Published in final edited form as:

ACS Infect Dis. 2016 November 11; 2(11): 746–748. doi:10.1021/acsinfecdis.6b00174.

Redefining the Defensive Line: critical components of the innate immune system

Mackenzie L. Lauro, Ching-Wen Hou, and Catherine L. Grimes*

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, 19716

Abstract

The human body harbors over a trillion microorganisms; the innate immune system is charged with a tremendous task, to recognize “the needle in the haystack” or in other words to sense the pathogen in this milieu. In this viewpoint, three recent discoveries in the field of innate immunity are discussed and we highlight how in each case multiple disciplines worked together to expand the elements of the innate immune system.

Nearly 100 years ago, Sir Alexander Fleming revealed to the world that our bodies produce an enzyme called lysozyme which functions as an antibiotic by destroying the walls of many bacterial cells¹. Today, we recognize that lysozyme is just one of the many mechanisms that our bodies have to protect ourselves against infectious diseases as part of the innate immune system. This seemingly simple “first line of defense” has proven to be much more sophisticated than was originally suggested. Janeway and Medzhitov proposed the idea that our bodies contain innate immune receptors that specifically recognize unique structures from microbes; but more importantly they hypothesized that these receptors subsequently informed the adaptive immune response^{2–5}. Seminal work by Hoffman and Beutler identified the first of these receptors, the Toll- like receptors (TLRs). In his Nobel winning work, Hoffman demonstrated that the TLRs were responsible for sensing a fungal infection in dropsholia⁶ and shortly after, Beutler published his Nobel manuscript, describing that TLR4 is responsible for sensing lipopolysaccharide (LPS) from bacteria⁷. The past twenty years has seen the field of innate immunity explode and we now know that there are many different types of innate immune receptors responsible for pathogen sensing including TLRs, Nod-like receptors (NLRs), C-type lectin receptors, RIG-1 like receptors, and DNA sensors^{8–9}. These innate immune receptors recognize conserved structures in a pathogen, pathogen-associated molecular patterns (PAMPs), and induce inflammatory and antimicrobial responses. Recognition also leads to the expression of protective proteins, including chemokines, cytokines, defensins, immunoreceptors, and cell adhesion molecules, to help us to defend against infection and send signals to the adaptive immune system^{9–10}. However, PAMPs do not only exist in pathogens, they are generally in all microorganisms. Thus, these molecules have been renamed to microbe-associated molecular patterns (MAMPs)¹¹.

So how does the innate immune system distinguish between pathogens and commensals? The problem is complex as many of the molecular calling cards of pathogenic bacteria are

*Corresponding author: cgrimes@udel.edu.

contained in the commensal bacteria that naturally reside in our bodies. One theory is that the innate immune receptors work as a network, creating what amounts to checks and balances on each other and only trigger when more than one sensor is activated^{12–15}. Together they survey the landscape of the cell, residing in extracellular membranes, intracellular membranes and the cytoplasm – constantly scanning the environment for signs of a pathogen. In order to provide this protective role, they must distinguish the pathogenic microbes and viruses from the symbiots^{16–19} and we now understand that if our innate immune system fails in distinguishing between the two, drastic consequences can ensue, such as diabetes, inflammatory bowel disease, pulmonary disorders, obesity, atherosclerosis and cancer²⁰. The exact mechanism by how the body differentiates the origin of the MAMPs is not well understood and more experiments are necessary to identify the methods the body uses to distinguish between commensal and pathogenic organisms.

A major step in understanding this conundrum is first identifying all of the receptors and functional proteins in the innate immune system, and delineating their mechanisms of activation and action. In the past year, major advances have been made to achieve this. In this *viewpoint*, we highlight three reported novel functions of proteins in the innate immune system: (1) the identification that a major enzyme in glucose metabolism, hexokinase, is an innate immune receptor²¹, (2) the recently elucidated crystal structure of one of the first NLRs identified, Nucleotide-binding oligomerization domain-containing protein 2 (Nod2)²², and (3) an intriguing report of a lectin binding protein that preferentially recognizes bacterial glycans over human²³. These advances are critical in broadening our understanding of the body's defensive line and how it functions in the complex environment of pathogens and the native microbiota.

