Airway Surface Liquid and Impaired Antiviral Defense in Cystic Fibrosis

Respiratory viral infections, including parainfluenza, respiratory syncytial virus, and influenza, are strongly associated with lung disease progression in patients with cystic fibrosis (CF) (1, 2). Human rhinovirus, the most common viral pathogen in CF, increases respiratory symptoms, airway inflammation, and the number of pulmonary exacerbations (3). Respiratory viral infections predispose patients with CF to increased prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections (4). These reports highlight the importance of respiratory viral infections as risk factors for CF lung disease progression.

Although protection against respiratory viral infections requires an intact humoral and cell-mediated immune response, the airway surface liquid (ASL), in concert with the airway epithelium, comprises the initial defensive barrier against infections. The CF pig has been an important model to identify primary events related to onset of CF lung disease. The CF pig develops human-like CF lung disease and respiratory bacterial infections due to loss of CFTR (cystic fibrosis transmembrane conductance regulator) bicarbonate secretion, resulting in adherent mucus with failure of mucociliary clearance (5, 6) and defective innate immune clearance of microbes (7). Importantly, the ASL is more acidic in CF pigs than in non-CF pigs, and correcting the CF ASL pH improves both *in vivo* and *in vitro* bacterial killing. Finally, several antimicrobial proteins in ASL, including lysozyme, lactoferrin (7), human β-defensin-3, and LL-37 (8), have pH-dependent antibacterial activity *in vitro*.

On the basis of these studies, Berkebile and colleagues (9) tested whether newborn CF pig nasal ASL has deficient antiviral activity. The rationale for using newborn pigs is to avoid any secondary effects on the innate immune system due to early infections or inflammation, which would degrade critical antimicrobial proteins (10). In this issue of the *Journal* (pp. 104–111), Berkebile and colleagues report that healthy adult human nasal ASL and healthy newborn pig nasal and tracheal ASL inhibit viral replication for Sendai virus (SeV), respiratory syncytial virus, influenza A, and replication-deficient adenovirus (9). Importantly, CF pig nasal ASL has impaired *in vitro* killing for SeV compared with non-CF pig nasal ASL. This is the first report, to our knowledge, that CF ASL has deficient antiviral activity. As part of the characterization of the nasal ASL antiviral activity, the authors show that human and pig nasal ASL have a broad antiviral repertoire *in vitro* against both RNA and DNA viruses. They also demonstrate that heat inactivation decreases the anti-SeV activity of pig nasal ASL to a small residual level, supporting the role for proteins and possibly other nonpeptide factors as part of the ASL antiviral capacity.

However, the mechanisms for deficient antiviral activity in CF ASL are complex and are not fully identified in this report. Compared with ASL antibacterial activity (7), ASL antiviral activity is not as dependent on pH. There is no significant change in anti-SeV activity over the pH range of 6.8–8.0 in non-CF or CF pig ASL (9), whereas antibacterial activity in ASL is significantly increased at pH 8.0 (7). Specific innate immune proteins—LL-37, a human cathelicidin; protegrin-1, a porcine cathelicidin; and human β-defensin 3—have minimal pH-related changes in anti-SEV activity, whereas, in contrast, lysozyme, lactoferrin, human β-defensin-3, and LL-37 have marked pH-dependent changes in antibacterial killing (7, 8). Furthermore, by 3 months of age and older, the nasal ASL pH in infants with CF rises to the same levels as in healthy subjects (11). Given the lack of a definitive role for pH in regulating ASL antiviral activity, what other components of the ASL are responsible for the antiviral innate immune defect in CF?

The ASL reflects airway epithelial cell signaling and secretory function. One major defect in CF airway epithelial cells is the failure of IFN signaling via STAT1 (signal transducer and activator of transcription 1) to activate IFN-stimulated genes (12), including inducible nitric oxide synthase 2 (iNOS2) in epithelial cells (13). The absence of iNOS2 leads to increased parainfluenza 3 viral replication and augmented cytokine generation. Treatment with NO donors or NOS2 transfection improves antiviral activity of CF cells (13). Another important host antimicrobial factor that is deficient in CF ASL is hypothiocyanate. Hypothiocyanate is generated by oxidation of thiocyanate by lactoperoxidase in the presence of hydrogen peroxide. Loss of CFTR-regulated transport of thiocyanate (14, 15) results in hypothiocyanate deficiency in the CF airway. Hypothiocyanate has both antibacterial and antiviral activities; hypothiocyanate inactivates influenza A and B *in vitro* (16). Although these signaling and secreted proteins may play a role in susceptibility for bacterial and viral infections in patients with CF, we still await new therapies that target these pathways. The report by Berkebile and colleagues spurs the CF community to confirm their findings in human infants and to determine the identity and mechanism of key innate immune factors required for sufficient antiviral innate immunity and prevention of lung disease sequelae. ■

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