Precision Clinical Medicine, 1(2), 2018, 57–59

doi: 10.1093/pcmedi/pby012
Comments

COMMENTS

ALPK1: a pattern recognition receptor for bacterial ADP-heptose

Yingzi Cong[1,2*]

[1]Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX 77555, USA, and [2]Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA

*Correspondence: Yingzi Cong, yicong@utmb.edu

Editor's note

A commentary on "Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose".

Living in an environment rich in microbes, the host senses and responds to them through coordinated immune responses, which mount robust defense against invading pathogens but are not overly aggressive towards commensal microbes. Multiple receptors have evolved to recognize the conserved signatures present in microbes. These receptors form the group known as pattern recognition receptors (PRRs),[1] which includes the nucleotide binding and oligomerization domain leucine-rich repeat (LRR) containing (NLR) family, the C-type lectin receptors (CLRs), scavenger receptors, RIG-I-like receptors, and the toll-like receptors (TLRs) family of transmembrane receptors. Through these PRRs, the innate immune system senses both pathogenic and non-pathogenic foreign molecules by recognition of pathogenic-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan, flagellin, and nucleic acids. PRR activation initiates signaling cascades that lead eventually to activation of transcription factor nuclear factor (NF)-κB, and transcription of innate immune response genes, including proinflammatory cytokines, which are necessary to eliminate invading pathogens.[2]

TLRs are highly conserved PRRs. LPS, a well-known PAMP produced by Gram-negative bacteria, is recognized by TLR4.[3] The TLR4-LPS pathway is involved in many important regulatory mechanisms under both the steady state and during infection. Proper synthesis of LPS is crucial in maintaining the structural integrity of the bacterial outer membrane and for protection against environmental challenges. Interestingly, recent studies have demonstrated that innate cells can also detect and respond to the bacterial sugar d-glycero-β-d-manno-heptose heptose-1,7-bisphosphate (HBP), a highly conserved metabolic intermediate of LPS biosynthesis in Gram-negative bacteria, including invasive *Salmonella*, *Escherichia coli*, *Helicobacter pylori*, *Shigella flexneri*, and *Neisseria meningitidis*.[4–6] Detection of HBP in the host cytosol activates NF-κB, leading to innate and adaptive immune responses. This signaling cascade depends on α-kinase 1 (ALPK1)-TIFA-TRAF6. However, it is unclear whether HBP is a novel PAMP that stimulates ALPK1 and TIFA-dependent immunity to Gram-negative pathogens. It is also unknown whether HBP directly binds ALPK1 or TIFA, which could serve as its receptor(s). An elegant study

Received: 23 August 2018; **Accepted:** 25 August 2018

published in *Nature* by Zhou *et al.*[7] that used comprehensive approaches including transposon screening, biochemical analyses, CRISPR–Cas9 screening, high-performance liquid chromatography, and crystal structure, demonstrated that ADP-β-d-manno-heptose (ADP-Hep), but not other heptose metabolites, directly binds the N-terminal domain of ALPK1, and stimulates its kinase domain to phosphorylate TIFA and activate NF-κB. Thus, this study identified ADP-Hep as a PAMP and ALPK1 as its PRR.

Many Gram-negative bacteria, such as *Yersinia*, induce cytokine expression in a type III secretion system (T3SS)-dependent manner, which is mediated by activation of NF-κB.[8,9] By screening 21 000 *Y. pseudotuberculosis* transposon mutants in *Y. pseudotuberculosis Δ6*, which lack most T3SS effector genes, Zhou *et al.* identified 37 mutants that were defective in activation of NF-κB. They eventually selected *hlde*, which incorporated with GmhA and GmhB synthesizes ADP-d-glycero-β-d-manno-heptose (ADP-DD-Hep) from d-sedoheptulose 7-phosphate (S7P) through d-glycero-β-d-manno-heptose 7-phosphate (H7P), HBP, and d-glycero-β-d-manno-heptose 1-phosphate (H1P). Interestingly, although HBP, ADP-DD-Hep, and ADP-LD-Hep, but not S7P, stimulated NF-κB activation when electroporated into the cells, only ADP-DD-Hep and ADP-LD-Hep stimulated NF-κB activation and cytokine production when added directly to the cells, indicating that ADP-Hep is a potent and versatile PAMP.

To investigate the possible receptor(s) for ADP-Hep, Zhou *et al.* used a genome-wide CRISPR–Cas9 screen, and identified ALPK1, TIFA, and TRAF6 mediating ADP-LD-Hep induction of NF-κB activation. Deletion of ALPK1 or TIFA abolished ADP-LD-Hep-induced NF-κB activation and cytokine expression. They further showed that ALPK1 kinase activity is required for ADP-LD-Hep-induced phosphorylation of TIFA, and that ADP-LD-Hep stimulated coimmunoprecipitation of TIFA with ALPK1 and TRAF6. Sensing of ADP-Hep by ALPK1 is not just limited to bacteria with T3SS. *Burkholderia cenocepacia*, enterotoxigenic *E. coli*, and diffuse-adhering *E. coli*, also stimulated NF-κB activation through the ALPK1-TIFA axis in an *hldE*-dependent manner. Zhou *et al.*, thereby, demonstrated that ADP-Hep activation of NF-κB is mediated specifically by the ALPK1-TIFA-TRAF6 axis.