It is well established that the protective polymer, bacterial peptidoglycan, is an excellent structure for the human body to use to recognize bacteria. Peptidoglycan is composed of linear chains of beta-(1,4)-N-acetyl-muramic acid (NAM) and N-acetyl-glucosamine (NAG) units joined by peptide crosslinks. As human cells do not cloak themselves in these jackets, the human innate immune systems recognize the presence of bacteria by taking advantage of peptidoglycan fragments. To date, the two major receptor classes thought to be responsible for sensing peptidoglycan have been the NLRs and TLRs⁹. However, Underhill and co-workers observed that hexokinase, an enzyme in glycolysis, is an innate receptor capable of recognizing a peptidoglycan fragment²¹. Their data showed that NAG, a minimal component of peptidoglycan, activates the IL-1 β secretion through the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome. This is surprising as all fragments of bacterial peptidoglycan have focused on the NAM moiety -- the most well documented being the synthetic fragment, muramyl dipeptide (MDP). Intrigued that NAG was able to activate the NLRP3 inflammasomes, they further showed that NAG inhibition of hexokinases induces its dissociation from the mitochondria membrane and triggers NLRP3 activation. In addition, they demonstrate that other known inhibitors of hexokinase, such as intermediates of glycolysis, are capable of activating an inflammatory response. Although the mechanism of how NAG induced release of hexokinase from the mitochondrial membrane activates the inflammasome is not understood, this manuscript supports the developing hypothesis that two fundamental processes, innate immune signaling and metabolism, are closely linked²⁴.

With regard to MAMP sensing, a major achievement was reported by Shimizu and workers, with the report of the crystal structure of Nod2. Nod2 was first identified in 2001 as a protein associated with Crohn's disease and later described as an innate immune receptor. Like the TLRs, Nod2 contains a leucine-rich repeat (LRR) domain that is thought to be responsible for ligand binding. This manuscript provides the first molecular snapshot of this protein. The authors demonstrate that ADP binding is critical for locking Nod2 into a closed conformation, which is similar to the homologous proteins, Apaf-1 and NLRC4. A putative binding region for the bacterial ligands was hypothesized to include a highly conserved set of amino acids and unassigned electron density, similar to the size of the carbohydrate found in bacterial peptidoglycan. In addition, the mutations of a variety of conditions were highlighted. They demonstrated gain of function mutants that leads to Blau syndrome or early-onset sarcoidosis are on the interface of the interdomain contacts. It is believed these mutants prevent the formation of the "ADP closed conformation" that is the inactive state, leading to more activation. The Crohn's disease mutants are predicted to interfere with oligomerization, recruitment to the membrane or protein-protein interactions leading to loss of function; they do not appear to be near the putative ligand-binding site. This manuscript is important for the development of specialized treatments of Nod2 associated diseases, for example those that would be able to confer corrective conformational changes, thereby restoring proper function for the Crohn's and Blau associated mutations.

Finally, Kiessling and co-workers have identified the mechanism by which lectins are able to distinguish human glycans from bacterial glycans. They investigated the binding of human intelectin-1 (hIntL-1) to a microbial glycan array. To their surprise, exocyclic, terminal 1,2-diols presented on glycans found preferentially on bacterial surfaces were able to bind to hIntL-1. The exocyclic, terminal 1,2-diol is not sufficient for selection and through protein crystallography they demonstrated that additional features of the glycan such as functionality and conformation of the anomeric position are necessary to distinguish from the host. Additionally, specificity was dictated by the ability to coordinate a protein bound calcium ion, which is similar to the mechanism C-type lectins use to preferentially bind glycans. Yet, the C-type lectins and the X-type lectins, which intelectin-1 belongs, bind the calcium ion differently: the C-type lectin coordinates carbohydrate hydroxyl groups while the X-type lectin binding necessitates an exocyclic, terminal 1,2-diol. Since hIntL-1 is not identified as a member of the innate immune the authors evaluated homologous hIntL-1 from another mammalian source, mouse, to determine if the preferred recognition motif is conserved. The mouse homolog retained the same preference to bacterial ligands supporting an evolutionarily conserved recognition. Together these findings suggest that this protein could provide a protective function, but the exact role of hIntL-1 during bacterial invasion is still undefined.

What makes an innate immune receptor, a receptor? These manuscripts highlight that the definition needs to be broadened, as it appears that many proteins that have roles distant to the innate immune system, are actually integral components of this sophisticated system. Key events in how an innate immune receptor recognizes its target include binding, activation, and molecular signaling; but should other functional outputs also qualify a protein as an immune receptor? More over, it is essential to understand how the immune receptors and functional proteins integrate and decode the multitude of signals the system

receives. Just as this system must work jointly, we believe it is essential that many fields including but not limited to chemistry, structural biology, molecular biology and immunology continue to be combined to reveal how the innate immune system is able to mount the proper immune response to a pathogen or a commensal.

