

Digging through the Obstruction: Insight into the Epithelial Cell Response to Respiratory Virus Infection in Patients with Cystic Fibrosis

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Respiratory virus infections are common but generally self-limiting infections in healthy individuals. Although early clinical studies reported low detection rates, the development of molecular diagnostic techniques by PCR has led to an increased recognition that respiratory virus infections are associated with morbidity and acute exacerbations of chronic lung diseases, such as cystic fibrosis (CF). The airway epithelium is the first barrier encountered by respiratory viruses following inhalation and the primary site of respiratory viral replication. Here, we describe how the airway epithelial response to respiratory viral infections contributes to disease progression in patients with CF and other chronic lung diseases, including the role respiratory viral infections play in bacterial acquisition in the CF patient lung.

Respiratory viral infections significantly contribute to the morbidity and progressive decline in lung function experienced by patients with chronic lung diseases such as cystic fibrosis (CF). In early studies of CF patients, respiratory viral infections were associated with approximately 40 to 50% of pulmonary exacerbations (1, 2), although these values likely underestimate the true impact respiratory viral infections have on disease progression because they are based upon insufficiently sensitive detection methods. Not surprisingly, with newer PCR techniques, the more recently reported rates of detection of respiratory viral infections during periods of pulmonary exacerbations are between 50 and 60% (3, 4). The RNA viruses influenza virus, rhinovirus (RV), and respiratory syncytial virus (RSV) are the most common viral infections detected in CF patients, with RSV promoting early respiratory tract morbidity and RV being the most common causative viral agent in pulmonary exacerbation. Although the incidence of viral infections is not greater in patients with CF than in healthy controls, the severity and length of viral infection are amplified in patients with CF and they are associated with increased antibiotic use, deterioration of pulmonary function, and longer durations of hospitalization. The mechanisms underlying the increased severity of respiratory viral infections in patients with CF are incompletely understood. Here, we highlight recent research characterizing the mechanisms by which the airway epithelium contributes to the severity of respiratory viral infection in the lungs of patients with CF and expand these findings to viral infections in other chronic lung diseases.

IMPAIRED ANTIVIRAL RESPONSES CONTRIBUTE TO RESPIRATORY VIRUS-INDUCED CF EXACERBATIONS

The airway epithelium is the first line of defense against inhaled pathogens and thus is a critical component of the innate immune system. In addition, the airway epithelium is the primary site of virus replication during respiratory virus infection and plays a critical role in viral pathogenesis in the CF patient lung. When microbial ligands engage various families of pattern recognition receptors (PRRs), distinct signaling cascades are activated within airway epithelial cells (AECs) that coordinate the host's response to the invading microbe (Fig. 1). During respiratory viral infec-

tion, the most relevant PRRs are Toll-like receptors and retinoic acid-inducible gene 1-like receptors, which detect double-stranded RNA (dsRNA), a by-product of virus replication, and activate signaling cascades that culminate in the production of antiviral molecules. Type I and III interferons (IFN- β and - λ , respectively) are the major antiviral substances produced by AECs, and they act in an autocrine and paracrine manner to induce an antiviral state within AECs mediated by IFN-stimulated genes (ISGs).

Studies of primary human AECs have shown that primary CF AECs produce reduced levels of antiviral mediators downstream of IFN, specifically, nitric oxide synthase 2 (NOS2), 2',5'-oligoadenylate synthetase 1 (OAS1), and signal transducer and activator of transcription 1 (STAT1), in response to human parainfluenza virus 3 (HPIV3), resulting in greater release of infectious HPIV3 than in non-CF AECs (5). In addition, a recent clinical study has demonstrated that the lower airway RV burden is greater in patients with CF than in healthy controls and that the greater RV load in CF patients is negatively associated with type I IFN levels in the BAL fluid of the patients (6). These studies raise the question of whether CF AECs are inefficient at producing IFN in response to virus infection, are unable to respond to IFN, or respond to IFN stimulation but do not produce particular antiviral mediators. Recent work has demonstrated that CF AECs produce levels of type I and III IFNs similar to those of non-CF AECs in response to virus or the dsRNA analog poly(I-C), suggesting that CF AECs can produce and respond to IFN and thus, any defect in ISG production may be specific to certain ISG pathways (7, 8). It is important to point out that inflammatory cytokine secretion is similar in CF

