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Sensing the enemy within: how macrophages detect intracellular Gram-negative bacteria

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Caspase-11 contributes to host defense against Gram-negative bacterial pathogens by inducing an inflammatory form of programmed cell death in infected cells. Lipopolysaccharides (LPS) have been identified as the microbial agents that stimulate caspase-11 activation; however, the mechanism of LPS detection has been unknown. In a recent study, Shao and colleagues demonstrate that caspase-11 and its human homologues, caspases -4 and -5, unexpectedly act as direct sensors of cytosolic LPS.

Lipopolysaccharides (LPS) are major structural constituents of the outer membrane of Gram-negative bacteria. They consist of three major components, namely a serotype-specific O-antigen polysaccharide that extends from the cell surface, a centrally located core oligosaccharide, and a lipidated dissaccharide known as lipid A that anchors the LPS molecule in the bacterial membrane. LPS is a prototypical pathogen-associated molecular pattern (PAMP) that readily stimulates the immune system of metazoan hosts. Extracellular LPS triggers the pattern recognition receptor Toll-like receptor (TLR)4 to signal from the plasma membrane and endosomes, which was long held responsible for inducing lethality in metazoan hosts. TLR4 signaling is initiated when the lipid A component of LPS is captured by circulating LPS binding protein (LBP), which transfers it to soluble or membrane-bound CD14 (Figure 1). LPS is then relayed to myeloid differentiation factor 2 (MD2) molecules that are associated with TLR4. Recombinant CD14, MD2, and TLR4 bind LPS *in vitro* with distinct on/off kinetics, in agreement with their differential functions in the capture and serial transfer of LPS [1]. Ultimately, binding of LPS to TLR4/MD2 results in dimerization of the receptor complex and initiation of an intracellular signaling cascade that drives broad myeloid differentiation primary response gene 88 (MyD88)- and TIR domain-containing adaptor inducing IFN-β (TRIF)-dependent gene transcription [2].

In addition to the well-established roles of TLR4 in the detection of extracellular LPS, a body of recent work revealed an intriguing and unsuspected mechanism by which macrophages detect and respond to LPS found in the cytosol (Figure 1). Important insight came from the observation that mice and macrophages lacking TLR4 respond to cytosolic LPS and intracellular Gram-negative bacteria by activating caspase-11, a protease that induces an inflammatory programmed cell death mode and promotes secretion of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 [3–5]. The importance of this mechanism for host defense against Gram-negative infections is highlighted by the increased susceptibility of caspase-11-deficient mice to *Burkholderia thailandensis* and *Burkholderia pseudomallei* infection [3,6,7].

Activation of caspase-11 was believed to be regulated by large protein assemblies, analogous to the inflammasome complexes that activate the related caspase-1 [8]. However, Shao and coworkers revealed in their recent report that mouse caspase-11 and its human homologues, caspases -4 and -5, bind LPS *in vitro* with high affinity and specificity through their amino-terminal caspase recruitment domain (CARD) motifs [9]. This process is sufficient to induce oligomerization and activation of the respective caspases. In agreement, expression of recombinant caspase-4 and -11 in *Escherichia coli* was sufficient to induce oligomerization, whereas expression in Sf9 insect cells was not [9], suggesting that a bacterial factor triggered caspase-11 activation. Furthermore, incubating the Sf9-purified caspases with lysates of Gram-negative (but not Gram-positive) bacteria or LPS directly prompted their oligomerization. Surface plasmon resonance (SPR) assays confirmed direct and high-affinity interactions between caspases -4 and -11 and either LPS or lipid A, but not other bacterial PAMPs [9]. SPR and gel filtration assays further illustrated that the CARD motif was necessary and sufficient for caspase-11 oligomerization and binding to LPS, suggesting that LPS–caspase-11 CARD interactions were imperative for proximity-induced activation of this caspase. Importantly, the absence of high-affinity binding between LPS and caspases -1 and -9 demonstrated that LPS binding is not a general feature of CARD-containing caspases, but a rather specific property of caspase-11 and its human orthologs [9]. Nevertheless, this observation raises the intriguing possibility that the CARD, pyrin, death domain and death effector domain motifs of other proteins implicated in innate immunity and apoptosis signaling may directly and specifically associate with particular PAMPs.

