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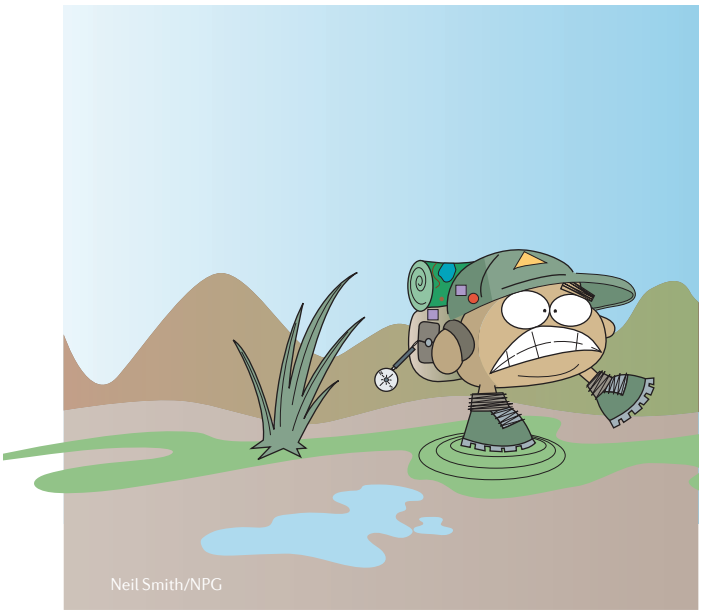
HOST RESPONSE

New LPS receptors discovered

> these data uncover a new role for inflammatory caspases as cytoplasmic LPS receptors

Lipopolysaccharide (LPS) is an outer membrane component of Gram-negative bacteria that is classically recognized by immune cells via the pattern recognition receptor Toll-like receptor 4 (TLR4). However, cytoplasmic recognition of LPS has recently been shown to activate the caspase 11-dependent non-canonical inflammasome in a TLR4-independent manner, but the cytoplasmic receptor for LPS is unknown. Now, Shi, Zhao *et al.* show that caspase 11 binds to LPS directly, which results in caspase oligomerization and inflammasome activation.

Inflammasomes are oligomeric multiprotein complexes that assemble in the cytoplasm in response to danger- or pathogen-associated molecular patterns, which leads to caspase-dependent activation of interleukin-1β (IL-1β) and IL-18 and pyroptotic cell death. Inflammasome assembly is canonically triggered by NOD-like receptors or by the DNA sensor AIM2, and it results in the activation of caspase 1. A non-canonical pathway has recently been described in mice, which involves an unidentified cytosolic LPS receptor and results in activation of caspase 11. As humans lack caspase 11, Shi, Zhao *et al.* investigated the role of the human inflammatory caspase, caspase 4, which is homologous to mouse caspase 11, in non-canonical inflammasome activation. LPS transfection into human cells induced caspase 1-independent pyroptosis, which was inhibited by knocking down *CASP4* expression. Furthermore, ectopic expression of caspase 4, but not of a protease-deficient caspase 4 mutant, resulted in LPS-induced pyroptosis in epithelial 293T cells, which do not express caspase 4. Collectively, these data show that cytoplasmic LPS sensing in human cells activates the non-canonical caspase 4-dependent inflammasome.



To investigate the molecular partners of human caspase 4 and mouse caspase 11 that are responsible for LPS recognition, the authors purified recombinant caspase 4 and caspase 11 from *Escherichia coli* and from insect cells. Intriguingly, expression of caspase 4 and caspase 11 in *E. coli* resulted in their purification as oligomers, whereas monomers were obtained when the caspases were expressed in insect cells, which suggests that the caspases might directly bind to the LPS that is present in *E. coli* (but absent from insect cells), triggering caspase oligomerization. Indeed, pulldown assays using biotinylated LPS or biotinylated lipid A (which is a core component of LPS that has previously been shown to be sufficient to trigger non-canonical inflammasome activation) precipitated caspase 4 and caspase 11, and the direct binding of these caspases to LPS was confirmed by surface plasmon resonance. Furthermore, addition of LPS or lipid A to caspase 4 and caspase 11 monomers that were purified from insect cells was sufficient to induce caspase oligomerization and activation. Interestingly, a caspase 11 mutant that lacked the amino terminus, which contains the caspase-activation and recruitment domain (CARD), was incapable of binding to LPS or oligomerization, suggesting that the CARD mediates both processes. To confirm this hypothesis, the authors identified three CARD mutations that disrupted LPS binding and lipid A-induced oligomerization. Notably, the three mutant proteins were unable to restore LPS-induced pyroptosis following expression in 293T cells or in *Casp11*$^{-/-}$ mouse macrophages.

Taken together, these data uncover a new role for inflammatory caspases as cytoplasmic LPS receptors and describe a novel mechanism of inflammasome activation, in which direct LPS binding results in caspase oligomerization and activation, leading to the induction of pyroptosis.

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ORIGINAL RESEARCH PAPER Shi, J., Zhao, Y. *et al.* Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* http:dx.doi.org/10.1038/nature13683 (2014)