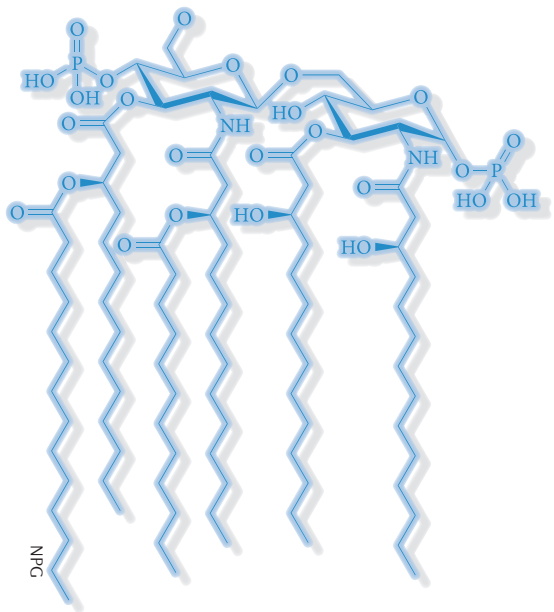
HOST RESPONSE

LPS goes non-canonical

For many years, it has been accepted that host innate immune cells recognize lipopolysaccharide (LPS) through the pattern recognition receptor Toll-like receptor 4 (TLR4). Now, new work focused on identifying the activation signal for the non-canonical inflammasome has revealed that LPS is also recognized in a TLR4-independent, caspase 11-dependent manner.

Inflammasomes are cytoplasmic molecular signalling platforms that activate caspase 1 in response to pathogen-associated molecular patterns (PAMPs), leading to the activation of interleukin-1β (IL-1β) and IL-18, and pyroptotic cell death. Canonical inflammasomes involve NOD-like receptors (NLRP3 or NLRC4) or the nucleotide sensor AIM2, and the adaptor protein ASC. Recently, a caspase 11-dependent non-canonical inflammasome that recognizes Gram-negative bacteria was identified in mice. This pathway uses components of the canonical pathway (caspase 1, NLRP3 and ASC) to activate IL-1β and IL-18, and caspase 11 alone is sufficient to mediate pyroptosis, but the identity of the specific activation signal was unclear.



Previous work had shown that activation is a two-step process, involving a priming signal (such as cholera toxin B subunit (CTB)) and an activation signal. As Gram-negative but not Gram-positive bacteria stimulate this inflammasome, much research has focused on the potential role of LPS, which constitutes the outer leaflet of the outer membrane in the Gram-negative cell wall. Kayagaki *et al.* noticed that activation of the non-canonical inflammasome in macrophages occurred in response to CTB plus *Escherichia coli* LPS, but interestingly, this activation was serotype specific, occurring in response to only one (*E. coli* O111:B4) of the three serotypes tested. Further analysis revealed that only *E. coli* O111:B4 LPS could bind CTB and so was taken up into host cells, and that the requirement for CTB priming could be by-passed by direct transfection into host cells.

LPS comprises three main components: lipid A, the core oligosaccharide and O antigen. As the lipid A component is recognized by TLR4, many Gram-negative bacteria produce a modified lipid A to evade innate immune detection. The authors found that transfection of mouse macrophages with synthetic unmodified lipid A or LPS from *Salmonella enterica* subsp. *enterica* serovar Typhimurium (which has an identical lipid A structure to that of *E. coli*) resulted in activation of the non-canonical inflammasome, whereas transfection of the tetra-acylated form of lipid A that is produced by *Helicobacter pylori* did not. To check whether TLR4 was involved, *E. coli* LPS or lipid A was transfected into primed mouse macrophages lacking TLR4, the TLR4-associated lipid A-binding proteins MD1 and MD2 or the key downstream signalling proteins TRIF and type I interferon receptor. In each case, the level of caspase 11-dependent activation observed was similar to that observed in wild-type cells, suggesting that non-canonical inflammasome activation is TLR4 independent. Finally, the authors investigated the relevance of these findings *in vivo* and found that in a mouse model of LPS-induced lethal sepsis, primed TLR4-null mice were as susceptible as primed wild-type mice to a lethal dose of *E. coli* LPS, whereas primed caspase 11-null mice showed a greater level of resistance than wild-type mice.

Taken together, the authors conclude that activation of the caspase 11-dependent non-canonical inflammasome proceeds via recognition of intracellular lipid A in a TLR4-independent manner. Future work is sure to focus on identifying the lipid A sensor.

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ORIGINAL RESEARCH PAPER Kayagaki, N. *et al.* Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science* http://dx.doi.org/10.1126/science.1240248 (2013)