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Switching Rho GTPase activation into effective antibacterial defenses requires the caspase-1/IL-1beta signaling axis

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The monitoring of the activation state of Rho GTPases has emerged as a potent innate immune mechanism for detecting pathogens. In the March issue of *PLOS Pathogens*, we show that the activation of Rho GTPases by the CNF1 toxin during *E. coli*-triggered bacteremia leads to a GR1$^+$cell-mediated efficient bacterial clearing and improves host survival. Host alarm requires the Caspase-1/IL-1beta signaling axis. Furthermore, we discover that pathogenic bacteria have the capacity to block immune responses via the expression of the α-hemolysin pore-forming toxin. In this commentary, we will comment on these findings and highlight the questions raised by this example of attack-defense mechanisms used alternatively by the pathogen and the host during blood infection.

Keywords: antibacterial defenses, bacterial effectors, bacterial toxins, caspase-1, effector-triggered immunity, innate immunity, Il-1beta

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Introduction

The high incidence of human infections by *Escherichia coli* and the ensuing deadly sepsis are critical public health issues for developed countries. *E. coli* is a versatile bacteria that exists as either a commensal or a pathogen responsible for both intra- and extra-intestinal infections. The pathotypes of *E. coli* have been defined by their capacity to express various combinations of virulence factors encoded by genes found on plasmids and/or large genomic pathogenicity islands.[1] Among them, uropathogenic *E. coli* (UPEC) are responsible for urinary tract infections (UTI), including pyelonephritis, which is a frequent cause of bacteremia.[2] The presence of bacteria in the blood of patients is a medical emergency associated with high mortality rates.[3]

The virulence potential of UPEC has been characterized. This virulence potential encompassed specific factors to colonize the urinary tract and to promote bacterial survival and inflammatory-based damages to urinary tract epithelia. Several types of virulence factors have been found to be associated with UPEC, including adhesins, flagellin, polysaccharide capsule, iron uptake systems, and secreted toxins.[1] Among these virulence determinants, the 2 highly prevalent toxins α-Hemolysin (HlyA) and the Cytotoxic Necrotizing Factor 1 (CNF1) have been characterized. CNF1 is a Rho GTPases-targeting toxin that deamidates the glutamine in position 61 of Rac and Cdc42, as well as the equivalent glutamine 63 of RhoA, destroying the GTPase activity and thereby locking them into an active form.[4-6] CNF1 shows structural and functional homologies with factors found in other pathogenic bacterial species. This includes CNF2 from *E. coli*, CNFy from *Yersinia pseudotuberculosis* and DNT from *Bordetella* spp.[4]. More recently, the effector VopC from *Vibrio parahaemolyticus* that is injected through a TTS3 secretion system was found to deamidate Rac1 and Cdc42.[7]

In vivo, CNF1 promotes the persistence of UPEC and exacerbates the inflammatory reaction during urinary tract infection (UTI).[8,9] Additionally, *cnf1* is found with high prevalence in uroseptic strains of UPEC, raising the question of its function during bacteremia.[10] CNF1 and HlyA are found within the same pathogenicity island (PAI) and are co-transcribed under control of the same master regulator RfaH.[11] This genetic link

between HlyA and CNF1 raises the question of a possible concerted action of both factors.

Rho GTPases are not only master regulators of the actin cytoskeleton but also central elements of the host responses against pathogens.[12] CNF1 has been shown to induce NF-κB activation and to promote cytokine secretion from uroepithelial and endothelial cells.[13-15] A series of recent studies points to the capacity of host cells to perceive the activity of toxin-targeting Rho GTPase, leading to inflammatory responses that are likely detrimental to the persistence of bacteria.[16-19] This system of recognition of the activity of toxins targeting Rho GTPase is related to Effector–Triggered Immunity (ETI).[19-21] Major innate immune signaling hubs comprising NOD and RIP proteins are essential in relaying Rho GTPase signaling to NF-kB. As discussed below, our recent data answer major questions raised by all these findings.

Host Responses to Effector-Triggered Rho GTPases Modification During Bacteremia: Anti-virulence Immunity

We established the anti-virulence effect of the activity of a toxin-activating Rho GTPase in the context of bacteremia and the major consequences on resistance of the host to infection.[22] Several studies indicate that the activation of Rho GTPases engages effective innate immune responses.[19] To address the relevance of ETI during infection, we chose the prototypic uropathogenic strain of *E. coli* UTI89 in which we deleted the HlyA gene to study the role of CNF1 without the interference of HlyA. We then established the kinetics of bacterial persistence in the blood. Our initial observation was that the CNF1-expressing strain of *E. coli* is cleared from the blood faster than the isogenic *cnf1*-deleted mutant. Complementation of the CNF1-deleted strain with a plasmid encoding either CNF1 or the catalytic inactive mutant allowed us to link the bacterial clearing effect to the activity of the CNF1 toxin. Importantly, we measured that this rapid clearing of *E. coli*-expressing CNF1 was associated with a dramatic increase of the survival of mice during bacteremia. Because CNF1 is a virulence factor, we named this phenomenon Anti-Virulence Immunity (AVI). We moved on to search for host factors that mediate the anti-bacterial clearing upon the detection of CNF1 activity.