ALPK1 contains a kinase domain and an α-helical domain linked by an unstructured region, which raises the question of which domains are crucial in mediating ADP-LD-Hep activation of NF-κB? Zhou *et al.* found that co-expression of ALPK1-N492 and ALPK1-ΔN492 was sufficient for ADP-LD-Hep to activate NF-κB and phosphorylation of TIFA. By using high-performance liquid chromatography, Zhou *et al.* identified from small molecule extracts one active fraction that contained the presumed ADP-Hep ion, and detected apo-ALPK1-(N+K) binding to ADP-LD-Hep. To further confirm ADP-Hep binding to ALPK1, Zhou *et al.* determined the ALPK1-NTD crystal structure. They found a narrow pocket in ALPK1-NTD, which directly binds ADP-LD-Hep. They also found that mutant forms in respective residues of ALPK1 failed to activate ADP-LD-Hep-induced NF-κB, and that Apo-ALPK1-(N+K) did not phosphorylate TIFA without binding ADP-LD or DD-Hep. Thus, Zhou *et al.* demonstrated that ALPK1 is a specific receptor for ADP-Hep.

What is the biological significance of ALPK1-ADP-Hep signaling *in vivo*? Zhou *et al.* took a step further to determine how ADP-Hep functions *in vivo* and the role of ALPK1 in this process. They showed that injection of ADP-LD-Hep induced massive neutrophil recruitment with increased production of several NF-κB-dependent cytokines and chemokines in WT, but not in Alpk1$^{-/-}$, mice. Furthermore, on infection with *B. cenocepacia*, which triggers lung inflammation, WT, but not Alpk1$^{-/-}$, mice showed increased expression of NF-κB-dependent cytokines and chemokines in the lungs, which led to higher bacterial load in the lungs of Alpk1$^{-/-}$, but not WT, mice. These results demonstrated the biological significance of recognition of ADP-Hep by ALPK1 *in vivo*.

The study by Zhou *et al.* identified ALPK1 as a PRR for recognition of ADP-Hep, a metabolic intermediate of LPS biosynthesis, but not LPS itself. This study provides great insights into how hosts respond to Gram-negative bacterial infection, as well as novel avenues for the development of vaccines. It also provides new targets for precision medicine by specifically targeting ALPK1-ADP-Hep signaling to develop therapeutics in the treatment of infectious diseases and other diseases. The large amounts of Gram-negative bacteria present in gut microbiota mean that these findings also raise the question of the importance of the ALPK1-ADP-Hep axis in regulation of intestinal homeostasis. ALPK1 has been identified as a susceptible gene of colitis in animal models. In IL-10$^{-/-}$ mice, which spontaneously develop colitis, a major susceptibility locus (26 Mb) was found on chromosome 3 (termed Cdsc1) by comparing the susceptible C3H/HeJBir.IL-10$^{-/-}$ and the resistant C57BL/6.IL-10$^{-/-}$ strains.[10] A recent work further identified a short interval (1.71–Mb, designated Hiccs) within the same region as a major susceptibility locus for colitis and associated colon cancer induced by *H. hepaticus*.[11] Interestingly, ALPK1 is one of the best candidate genes in both Cdsc1 and Hiccs, thus suggesting ALPK1 as a susceptible colitis gene. It is of great interest for the purpose of precision medicine to define the role of the ALPK1-ADP-Hep axis in regulation of intestinal homeostasis and the pathogenesis of inflammatory bowel diseases and other autoimmune inflammatory diseases.

Conflict of interest statement

None declared.

References

1. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;**140**:805–20. doi:10.1016/j.cell.2010.01.022.

2. Chen G, Shaw MH, Kim YG, *et al*. NOD-like receptors: role in innate immunity and inflammatory disease. *Annu Rev Pathol* 2009;**4**:365–98. doi:10.1146/annurev.pathol.4.110807.092239.
3. Poltorak A, He X, Smirnova I, *et al*. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998;**282**:2085–8.
4. Gaudet RG, Guo CX, Molinaro R, *et al*. Innate Recognition of Intracellular Bacterial Growth Is Driven by the TIFA-Dependent Cytosolic Surveillance Pathway. *Cell Rep* 2017;**19**:1418–30. doi:10.1016/j.celrep.2017.04.063.
5. Milivojevic M, Dangeard AS, Kasper CA, *et al*. ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1,7-bisphosphate of gram-negative bacteria. *PLoS Pathog* 2017;**13**:e1006224. doi:10.1371/journal.ppat.1006224.
6. Gaudet RG, Sintsova A, Buckwalter CM, *et al*. INNATE IMMUNITY. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity. *Science* 2015;**348**:1251–5. doi:10.1126/science.aaa4921.
7. Zhou P, She Y, Dong N, *et al*. Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. *Nature* 2018. doi:10.1038/s41586-018-0433-3.
8. Litvak Y, Sharon S, Hyams M, *et al*. Epithelial cells detect functional type III secretion system of enteropathogenic Escherichia coli through a novel NF-kappaB signaling pathway. *PLoS Pathog* 2017;**13**:e1006472. doi:10.1371/journal.ppat.1006472.
9. Auerbuch V, Golenbock DT, Isberg RR. Innate immune recognition of Yersinia pseudotuberculosis type III secretion. *PLoS Pathog* 2009;**5**:e1000686. doi:10.1371/journal.ppat.1000686.
10. Beckwith J, Cong Y, Sundberg JP, *et al*. Cdcs1, a major colitogenic locus in mice, regulates innate and adaptive immune response to enteric bacterial antigens. *Gastroenterology* 2005;**129**:1473–84. doi:10.1053/j.gastro.2005.07.057.
11. Boulard O, Kirchberger S, Royston DJ, *et al*. Identification of a genetic locus controlling bacteria-driven colitis and associated cancer through effects on innate inflammation. *J Exp Med* 2012;**209**:1309–24. doi:10.1084/jem.20120239.