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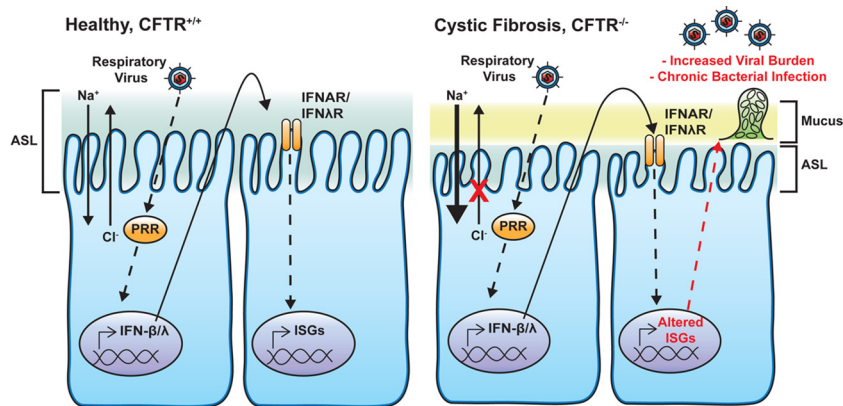


FIG 1 Epithelial responses to respiratory virus infection in AECs from healthy persons and cystic fibrosis (CF) patients. In CF patient AECs, mutations in the CF transmembrane conductance regulator (CFTR) ion channel lead to reduced chloride (Cl⁻) secretion and increased sodium (Na⁺) uptake, resulting in a depleted airway surface liquid (ASL) level and increased mucus buildup, impairing mucociliary clearance of pathogens from the airway. CF and non-CF AECs recognize viral infection through PRRs, which lead to the production of type I and III IFNs (IFN-β and -λ, respectively). These molecules are secreted from AECs and act in an autocrine and paracrine manner to signal through their cognate receptors, the IFN-α/β receptor (IFNAR) and the IFN-λ receptor (IFNλR), respectively, to induce the expression of antiviral mediators (i.e., ISGs). CF patient AECs have defects in ISG production, resulting in an increased viral burden. The innate immune response to respiratory viral infection also enhances bacterial colonization and *P. aeruginosa* biofilm growth.

and non-CF primary AECs during viral infection, and thus, the inflammatory response induced by virus infection in CF patients does not contribute to increased morbidity (9). Because of the negative correlation between virus-induced cytotoxicity and inflammatory cytokine production in CF AECs (9, 10), a possible explanation for the lack of an exaggerated inflammatory response during viral infection could be the increased virus-induced death of AECs in CF. Taken together, these studies suggest that reduced antiviral responses to respiratory viral infections result in uncontrolled viral replication and increased viral burdens, but not exaggerated inflammation, in the CF patient lung (Fig. 1). Further research is needed to clearly define what defects exist in antiviral networks in the CF airway.

BACTERIAL COINFECTION DURING RESPIRATORY VIRAL INFECTIONS

Beyond the morbidity linked to respiratory viral infections alone, clinical studies have linked respiratory viral infections with the development of chronic infections with the Gram-negative bacterium *Pseudomonas aeruginosa* in CF patients. *P. aeruginosa* is the most common bacterial pathogen in patients with CF and is well known to have deleterious effects on lung function. Seasonal trends have been noted in which the majority of patients with CF were initially infected with *P. aeruginosa* during respiratory viral seasons (11). In addition, up to 85% of new *P. aeruginosa* colonization in CF patients occurred within 3 weeks following a respiratory viral infection (12). RSV has been reported to be the most common respiratory virus associated with the development of chronic *P. aeruginosa* infections in CF patients (13). However, the mechanisms underlying how respiratory viruses enhance the development of chronic *P. aeruginosa* infections remain poorly understood. Studies have shown that RSV infection strongly enhanced *P. aeruginosa* binding to AECs *in vitro* (14). RSV infection has also been noted to increase *Streptococcus pneumoniae* adherence to AECs (15). Furthermore, RSV infection increased *P. aeruginosa* burdens in the lungs of mice coinfecting with RSV and *P. aeruginosa*, further suggesting that RSV infection facilitates acute *P. aeruginosa* infection (16).