IL-1beta is Critical for The CNF1-Triggered Bacterial Clearing During Bacteremia

IL-1beta is a potent pro-inflammatory cytokine that is produced by innate immune cells as a precursor that has to be cleaved by caspase-1 or caspase-11 to form mature IL-1beta.[23] Mature IL-1beta is involved in a variety of cellular processes, such as immune cell activation, proliferation, differentiation and pyroptosis.[23] We observed that Caspase-1/11 impaired mice have a decreased AVI, resulting in an increased bacterial load in the blood. Moreover, the injection of IL-1 Receptor antagonist (KINERET) was found to block the clearing of bacteria-expressing CNF1, pointing to the important role of IL-1beta. KINERET treatment of rabbits infected with community-acquired *Staphylococcus aureus* also increased the bacterial burden in the lung.[24] Together, these results suggest that KINERET may be more adaptable to local rather than systemic treatments.

Rho GTPases: Linking TLR and Inflammasome Signaling?

Rho GTPases are master regulators of the cell cytoskeleton and key elements of the host responses against pathogens. Indeed, they control leukocyte phagocytosis, migration and the production of reactive oxygen species.[12,25] They also control expression of inflammatory cytokine and chemokine.[15] Recent reports have revealed the crucial role of Rho GTPases and notably Rac as drivers of the innate immune signaling pathways.[26] Our work previously established that the activation of Rho GTPases engages protective immune responses in Drosophila.[16] Now, we show that the simultaneous activation of Rho GTPases and TLR signaling pathways engages protective immune responses during bacteremia in mammals.[22] We also show that IL-1beta secretion is synergistically induced by macrophages when CNF1 and LPS treatments are combined. This synergy is also found when CNF1 is combined with TLR2 agonists such as PAM3CSK4 or FSL-1. These data suggest that TLR4 or TLR2 signaling pathways synergize with the signaling pathways engaged by CNF1 activity to promote efficient inflammatory responses.[22] The increase of immune responses observed when the host interacts with strain-expressing virulence factors activating Rho GTPases could be an innate immune mechanism to gauge the effective virulence of bacterial strains and to adapt commensurate anti-bacterial responses. Following this idea, we speculate that the acquisition by the host of multiple immune detection pathways acting in synergy is necessary to confer immunity to the host. In support of this idea, the collaboration of TLR and NLR signaling pathways was found to be critical for the maturation of haematopoietic cells and T cell effector function.[27,28] The combining of agonists of immune sensors is likely a promising strategy for the development of the next generation of vaccination adjuvant.[29]

Neutralizing Rho GTPases Downstream Effects to Block AVI and Promote Virulence

More than one third of UPEC are positive for *cnf1*. How can one reconcile the presence of CNF1 in pathogenic strains with our findings that the toxin triggers effective anti-bacterial responses? Genetic studies have also revealed that *cnf1* is always associated with the α-hemolysin operon and that both toxin-encoding genes are co-transcribed. We now show that this genetic link is translated into a cooperative mode of action between the toxins favoring the persistence of pathogenic bacteria during bacteremia. We demonstrate that the strains that express HlyA have a reduced expression of IL-1beta cytokine that is associated with a lower persistence in the blood. Thus, HlyA dampens the innate immune

