenicity seen in RSV infections may be caused by host inflammatory responses (193). Studies have found that the reduction in cilia number following RSV infection is independent of cell death, indicating ciliated AECs become denuded, dedifferentiated into squamous cells, or transdifferentiated into secretory cells following infection rather than being shed off the epithelium (98, 128, 175). A possible explanation is a marked downregulation of cilia-associated genes following RSV infection similarly seen with influenza (128). However, it is yet unknown how viral factors can directly interfere with ciliogenesis pathways. Interestingly, the addition of the free radical scavenger N-acetyl-cysteine (NAC) to RSV-infected AECs was able to restore gene expression levels and ciliary activity, suggesting that the detrimental effects on cilia may be partially mediated by oxidative stress (128). Although not directly associated with viral-induced ciliary dysfunction, a study by Simet and colleagues (172) showed that sustained alcohol exposure in mice increased oxidative stress in the lungs that led to ciliary dysfunction and slowing of CBF. Dietary supplementation with antioxidants such as NAC and procysteine prevented alcohol-induced ciliary dysfunction and restored CBF. It would be revealing to test whether the use of NAC also restores ciliary function during infection by other respiratory viruses.

Structurally, transmission electron microscopy (TEM) of RSV-infected AECs shows abnormalities of the cilium ultrastructure. In 15-day postinfected ciliated bronchial AECs, Mata and colleagues (128) identified disorganization of cilia microtubules and a general loss in cilia number. In a separate study of RSV-infected AECs at 5 days after infection, the number of cilia was also significantly reduced, although cilia length was normal (98). Finally, Smith et al. (175) looked at AEC cultures at 3 days after infection and similarly observed a decrease in cilia number. Interestingly, TEM analysis found that infected AECs also displayed significant mitochondrial damage. Thus, although airway epithelial integrity following infection was largely normal in all studies, MCC is predicted to be significantly impaired due to reduced cilia number and impaired MCC. This would explain why RSV infections predispose the airways for secondary bacterial infections.

**Rhinovirus**

Human rhinoviruses (HRV) are the predominant cause of the common cold and are responsible for ≤40% of all viral respiratory tract infections (2). Associated clinical manifestations of HRV infection include acute sinusitis, exacerbation of asthma and COPD symptoms, and pneumonia in individuals who are immunocompromised (64, 85, 100, 198, 206). They are members of the picornaviridae family that also includes enteroviruses and hepatitis A virus. Three HRV species (A, B, and C) have been identified and are classified based on sequence alignment data of the major HRV structural proteins (VP1 or VP4/2; Refs. 106, 207). HRVs are genetically heterogeneous, encompassing 168 recognized subtypes to date (7, 223).

HRVs are nonenveloped positive-sense, single-stranded RNA viruses. The majority of HRV-A (~90%) and all HRV-B utilize the intracelluar adhesion molecule (ICAM-1; major group) to gain cell entry (14, 190), whereas the remaining subset of HRV-A uses the low-density lipoprotein receptor (minor group; Refs. 75, 85). The more recently identified HRV-C uses cadherin-related family member 3 (CDHR3) as its entry factor (15, 60). ICAM-1 expression has been identified on the apical surface of both ciliated and nonciliated AECs cultured under ALI conditions, with experiments demonstrating HRV16 (major group, species HRV-A) capable of infecting both ciliated and secretory cell populations (89, 107). Moreover, Warner and colleagues (199) show that HRV16 replication occurs exclusively in ciliated cells. As for HRV-C, CDHR3 was found to localize exclusively on ciliated AECs, and its expression was strongly correlated with both HRV-C binding and replication (60).

Studies investigating cilia-specific defects associated with HRV infections are limited. An early study of volunteers experimentally inoculated with HRV showed significant reduction in MCC compared with control subjects (159). Interestingly, in vitro studies in ALI-differentiated AECs show that HRV infections have minimal effects on epithelial morphology (40, 199), suggesting that most observed pathology in clinical subjects may be due to inflammatory processes mediated by immune cells (126, 159, 189). Indeed, transepithelial electrical resistance (TEER) measurements of ALI-differentiated primary AECs, which are a function of epithelial integrity, were also not significantly affected by HRV at 2 and 5 days after infection (40). Although airway epithelial integrity remains intact following HRV infection, in vitro inoculation of primary AECs nonetheless caused loss of apical cilia (89) and CBF slowing (40). At the transcriptomic level, HRV infection of ALI-differentiated AECs reduced expression of multiple genes involved in cilia motility, cytoskeletal structure, and multilamellar ciliation expected to translate to reduced cilia expression and function (40, 89).

**Coronavirus**

Human coronaviruses (HCoV) belong to the coronaviridae family of viruses with seven members to date that are known to infect humans, including HCoV-229E, HCoV-OC43, severe acute respiratory syndrome coronavirus (SARS-CoV or SARS-
CoV1), SARS-CoV2, HCoV-NL63, HCoV-HKU1, and Middle East respiratory syndrome coronavirus (MERS-CoV; Refs. 145, 176, 180). HCoV-229E and HCoV-OC43 are the most well-studied members, and infections typically result in mild flulike symptoms. Both members account for ≈29% of all common colds (151). However, infection by other members such as SARS-CoV, SARS-CoV2, and MERS-CoV can lead to more serious disease such as respiratory distress and pneumonia that can be fatal in individuals with underlying health conditions (127, 176, 180). More recently, the highly pathogenic SARS-CoV2, responsible for COVID-19 disease, has emerged as a global pandemic in 2019–2020 (39a).

Coronaviruses are positive-sense, single-stranded RNA enveloped viruses of the coronaviridae family (180). Entry receptors vary across family members, with HCoV-229E utilizing human amino peptidease N (hAPN/CD13; Refs. 18, 197), HCoV-OC43 utilizing either human leukocyte antigen (HLA) class I or sialic acids (31, 147), SARS-CoV and SARS-CoV2 utilizing angiotensin-converting enzyme 2 (ACE2; Refs. 173, 181), and MERS-CoV utilizing dipeptidyl peptidase 4 (DPP4/CD26; Refs. 147, 176, 180). This review will discuss studies of HCoV-229E and HCoV-OC43 given their prevalence, with attention also given to SARS-CoV and SARS-CoV2, as the latter has recently risen as a global pandemic.

Both HCoV-229E and HCoV-OC43 have different cell tropisms in the airway epithelium based on the localization of their respective host receptors. The host receptor for HCoV-229E, CD13, is expressed on the apical surface of nonciliated cells. IF studies conducted in ALI-differentiated primary AECs show HCoV-229E colocalization with CD13 (34, 197). HCoV-OC43, on the other hand, infects ciliated AECs as its host receptor (HLA class I and sialic acid) localizes to the apical membrane of ciliated AECs (31, 188). Interestingly, in vitro infections with either HCoV-229E or HCoV-OC43 in ALI-differentiated AECs show little cytopathic effects. Dijkman and colleagues (34) noted minimal morphological differences between AEC cultures infected with either HCoVs-229E or -OC43. However, the specific effects of HCoV on CBF were not assessed in the study.

A separate study by Essaidi-Laziosi and colleagues (40) investigated the effects of HCoV-OC43 infection in vitro and noted a similar lack of cytopathology as well as no changes in CBF compared with mock infected controls. Moreover, Wang and colleagues (197) determined that in vitro infections of ALI-differentiated AECs with HCoV-229E did not reduce TEER, demonstrating intact epithelial integrity. Findings from an ex vivo study conducted by Chilvers and colleagues (24) paint a slightly different picture of HCoV-229E infections. Nasal brushings from experimentally inoculated volunteers showed epithelial damage and cilia loss, with TEM revealing loss of cilia on ciliated AECs, microtubular defects, and mitochondrial damage. Although they saw no reduction in CBF after inoculation, cilia dyskinesia was noted. These findings strongly suggest that HCoV-229E and HCoV-OC43 infections in AECs in vitro are generally mild and self-limited, whereas cellular and cilia damage can occur in patients resulting from secondary inflammatory processes.