It is widely recognized that oligomerization of initiator caspases results in their proximity-induced autoactivation [10]. Activation of caspase-1, the prototype inflammatory caspase, occurs upon its recruitment to inflammasomes

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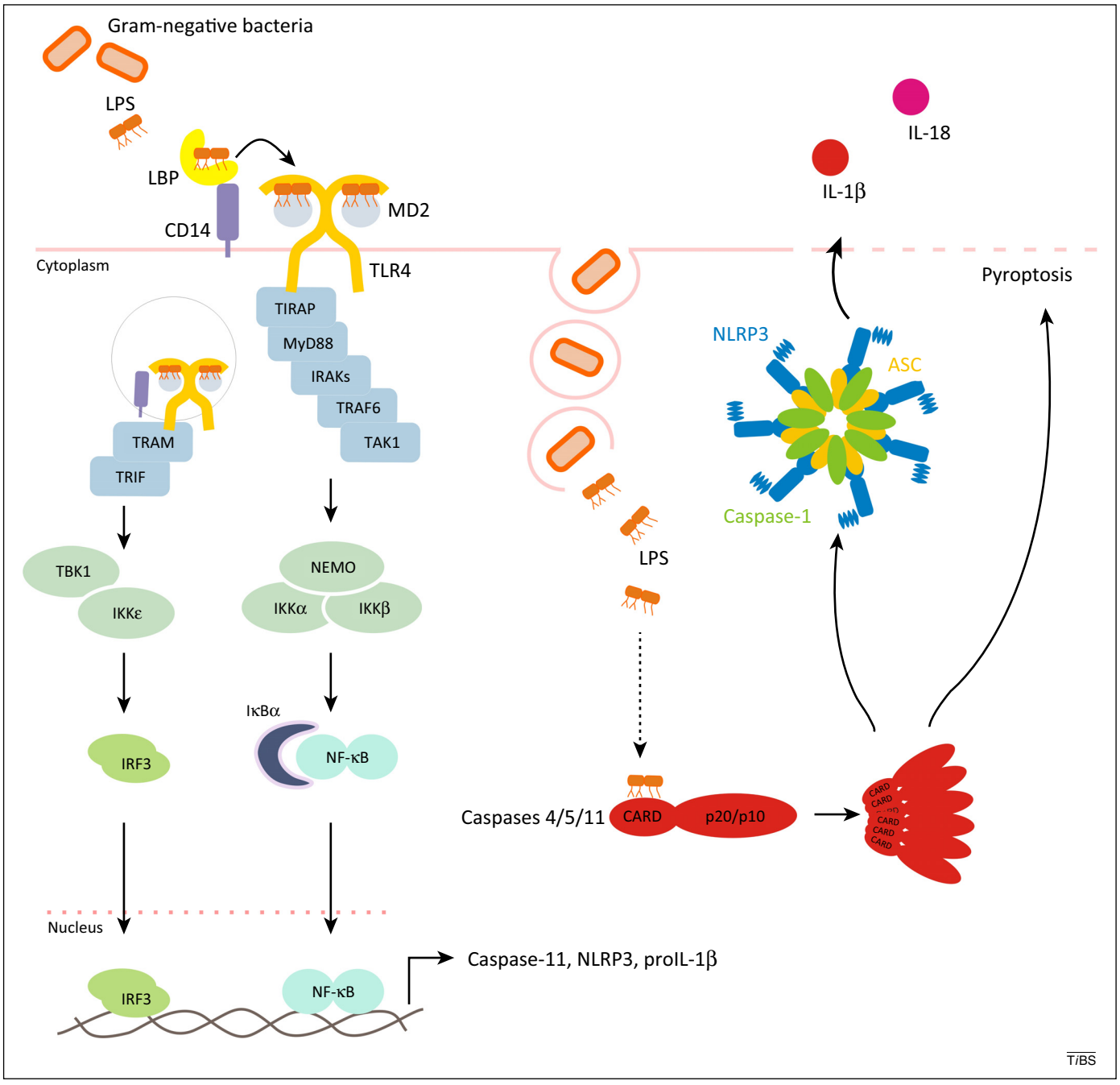

