Designer Dendrons To Dissect Innate Immune Signaling

Pamela V. Chang† and Catherine L. Grimes‡

†Department of Microbiology and Immunology, Cornell University, 930 Campus Road VMC C4-185, Ithaca, New York 14853, United States
‡Department of Chemistry and Biochemistry, University of Delaware, 134 Brown Laboratories, Newark, Delaware 19716, United States

Structure-based design of new polymeric inflammasome activators that control immune responses could lead to better vaccines and immunotherapies.

Chemists have the unique ability to synthesize small molecules to their heart’s delight. Small molecule synthesis continues to revolutionize the development of pharmaceuticals and improve the understanding of vital biochemical and cellular processes. Here, Esser-Kahn and co-workers describe the design of well-defined, tunable dendritic polymers to better understand the activation of innate immune system via the inflammatory response protein complexes (inflammasomes).1 The authors focused on the inflammasome called NLRP3, a pattern recognition receptor whose activation mechanism has eluded scientists since its discovery.2 NLRP3 inflammasomes belong to a family of pattern recognition receptors that initiate the innate immune system. Over the past 20 years, we have learned that the innate immune system uses a series of various receptors and DNA sensors to drive immune responses.3 These innate immune receptors recognize pathogen-associated molecular patterns and induce inflammatory and antimicrobial responses.4 Working in concert, the system can mount an immune response by sending signals to the adaptive immune system via signaling proteins and cell adhesion molecules.5 Although we have learned a lot about the immune system, its complexity has often evaded scientists, in particular, the specific activation mechanism(s) of individual receptors. This uncertainty is especially true for the NLRP3 inflammasome.

NLRP3 can be activated by a large number of molecules, including adenosine triphosphate (ATP), various pore-forming toxins, particulate crystals and aggregates, and microbial molecules.6 However, before NLRP3 can be activated, the levels of the protein must first be increased by activation of other immune pathways.7 Esser-Kahn and co-workers wanted to know whether there are other substances that can activate NLRP3 inflammasomes. They designed new molecular entities with discrete chemical properties that can stimulate the innate immune system via NLRP3 activation. The authors show that these molecules can specifically and precisely activate the NLRP3 inflammasome by the rupture of lysosomes, the membrane-bound organelles commonly found in animal cells.8

The two main players in the investigation were a matched pair of dendritic polymers, denoted LR+ and LR−, that have identical dendrons but differ in the dendron’s spacing. As a result, only one of the polymers (LR+) can cause lysosome rupture (Figure 1). The polymers consist of an L-lysine-dicysteine backbone, which is decorated with multiple dendritic lysine residues. These lysine residues are capped with histidine and tryptophan in specific molar ratios. The protonation of histidine in the acidic environment of lysosomes facilitates lysosomal rupture (Figure 1a), while tryptophan assists in cellular uptake. The spacing of the lysines in the backbone is critical. If too many spacers are added, the properties of the polymer change. The team has

Published: August 2, 2018

Figure 1. Effect of LR+ (A) and LR− (B) polymers on lysosomes. Acidification of LR+ polymer inside lysosomes causes their rupture and activation of inflammasomes. LR− polymer accumulates in lysosomes without causing the rupture. Reprinted with permission from ref 1. Copyright 2018 American Chemical Society.

acscentssci.org

ACS Publications © 2018 American Chemical Society

948

DOI: 10.1021/acssentsci.8b00430
ACS Cent. Sci. 2018, 4, 948–949

First Reactions

This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.

acscentssci.org

ACS Publications © 2018 American Chemical Society

948

DOI: 10.1021/acssentsci.8b00430
ACS Cent. Sci. 2018, 4, 948–949

First Reactions

This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.
found that if the ratio exceeds 34%, the ability to cause lysosomal rupture decreases (Figure 1b). Using these critical probes the researchers systematically investigated how lysosomal rupture activates NLRP3 inflammasome formation.