The pathology of SARS-CoV and SARS-CoV2 infections is considerably more severe. Both viruses share the same host receptor, ACE2, which may localize to the apical surface of ciliated AECs (145, 181). A recent preprint suggests expression of ACE2 within airway motile cilia by immunofluorescence microscopy (109). A study by Sims and colleagues (173) showed that SARS-CoV only infects ciliated AECs differentiated under ALI conditions and not undifferentiated primary AECs grown in submersion. Another study showed ACE2 expression increases with AEC differentiation in vitro (94) and that infection is more efficient when virus is applied to the apical surface rather than the basolateral side. In vivo studies of SARS-CoV2-infected macaques showed viral replication in type I/II pneumocytes and in ciliated airway epithelial cells (157). In postmortem trachea and lungs of patients with COVID-19, shedding of ciliated cells into the airway lumen was observed, exposing underlying basal cells (43). These data all implicate cilia as potentially important sites of SARS-CoV1 and/or SARS-CoV2 infection.

Interestingly, recent high-sensitivity RNA in situ mapping and quantitative PCR analysis demonstrated higher levels of ACE2 expression in human nasal epithelial cells compared with bronchial epithelial cells. Notably, progressively reduced levels of ACE2 expression were observed in the distal airways (79). These findings reveal a degree of heterogeneity within the ciliated cell population of the airways that correlated with higher SARS-CoV2 infectivity in the nose versus the lower airways. It would be revealing to also examine whether such heterogeneity in host receptor expression exists for other respiratory viruses. Moreover, the study also demonstrated significantly higher levels of ACE2 receptor expression in AECs isolated from patients with CF, although the clinical implications of this feature remain unclear (79). ACE2 expression may also be upregulated in the lower airways in cigarette smokers, perhaps increasing susceptibility to infection (216). It is important to note that other factors beyond ACE2 are important for SARS-CoV and SARS-CoV2 infections, including the surface activating protease Tmprss2 (163), and thus relative ACE2 expression level alone may not necessarily correlate with relative susceptibility of cells to infection.

Unlike the milder symptoms with HCoV-229E and HCoV-OC43, SARS-CoV inoculation of ALI-differentiated AECs results in disruption of tight junctions and shedding of ciliated AECs within 3 days (173). Peer-reviewed, detailed studies of SARS-CoV2 pathobiology in AECs are limited due to its recent emergence and lack of antibody tools. Preliminary findings show that much like SARS-CoV, SARS-CoV2 can infect and replicate in ciliated AECs (134). Histology of lung biopsies from a 72-yr-old man with COVID-19 showed extensive damage to the airway epithelium, with loss of ciliated AECs and a denuded epithelium (217). The effects of SARS-CoV and SARS-CoV2 on CBF have not been reported, although disruption of MCC is expected due to shedding of ciliated AECs both in vitro and in vivo (173, 217, 220).

**BACTERIAL INFECTIONS**

Bacteria such as H. influenzae, P. aeruginosa, and S. pneumoniae are opportunistic pathogens that cause serious respiratory illness in disease settings where innate MCC is perturbed. A prime example of this is CF and PCD, where the principal cause of morbidity and mortality is bacterial infections of the airways (30, 115, 119, 201). In the asthmatic airways, 16S rRNA sequencing reveals a significant increase in opportunistic bacterial numbers that correlated with asthma exacerbations.

**L608 HOST-PATHOGEN INTERACTIONS WITH RESPIRATORY CILIA**

ARJ-Lung Cell Mol Physiol • doi:10.1152/ajplung.00283.2020 • www.ajplung.org
As previously detailed, infection by respiratory viruses significantly impacts cilia function and MCC, which promotes bacterial colonization. In fact, during the 1918 flu pandemic, most fatalities were due to secondary bacterial pneumonia by *S. pneumoniae* or *H. influenzae* (139, 140).

Given the prevalence of bacterial airway infections, especially following viral infections, a better understanding of bacterial mechanisms to evade MCC will aid in understanding disease pathology and may reveal new treatment strategies to reduce bacterial infections without antibiotics. Upper respiratory infections, including acute and chronic rhinosinusitis, account for ≥20% of adult antibiotic prescriptions in the United States (179). Thus respiratory infections are a major contributor to the emergence of antibiotic-resistant bacteria (121), called “arguably the greatest risk...to human health” by the World Economic Forum (177). Stimulating endogenous innate defenses like MCC may combat infections without selecting for resistance. This requires a better knowledge of how bacteria thwart these defenses. When bacteria adhere to the ciliated epithelium, they can form biofilms that are notoriously difficult to eradicate (Fig. 3). The following sections will detail the effects of infection by common respiratory bacteria on cilia function and methods used to bypass MCC. Bacterial virulence factors and effects are summarized in Table 2.

*S. pneumoniae*

*S. pneumoniae* is a gram-positive bacterium normally part of the commensal upper respiratory microbiota but can become pathogenic if allowed to colonize the lower respiratory tract (19, 125). Early studies on agar-embedded human ciliated nasal turbinate tissue show that addition of *S. pneumoniae* decreased CBF by 24% (7.3 vs. 9.6 Hz) compared with controls (46). Interestingly, the study also observed no evidence of physical association between *S. pneumoniae* with AECs, suggesting that secreted factors primarily reduced CBF (46). It was eventually determined that CBF slowing by *S. pneumoniae* is mediated by two primary virulence factors, pneumolysin and hydrogen peroxide (H₂O₂). The polypeptide pneumolysin is a pore-forming cytolytic toxin that can slow CBF and cause cellular damage (44, 178). H₂O₂ is a potent oxidant that also slows CBF (45, 77).

Pneumolysin is the key virulence factor of *S. pneumoniae* infections and is critically involved in the pathogenesis of pneumococcal pneumonia. It possesses hemolytic activity and is cytotoxic at higher concentrations. At lower concentrations, it slows CBF without significantly altering cell morphology (44, 178), indicating that pneumolysin can exert direct effects on cilia function. TEM analysis of nasal AECs exposed to pneumolysin for 4 h show cytoplasmic blebbing and mitochondrial damage, although surprisingly, ciliary ultrastructure remained intact (178). Interestingly, a more recent study by Fliegauf and colleagues (49) in murine ciliated AECs proposed that *S. pneumoniae* can disrupt MCC through pneumolysin-independent means. In this study, *S. pneumoniae* infection did not decrease CBF but instead disrupted cellular integrity via F-actin and caused ineffective MCC. Infected cultures displayed significant loss in structural integrity, weakened cilia strokes, and deformation of the planar surface of the ciliated epithelium. Thus, although CBF was normal, cilia beating generated turbulent fluid flow that impaired MCC. Interestingly, the authors also infected AECs with pneumolysin-deficient *S. pneumoniae* and observed similar effects in vitro. They speculated that other bacterial toxins targeting the actin cytoskeleton (i.e., glycosyltransferases toxin A/B, cytchalasins) may be responsible. The lack of effects on CBF following *S. pneumoniae* infection may be due to species-dependent differences as the previous studies demonstrating CBF reduction by *S. pneumoniae* utilized human cultures.

