Gain-of-Function Polymorphisms in Human Inflammasomes: Implications for Cystic Fibrosis

Cystic fibrosis (CF) is an inherited disease involving chronic infection and inflammation of the lungs (1). CF results from mutations in the *CFTR* (CF transmembrane conductance regulator) gene (2) and is the most common inherited genetic disease in white individuals, affecting ~1 in 3,000 newborns (3). *Pseudomonas aeruginosa* is a common cause of morbidity in patients with CF, triggering excessive inflammation, lung damage, and eventual respiratory failure (4). Several therapeutic approaches have been attempted or are currently in use, including antiinflammatory drugs, antioxidants, antibiotics, CFTR modulators, and gene therapy (1, 5). However, management of infection and lung damage because of chronic inflammation remains a major challenge for patients with CF.

CF lung disease involves elevated expression of proinflammatory cytokines because of dysregulation of the immune response (6). Central regulators of innate immunity are pattern recognition receptors, including TLRs (Toll-like receptors) and NLRs (nod-like receptors) that recognize pathogen-associated molecular patterns and damage-associated molecular patterns. Some NLRs, such as inflammasomes, are self-oligomerizing protein complexes that consist of nucleotide-binding and oligomerization domains, and the ASC protein, which recruits procaspase-1, leading to its activation (7–9). Active caspase-1 is responsible for proteolytic activation of proinflammatory cytokines, IL-1β and IL-18. The release of these proinflammatory cytokines and subsequent pyroptosis of the cell can lead to inflammatory lung damage (6, 7). In a recent study of a murine CF model using *P. aeruginosa*, the inhibition of the NLRP3 inflammasome was linked to improved bacterial clearance from the lung (10). Unlike animal models, the complexity of inflammasome biology in the human population arises from SNPs of these genes. These SNPs can give rise to inflammasome variants, which alter their activity, leading to changes in proinflammatory cytokine expression. In fact, it has been known for some time that mutations in the gene encoding *NLRP3* can induce a number of autoinflammatory disorders known as cryopyrin-associated periodic syndromes (11). Studies are now beginning to delve into the mechanistic action of specific inflammasome genetic variants. For instance, a recent report on the NLRP3 (Q705K) variant demonstrated an increase in inflammasome activation for the variant over wild-type NLRP3 (12). However, a variant in the *NLRC4* gene was found to correlate with decreased expression of IL-18 and improved lung function in patients with human immunodeficiency virus (HIV) and tuberculosis coinfection (13). In another study, a missense variant of NLRC4 was also associated with decreased expression of IL-18 (14).

In this issue of the *Journal*, Graustein and colleagues (pp. 157–166) identify inflammasome SNPs associated with *P. aeruginosa* lung colonization and lung function in CF (15). For this study, the authors genotyped variants from patients in the EPIC (Early *Pseudomonas* Infection Control) observational study. Previous investigations have described SNPs for *NLRP3* and *NLRC4* that alter the activity of these inflammasomes (12–14, 16, 17). However, Graustein and colleagues now show for the first time a correlation between inflammasome SNPs and the clinical outcomes of patients with CF carrying these genetic variants. The authors demonstrate that the NLRP3 (Q705K) variant correlated with an increased rate of *P. aeruginosa* lung infection and decreased lung function over time. However, only children who had previously been infected with *P. aeruginosa* demonstrated this effect. The authors note that a group of older children who were never infected with *P. aeruginosa* could exert a cohort bias against containing the NLRP3 (Q705K) variant, possibly explaining why the effects were only observed in children previously infected.

The authors also identify the novel *NLRC4* (A929S) variant as an SNP associated with protection of lung function for children with CF never infected with *P. aeruginosa*. However, because of the small sample size, the effect of the *NLRC4* (A929S) SNP on *P. aeruginosa* lung colonization could not be directly investigated. Instead, the authors analyzed a group of variants from multiple genes in the NLRC4 pathway as a single variable and found a protective role against *P. aeruginosa* lung colonization for these alterations. The lung colonization findings include only patients never infected with *P. aeruginosa* before enrollment. As with the *NLRP3* SNP cohorts, this protective *NLRC4* variant could reflect cohort bias against including children previously infected with *P. aeruginosa*. The authors also note that the *NLRC4*-related variants studied were only associated with protection in children with wild-type *CAV2* (caveolin 2), a gene associated with facilitating *P. aeruginosa* infection (18). This finding suggests that the NLRC4 pathway variants may antagonize the activity of CAV2.

To better understand the importance of *NLRP3* and *NLRC4* SNPs in chronic inflammation, the authors used human macrophage-like cell lines expressing wild-type or variant inflammasome constructs for *in vitro* studies. Using these cell lines, the authors demonstrate that the NLRP3 (Q705K) variant-expressing cells are more responsive to

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NLRP3-specific stimulation (nigericin) than wild-type cells. On the other hand, when cells were infected with *P. aeruginosa* strain PAO1, there was no difference in the response between NLRP3 wild-type and variant-expressing cells. The authors were unable to detect any statistical differences in cytokine expression from NLRC4 (A929S) variant-expressing cells. These findings may be due to the properties of the cell line or the *P. aeruginosa* strain used. A study using neutrophils or lung epithelial cells would likely help to further elucidate the mechanism of the NLRP3 (Q705K) and NLRC4 (A929S) variants. Furthermore, it has been reported that NLRP3 is predominantly responsible for IL-1β processing in CF neutrophils (10). It was also found that patients carrying the *NLRP3* (Q705K) and *CARD-8* (C10X) SNPs have delayed neutrophil apoptosis, increased IL-1β levels, and low macrophage phagocytic capacity, which increases the risk of damaging inflammation (17). The release of IL-18 from cells expressing the NLRP3 (Q705K or V198M) variants or the NLRC4 (A929S) variant represents another avenue to investigate inflammasome gain or loss of function. In fact, a previous study with a monocytic cell line expressing wild-type and the Q705K variant of NLRP3 has demonstrated an enhanced expression of IL-18 and IL-1β in the variant-expressing cells over wild-type in response to stimulation by alum (12).

In conclusion, Graustein and colleagues provide compelling clinical insights into the roles of *NLRP3* and *NLRC4* SNPs in CF. Importantly, the clinical data presented in this paper are accompanied by mechanistic *in vitro* studies. Together, these analyses shed light on the role of inflammasome variants in altering the balance between beneficial and detrimental excessive inflammation. Specifically, their clinical and *in vitro* findings identify the NLRP3 (Q705K) inflammasome variant as a biomarker for increased inflammation, earlier *P. aeruginosa* colonization, and accelerated disease progression in CF. Finally, the authors provide evidence in support of the concept that NLRP3 inflammasome inhibition is a viable approach to slow the progression of CF and potentially other pulmonary inflammatory diseases. ■

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Duane Jeansonne, Ph.D.
Center for Lung Biology and Disease
and
Department of Pathobiological Sciences Louisiana State University School of Veterinary Medicine
Baton Rouge, Louisiana

Samithamby Jeyaseelan, D.V.M., Ph.D.
Center for Lung Biology and Disease
Department of Pathobiological Sciences Louisiana State University School of Veterinary Medicine
Baton Rouge, Louisiana
and
Department of Medicine Louisiana State University Health Sciences Center New Orleans, Louisiana

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