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8 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 damageassociated 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-1 β 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 NLRC4related 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) variantexpressing 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-1B 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-1ß 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-1 β 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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References

- Ralhan A, Laval J, Lelis F, Ballbach M, Grund C, Hector A, et al. Current concepts and controversies in innate immunity of cystic fibrosis lung disease. J Innate Immun 2016;8:531–540.
- Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO3- transport in mutations associated with cystic fibrosis. *Nature* 2001;410:94–97.
- O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet 2009;373:1891– 1904.
- 4. Davies JC, Alton EW, Bush A. Cystic fibrosis. BMJ 2007;335:1255-1259.
- Almughem FA, Aldossary AM, Tawfik EA, Alomary MN, Alharbi WS, Alshahrani MY, *et al.* Cystic fibrosis: overview of the current development trends and innovative therapeutic strategies. *Pharmaceutics* 2020; 12:616.
- Iannitti RG, Napolioni V, Oikonomou V, De Luca A, Galosi C, Pariano M, et al. IL-1 receptor antagonist ameliorates inflammasome-dependent inflammation in murine and human cystic fibrosis. Nat Commun 2016;7: 10791.
- Ravi Kumar S, Paudel S, Ghimire L, Bergeron S, Cai S, Zemans RL, et al. Emerging roles of inflammasomes in acute pneumonia. Am J Respir Crit Care Med 2018;197:160–171.
- Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. *Cell Discov* 2020;6:36.
- Leissinger M, Kulkarni R, Zemans RL, Downey GP, Jeyaseelan S. Investigating the role of nucleotide-binding oligomerization domain-like receptors in bacterial lung infection. *Am J Respir Crit Care Med* 2014; 189:1461–1468.
- McElvaney OJ, Zaslona Z, Becker-Flegler K, Palsson-McDermott EM, Boland F, Gunaratnam C, et al. Specific inhibition of the NLRP3 inflammasome as an antiinflammatory strategy in cystic fibrosis. Am J Respir Crit Care Med 2019;200:1381–1391.
- 11. Kastner DL. Hereditary periodic fever syndromes. *Hematology Am Soc Hematol Educ Program* 2005;2005(1):74–81.
- Verma D, Särndahl E, Andersson H, Eriksson P, Fredrikson M, Jönsson JI, et al. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1β and IL-18 production. PLoS One 2012;7:e34977.
- Ravimohan S, Maenetje P, Auld SC, Ncube I, Mlotshwa M, Chase W, et al. A common NLRC4 gene variant associates with inflammation and pulmonary function in human immunodeficiency virus and tuberculosis. *Clin Infect_Dis* 2020;71:924–932.
- 14. Johansson Å, Eriksson N, Becker RC, Storey RF, Himmelmann A, Hagström E, et al.; PLATO Investigators. NLRC4 inflammasome is an important regulator of interleukin-18 levels in patients with acute coronary syndromes: genome-wide association study in the PLATelet inhibition and patient outcomes trial (PLATO). Circ Cardiovasc Genet 2015;8:498–506.
- Graustein AD, Berrington WR, Buckingham KJ, Nguyen FK, Joudeh LL, Rosenfeld M, et al. Inflammasome genetic variants, macrophage function, and clinical outcomes in cystic fibrosis. Am J Respir Cell Mol Biol 2021;65:157–166.
- Verma D, Lerm M, Blomgran Julinder R, Eriksson P, Söderkvist P, Särndahl E. Gene polymorphisms in the NALP3 inflammasome are associated with interleukin-1 production and severe inflammation: relation to common inflammatory diseases? *Arthritis Rheum* 2008;58: 888–894.
- Blomgran R, Brodin VP, Verma D, Bergström I, Söderkvist P, Sjöwall C, et al. Common genetic variations in the NALP3 inflammasome are associated with delayed apoptosis of human neutrophils. *PLoS One* 2012;7:e31326.
- Zaas DW, Swan ZD, Brown BJ, Li G, Randell SH, Degan S, et al. Counteracting signaling activities in lipid rafts associated with the invasion of lung epithelial cells by Pseudomonas aeruginosa. J Biol Chem 2009;284:9955–9964.