

CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Making Asthma Crystal Clear

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Charcot–Leyden crystals have been observed in the airway mucus of patients with allergic airway disease since the 19th century, but their role in the pathogenesis of allergy has been unclear. Charcot–Leyden crystals and lysophospholipase are abundant in eosinophils and basophils, and originally, it was thought that lysophospholipase was the constituent protein of Charcot–Leyden crystals. Subsequent elucidation of the genomic organization, sequence, and structure of the constituent protein revealed the characteristic carbohydrate-recognition domain of the galectins, a finding that led to the designation of the protein as galectin-10.¹ Galectins are evolutionarily conserved glycan-binding proteins, or lectins, with canonical affinity for beta-galactose–modified glycoconjugates. They regulate protein–protein interactions both within the cell and in the extracellular matrix (Fig. 1A).³ Among the approximately 20 known human galectin proteins generated through alternative splicing of pre-messenger RNAs transcribed from 11 genes, only galectin-10 is known to crystallize rapidly and spontaneously under certain conditions, including after exocytosis from the eosinophil into the extracellular milieu.

Persson and colleagues,² intent on gaining more insights into the biologic mechanisms of

galectin-10 in the airway, recently verified that Charcot–Leyden crystals are abundant in tissue samples obtained from patients who had chronic rhinosinusitis with nasal polyps — a disease associated with eosinophil infiltration — and used structural data from radiographs to confirm the biosimilarity of in situ Charcot–Leyden crystals and recombinantly expressed galectin-10. When the authors removed a strategically placed histidine tag from the recombinant protein, spontaneous crystallization of the galectin-10 dimers occurred, and a combination of structural and mutational analyses led them to engineer a dimeric mutant galectin-10, in which the tyrosine amino acid at position 69 was replaced with glutamic acid (Tyr69Glu). The mutant protein was unable to crystallize owing to disruption of aromatic ring-stacking at the critical Tyr⁶⁹ “crystal-packing hotspot.” Inoculation of native Charcot–Leyden crystals into the lungs of mice caused induction of innate cytokines interleukin-6 and tumor necrosis factor α and was accompanied by tissue infiltration by neutrophils and monocytes; however, inoculation of mutant non-crystallizable dimers did not have a similar effect.

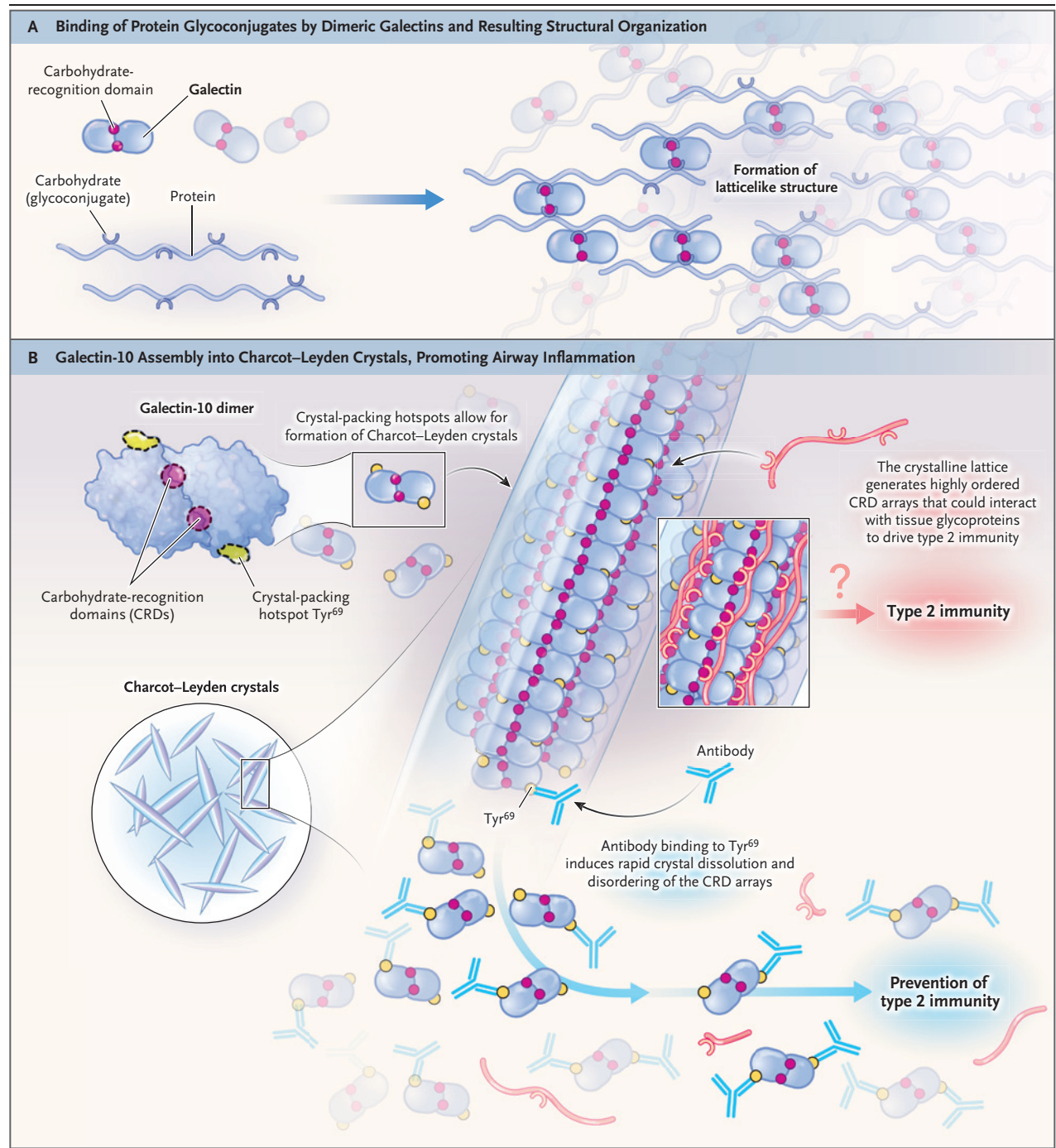
Exposure of the polar Tyr⁶⁹ residues at the tips of the galectin-10 dimers, away from the carbohydrate-recognition domain, created an immuno-

Figure 1 (facing page). Charcot–Leyden Crystals and Airway Inflammation.