responses triggered by CNF1 with little effect on the strain deleted from CNF1. The details regarding the molecular mechanisms that withstand the inhibition of innate immune responses in this context have yet to be determined. One possible explanation is that HlyA disrupts NF-kB signaling via activation of host serine proteases as described during UTI.[30] Such a hypothesis is consistent with our findings that HlyA acts downstream from the activation of Rac and upstream from the secretion of IL-1beta. This provides further evidence of the importance of pathogens to target the caspase-1/IL-1beta signaling axis.[31-34] Furthermore, this ascribes a new virulence function to HlyA pore forming toxin with dramatic consequences for host survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol 2004; 2:123-40; PMID:15040260; http://dx.doi.org/10.1038/nrmicro818
2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015; 13:269-84; PMID:25853778; http://dx.doi.org/10.1038/nrmicro3432
3. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes Infect 2003; 5:449-56; PMID:12738001; http://dx.doi.org/10.1016/S1286-4579(03)00049-2
4. Lemonnier M, Landraud L, Lemichez E. Rho GTPase-activating bacterial toxins: from bacterial virulence regulation to eukaryotic cell biology. FEMS Microbiol Rev 2007; 31:515-34; PMID:17680807; http://dx.doi.org/10.1111/j.1574-6976.2007.00078.x
5. Flatau G, Lemichez E, Gauthier M, Chardin P, Paris S, Fiorentini C, Boquet P. Toxin-induced activation of the G protein p21 Rho by deamidation of glutamine. Nature 1997; 387:729-33; PMID:9192901; http://dx.doi.org/10.1038/42743
6. Schmidt G, Sehr P, Wilm M, Selzer J, Mann M, Aktories K. Gln 63 of Rho is deamidated by Escherichia coli cytotoxic necrotizing factor-1. Nature 1997; 387:725-9; PMID:9192900; http://dx.doi.org/10.1038/42735
7. Zhang L, Krachler AM, Broberg CA, Li Y, Mirzaei H, Gilpin CJ, Orth K. Type III effector VopC mediates invasion for Vibrio species. Cell Rep 2012; 1:453-60; PMID:22787576; http://dx.doi.org/10.1016/j.celrep.2012.04.004
8. Rippere-Lampe KE, Lang M, Ceri H, Olson M, Lockman HA, O'Brien AD. Cytotoxic necrotizing factor type 1-positive Escherichia coli causes increased inflammation and tissue damage to the prostate in a rat prostatitis model. Infect Immun 2001; 69:6515-9; PMID:11553597; http://dx.doi.org/10.1128/IAI.69.10.6515-6519.2001
9. Rippere-Lampe KE, O'Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor type 1 (cnf(1)) attenuates the virulence of uropathogenic Escherichia coli. Infect Immun 2001; 69:3954-64; PMID:11349064; http://dx.doi.org/10.1128/IAI.69.6.3954-3964.2001
10. Dubois D, Delmas J, Cady A, Robin F, Sivignon A, Oswald E, Bonnet R. Cyclomodulins in urosepsis strains of Escherichia coli. J Clin Microbiol 2010; 48:2122-9; PMID:20375237; http://dx.doi.org/10.1128/JCM.02365-09
11. Landraud L, Gibert M, Popoff MR, Boquet P, Gauthier M. Expression of cnf1 by Escherichia coli J96 involves a large upstream DNA region including the hlyCABD operon, and is regulated by the RfaH protein. Mol Microbiol 2003; 47:1653-67; PMID:12622819; http://dx.doi.org/10.1046/j.1365-2958.2003.03391.x
12. Boquet P, Lemichez E. Bacterial virulence factors targeting Rho GTPases: parasitism or symbiosis? Trends Cell Biol 2003; 13:238-46; PMID:12742167; http://dx.doi.org/10.1016/S0962-8924(03)00037-0
13. Falzano L, Quaranta MG, Travaglione S, Filippini P, Fabbri A, Viora M, Donelli G, Fiorentini C. Cytotoxic necrotizing factor 1 enhances reactive oxygen species-dependent transcription and secretion of proinflammatory cytokines in human uroepithelial cells. Infect Immun 2003; 71:4178-81; PMID:12819113; http://dx.doi.org/10.1128/IAI.71.7.4178-4181.2003
14. Boyer L, Travaglione S, Falzano L, Gauthier NC, Popoff MR, Lemichez E, Fiorentini C, Fabbri A. Rac GTPase instructs nuclear factor-kappaB activation by conveying the SCF complex and IkBalpha to the ruffling membranes. Mol Biol Cell 2004; 15:1124-33; PMID:14668491; http://dx.doi.org/10.1091/mbc.E03-05-0301
15. Munro P, Flatau G, Doye A, Boyer L, Oregioni O, Mege JL, Landraud L, Lemichez E. Activation and proteasomal degradation of rho GTPases by cytotoxic necrotizing factor-1 elicit a controlled inflammatory response. J Biol Chem 2004; 279:35849-57; PMID:15152002; http://dx.doi.org/10.1074/jbc.M401580200