### Table 2. Ciliary defects caused by respiratory bacteria and their associated virulence factors

| Bacteria                        | Virulence Factors                                                                 | Associated Ciliary Defects                                      | References                                                                 |
|---------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------|
| Nontypeable *Hemophilus influen*| Lipoooligosaccharide, protein D                                                  | CBF slowing, loss of cilia, ultrastructural abnormalities       | Gregg et al. (59), Johnson and Inzana (95), Wilson et al. (204), Yamanaka et al. (211), Janson et al. (91), Bailey et al. (9) |
| *Pseudomonas aeruginosa*        | 1-Hydroxyphenazine, 2-alkyl-4-hydroxyquinoline, hydrogen cyanide, pyocyanin, PA-IL/II, rhamnolipid | CBF slowing, ciliary membrane disruption, loss of dynein arms, loss of airway epithelial integrity | Hingley et al. (72), Wilson et al. (203), Munro et al. (142), Jackowski et al. (84), Read et al. (156), Mewe et al. (132), Nair et al. (143) |
| *Streptococcus pneumoniae*      | Pneumolysin, hydrogen peroxide                                                   | CBF slowing, disrupted surface fluid flow, ultrastructural abnormalities | Steinfort et al. (178), Feldman et al. (46), Duane et al. (36), Feldman et al. (44, 45), Fliegauf et al. (49), Honda et al. (77) |

CBF, cilia beat frequency; PA-IL/II, *P. aeruginosa* surface carbohydrate lectins.
H. influenzae was the major nonprotein virulence factor produced by S. pneumoniae that causes cellular damage and, importantly, slows ciliary beating in a dose-dependent manner (44, 104). Colonizing S. pneumoniae releases H2O2 at similar concentrations to that produced by activated neutrophils that significantly diminishes CBF (36, 44, 73). The mechanisms behind H2O2-mediated CBF slowing may be caused by protein kinase C (PKC) activation, which phosphorylates various ciliary proteins (160, 162). Kobayashi and colleagues (104) demonstrated that in sheep tracheal explants, H2O2 decreased CBF and addition of a PKC inhibitor fully restored CBF. Collectively, the combined inhibitory effects of pneumolysin and H2O2 on CBF may enhance initial colonization and enables spread of S. pneumoniae to the lower airways.

H. influenzae

H. influenzae is a gram-negative bacterium normally part of the commensal respiratory flora. However, it is highly prevalent and pathogenic in lower respiratory tract diseases like COPD, CF, PCD, and respiratory viral infections (74, 101, 164, 182, 194). H. influenzae is categorized into typeable and nontypeable strains, with the former possessing an outer polysaccharide capsule, and the latter lacking it (50, 101, 102). Typeable H. influenzae often causes systemic disease such as meningitis. In contrast, nontypeable H. influenzae (NTHi) causes infections of the respiratory tract and is primarily a mucosal pathogen (50, 101). This review will focus only on NTHi pathology in the respiratory tract.

As an opportunistic bacterium, NTHi infections typically occur when the integrity of the airway epithelium has been previously compromised. Its high prevalence in respiratory infections may be related to an ability to bypass host MCC. To initiate colonization, NTHi expresses a variety of surface adhesion proteins enabling direct adherence to airway mucus and to the airway epithelium (50, 101). Regarding its effects on CBF, inoculation of human ciliated AECs with NTHi led to a significant reduction in CBF and eventual loss of cilia (91, 204). Addition of bacterial filtrate was able to similarly reduce CBF, suggesting this effect is mediated by secreted factors (9, 211). Interestingly, studies show that the addition of NTHi filtrate to ciliated tissue specimens did not cause cell death despite slowing CBF, indicating that this was not due to cytotoxicity (91). Other studies of NTHi filtrate showed that it was not cytotoxic but causes cilia abnormalities and detachment (9, 211). Because NTHi culture supernatants contain a myriad of soluble factors, identification of specific factors that slow CBF is challenging. Current findings point toward two primary NTHi virulence factors that slow CBF. One is lipo-oligosaccharide, a surface antigen functionally similar to LPS (59, 95), and the other is protein D, a 42-kDa surface lipoprotein encountered in all H. influenzae strains (91). Although the mechanism for CBF slowing is unknown, Bailey and colleagues (9) suggested it may be PKCe-dependent. They showed direct, dose-dependent activation of PKCe in response to NTHi filtrate. In addition, NTHi filtrate that had been heat-inactivated or freeze-thawed failed to cause PKCe activation, suggesting that a temperature labile component activates PKCe (9).

P. aeruginosa

P. aeruginosa is an opportunistic gram-negative bacterium that causes serious respiratory disease in patients with underlying conditions (e.g., bronchiectasis, COPD, CF) or who are immunocompromised (22, 30, 119, 201). It is also the single most important cause of mortality in CF, where P. aeruginosa infections cause significant epithelial damage and progressive decline in lung function (66). Once colonization is established, the bacterium is rarely eradicated in patients with preexisting respiratory conditions despite antibiotics (138). This is likely due to a multitude of secreted virulence factors enabling P. aeruginosa to evade MCC and host innate defenses (Table 2).

In the late 1980s, studies noted that P. aeruginosa culture filtrate slowed CBF in both human and animal models. Chloroform extraction of heat-stable factors from cultured supernatants followed by mass spectrometry identified multiple potentially cilioinhibitory components, some also found in clinical sputum samples (143, 154, 205). These include the phenazine pigment pyocyanin and its oxidative metabolite, 1-hydroxyphenazine (1-HP), 2-alkyl-4-hydroxyquinolines, rhamnolipid hemolysin, and hydrogen cyanide (72, 143, 203). In vitro studies by Wilson and colleagues (203) in human nasal ciliated epithelial brushings showed pyocyanin had a slow onset of cilioinhibition, whereas 1-HP caused rapid CBF slowing. Both compounds slowed CBF dose-dependently. Subsequently, an in vivo study on guinea pigs by Munro and colleagues (142) showed that tracheal mucus velocity was dramatically slowed by either compound in a dose-dependent manner. Similarly, pyocyanin demonstrated slower onset CBF reduction, whereas 1-HP rapidly inhibited CBF. Rhamnolipid, a hemolytic detergent-like compound, can slow CBF and, in higher concentrations, cause cell lysis (171). Addition of rhamnolipid to human nasal brushings at concentrations below 125 μg/mL significantly slowed CBF, whereas concentrations above this disrupted cellular membranes and dissociated AECs (156).

Interestingly, work by Zhao and colleagues (222) showed inherent differences between murine nasal versus tracheal ciliated AECs in response to P. aeruginosa inoculation. The study demonstrated that nasal AECs were more sensitive to P. aeruginosa ciliotoxins, whereas tracheal cultures were more resistant. Application of P. aeruginosa-conditioned medium to nasal AECs, but not tracheal AECs, significantly decreased basal CBF and blunted CBF increase in response to stimulation. Whether these findings translate similarly to human airways remains to be investigated. However, they suggest differences may exist between nasal and bronchial cilia that might have relevance to localized susceptibility of various types of infections.

More recently, cyanide production by P. aeruginosa was also determined to be a potent inhibitor of CBF in both ALI-differentiated nasal AECs and nasal brushings (143). Supernatants from a cyanide-deficient strain of P. aeruginosa did not significantly slow CBF, whereas supernatants from a pyocyanin-deficient strain did. This suggested that cyanide, and not pyocyanin, was the primary inhibitor of CBF, at least under their study conditions. Furthermore, another study demonstrated that P. aeruginosa surface carbohydrate lectins PA-IL and PA-IIL, which facilitate bacterial adherence to host cells, are also cilioinhibitory (132). PA-IL or PA-IIL alone or in combination slowed CBF. Interestingly, immunohistochemi-
Fungal infections of the respiratory tract are less common than viral and bacterial infections, although pathogenic fungi belonging to the Aspergillus genus can colonize the airways. A. flavus and A. fumigatus are opportunistic pathogens that invade the human respiratory tract through inhalation of spores, causing serious respiratory disease in patients who are immunocompromised or those with preexisting lung conditions (32, 158). Sensitization to Aspergillus spores causes allergic bronchopulmonary aspergillosis or allergic fungal rhinosinusitis (23, 169). Worryingly, recent studies observed Aspergillus colonization in individuals who are immunocompetent with severe influenza (80, 166, 192).

