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INSIGHTS

Breathe—Your immune system is counting on it

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Always, but especially in these times of COVID pandemic, we know the dangers of breathing into our lungs a deadly pathogen. Fortunately, healthy lungs are equipped with an innate immune system that works to clear those pathogens. A study in this issue (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20201831) shows, for the first time, that breathing-induced changes in the pH of the airway surface contribute to bacterial killing, pointing to new therapeutic strategies for maintaining pulmonary health.

The airway environment is complex, even when considered in a snapshot of time (Olivença et al., 2021). Multiple epithelial cell types contribute to the conductive airway represented by the trachea, bronchi, and bronchioles, including goblet cells, ciliated cells, club cells, ionocytes, and basal cells. At the level of the alveolus, we have Type I and Type II alveolar epithelial cells, each with distinct functions. In addition to the epithelial cells that line the airway, several subsets of immune cells also contribute to the airway ecosystem. This is dominated by the scavenging macrophages in the healthy lung, but neutrophils often become dominant during pulmonary infections. Various other immune cells, including dendritic cells and T lymphocytes, contribute at lower abundances.

Of course, a description of the lung ecosystem would be woefully incomplete without inclusion of the many pulmonary pathogens to which people are exposed every day. While the lung was long thought to be a sterile environment, it is becoming clear that even overtly healthy individuals experience transient invasion of the airway by viruses (SARS-CoV-2, influenza, respiratory syncitial virus, etc.) and a wide variety of bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, etc.) and fungi (e.g., *Aspergillus* sp.). This should come as no surprise whatsoever, given that humans sample their environment with every breath, everywhere we go. Fortunately, the epithelial and immune cells of the airway in most people collaborate, serving as sentries to repel or kill and clear such invading pathogens, in part by maintaining conditions that are not conducive to establishment of chronic infection (Kuek and Lee, 2020). This delicate balance is lost in people affected by some transient disorders, such as acute respiratory distress syndrome, or chronic disorders, such as cystic fibrosis (CF). In the latter case, the interactions between the host cells comprising the airway are disrupted due to the loss of functional CFTR (the CF transmembrane conductance regulator), a chloride/bicarbonate ion channel expressed in several of these cell types.

Unlike the single tube of the intestine, with essentially one-way traffic, the human airway is a closed system with much more complex architecture. Due to the branching of large airways leading to small airways terminating in half a billion alveoli, the adult human airway comprises an estimated 50 m^2 of surface area (Fröhlich et al., 2016). Depending upon posture and level of activity, different regions of the lung are recruited into active breathing and experience regulated changes in arterial perfusion, resulting in increased oxygen delivery to exercising tissues.

Importantly, the airway is not a static environment, but rather quite dynamic, exposed to environmental conditions on the order of 15 times per minute during tidal breathing in adult humans at rest. Furthermore, the composition of the gases entering and exiting the lung differ, being altered by gas exchange at the alveoli. One may wonder, then, how inspired and expired gases alter the ecosystem of the lung, and how this impacts the defense mechanisms we rely upon to keep invading pathogens from establishing a foothold.

Insights from Nael A. McCarty.

In a highly mechanistic study reported here (Kim et al., 2021), an investigative team led by Dr. John Hanrahan (McGill University) has explored an important physiological function not previously considered but likely to be relevant to CF and a variety of

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other pulmonary diseases: respiratory cycling of airway surface pH, which contributes to host defense mechanisms.

The authors asked whether the changes in CO_2 abundance within the airway during tidal breathing may contribute to changes in pH of the airway surface liquid (ASL), whether these functions are altered in the CF airway, and whether this contributes to host defense. The methods applied are technically demanding, and the results are compelling.