The formation of chronic bacterial infections often involves the formation of bacterial biofilms, which are surface-associated communities of bacteria with characteristic upregulation of antibiotic resistance genes and polymeric matrix production that serve to protect bacteria from host immune mechanisms and clearance (17). We have recently demonstrated that RSV, RV, and adenovirus infections and the resulting type I and III IFN responses enhance *P. aeruginosa* biofilm growth on CF AECs (18). Moreover, RSV infection promotes the release from CF AECs of the host iron-binding protein transferrin, which is required for RSV-mediated biofilm growth (18). Iron is a necessary nutrient for *P. aeruginosa* biofilm growth on AECs, and a strong positive correlation between increased airway iron and disease severity in the CF patient lung has been reported (19). These studies provide further evidence that respiratory viral infections negatively affect disease severity in CF. It is unlikely that IFN-mediated bacterial growth is specific to CF lung disease, as previous studies have demonstrated that in models of acute influenza virus-bacterial coinfections, the type I IFN response to influenza virus is responsible for increased bacterial burdens in the lung (20–22). Type III IFN signaling also has adverse effects on bacterial clearance from the airways. In acute models of *P. aeruginosa* and *Staphylococcus aureus* infections, mice lacking interleukin-28Rα, one component of the heterodimeric type III IFN receptor, have better bacterial clearance than wild-type mice (23). Collectively, these results suggest that respiratory viral infections alter the environmental conditions in the lung, creating favorable conditions for bacterial coinfection and disease progression in CF patients (Fig. 1).

RESPIRATORY VIRAL EXACERBATION OF OTHER CHRONIC LUNG DISEASES

In other chronic lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD), respiratory virus infections are also recognized as major contributors to exacerbations. Respiratory viruses are present in up to approximately 80 and 60% of asthma and COPD exacerbations, respectively, with influenza virus, RV, and RSV being the most commonly identified viruses (24). As in CF patients, the virus loads in asthma and COPD pa-

tients are higher following respiratory viral infection than in healthy controls (25, 26). Interestingly, studies with primary AECs have demonstrated that the innate immune response to viral infections is impaired in these patients as well, with AECs producing reduced levels of type I and III IFNs (26–28). Recently, it was shown that suppressor of cytokine signaling 1 (SOCS1), a negative regulator of the IFN response to respiratory viruses, is upregulated in primary AECs from asthmatic patient cells, suggesting a potential mechanism for the deficient antiviral responses observed during respiratory viral infections in asthmatic patients (29). In addition, the production of inflammatory mediators and increased neutrophil influx during respiratory viral infection in asthma and COPD patients has been reported and likely contributes to the pulmonary damage patients experience during viral infections (25, 26). Recent studies have also demonstrated an association between respiratory viral infections and bacterial infections in asthma and COPD patients, but the mechanism underlying this relationship remains unclear (30, 31).

CONCLUSIONS

In summary, respiratory viral infections are major contributors to acute exacerbations of chronic lung diseases. The majority of studies suggest that an impaired immune response to viral infection results in increased viral replication in the airways of patients, but the underlying reasons for a reduced immune response in the respiratory epithelium of CF patients remain incompletely defined and require further investigation. In addition, emerging evidence also suggests that respiratory viral infection promotes pulmonary bacterial colonization in chronic lung diseases. Although we focused on the airway epithelial response to respiratory viral infection, many other cell types, such as phagocytes and T cells, are pivotal for the host to effectively respond to respiratory viral infection. We refer readers to a recent review that highlights the advances made in understanding how these innate and adaptive responses also contribute to viral pathogenesis and bacterial coinfection in the lung (32). Given the association between viral infection and pulmonary exacerbation in CF, as well as other chronic lung diseases, it is important to understand the mechanisms underlying the increased severity of respiratory virus infections to better develop therapeutics targeting viral infections and their associated comorbidities, including the bacterial coinfections that may develop as a result of viral infection.

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