16. Boyer L, Magoc L, Dejardin S, Cappillino M, Paquette N, Hinault C, Charriere GM, Ip WK, Fracchia S, Hennessy E, et al. Pathogen-derived effectors trigger protective immunity via activation of the Rac2 enzyme and the IMD or Rip kinase signaling pathway. Immunity 2011; 35:536-49; PMID:22018470; http://dx.doi.org/10.1016/j.immuni.2011.08.015
17. Keestra AM, Winter MG, Auburger JJ, Frassle SP, Xavier MN, Winter SE, Kim A, Poon V, Ravesloot MM, Waldenmaier JF, et al. Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. Nature 2013; 496:233-7; PMID:23542589; http://dx.doi.org/10.1038/nature12025
18. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S, et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature 2014; 513:237-41; PMID:24919149; http://dx.doi.org/10.1038/nature13449
19. Stuart LM, Boyer L. RhoGTPases–NODes for effector-triggered immunity in animals. Cell Res 2013; 23:980-1; PMID:23689278; http://dx.doi.org/10.1038/cr.2013.68
20. Jones JD, Dangl JL. The plant immune system. Nature 2006; 444:323-9; PMID:17108957; http://dx.doi.org/10.1038/nature05286
21. Staskawicz B, Mudgett MB, Dangl JL, Galan JE. Common and contrasting themes of plant and animal diseases. Science 2001; 292:2285-9; PMID:11423652; http://dx.doi.org/10.1126/science.1062013
22. Diabate M, Munro P, Garcia E, Jacquel A, Michel G, Obba S, Goncalves D, Luci C, Marchetti S, Demon D, et al. Escherichia coli α-Hemolysin Counteracts the Anti-Virulence Innate Immune Response Triggered by the Rho GTPase Activating Toxin CNF1 during Bacteremia. PLoS Pathog 2015; 11:e1004732; PMID:25781937; http://dx.doi.org/10.1371/journal.ppat.1004732
23. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. Immunity 2013; 39:1003-18; PMID:24332029; http://dx.doi.org/10.1016/j.immuni.2013.11.010
24. Labrousse D, Perret M, Hayez D, Da Silva S, Badiou C, Couzon F, Bes M, Chavanet P, Lina G, Vandenesch F, et al. Kineret(R)/IL-1ra blocks the IL-1/IL-8 inflammatory cascade during recombinant Panton Valentine Leukocidin-triggered pneumonia but not during S. aureus infection. PLoS One 2014; 9:e97546; PMID:24905099
25. Bokoch GM. Regulation of innate immunity by Rho GTPases. Trends Cell Biol 2005; 15:163-71; PMID:15752980; http://dx.doi.org/10.1016/j.tcb.2005.01.002
26. Stuart LM, Paquette N, Boyer L. Effector-triggered versus pattern-triggered immunity: how animals sense pathogens. Nat Rev Immunol 2013;; PMID:23411798
27. Burberry A, Zeng MY, Ding L, Wicks I, Inohara N, Morrison SJ, Nunez G. Infection mobilizes hematopoietic stem cells through cooperative NOD-like receptor and Toll-like receptor signaling. Cell Host Microbe 2014; 15:779-91; PMID:24882704; http://dx.doi.org/10.1016/j.chom.2014.05.004
28. O'Donnell H, Pham OH, Li LX, Atif SM, Lee SJ, Ravesloot MM, Stolfi JL, Nuccio SP, Broz P, Monack DM, et al. Toll-like receptor and inflammasome signals converge to amplify the innate bactericidal capacity of T helper 1 cells. Immunity 2014; 40:213-24; PMID:24508233; http://dx.doi.org/10.1016/j.immuni.2013.12.013
29. Maisonneuve C, Bertholet S, Philpott DJ, De Gregorio E. Unleashing the potential of NOD- and Toll-like agonists as vaccine adjuvants. Proc Natl Acad Sci U S A 2014; 111:12294-9; PMID:25136133; http://dx.doi.org/10.1073/pnas.1400478111
30. Dhakal BK, Mulvey MA. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. Cell Host Microbe 2012; 11:58-69; PMID:22264513; http://dx.doi.org/10.1016/j.chom.2011.12.003
31. Finlay BB, McFadden G. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell 2006; 124:767-82; PMID:16497587; http://dx.doi.org/10.1016/j.cell.2006.01.034
32. Rahman MM, McFadden G. Modulation of NF-kappaB signalling by microbial pathogens. Nat Rev Microbiol 2011; 9:291-306; PMID:21383764; http://dx.doi.org/10.1038/nrmicro2539
33. Brodsky IE, Medzhitov R. Targeting of immune signalling networks by bacterial pathogens. Nat Cell Biol 2009; 11:521-6; PMID:19404331; http://dx.doi.org/10.1038/ncb0509-521
34. Shin S, Brodsky IE. The inflammasome: Learning from bacterial evasion strategies. Semin Immunol 2015; 27:102-10; PMID:25914126; http://dx.doi.org/10.1016/j.smim.2015.03.006