understanding the implications of what is observed in the blood with regard to changes in the lungs would require additional sampling by sputum induction or bronchoscopy. Furthermore, whole-blood analysis, even when corrected for cell types, is limited because it gives insight into what could be viewed as the “total transcriptome” response and tells us little or nothing about individual cells. Thus, future studies should attempt to localize the differential expression to the different cell types.

The wider question with respect to this study is the relevance of its findings to clinical asthma. Allergen challenge has little or no place in clinical practice (18). It cannot be performed in patients with more severe asthma who are receiving inhaled corticosteroids, long-acting bronchodilators, or biologics. However, there are existing data on blood transcriptomes in individuals with severe asthma (3) that could serve as the first step in linking the biomarkers in this allergen challenge study to cohorts that are more clinically relevant. It would also be valuable to see whether, to what extent, and how the identified biomarkers predict the risk of asthma exacerbation and its severity, and whether these biomarkers are responsive to any treatment. These types of validation could ultimately make the allergen challenge more valuable as a predictive model for drugs in development. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Sequestering Damage-associated Molecular Patterns in Critical Illness

A Novel Homeostatic Role for the Erythrocyte

In the last decade, it has been established that cell-free mitochondrial DNA (cf-mtDNA) is an important damage-associated molecular pattern (DAMP) that is fundamental to the pathophysiology of many critical illnesses (1). High levels of circulating cf-mtDNA have been

associated with severity of illness in trauma (2, 3), acetaminophen-related liver injury (4), and sepsis (5–7). Accordingly, cf-mtDNA levels have been proposed to be more powerful predictors of clinical outcomes than lactate levels in sepsis (6). Their relevance to human disease has been attributed to their release into the bloodstream after cellular injury and their consequent ability to activate neutrophils through interaction with Toll-like receptor 9 (TLR9) and formyl peptide receptor-1. This TLR9-related neutrophil activation in turn

Originally Published in Press as DOI: 10.1164/rccm.201710-2094ED on November 9, 2017

leads to multiorgan injury (8), and plays a clear role in acute lung injury (9) and sepsis-related acute kidney injury (10).

Given this burgeoning recognition of circulating cf-mtDNA as a putative “enemy within” (8), it follows that homeostatic mechanisms likely exist to regulate these DAMPs. To date, the primary known mechanism of circulating cf-mtDNA clearance is intracellular degradation by the lysosomal enzyme DNase II (11). The report in this issue of the *Journal* by Hotz and colleagues (pp. 470–480) therefore represents an exciting advance in our understanding of circulating cf-mtDNA homeostasis, describing an emerging role for red blood cells (RBCs) in human health and disease (12). Through an elegant series of experiments and observations, this group demonstrates that a subset of TLR9-expressing RBCs function as a sink for circulating cf-mtDNA. Identification of this novel mechanism of DAMP clearance, which expands upon the known ability of erythrocytes to regulate chemokine availability (13, 14), offers a promising new conceptual framework and therapeutic approach to critical illness.

Hotz and colleagues observed that 99% of circulating cf-mtDNA in healthy humans and mice is found within the RBC fraction of blood, rather than plasma. Interestingly, in conditions marked by increased release of cf-mtDNA (for example, in mice after experimentally induced inflammation or in humans after trauma), this blood compartmentalization reverses, with cf-mtDNA primarily localizing to the plasma fraction of blood. The authors speculate that this shift arises from both a saturation of homeostatic RBCs’ “buffering” capacity and a qualitative impairment of RBCs’ ability to scavenge cf-mtDNA. In support of the latter, Hotz and colleagues identified impairment of *ex vivo* cf-mtDNA scavenging in erythrocytes collected from patients with trauma. The precise mechanism that underlies this qualitative dysfunction during critical illness requires further exploration.

This observed scavenging ability of RBCs is a dramatic departure from the classic dogma that erythrocytes function simply as vessels for oxygen delivery. Indeed, by using adoptive cell transfer of RBCs isolated from transgenic mice, Hotz and colleagues identified that TLR9-containing erythrocytes are required for this buffering capability. Given the absence of transcriptional machinery in RBCs, activation of TLR9 on erythrocytes would be expected to have little inflammatory consequence, enabling these receptors to serve a primarily “scavenging” function. An evolutionary benefit of RBC scavenging, however, is somewhat contradicted by the finding that erythrocyte TLR9 is primarily intracellular—a maximally efficient scavenger would be expected to display this DAMP receptor on the cell surface. Furthermore, TLR9 activation of RBCs was not without downstream consequences, as the authors observed morphological changes (such as RBC crenation) in RBCs treated with CpG-containing DNA (representative of cf-mtDNA).

Although cf-mtDNA buffering may appear to be a novel function of RBCs, it is surprising that only a small percentage of circulating erythrocytes actually express TLR9. However, given that the “denominator” of total RBCs numbers in the trillions, even a small (~5%) percentage of TLR9-expressing RBCs represents a substantial circulating pool with a cf-mtDNA buffering capability. Most exciting, however, is that this finding clearly demonstrates functional RBC heterogeneity. Although we have long recognized different subsets of circulating leukocytes (e.g., monocytes and lymphocytes), these findings suggest a yet-undescribed division of labor within the erythrocyte pool. This heterogeneity may be teleologically driven by selective environmental pressures, supporting the maintenance of a

pool of TLR9-expressing erythrocytes that could be employed in the host response to erythrocyte pathogens such as malaria.