Uncategorized References

1. Fleming A. On a remarkable bacteriolytic element found in tissues and secretions. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character*. 1922; 93(653): 306–317. DOI: 10.1098/rspb.1922.0023
2. Janeway CA. APPROACHING THE ASYMPTOTE - EVOLUTION AND REVOLUTION IN IMMUNOLOGY. *Cold Spring Harbor Symposia on Quantitative Biology*. 1989; 54:1–13.
3. Medzhitov R, Janeway CA. On the semantics of immune recognition. *Research in Immunology*. 1996; 147(4):208–214. DOI: 10.1016/0923-2494(96)87222-1 [PubMed: 8844897]
4. Medzhitov R, PrestonHurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature*. 1997; 388(6640):394–397. [PubMed: 9237759]
5. Medzhitov R. Approaching the asymptote: 20 years later. *Immunity*. 2009; 30(6):766–75. DOI: 10.1016/j.immuni.2009.06.004 [PubMed: 19538928]
6. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell*. 1996; 86(6):973–983. DOI: 10.1016/s0092-8674(00)80172-5 [PubMed: 8808632]
7. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*. 1998; 282(5396):2085–8. [PubMed: 9851930]
8. Brubaker SW, Bonham KS, Zanoni I, Kagan JC. Innate immune pattern recognition: a cell biological perspective. *Annual review of immunology*. 2015; 33:257–90. DOI: 10.1146/annurev-immunol-032414-112240
9. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006; 124(4): 783–801. DOI: 10.1016/j.cell.2006.02.015 [PubMed: 16497588]
10. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nature immunology*. 2015; 16(4):343–53. DOI: 10.1038/ni.3123 [PubMed: 25789684]
11. Mackey D, McFall AJ. MAMPs and MIMPs: proposed classifications for inducers of innate immunity. *Molecular microbiology*. 2006; 61(6):1365–71. DOI: 10.1111/j.1365-2958.2006.05311.x [PubMed: 16899081]
12. Beutler E, Gelbart T, West C. Synergy between TLR2 and TLR4: A safety mechanism. *Blood Cells Molecules and Diseases*. 2001; 27(4):728–730. DOI: 10.1006/bcmd.2001.0441
13. Thaïss CA, Levy M, Itav S, Elinav E. Integration of Innate Immune Signaling. *Trends in Immunology*. 2016; 37(2):84–101. DOI: 10.1016/j.it.2015.12.003 [PubMed: 26755064]
14. Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(25):13766–13771. DOI: 10.1073/pnas.250476497 [PubMed: 11095740]
15. Underhill DM. Collaboration between the innate immune receptors dectin-1, TLRs, and Nods. *Immunological Reviews*. 2007; 219:75–87. DOI: 10.1111/j.1600-065X.2007.00548.x [PubMed: 17850483]
16. Duerkop BA, Hooper LV. Resident viruses and their interactions with the immune system. *Nature Immunology*. 2013; 14(7):654–659. DOI: 10.1038/ni.2614 [PubMed: 23778792]
17. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, Gordon JI. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature*. 2010; 466(7304):334–U81. DOI: 10.1038/nature09199 [PubMed: 20631792]

18. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature*. 2007; 449(7164):804–10. DOI: 10.1038/nature06244 [PubMed: 17943116]
19. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001; 291(5505):881–4. DOI: 10.1126/science.291.5505.881 [PubMed: 11157169]
20. Thaïss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature*. 2016; 535(7610):65–74. DOI: 10.1038/nature18847 [PubMed: 27383981]
21. Wolf AJ, Reyes CN, Liang WB, Becker C, Shimada K, Wheeler ML, Cho HC, Popescu NI, Coggeshall KM, Arditi M, Underhill DM. Hexokinase Is an Innate Immune Receptor for the Detection of Bacterial Peptidoglycan. *Cell*. 2016; 166(3):624–636. DOI: 10.1016/j.cell.2016.05.076 [PubMed: 27374331]
22. Maekawa S, Ohto U, Shibata T, Miyake K, Shimizu T. Crystal structure of NOD2 and its implications in human disease. *Nature communications*. 2016; 7:11813.doi: 10.1038/ncomms11813
23. Wesener DA, Wangkanont K, McBride R, Song X, Kraft MB, Hodges HL, Zarling LC, Splain RA, Smith DF, Cummings RD, Paulson JC, Forest KT, Kiessling LL. Recognition of microbial glycans by human intelectin-1. *Nature structural & molecular biology*. 2015; 22(8):603–10. DOI: 10.1038/nsmb.3053
24. Jin C, Henao-Mejia J, Flavell RA. Innate immune receptors: key regulators of metabolic disease progression. *Cell metabolism*. 2013; 17(6):873–82. DOI: 10.1016/j.cmet.2013.05.011 [PubMed: 23747246]