Studies investigating specific interactions of A. flavus and A. fumigatus with AECs are limited. However, both pathogens secrete known virulence factors that damage the airway epithelium and/or slow CBF (Table 3). Culture filtrates from spumtum of patients with A. fumigatus pulmonary aspergillosis caused significant CBF slowing and dyskinesia in human ciliated epithelium from nasal brushings (3). CBF slowing was gradual, starting at 2 h and continuing over 6 h. Epithelial damage was also observed in most samples treated with the A. fumigatus culture filtrates (3). Purification and characterization of culture filtrates later identified several low-molecular-weight, heat-labile factors with cilioinhibitory properties (4). Of these, gliotoxin, fumagillin, and helvolic acid were confirmed to cause CBF slowing in a dose-dependent manner. Gliotoxin was most potent, being active at 0.2 μg/mL, whereas fumagillin and helvolic acid required much higher concentrations. High-molecular-weight fractions of the culture filtrate were also cilioinhibitory but were not characterized (4). Gliotoxin is an oxidation-reduction-active compound that causes oxidative damage through the generation of reactive oxygen species such as H$_2$O$_2$ (39). Although unconfirmed, gliotoxin-mediated production of H$_2$O$_2$ may cause cilioinhibition via PKC activation (8, 104, 153).

A. flavus is an important fungal pathogen due to its ability to produce aflatoxins, which are highly potent mycotoxins known to cause severe acute and chronic health effects.

### Table 3. Ciliary defects caused by pathogenic respiratory fungi and their associated virulence factors

| Fungi             | Virulence Factors                | Associated Ciliary Defects     | References         |
|-------------------|----------------------------------|--------------------------------|--------------------|
| Aspergillus fumigatus | Gliotoxin, fumagillin, helvolic acid | CBF slowing, epithelial damage | Amitani et al. (3, 4) |
| A. flavus         | Aflatoxins (AFB$_1$/AFB$_2$)     | CBF slowing                   | Lee et al. (112)   |

CBF, cilia beat frequency.
to cause cancer, immune suppression, and teratogenicity (13, 123). Of these, aflatoxin B₁ (AFB₁) is the most harmful and is one of the few mycotoxins that has been weaponized as a chemical warfare agent (13). In the respiratory tract, inhalation of AFB₁ was associated with higher incidences of lung carcinoma (123). The carcinogenicity of aflatoxins is attributed to PKC activation, which consequently leads to aberrant activation of DNA-synthesizing enzymes. As PKC activation is a known inhibitor of CBF, Lee and colleagues (112) investigated the effects of AFB₁ and AFB₂ on ALI-cultured nasal primary AECs and found the mycotoxins caused a rapid and significant decline in CBF. Moreover, pretreatment of cultures with PKC inhibitors blocked CBF slowing by AFB₁ and AFB₂, further demonstrating that the cilioinhibitory effects were PKC-dependedent. Subsequent experiments determined that CBF slowing by AFB₁/₂ was independent of changes in calcium (112). Interestingly, AFB₂ also impaired airway bitter taste receptor (T2R)-mediated production of nitric oxide (NO) in response to bacterial quorum-sensing molecules (112). NO is an important mediator of innate immunity with potent antimicrobial properties in addition to its ability to increase CBF through cGMP production and activation of protein kinase G (PKG; Refs. 86, 111, 195). The combined inhibitory effects of aflatoxins on CBF and NO production may significantly impair host innate defenses to enhance A. flavus survival. As with previously described pathogens, this impairment may also promote coinfection with other pathogens.

CHEMOSENSORY FUNCTIONS OF AIRWAY CILIA

It is increasingly apparent that common respiratory pathogens have evolved intricate means to neutralize and bypass host MCC mechanisms to successfully colonize the airways. To date, most studies investigating interactions between respiratory pathogens and the ciliated airway epithelium highlight detrimental associations with CBF and/or MCC. Although defects associated with MCC will result in poor clearance of inhaled pathogens, emerging roles of motile cilia involving intracellular signaling and immune regulation should also be taken into consideration.

Although primarily viewed as mechanical organelles, only recently are we beginning to discover that motile cilia also possess sensory capabilities, once thought to be unique to nonmotile primary cilia. Unlike motile cilia, primary cilia are found on nearly every cell as hairlike projections extending from the cell membrane. They sense and transduce environmental cues to maintain cellular homeostasis and are involved in various cellular functions ranging from proliferation, differentiation, regulation of inflammation, and embryonic development (5, 165). Recently, the delineation between motile cilia and primary cilia functions have blurred. Key signaling proteins such as hedgehog and smoothened, once thought unique to primary cilia, are also expressed in motile cilia (122). Moreover, in the airway epithelium, the expression of primary cilia is required for subsequent development of motile cilia (88).

As motile cilia represent the very first point of contact with invading pathogens, it makes sense for these organelles to be equipped with surface receptors granting sensory abilities to rapidly detect and respond to infectious microbes. For example, several members of the T2R family of G protein-coupled receptors localize to the respiratory cilia to increase CBF and NO production in response to bacterial acyl-homoserine lactone and quinolone quorum-sensing molecules (51, 111). Toll-like receptors, which are microbial pattern recognition receptors found on immune cells such as macrophages and dendritic cells, have also been identified in airway motile cilia (83, 221). The transient receptor potential channel subfamily V member 4 (TRPV4) ion channel also localizes to motile cilia in mice. Stimulation with TRPV4 agonists induced Ca²⁺ influx that increased CBF (118). Interestingly, TRPV4 was also implicated in the regulation of innate airways defense in response to bacterial LPS. LPS activation of TRPV4 in mouse tracheobronchial AECs rapidly increased both CBF and NO production (1). Findings by Nordgren et al. (146) lend further credence to the signaling function of motile cilia by demonstrating that cilia regulate signal transduction through the transcription factor serum response factor (SRF). SRF localizes to motile cilia in differentiated murine AECs and regulates interleukin (IL)-8 expression and CBF following organic dust exposure. A recent study also showed that ciliary proteins possess immunoregulatory functions, with siRNA-mediated knockdown of IFT88 altering NF-κB signaling and the expression of multiple downstream inflammatory cytokines (131).

Combined, these findings bear significant implications to the way we view respiratory cilia and their contributions to airway pathology during infections. Their loss not only affects MCC, but also may blunt chemosensory and immunoregulatory functions of AECs. This often overlooked aspect of cilia function brings significant ramifications to current interpretations of AEC studies as these organelles are an integral component of the differentiated airway epithelium in vivo. However, the majority of in vitro AEC models rely on undifferentiated AEC cultures that do not express motile cilia. Bearing this in mind, the choice of in vitro models should be taken into greater consideration in future studies of the airways. However, the use of primary differentiated cells for mechanistic studies is often hampered by difficulties performing genetic manipulations due to the limited numbers of passages primary airway basal cells can be cultured as well as the innate resistance of differentiated airway cells to viral transduction or other means of transfection. Methodologies like clustered regularly interspaced short palindromic repeats (CRISPR) knockout would be greatly facilitated by more efficient technologies to deliver recombinant DNA or RNA to primary airway cells without disrupting normal cilia differentiation. Such advances may come from the field of gene therapy, where work has been ongoing for many years to deliver expression vectors to airway cells to treat diseases like CF (212).