Dimeric galectins bind protein glycoconjugates and confer structural organization (Panel A). The prototypic galectins use carbohydrate-recognition domains to crosslink glycoconjugates on intracellular and extracellular proteins, thus creating latticelike structures that are critical to the function of tissues. Galectin-10 assembly into Charcot–Leyden crystals promotes airway inflammation (Panel B). The tyrosine residue at amino acid position 69 (Tyr⁶⁹) of galectin-10 promotes spontaneous symmetric organization into Charcot–Leyden crystals. The crystalline lattice generates highly ordered arrays of carbohydrate-recognition domains with the potential for high-avidity interactions with tissue glycoproteins. Persson et al. reported recently that antibody binding to apically exposed Tyr⁶⁹ disrupts the crystal-packing hotspot, which leads to dissolution of the lattice and disordering of the carbohydrate-recognition domain arrays.² The authors then used mouse models to show that Charcot–Leyden crystals elicit type 2 immunity, whereas the dimers do not have such an effect, and that dissolution of the crystals decreases airway inflammation — findings that create new potential therapeutic strategies. Elucidating the putative tissue-binding partners for the carbohydrate-recognition domain of galectin-10 may shed additional light on host inductive pathways that trigger type 2 immunity.

genic site on the crystal interface that was exploited to raise nanobodies in llamas. Nanobodies are therapeutic proteins that leverage the fact that camelidae (e.g., llamas and camels) possess fully functional antibodies that contain only heavy chains. The galectin-10-specific nanobodies were placed onto mouse and human IgG

scaffolds to create antibodies that were selected for their ability to dissolve galectin-10 crystals. The authors found that anti-galectin-10 monoclonal antibodies precisely targeted the critical exposed Tyr⁶⁹ polar tips on Charcot-Leyden crystals, and in mucus samples from patients with chronic rhinosinusitis with nasal polyps, these



antibodies dissolved the crystals. Furthermore, in immunodeficient mice that were reconstituted with peripheral-blood mononuclear cells from patients with allergy to house dust mites, airway mucus hyperplasia and increased IgE and airway resistance developed when they were exposed to the allergen in the presence of Charcot–Leyden crystals. These changes were abrogated by co-administration of the Charcot–Leyden crystal antibody. Thus, the authors have revealed a role for Charcot–Leyden crystals in pathogenesis and a foundation for an experimental therapeutic approach: the dissolution of Charcot–Leyden crystals in affected tissues.

Mice do not express galectin-10, but they do express galectin-related proteins with altered glycan specificities, a property now known to be shared with galectin-10. One such protein is prominent in the eye lens in rodents, where it associates with crystalline proteins that establish the transparent, organelle-free environment of the curved lens. The capacity for dimeric galectins to establish latticelike structures enables increased avidity for otherwise low-affinity binding by increasing the number of interaction domains through an ordered process that resembles crystal formation.

In a galectin-related protein found in mushrooms, the key residue in the carbohydrate-recognition domain (that would normally mediate binding with galactosidase) is variant, which enables recognition of chitooligosaccharides, repetitive cell-wall components of fungal and insect predators. Chitin is an insoluble structural scaffold of parasites, insects, and fungi, and it arrays numerous glycoproteins that can become allergens when they enter or invade vertebrate mucosa. The immune response to inhaled chitin particles in mice⁴ resembles the mixed inflammatory response to Charcot–Leyden crystals in mice, described by Persson et al.,² which suggests a generalized pathway for confronting indigestible particles. Perhaps crystallization in mammalian cells represents a response analogous to chitin formation in invertebrates, a process that uses reiterative assembly to generate a robust structural element while creating repetitive high-avidity sites that can be recognized by host immune cells and tissues as part of a specialized barrier reaction (Fig. 1B).

Dissolution of Charcot–Leyden crystals in patients with asthma and in patients with chronic rhinosinusitis with nasal polyps may have therapeutic benefits. Treatments that target eosinophils currently exist; however, in some cases, Charcot–Leyden crystals might persist in tissues or may be derived from basophils, and the availability of treatments that target these crystals is lacking. To the extent that Charcot–Leyden crystals represent an influential biophysical component in pathologic airway mucus, dissolution of the crystals could lower elasticity and improve clearance. Persistent mucus plugs, which are rich in eosinophils, occur commonly in severe asthma,⁵ and therapeutic agents that target this pathologic condition are needed. In addition, activation of innate and adaptive immune responses in the airways by Charcot–Leyden crystals may be decreased by dissolution of the crystals, which could result in antiinflammatory effects independent of mucolytic effects. To this end, camelid-derived nanobodies present several advantages as inhaled therapeutics, owing to their small size, thermal stability, high solubility, and short systemic half-life. Although further work is needed to ascertain the role of Charcot–Leyden crystals in disease, the story of these crystals is a fascinating one, and such research may yet yield additional insights into the basic mechanisms underpinning type 2 immunity.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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