Figure 1. Lipopolysaccharide (LPS) sensing by Toll-like receptor (TLR)4 and the caspase recruitment domain (CARD) motif of caspases -4, -5, and -11. Signaling through TLR4 by LPS (left) is initiated by the coordinated activation of LPS binding protein (LBP) and CD14, which transfer LPS to the TLR4/myeloid differentiation factor 2 (MD2) complex. Ultimately, TLR4 oligomerization induces an intracellular signaling cascade that drives myeloid differentiation primary response gene 88 (MyD88)- and TIR domain-containing adaptor inducing IFN-β (TRIF)-dependent gene expression of caspase-11, NOD-like receptor protein (NLRP)3, and the inactive proinflammatory cytokine prointerleukin (proIL)-1β. An alternative mechanism of LPS detection can occur when lysis of bacteria-containing vacuoles causes the release of LPS into the cytosol (right). Shao and colleagues demonstrate that cytosolic LPS is detected by the CARD motifs of caspases -4, -5, and -11, which induces their oligomerization and proteolytic activation. These caspases trigger programmed cell death of the infected cell and activate the NLRP3 inflammasome for maturation and secretion of the inflammatory cytokines IL-1β and IL-18.

[8]. Similarly, the apoptotic initiator caspases -8 and -9 are activated in the death-inducing signaling complex (DISC) and the apoptosome, respectively [10]. In these multi-protein complexes, the activating signal is captured by platform proteins. For inflammasomes, these platform proteins are of the NOD-like and HIN200 receptor families; Apaf-1 detects cytochrome c that has been released from mitochondria for assembly of the apoptosome; and ligation of plasma membrane-bound death receptors by their respective ligands triggers DISC formation [8,10]. Through their ability to sense LPS directly, caspases -4, -5, and -11 appear to escape (at least *in vitro*) the need for multi-protein complex assembly for their activation. Considering the hydrophobicity and large molecular mass of LPS (which varies greatly, but is at least 10 kDa), it is tempting to speculate that LPS may form large aggregates to which caspases -4, -5, and -11 are recruited to trigger their proximity-induced auto-activation. As such,

LPS may itself function as a molecular platform for recruitment and activation of these inflammatory caspases. Undoubtedly, the structures of uncleavable and catalytic mutants of caspases -4, -5, and -11 crystallized in the presence of LPS will shed more light on the structural rearrangements by which this PAMP promotes inflammatory caspase activation.

In conclusion, Shao and colleagues convincingly showed that the CARD motifs of caspases -4, -5, and -11 can directly and specifically bind LPS with high affinity *in vitro* and in cells. Nevertheless, it is tempting to speculate that mouse caspase-11, and its human orthologs caspases -4 and -5, may function *in vivo* as end-acceptors of cytosolic LPS in a serial LPS transfer mechanism analogous to the role of TLR4 in the well-characterized LBP/CD14/MD2-TLR4 signaling axis. Undoubtedly, these intriguing inflammatory caspases have more surprises in store for us.

References

1 Shin, H.J. *et al.* (2007) Kinetics of binding of LPS to recombinant CD14, TLR4, and MD-2 proteins. *Mol. Cells* 24, 119–124
2 Meissner, F. *et al.* (2013) Direct proteomic quantification of the secretome of activated immune cells. *Science* 340, 475–478
3 Aachoui, Y. *et al.* (2013) Caspase-11 protects against bacteria that escape the vacuole. *Science* 339, 975–978
4 Hagar, J.A. *et al.* (2013) Cytoplasmic LPS;1; activates caspase-11: implications in TLR4-independent endotoxic shock. *Science* 341, 1250–1253
5 Kayagaki, N. *et al.* (2013) Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science* 341, 1246–1249
6 Kayagaki, N. *et al.* (2011) Non-canonical inflammasome activation targets caspase-11. *Nature* 479, 117–121
7 Wang, S. *et al.* (1998) Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* 92, 501–509
8 Lamkanfi, M. and Dixit, V.M. (2012) Inflammasomes and their roles in health and disease. *Annu. Rev. Cell Dev. Biol.* 28, 137–161
9 Shi, J. *et al.* (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* 514, 187–192
10 Shi, Y. (2004) Caspase activation: revisiting the induced proximity model. *Cell* 117, 855–858