FUTURE DIRECTIONS AND UNANSWERED QUESTIONS

Based on current findings concerning the interplay between motile cilia and respiratory pathogens, many of these microorganisms have evolved intricate means to quickly overcome MCC mechanisms by specifically targeting aspects of cilia biology that encompass motility, beat coordination, ultrastructure, and expression. In nearly all types of infections described here, resulting MCC impairment places the conducting airways at increased risk of coinfection. However, despite an abundance of reports documenting various ciliopathies associated with infections, the pathogenesis remains poorly defined. Fur-
thermore, with new understanding that motile cilia may function as sensory organelles involved in immune surveillance, signaling by inflammatory pathways, and/or airway epithelial repair, loss of cilia during airway infections may bear even more significant consequences to airway epithelial homeostasis than previously thought. Recent data described above have challenged the long-standing concept that ciliated cells serve a purely mechanical role in MCC. Many signaling processes elucidated in nonmotile primary cilia in other tissues may also occur in motile cilia and have unknown implications for airway physiology. Further research is required to better understand how loss of motile cilia signaling contributes to the pathogenesis of airway diseases. Proper pharmacological therapeutic intervention may be able to modulate these signaling pathways even without restoration of cilia and might alleviate some aspects of disease.

Questions also remain regarding how compounds that modulate CBF and/or maintain cilia expression may influence clinical outcomes associated with airway infections as in vivo studies investigating this aspect remain sparse. Thus efforts to uncover the molecular mechanisms behind pathogen-induced ciliopathies may result in new therapeutic options that extend beyond enhancing the mucociliary escalator. More research on ciliated cells is also needed to understand potential ciliated cell heterogeneity, either between different anatomic regions or within the same regions of the airway. Such heterogeneity, although relatively unexplored, may underlie susceptibility of different airway regions to different pathogens like SARS-CoV2 or P. aeruginosa.

GRANTS

Writing of this review was supported, in part, by NIH/National Institute on Deafness and Other Communication Disorders Grant R01DC016309.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.E.K. and R.J.L. prepared figures; L.E.K. drafted manuscript; L.E.K. and R.J.L. edited and revised manuscript; L.E.K. and R.J.L. approved final version of manuscript.

REFERENCES

1. Alpizar YA, Boonen B, Sanchez A, Jung C, López-Requena A, Naert R, Steelant B, Luysts K, Plata C, De Vooght V, Vanoirbeek JAJ, Meseguer VM, Voets T, Alvarez JL, Hellings PW, Hoet PHM, Nemery B, Valverde MA, Talavera K. TRPV4 activation triggers protective responses to bacterial lipopolysaccharides in airway epithelial cells. Nat Commun 8: 1059, 2017. doi:10.1038/ncomms11201-3.
2. Ambrosioni J, Briveuxa PO, Wagner G, Mamin A, Kaiser L. Epidemiology of viral respiratory infections in a tertiary care centre in the era of molecular diagnosis, Geneva, Switzerland, 2011–2012. Clin Microbiol Infect 20: 0578–0584, 2014. doi:10.1111/1469-0691.12525.
3. Amirani R, Murayama T, Nawada R, Lee WJ, Niimi A, Suzuki K, Tanaka E, Kuze F. Aspergillus culture filtrates and sputum sols from patients with pulmonary aspergillosis cause damage to human respiratory ciliated epithelium in vitro. Eur Respir J 8: 1681–1687, 1995. doi:10.1183/09031936.95.08101681.
4. Amirani R, Taylor G, Elezis EN, Llewellyn-Jones C, Mitchell J, Kuze F, Cole PJ, Wilson R. Purification and characterization of factors produced by Aspergillus fumigatus which affect human ciliated respiratory epithelium. Infect Immun 63: 3266–3271, 1995. doi:10.1128/IAI.63.9.3266-3271.1995.
5. Anvarian Z, Myktykin K, Mukhopadhayay S, Pedersen LB, Christensen ST. Cellular signalling by primary cilia in development, organ function and disease. Nat Rev Nephrol 15: 199–219, 2019. doi:10.1038/s41581-019-0116-9.
6. Arbi M, Pefani DE, Kyrouri C, Lalioti ME, Kalogerotopoulou A, Papapanastasiou AD, Taraviras S, Lygerou Z. GemC1 controls multi-ciliogenesis in the airway epithelium. EMBO Rep 17: 400–413, 2016. doi:10.15252/embr.201540882.
7. Arden KE, Greer RM, Wang CYT, Mackay IM. Genotypic diversity, circulation patterns and co-detections among rhinoviruses in Queensland, 2001. Access Microbiol 2: 2020. doi:10.1099/acmi.0.000075.
8. Bailey KL, Kharbanda KK, Katafiasz DM, Sisson JH, Wyatt TA. Oxidative stress associated with aging activates protein kinase Cε, leading to cilia slowing. Am J Physiol Lung Cell Mol Physiol 315: L882–L890, 2018. doi:10.1152/ajplung.00033.2018.
9. Bailey KL, LeVan TD, Yanov DA, Pavlik JA, DeVasure JM, Sisson JH, Wyatt TA. Non-typeable Haemophilus influenzae decreases cilia beating via protein kinase Cε. Respir Res 13: 49, 2012. doi:10.1186/1465-9921-13-49.
10. Bakshani CR, Morales-Garcia AL, Althaus M, Wilcox MD, Pearson JP, Bythell JC, Burgess JG. Evolutionary conservation of the antimicrobial function of mucus: a first defence against infection. NPJ Biofilms Microbiomes 4: 14, 2018. doi:10.1038/s41522-018-0057-2.
11. Bals R. Epithelial antimicrobial peptides in host defense against infection. Respir Res 1: 5, 2000. doi:10.1186/RR5.
12. Barrera NP, Morales B, Villámon L. Plasma and intracellular membrane inositol 1,4,5-trisphosphate receptors mediate the Ca2+ increase associated with the ATP-induced increase in ciliary beat frequency. Am J Physiol Cell Physiol 287: C1114–C1124, 2004. doi:10.1152/ajpcell.00034.2003.
13. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 16: 497–516, 2003. doi:10.1128/CMR.16.3.497-516.2003.
14. Blaaas D, Fuchs R. Mechanism of human rhinovirus infections. Mol Cell Pediatr 3: 21, 2016. doi:10.1186/s40348-016-0049-3.
15. Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, Palmenberg AC, Gern JE. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad Sci USA 112: 5485–5490, 2015. doi:10.1073/pnas.1421781112.
16. Borchers AT, Chang C, Gershwin ME, Gershwin LJ. Respiratory syncytial virus–a comprehensive review. Clin Rev Allergy Immunol 45: 331–379, 2013. doi:10.1007/s12016-013-8368-9.
17. Brekman A, Walters MS, Tilley AE, Crystal RG. FOXJ1 prevents cilia growth inhibition by cigarette smoke in human airway epithelium in vitro. Am J Respir Cell Mol Biol 51: 688–704, 2014. doi:10.1165/rcmb.2013-0363OC.
18. Breslin J, Mork I, Smith MK, Vogek LG, Hemmilä EM, Bonavia A, Talbot PJ, Sjöström H, Norén O, Holmes KV. Human coronavirus 229E: receptor binding domain and neutralization by soluble receptor at 37°C. J Virol 77: 4435–4438, 2003. doi:10.1128/JVI.77.4.4435-4438.2003.
19. Brooks LRR, Mias GI. Streptococcus pneumoniae’s virulence and host immunity: aging, diagnostics, and prevention. Front Immunol 9: 1366, 2018. doi:10.3389/fimmu.2018.01366.
20. Bustamante-Marin XM, Ostrowski LE. Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol 9: a028241, 2017. doi:10.1101/cshperspect.a028241.
21. Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK, Boucher RC, Ruhlstein M. A pericytic brush promotes the lung health by separating the mucus layer from airway epithelia. Science 337: 937–941, 2012. doi:10.1126/science.1223012.
22. Campodónico VL, Gadjeva M, Paradis-Bleau C, Uluer A, Pier GB. Airway epithelial control of Pseudomonas aeruginosa infection in cystic fibrosis. Trends Mol Med 14: 120–133, 2008. doi:10.1016/j.molmed.2008.01.002.
23. Chaudhary N, Marr KA. Impact of Aspergillus fumigatus in allergic airway diseases. Clin Transl Allergy 1: 4, 2011. doi:10.1186/2045-7022-1-4.
24. Chilvers MA, McKeen M, Rutman A, Myint BS, Silverman M, O’Callaghan C. The effects of coronavirus on human nasal ciliated respiratory epithelium. Eur Respir J 18: 965–970, 2001. doi:10.1183/09031936.01.0009301.
25. Chilvers MA, O’Callaghan C. Analysis of ciliary beat pattern and beat frequency using digital high speed imaging: comparison with the photomultiplier and photodiode methods. Thorax 55: 314–317, 2000. doi:10.1136/thorax.55.4.314.
microdomains that are distinct from cilia. Virology 484: 395–411, 2015. doi:10.1016/j.virology.2015.05.014.