Based on the potential benefit of RBCs in cf-mtDNA scavenging, can we expect transfusions to be beneficial in critical illness? Clinical studies have clearly demonstrated the lack of such a benefit (15). Indeed, Hotz and colleagues demonstrate that any potential benefit from mtDNA buffering by exogenous RBCs is likely offset by the creation of new cf-mtDNA by transfusion-induced necroptosis. Thus, this work does not suggest (or support) the use of therapeutic transfusion in critical illness.

In summary, Hotz and colleagues substantially add to the DAMP literature by describing an endogenous mechanism that is responsible for DAMP clearance. Their work not only offers a new avenue for developing novel therapies that harness these natural homeostatic processes, it also raises the fascinating concept of RBC heterogeneity. These findings meaningfully advance our understanding of the erythrocyte’s role in critical illness. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Mucopurulent Triggering of the Airway Epithelium Implications in Health and Cystic Fibrosis

Muco-obstructive lung disease in cystic fibrosis (CF) is caused by dysfunction of the CF transmembrane conductance regulator (CFTR) in airway epithelial cells. In health, CFTR functions as a cAMP-dependent anion (Cl^- and bicarbonate) channel that plays a central role in the secretion of salt and water, which are essential for proper hydration and transportability of the thin film of mucus that covers airway surfaces (1). This mucus layer protects the airways by enabling the entrapment and mucociliary clearance of billions of bacteria, viruses, allergens, and other noxious stimuli that are deposited in the lungs every day during tidal breathing. In CF, impaired CFTR-mediated Cl^- /fluid secretion in combination with increased Na^+ /fluid absorption mediated by the epithelial Na^+ channel (ENaC) results in the depletion of airway surface liquid (ASL) (1). This airway surface dehydration has long been recognized as a key pathomechanism of mucociliary dysfunction that sets the stage for a vicious cycle of airway mucus plugging, bacterial infection, inflammation, and structural lung damage. More recently, *in vitro* studies in cultured airway epithelia provided additional insights into the link between airway surface dehydration caused by the primary CF ion transport defect and impaired mucus clearance (2). These studies demonstrated that increased mucus concentrations lead to an increase in the osmotic pressure generated by the mucus layer that, once a critical threshold is exceeded ($\geq 7\%$ solids), results in progressive compression of the subjacent periciliary layer. This in turn leads to a slowing of cilia-dependent mucus transport, and ultimately mucus stasis and adhesion to airway surfaces (2).

In addition to abnormal airway ion transport, chronic airway inflammation and infection in CF are associated with goblet cell metaplasia and overproduction of secreted mucins (MUC5AC and MUC5B) (3). This additional airway epithelial phenotype is also commonly observed in other chronic airway diseases and is triggered by a spectrum of factors produced in infected and inflamed airways, including neutrophil elastase released from activated neutrophils, proinflammatory cytokines released in type 1 and type 2 inflammation (such as IL-1 β and IL-13), and bacterial exoproducts (2–4). This mucus hypersecretion may aggravate mucus plugging in CF and may potentially lead to mucus hyperconcentration and mucociliary dysfunction in other chronic airway diseases, especially if it is not paralleled by a coordinate increase in epithelial fluid

secretion (2, 4–6). However, although the initial steps of the pathogenic cascade linking epithelial CFTR malfunction to airway surface dehydration and mucociliary dysfunction have been studied in detail, little is known about the impact of the chronic infectious and inflammatory airway milieu on epithelial mucus secretion and fluid transport, and the role of these “secondary hits” in the *in vivo* pathogenesis of CF lung disease.

In this issue of the *Journal*, Abdullah and colleagues (pp. 481–491) present a novel translational *in vitro* model for quantitative studies of the impact of infectious and inflammatory stimuli on mucin and fluid secretion by normal airway epithelia and maladaptive responses in CF (7). This model is based on the collection of mucopurulent material from the lungs of patients with CF who are infected with CF pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and exposure of primary well-differentiated normal and CF human bronchial epithelial cell cultures to supernatant of this material containing a mixture of soluble inflammatory and bacterial mediators that are present in chronically infected CF airways *in vivo*.

In a series of diligent experiments, Abdullah and colleagues (7) make several important observations that advance our current understanding of acute and chronic responses of the airway epithelium to infective and inflammatory stimuli. First, they demonstrate that acute exposure of primary airway epithelial cultures to supernatant of mucopurulent material (SMM) results in a rapid (within minutes) and dose-dependent release of intraepithelial mucin stores onto the apical surface (7). This rapid mucin secretory response may have important host defense functions under acute infective and/or inflammatory conditions. Second, Abdullah and colleagues show that prolonged exposure to SMM (i.e., conditions more reflective of chronic infection and inflammation *in vivo*) has profound stimulatory effects on the expression, secretion, and apical accumulation of MUC5AC and MUC5B in normal and CF airway cultures (7). This mucin hypersecretion is likely triggered by a mixture of potent stimuli of mucin synthesis and secretion, including neutrophil elastase, bacterial exoproducts, proinflammatory cytokines (IL-1 β and TNF- α), and extracellular nucleotides, that have been shown to be contained in SMM at high levels (3, 7). Of note, upregulation of the expression, secretion, and apical accumulation of MUC5AC and MUC5B did not differ between normal and CF cultures, indicating that mucin secretory responses to SMM stimuli are not altered in CF airway epithelia. Third, in contrast to the case with mucin secretion, Abdullah and colleagues show that SMM-induced fluid secretory responses differ substantially