Journal Highlight: Protein crystallization promotes type 2 immunity and is reversible by antibody treatment

Reviewed by Kiho Son

PhD Candidate, Firestone Institute of Respiratory Health, McMaster University, Canada

The biological nature of Charcot-Leyden crystals (CLCs) has perplexed asthma researchers for over one hundred years since their discovery in 1853. Although these extracellular structures have been consistently observed in eosinophil-rich mucus plugs in inflamed airways of asthmatic patients, their proteinaceous composition was not determined until the late 1900s. In essence, the cytoplasmic protein galectin-10 (Gal10) is released from activated eosinophils to spontaneously crystallize; therefore, CLCs have been historically utilized as a biomarker for eosinophil-based inflammation. Nevertheless, the functional role of CLCs and their contribution to the pathophysiology of chronic airway diseases has yet to be elucidated.

In this highlighted study by ***Persson et al. (2019)***, the authors generated recombinant Gal10 crystals that were biosimilar to patient-derived CLCs to study the immune response in asthma mouse models. The injection of the recombinant Gal10 crystals into the airways of naïve mice resulted in an innate immune response characterized by an influx of neutrophils and monocytes, and the increased production of pro-inflammatory cytokines such as IL-6 and TNFα. The authors found this response to be notably absent in mice injected with Gal10 muteins incapable of spontaneous crystallization. Furthermore, several antibodies were developed against Gal10 and screened for their ability to mitigate autocrystallization. The application of antibodies to pre-formed crystals *in vitro* resulted in complete dissolution within 90 minutes by disrupting a tyrosine residue critical for crystal-packing. The antibodies also prevented crystal induced airway disease. In summary, Persson et al. established CLCs as novel therapeutic target to combat crystallopathic components of asthma.

Questions and Answers with Dr. Bart Lambrecht (Senior Author)

Since you are predominantly known for your dendritic and epithelial cell research, I'm curious what the catalyst was for you to undertake this eosinophil-centric project?

About 10 years ago, we were studying aluminium hydroxide ($Al(OH)_3$) as an activation signal for dendritic cells (DCs) to subsequently polarize the immune response, and found that it triggered the production of uric acid, a compound known to crystallize in joints and cause gout. We then injected these uric acid crystals into mice and showed a Th2 immune response, for which we were using eosinophilia as a readout. Translationally however, I was positive that uric acid crystals would not be forming in our lungs, so perhaps other crystals could be causing the Th2 response? I remembered CLCs being described anecdotally from my medical studies, and thus began our eosinophil-intrinsic work.

Could you comment on any technical difficulties and/or advantages you've experienced working with eosinophils compared to other cell types you're familiar with?

DCs are notoriously difficult to work on because they are a very minor population. For example, we will usually obtain 2 million eosinophils from lavage in mouse models, but will only acquire 20 thousand

Article: Persson EK, Verstraete K, Heyndrickx I, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* 2019; 364(6442), eaaw4295

DCs for the same volume. You guys are spoiled in fact, that you can get so many cells! –*laughs*– It's definitely been a luxury for me, eosinophils are relatively easy cells to work with. The only backdrop I can think of is that single cell RNA analyses in eosinophils are still proving to be difficult. I'm hoping to apply my myeloid biology expertise to help find successful methods for doing this. We could thus uncover absolutely fascinating immune regulatory roles for these cells.

As mentioned in your article, you not only produced recombinant galectin-10 (Gal10) that autocrystallized into CLCs that were biosimilar to patient-derived CLCs, but also developed antibodies that solubilized the crystals. Did you expect such success from your bioengineering endeavours, or was there a serendipitous component to the outcome as well?

The original driving reason we had for generating antibodies against CLCs was to prevent crystal formation. We reasoned that an antibody binding to soluble galectin-10 would be bulky and prevent the protein to go into crystallization. However, in our initial control experiments, we solely administered the antibody to existing crystals and saw their subsequent dissolution. There are very few moments in your career where you think ***this is it***, and often these moments for me are very visual. This was not some abstract modeling, dissolution just happened under our eyes. This was beyond our wildest dreams.

In the current paradigm of asthma pathophysiology, a variety of factors contribute to the Th2 response. As your research has clearly shown a reduction in Th2 immunity upon adding antibodies to CLCs, what are your thoughts on the contributions of CLC formation to overall disease progression and/or severity?

Dr. Fahy at UCSF has proposed that severe asthmatics have localized regions of hyper Th2 immunity in the lung where they exhibit high induction of eosinophil activation and mucus plugging, in addition to a persistent stimulus to sustain the Th2 response. We think that CLCs accumulate in the mucus plugs, which are also packed with dying eosinophils. Even if the inflammation dissipates and the inflammatory cells die, the CLCs could remain stable in the mucus, allowing the plugs to remain in place and preserving the Th2 focus.

What are the implications of your findings in a clinical setting?

So far, the biologicals for asthma treatment (e.g., anti-IgE, anti-IL5) predominantly work to reduce exacerbation frequency, but have a modest effect on lung function. They are removing the acute inflammatory aspects of the disease, but likely not treating the smoldering mucus plugs that lead to airway narrowing. With every exacerbation, we think the mucus plugs grow like rings in a tree trunk that ultimately obliterates the lumen. Once patients reach this state, they require a therapy to eliminate the mucus plugs otherwise risking potential death. We are hoping this is preventable by new biologicals (such as our antibody) and maybe new mucolytics in conjunction with current therapies. In broader terms, CLCs are probably the tip of the iceberg. There are research groups claiming that up to 30 proteins in our body have the capacity to crystallize; however, protein crystallopathy has thus far been understudied. To our knowledge, our study is the first to show that protein crystallopathy could also be reversed by antibodies.

Dr. Bart Lambrecht would like to acknowledge all the co-authors of the article and their contributions.

N.B. **Associated "comment" paper:** Fahy JV, Locksley RM. Making Asthma Crystal Clear. N Engl J Med. 2019 Aug 29;381(9):882-884. doi: 10.1056/NEJMcibr1908064.

Article: Persson EK, Verstraete K, Heyndrickx I, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* 2019; 364(6442), eaaw4295