99. Kanthakumar K, Taylor G, Tsang KW, Cundell DR, Rutman A, Smith S, Jeffery PK, Cole PJ, Wilson R. Mechanisms of action of Pseudomonas aeruginosa pyocyanin on human ciliary beat in vitro. Infect Immun 61: 2848–2853, 1993. doi:10.1128/IAI.61.7.2848-2853.1993.

100. Kennedy JL, Turner RB, Braciale T, Heymann PW, Borish L. Pathogenesis of rhinovirus infection. Curr Opin Virol 2: 287–293, 2012. doi:10.1016/j.coiviro.2012.03.008.

101. King P. Haemophilus influenzae and the lung. (Haemophilus and the lung). Clin Transl Med 1: 10, 2012. doi:10.1002/1521-393X.10011–10013.

102. King PT, Sharma R. The lung immune response to nontypeable Haemophilus influenzae (lung immunity to NTHi). J Immunol Res 2015: 706756, 2015. doi:10.1155/2015/706756.

103. Klein MK, Haberberger RV, Hartmann P, Faulhammer P, Lüscher KS, Krain B, Wess J, Kummer W, König P. Muscarinic receptor subtypes in cilia-driven transport and airway epithelial development. Eur Respir J 33: 1113–1121, 2009. doi:10.1183/09031936.00015108.

104. Kobayashi K, Salathé M, Pratt MM, Cartagena NJ, Soloni F, Seybold ZV, Wanner A. Bacterial pneumonia as an influenza complication. Curr Opin Infect Dis 23: 201–207, 2010. doi:10.1093/cid/cip667.

105. Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine 26, Suppl 4: D59–D66, 2008. doi:10.1016/j.vaccine.2008.07.025.

106. Kuroda M, Niwa S, Sekizuka T, Tsukagoshi H, Yokoyama M, Ryo A, Kobayashi K, Salathé M, Pratt MM, Cartagena NJ, Soloni F, Seybold ZV, Wanner A. Human and avian influenza viruses target different cell types in cultures and are not increased by ACE inhibitors or angiotensin receptor blockers (Preprint). medRxiv: 2020.05.08.20092660, 2020. doi:10.1101/2020.05.08.20092660.

107. Lachowicz-Szroggings ME, Boushey HA, Finkbeiner WE, Widdicombe JH. Interleukin-13-induced mucus metaplasia increases susceptibility of human airway epithelium to rhinovirus infection. Am J Respir Cell Mol Biol 43: 652–661, 2010. doi:10.1165/rcmb.2009-0244OC.

108. Lee IT, Nakayama T, Wu CT, Golstev Y, Jiang S, Liao CK, Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Kuroda M, Niwa S, Sekizuka T, Tsukagoshi H, Yokoyama M, Ryo A, Kobayashi K, Salathé M, Pratt MM, Cartagena NJ, Soloni F, Seybold ZV, Wanner A. Bacterial pneumonia as an influenza complication. Curr Opin Infect Dis 23: 201–207, 2010. doi:10.1093/cid/cip667.

109. Lee N, Lui GC, Wong KT, Li TC, Tse EC, Chan JY, Yu J, Wong SS, King PT, Sharma R. Human rhinovirus infection: lessons from mouse models. J Aerosol Med Pulm Drug Deliv 21: 13–24, 2008. doi:10.1080/jamp.2007.0659.

110. Leczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev 15: 194–222, 2002. doi:10.1128/CMR.15.2.194-222.2002.

111. Mall MA. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J Aerosol Med Pulm Drug Deliv 21: 13–24, 2008. doi:10.1080/jamp.2007.0659.

112. Manes RP, Batra PS. Fungal aflatoxins and nasal epithelial cells damage and human rhinovirus infection: cytological findings. Acta Biomed 91: 146–147, 2020. doi:10.23750/abm.v91i1.8924.

113. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 55: 2000607, 2020. doi:10.1183/13993003.00607-2020.

114. Mata M, Arronido I, Armengot M, Carda C, Martinez I, Melero JA, Cortijo J. Respiratory syncytial virus inhibits ciliogenesis in differentiated normal human bronchial epithelial cells: effectiveness of N-acetylcysteine. PLoS One 7: e51027, 2012. doi:10.1371/journal.pone.0051027.

115. Matsuo TC, Manes RP, Batra PS. Fungal aflatoxins and nasal epithelial cells damage and human rhinovirus infection: cytological findings. Acta Biomed 91: 146–147, 2020. doi:10.23750/abm.v91i1.8924.

116. Me Fie M, Coneva L, Collins I, Covrey CR, Clube AM, Chanalaris A, Vincent TL, Bezbradica JS, Sanson SN, Wann AK. Ciliated cells specify the cell inflammatory response by tuning NFκB signaling, independently of primary cilia. J Cell Sci 133: jcs239871, 2020. doi:10.1242/jcs.239871.

117. Meuw E, Tieler D, Schönberg R, Schachner M, Jaeger KE, Schumacher U. Pseudomonas aeruginosa lactones I and II and their interaction with human airway cilia. J Laryngol Otol 119: 599–595, 2005. doi:10.1258/002221505S156313.

118. Milazzo G, Mercatelli D, Djukanovic R, Holgate ST, Roche WR. Ciliated cell damage in the bronchial epithelium of asthmatics and non-asthmatics. Clin Exp Allergy 23: 185–189, 1993. doi:10.1111/j.1365-2222.1993.tb00880.x.
137. Montefort S, Roche WR, Holgate ST. Bronchial epithelial shedding in asthmatics and non-asthmatics. Respir Med 87, Suppl B: 9–11, 1993. doi:10.1016/S0954-6110(96)80346-X.

138. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol 7: 59, 2017. doi:10.3389/fcimb.2017.00039.

139. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 198: 962–970, 2008. doi:10.1086/591970.

140. Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. Front Microbiol 10: 1041, 2019. doi:10.3389/fmicb.2017.01041.

141. Munkholm M, Mortensen J. Mucociliary clearance: pathophysiological aspects. Clin Physiol Funct Imaging 34: 171–177, 2014. doi:10.1111/cpf.12085.

142. Munro NC, Barker A, Rutman A, Taylor G, Watson D, McDonald-Gibson WJ, Towart R, Taylor WA, Wilson R, Cole PJ. Effect of procoyanin and 1-hydroxyphenazine on in vivo tracheal mucus velocity. J Appl Physiol (1985) 67: 316–323, 1989. doi:10.1152/jappl.1989.67.1.316.

143. Nair C, Shoemaker A, Chan M, Olsson S, Dixon M, Hogg C, Alton EF, Davies JC, Williams HD. Cyanide levels found in infected cystic fibrosis sputum inhibit airway ciliary function. Eur Respir J 44: 1255–1261, 2014. doi:10.1183/09031936.000794.2009.

144. Nemajerova A, Kramer D, Siller SS, Herr C, Shomroni O, Pena T, Gallinas Suazo C, Glaser K, Wildung M, Steffen H, Sriraman A, Oberle F, Wienken M, Hennion M, Vital D, Royen B, Alevra M, Sarna M, Maglione M, de Jong PA. Characterization of cellular transcriptomic signatures induced by different respiratory viruses in human reconstituted airway epithelia. Sci Rep 9: 11493, 2019. doi:10.1038/s41598-019-48013-7.