Prior work from other laboratories relied primarily upon measurements of apical fluid pH in airway cells ex vivo under static conditions, sometimes via indirect assays, which led to the conclusion that ASL in CF cells was at best slightly acidic compared with ASL in non-CF cells. In contrast, Hanrahan and colleagues used flexible, double-barreled, ultrafine capillary microelectrodes to directly measure pH in the ASL of human nasal mucosa in vivo (Kim et al., 2021). It is well accepted that nasal epithelium is a good surrogate for distal airway epithelium, at least with respect to CFTR function (Brewington et al., 2018). With this apparatus, the team showed that the pH of nasal mucosa of healthy subjects oscillated widely between ∼7.5 and 9 during exhalation and between ∼8.5 and 9 during inhalation. Following up on this surprising observation, Hanrahan and colleagues used fluorescent pH indicators to measure ASL pH in cultures of primary human bronchial epithelial cells (a mix of ciliated and goblet cells) exposed to gases in a waveform designed to mimic tidal breathing (Kim et al., 2021). They found that oscillations in CO_2 in this system, as seen in tidal breathing, resulted in reciprocal pH changes. The peak pH reached was dependent upon the concentration of bicarbonate in the apical fluid.

This is where the relevance to CF comes in. While the genetic complexity of CF is very wide, with at least several hundred mutations in the gene encoding CFTR being associated with disease, most people with CF express the F_{508}del mutation that results in loss of functional CFTR protein at the plasma membrane. Loss of CFTR results in greatly reduced capacity for bicarbonate secretion into the ASL. Indeed, Hanrahan and colleagues compared breathing-induced pH changes in the nasal mucosa of CF and non-CF subjects using their in vivo microelectrode system (Kim et al., 2021). They found that peak and trough pH values measured during inhalation and expiration were 1.3 and 0.5 pH units lower, respectively, in CF subjects compared with healthy controls. This difference is relevant because, as shown in this study, *P. aeruginosa*—a very common CF pathogen—grown in artificial ASL was sensitive to alkaline pH in the range reached during inhalation only in non-CF subjects, and this function required activation of epithelial CFTR. The authors concluded that the breathing-induced oscillations in CO_2 abundance produced large shifts in pH toward levels of alkalinity that are antimicrobial, which may make important contributions toward innate immunity in the lung.

For this mechanism to be functional, the tissue must be able to accommodate rapid CO_2 hydration and dehydration reactions at rates that are relevant to the respiratory cycle. These reactions are catalyzed by carbonic anhydrase, which the authors showed to be tethered to the epithelial surface (Kim et al., 2021). Inhibitors of carbonic anhydrase greatly dampened the rate of alkalinization of artificial ASL upon reduction of CO_2 abundance. Transcript levels for carbonic anhydrase isozymes showed that CA12 was the most abundant isozyme in freshly isolated airway cells, and expression was approximately twofold higher in non-CF primary bronchial epithelial cells compared with CF bronchial epithelial cells. The authors concluded that membrane-tethered CA12, with its extracellular catalytic domain, may catalyze oscillations in ASL pH in response to tidal breathing. This premise is supported by the fact that individuals expressing mutated CA12 exhibit lung phenotypes very similar to CF (Lee et al., 2016). The authors' calculations further suggest that the observed oscillations in pH would occur over the normal physiological range of breathing rates between rest and intense exercise.

It remains to be seen whether these findings extend to other pulmonary diseases, including chronic obstructive pulmonary disease. It is important to note that cigarette smoke can lead to a reduction in CFTR abundance in airway epithelial cells, resulting in what is often referred to as "acquired CF" (Raju et al., 2016). Hence, it would be interesting to know if cigarette smoke interferes with the breathing-induced oscillations in ASL pH and negatively impacts bacterial clearing. It is also true that many pulmonary diseases are considered polymicrobial, with chronic infections characterized by multiple bacterial species, some of which will have different preferred pH ranges. A future study, therefore, may ask whether similar effects on bacterial clearance are seen in cultures of *S. aureus* or *S. pneumoniae*, and whether the interactions between species in the polymicrobial environment of the CF lung, for instance, are impacted by oscillations in ASL pH that may have larger impact on the fitness of one species compared with another in the community. From a therapeutic standpoint, one may ask whether aerosolized treatments that lead to alkalinization of the airspace may help reduce chronic bacterial infections. Such therapeutics that alter the airway ecosystem in order to enhance the activity of the innate immune system may find benefit in the clinic.

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