A series of cell-based assays using human monocyte cancer cells were used to demonstrate that inflammasome activation caused by LR+ polymer can lead to the release of the inflammatory proteins via the NRLP3 inflammasome. If bone-marrow-derived dendritic cells that lack NRLP3 were used, no lysosomal rupture was observed in the presence of LR+. These experiments led the team to believe that the lysosomal rupture caused by LR+ is driving the synthesis of inflammatory proteins in cells by activating NRLP3. Unlike inflammasome activation caused by many known activators, however, the activation by the LR+ polymer only activates 10% of the cell population. With this astute observation in mind, Esser-Kahn and colleagues designed experiments to probe the mechanism of lysosomal rupture using inhibitors of both phagocytosis (a response by specific immune cells) and lysosomal acidification. Each of the inhibitors reduced the inflammatory protein secretion by immune cells after incubation with the LR+ polymer. However, reduction of this protein secretion proceeded in different ways: some inhibitors affected both lysosomal acidification and phagocytosis, while other inhibitors affected phagocytosis only. These experiments demonstrate that phagocytosis, acidification of lysosomes, and subsequent lysosomal rupture are all important for the mechanism of NRLP3 activation by LR+.

The goal of this study was to gain molecular control of NRLP3 activation via lysosomal rupture. After the cellular mechanism was thoroughly characterized, the authors returned to this original goal and found that, by tuning the properties (e.g., percentage of spacers between dendrons, the ratio of histidine to tryptophan residues, and concentration of dendrons) of polymers, the cellular response (i.e., inflammatory protein release) could be altered in immune cells. The high level of precision sets this work apart from previous studies on NRLP3 innate immune system activators.9 With the ability to systematically tune properties of the materials, the research team is now in a position to generate a desired outcome: the activation or inhibition of an inflammatory response.

While this work pushes the field of inflammasome biology and immunology forward, the polymers have not been tested in mice. The question of whether the materials will live up to their potential in animal models, the true setting for complex immune responses, remains open. However, this should not dampen your enthusiasm for the excellent work. After all, the team is derived from chemists who have the ability to synthesize new molecules and alter the properties of the reagents to ultimately tune and control activation of this important innate immune receptor.

Author Information
(P.V.C.) E-mail: pamela.chang@cornell.edu.
(C.L.G.) E-mail: cgrimes@udel.edu.

ORCID
Catherine Grimes: 0000-0002-0586-2879

REFERENCES

(1) Manna, S.; Howitz, W. J.; Oldenhuis, N. J.; Eldredge, A. C.; Shen, J.; Nihesh, F. N.; Lodoen, M. B.; Guan, Z.; Aaron, P.; Esser-Kahn, A. Immunomodulation of the NLRP3 Inflammasome through Structure-Based Activator Design and Functional Regulation via Lysosomal Rupture. ACS Cent. Sci. 2018, DOI: 10.1021/acscentsci.8b00218.
(2) Broz, P.; Dixit, V. M. Inflammasomes: mechanism of assembly, regulation and signalling. Nat. Rev. Immunol. 2016, 16 (7), 407−420.
(3) Takeuchi, O; Akira, S. Pattern Recognition Receptors and Inflammation. Cell 2010, 140 (6), 805−820.
(4) Kieser, K. J.; Kagan, J. C. Multi-receptor detection of individual bacterial products by the innate immune system. Nat. Rev. Immunol. 2017, 17 (6), 376−390.
(5) Medzhitov, R. Inflammation 2010: New Adventures of an Old Flame. Cell 2010, 140 (6), 771−776.
(6) Schroder, K; Tschopp, J. The Inflammasomes. Cell 2010, 140 (6), 821−832.
(7) Franchi, L; Muñoz-Planillo, R; Núñez, G. Sensing and reacting to microbes through the inflammasomes. Nat. Immunol. 2012, 13 (4), 325−332.
(8) Okada, M.; Matsuzawa, A.; Yoshimura, A.; Ichijo, H. The lysosome rupture-activated TAK1-JNK pathway regulates NLRP3 inflammasome activation. J. Biol. Chem. 2014, 289 (47), 32926−32936.
(9) Guo, H.; Callaway, J. B.; Ting, J. P. Y. Inflammasomes: Mechanism of action, role in disease, and therapeutics. Nat. Med. 2015, 21, 677.