145. Nordgren TM, Wyatt TA, Sweeter J, Bailey KL, Poole JA, Heires AJ, Sisson JH, Romberger DJ. Motile cilia harbor serum response factor as a mechanism of environment sensing and injury response in the airway. Am J Physiol Lung Cell Mol Physiol 306: L829–L839, 2014. doi:10.1152/ajplung.00097.2013.

146. Park KS, Wells JM, Zorn AM, Wert SE, Laubach VE, Fernandez LG, Whitsett JA. Transdifferentiation of ciliated cells during repair of the respiratory epithelium. Am J Respir Cell Mol Biol 45: 151–157, 2006. doi:10.1165/rcmb.2005-0320OC.

147. Park SA, Yoo H, Seol JH, Rhee K. HDAC3 and HDAC8 are required for cilia assembly and elongation. Biomed Open 8: bio43828, 2019. doi:10.1242/bio.43828.

148. Park SY, Kim JS. A short guide to histone deacetylases including recent progress on class II enzymes. Exp Mol Med 52: 204–212, 2020. doi:10.1038/s41679-020-0382-4.

149. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yung RW, Ng TK, Yuen KY; SARS study group. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361: 1319–1325, 2003. doi:10.1016/S0140-6736(03)13077-2.

150. Pittet LA, Hall-Stoodley L, Rutkow MW, Harmsen AG. Influenza virus infection decreases tracheal mucociliary velocity and clearance of Streptococcus pneumoniae. Am J Respir Cell Mol Biol 42: 450–460, 2010. doi:10.1165/rcmb.2007-0417OC.

151. Price ME, Sisson JH. Redox regulation of motile cilia in airway disease. Redox Biol 21: 2014, 2019. doi:10.1016/j.redox.2013.09.004.

152. Rada B, Leto TL. Procoyanin effects on respiratory epithelium: relevance in Pseudomonas aeruginosa airway infections. Trends Microbiol 21: 73–81, 2013. doi:10.1016/j.tim.2012.10.004.

153. Rawlins EL, Ostrowski LE, Randall SH, Holland BL. Lung development and repair: contribution of the ciliated lineage. Proc Natl Acad Sci USA 104: 410–417, 2007. doi:10.1073/pnas.0610770104.

154. Read RC, Roberts P, Munro N, Rutman A, Hastele R, Shroyack T, Hall R, McDonald-Gibson W, Lund V, Taylor G, Cole PJ, Wilson R. Effect of Pseudomonas aeruginosa rhamnolipids on mucociliary transport and ciliary beating. J Appl Physiol (1955) 72: 2271–2277, 1992. doi:10.1152/jappl.1992.72.6.2271.

155. Rockx B, Kuiken T, Herfst S, Bestebroer T, Lamers MM, Oude Munnink BB, de Meulder D, van Amerongen G, van den Brand J, Okba NMA, Schipper D, van Run P, Leijten L, Sikkema R, Verhoef E, Verstrepen B, Bogers W, Langermans J, Drosten C, Fentener van Vlissingen F, Moutier D, van Swart R, Koopmans M, Haagmans BL. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. Science 368: 1012–1015, 2020. doi:10.1126/science.abh7314.

156. Rudramurthy SM, Paul RA, Chakrabarti A, Mouton JW, Meis JF. Invasive aspergillosis by Aspergillus flavus: epidemiology, diagnosis, antifungal resistance, and management. J Fungi (Basel) 5: 55, 2019. doi:10.3390/jof5030055.

157. Sakakura Y, Sasaki Y, Togo Y, Wagner HN Jr, Hornick RB, Schwartz AR, Proctor DF. Mucociliary function during experimentally induced rhinovirus infection in man. Am Rev Rhinol Laryngol 82: 203–211, 1973. doi:10.1177/01403490734970300201.

158. Salathe M. Regulation of mammalian ciliary beating. Ann Rev Physiol 69: 401–422, 2007. doi:10.1146/annurev.physiol.69.040705.141253.

159. Salathe M, Lipson EJ, Ivonnet PI, Bookman RJ. Muscarinic signaling in ciliated tracheal epithelial cells: dual effects on Ca2+ and ciliary beating. Am J Physiol Lung Cell Mol Physiol 272: L301–L310, 1997. doi:10.1152/ajplung.1997.272.1.L301.

160. Salathe M, Pratt MM, Wanner A. Protein kinase C-dependent phosphorylation of a ciliary membrane protein and inhibition of ciliary beating. J Cell Sci 106: 1211–1220, 1993.

161. Sanchez-David RS, Scwann OC, Peacock TP, Barclay WS. ACE2: the only thing that matters? Am J Respir Crit Care Med 202: 161–163, 2020. doi:10.1164/rccm.202006-2812DD.

162. Santos M, Montella S, Tiddens HAWM, Guidi G, Casotti V, Sanmarti S, Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vandermeersch T, Van de Veerdonk FL, Haagmans BL. Host-pathogen interactions with respiratory cilia. J Gen Physiol 158: 97–115, 2021. doi:10.1085/jgp.202011681.

163. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vandermeersch T, Van de Veerdonk FL, Haagmans BL. Host-pathogen interactions with respiratory cilia. J Gen Physiol 158: 97–115, 2021. doi:10.1085/jgp.202011681.

164. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vandermeersch T, Van de Veerdonk FL, Haagmans BL. Host-pathogen interactions with respiratory cilia. J Gen Physiol 158: 97–115, 2021. doi:10.1085/jgp.202011681.

165. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vandermeersch T, Van de Veerdonk FL, Haagmans BL. Host-pathogen interactions with respiratory cilia. J Gen Physiol 158: 97–115, 2021. doi:10.1085/jgp.202011681.
HOST-PATHOGEN INTERACTIONS WITH RESPIRATORY CILIA

174. Smith CM, Do Hyang Lee J, Kulkarni H, Radhakrishnan P, Hirst R, Easton A, O’Callaghan C. Influenza virus infection of well-differentiated human airway epithelial cells by infectious aerosols: insights into the earliest stages of infection. F1000 Res 8: 337. 2019. doi: 10.12688/f1000research.18513.1.

175. Williams G, Hirst RA, Easton AJ, Andrew PW, O’Callaghan C. Ciliary dyskinesia is an early feature of respiratory syncytial virus infection. Eur Respir J 43: 485–496, 2014. doi: 10.1183/09031936.00205312.

176. Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 11: 59. 2019. doi: 10.3390/v11010059.

177. Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. N Engl J Med 363: 299–302, 2015. doi: 10.1056/NEJMp1215093.

178. Steinfort C, Wilson R, Mitchell T, Feldman C, Rutman A, Todd H, Steinfeld C, Wilson R, Mitchell T, Feldman C, Rutman A, Todd H, Uncapher CR, Dewitt CM, Colonno RJ. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus A. J Allergy Clin Immunol 137: 1766–1775, 2015. doi: 10.1016/j.jaci.2015.09.046.

179. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 24: 490–502, 2016. doi: 10.1016/j.tim.2016.03.006.

180. Sungnak W, Huang N, Bécair C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JI; HCA Lung Biological Network. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med 26: 681–687, 2020. doi: 10.1038/s41591-020-0868-6.

181. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med 372: 351–362, 2015. doi: 10.1056/NEJMr1300109.

182. Tamashiro E, Xiong G, Anselmo-Lima WT, Kreindler JL, Palmer Smith CM, Do Hyang Lee J, Kulkarni H, Radhakrishnan P, Hirst R, Williams G, Hirst RA, Easton AJ, Andrew PW, O’Callaghan C. Ciliary dyskinesia in critically ill patients. Am J Respir Crit Care Med 196: 524–527, 2017. doi: 10.1164/rccm.201612-2540LE.

183. Millen AE, Shields MD, Power UF. Respiratory syncytial virus interaction with human airway epithelium. Trends Microbiol 21: 238–244, 2013. doi: 10.1016/j.tim.2013.02.004.

184. Walker WT, Jackson CL, Allan RN, Collins SA, Kelso MJ, Rineh A, Yenpi NR, Nicholas B, Lau L, Johnston D, Lackie P, Faust SN, Lucas JSA, Hall-Stoodley L. Primary ciliary dyskinesia ciliated airway cells show increased susceptibility to Haemophilus influenzae biofilm formation. Eur Respir J 50: 1700612, 2017. doi: 10.1183/13993003.00612-2017.

185. Walker WT, Jackson CL, Lackie PM, Hogg C, Lucas JS. Nitric oxide in primary ciliary dyskinesia. Eur Respir J 40: 1024–1032, 2012. doi: 10.1183/09031936.00176111.

186. Walsh EE, Falsey AR. Respiratory syncytial virus infection in adult populations. Infect Drug Res Drug Targets 12: 98–102, 2012. doi: 10.2174/187152612800010116.

187. Wang G, Deering C, Macke M, Shao J, Burns R, Blau DM, Holmes KV, Davidson BL, Perlman S, McCray PB Jr. Human coronavirus 229E infects polarized airway epithelia from the apical surface. J Virol 74: 9234–9239, 2000. doi: 10.1128/JVI.74.19.9234-9239.2000.

188. Wark PA, Johnston SL, Bucchiere F, Powell R, Puddicombe S, LaJoy-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J MedExp 201: 937–947, 2005. doi: 10.1084/jem.20041901.

189. Warner SM, Wielher S, Michi AN, Proud D. Rhinovirus replication and innate immunity in highly differentiated human airway epithelial cells. Respir Res 20: 150, 2019. doi: 10.1186/s12931-019-1120-9.

190. Whitsett JA. Airway epithelial differentiation and multiciliary clearance. Am J Respir Crit Care Med 195: Suppl 3: S143–S148, 2018. doi: 10.1164/ansats.201802-128AW.

191. Wijers CD, Chmiel JF, Gaston BM. Bacterial infections in patients with primary ciliary dyskinesia: comparison with cystic fibrosis. Chron Respir Dis 14: 392–406, 2017. doi: 10.1177/147997317694621.

192. Wilson R, Pitt T, Taylor G, Watson D, MacDermot J, Sykes D, Roberts D, Cole P. Pyocyanin and 1-hydroxyphenazine produced by Pseudomonas aeruginosa inhibits the beating of human respiratory cilia in vitro. J Clin Invest 79: 221–229, 1987. doi: 10.1172/JCI112787.

193. Wilson R, Read R, Cole P. Interaction of Haemophilus influenzae with mucus, cilia, and respiratory epithelial cell membranes. J Infect Dis 165, Suppl 1: S100–S102, 1992. doi: 10.1093/infdis/165.Supplement_1-S100.

194. Wilson R, Sykes DA, Watson D, Rutman A, Cole PJ, Taylor GW, Cole P. Measurement of Pseudomonas aeruginosa phenazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelial cells. Cell Death Differ 26: 2740–2757, 2019. doi: 10.1038/s41418-019-0332-7.

195. Wu NH, Yang W, Beineke A, Dijkman R, Matrosovich M, van de Veerdonk FL, Kolvijk E, Leodrappe PO, Hodiamont CJ, Rijnders BJ, van Paassen J, Haas PJ, Oliveira Dos Santos C, Kampinga GA, Bergmans DC, van Dijk K, de Haan AF, van Dissel J, van der Hoeven HG, Verweij PE, Rahamat-Langendooij JC, Kullberg B-J, Netea MG, Brüggemann RJ, Hoedemaekers AW, Melchers WJG, Freudenburg W, Roescher N, Wiersinga WJ, van der Berg CHSV, Van AK, of Tien, C van der Hoven B, of Beek MT, Derde LPG, of Leer C, Aardema H, Lashof AO, Ang CW, Dutch Mycoses Study Group. Influenza-associated aspergillosis in critically ill patients. Am J Respir Crit Care Med 196: 524–527, 2017. doi: 10.1164/rccm.201612-2540LE.

196. Wu NY, Wang Y, Beineke A, Dijkman R, Matrosovich M, Bankert W, Thijs A, van Heijst R, Ment P, Herrler G. The differentiated airway epithelial infected by influenza viruses maintains the barrier function despite a dramatic loss of ciliated cells. Sci Rep 6: 39668, 2016. doi: 10.1038/srep39668.
209. Yaghi A, Dolovich MB. Airway epithelial cell cilia and obstructive lung disease. Cells 5: 40, 2016. doi: 10.3390/cells5040040.

210. Yaghi A, Zaman A, Cox G, Dolovich MB. Ciliary beating is depressed in nasal cilia from chronic obstructive pulmonary disease subjects. Respir Med 106: 1139–1147, 2012. doi: 10.1016/j.rmed.2012.04.001.

211. Yamanaka N, Ogra PL, Fujihara K, Bernstein JM, Hard R. Morphologic and motility changes of nasal cilia in primary culture caused by Haemophilus influenzae. Ann Otol Rhinol Laryngol 105: 452–457, 1996. doi: 10.1177/000348949610500606.

212. Yan Z, McCray PB Jr, Engelhardt JF. Advances in gene therapy for cystic fibrosis lung disease. Hum Mol Genet 28: R88–R94, 2019. doi: 10.1093/hmg/ddz139.

213. Yu F, Ran J, Zhou J. Ciliopathies: does HDAC6 represent a new therapeutic target? Trends Pharmacol Sci 37: 114–119, 2016. doi: 10.1016/j.tips.2015.11.002.

214. Yu X, Ng CP, Habacher H, Roy S. Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat Genet 40: 1445–1453, 2008. doi: 10.1038/ng.263.

215. Zagoory O, Brainan A, Priel Z. The mechanism of ciliary stimulation by acetylcholine: roles of calcium, PKA, and PKG. J Gen Physiol 119: 329–339, 2002. doi: 10.1085/jgp.20028519.

216. Zhang H, Rostami MR, Leopold PL, Mezy JG, O’Beirne SL, Struloviczi-Barel Y, Crystal RG. Expression of the SARS-CoV-2 ACE2 receptor in the human airway epithelium. Am J Respir Crit Care Med 202: 219–229, 2020. doi: 10.1164/rcrm.202003-0541OC.

217. Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, Zhang S, Cao T, Yang C, Li M, Guo G, Chen X, Chen Y, Lei M, Liu H, Zhao J, Peng P, Wang CY, Du R. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. Ann Intern Med 172: 629–632, 2020. doi: 10.7326/M20-0533.

218. Zhang L, Sanderson MJ. Oscillations in ciliary beat frequency and intracellular calcium concentration in rabbit tracheal epithelial cells induced by ATP. J Physiol 546: 733–749, 2003. doi: 10.1113/jphysiol.2002.028704.

219. Zhang L, Sanderson MJ. The role of cGMP in the regulation of rabbit airway ciliary beat frequency. J Physiol 551: 765–776, 2003. doi: 10.1113/jphysiol.2003.041707.

220. Zhao KQ, Goldstein N, Yang H, Cowan AT, Chen B, Zheng C, Palmer JN, Kreindler JL, Cohen NA. Inherent differences in nasal and tracheal ciliary function in response to Pseudomonas aeruginosa infection. Am J Rhinol Allergy 25: 209–213, 2011. doi: 10.2500/ajra.2011.25.3614.

221. Zhao Y, Shen J, Wu B, Liu G, Lu R, Tan W. Genotypic diversity and epidemiology of human rhinovirus among children with severe acute respiratory tract infection in Shanghai, 2013–2015. Front Microbiol 9: 1836, 2018. doi: 10.3389/fmicb.2018.01836.