The Family of LPS Signal Transducers Increases: the Arrival of Chanzymes

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https://doi.org/10.1016/j.immuni.2017.12.016

After LPS recognition, the MyD88-dependent and the TRIF-dependent pathways are consecutively activated in macrophages. Schappe et al. (2018) show that the chanzyme TRPM7 is required for an efficient LPS receptor complex endosomal relocation and the activation of the TRIF pathway.

Sensing of invading microorganisms by the innate immune system is mediated by pattern-recognition receptors (PRRs), proteins specialized for the recognition of microbe-associated molecular patterns (PAMPs). PRR engagement by PAMPs activates complex intracellular signaling pathways that induce the production of inflammatory mediators to fight the infections. One of the best-characterized PRR is the Toll-like receptor 4 (TLR4), which recognizes lipopolysaccharide (LPS), the main component of the outer membrane of Gram-negative bacteria. Besides TLR4, other proteins have been shown to be required for LPS recognition. In particular, the plasma LPS binding protein (LBP), MD-2 and CD14 form with TLR4 the LPS multi-receptor complex (Brubaker et al., 2015). In this issue of *Immunity*, Schappe et al. (2018) identify the chanzyme transient receptor potential melastatin-like 7 (TRPM7) as an additional molecule required by myeloid cells to respond to LPS.

In the classical view of the LPS recognition pathway, LPS single molecules are engaged by LBP and transferred to CD14, a glycosylphosphatidylinisitol (GPI)-anchored protein abundantly expressed on monocyte and macrophages, mouse dendritic cells (DCs), and a subpopulation of human inflammatory DCs. CD14, in turn, presents LPS to the TLR4-MD2 complex that dimerizes and initiates, from the plasma membrane, the TIRAP-MyD88-dependent pathway leading to a first wave of NF-κB and AP-1 activation (O'Neill and Bowie, 2007). Subsequently, the entire receptor complex is internalized and redirected to the endosomal compartment. Here, the TRAM-TRIF pathway is activated and induces a second wave of NF-κB nuclear translocation and the activation of IRF3 transcription factor for type I interferon (IFN) production (Rosadini and Kagan, 2017) (Figure 1). The second wave of NF-κB activation is fundamental to sustain NF-κB dependent transcription of inflammatory cytokine genes, particularly in macrophages. CD14 controls the entire LPS receptor complex endosomal relocation (Zanoni et al., 2011). Downstream of CD14, ITAM-bearing molecules, the tyrosine kinase Syk and its effector phospholipase Cγ2 (PLCγ2) are important regulators of TLR4 endocytosis and signaling (Chiang et al., 2012; Zanoni et al., 2011). Other molecules, including CD13, a negative controller of TLR4 endocytosis in DCs, and CD300b, a promoter of the TRIF pathway, have been added to the factors composing the LPS receptor complex. CD14 is required for both smooth and rough LPS-induced activation of the TRAM-TRIF pathway and type I IFN production, whereas it is required for the activation of the membrane TIRAP-MyD88 dependent pathway only in the presence of smooth but not rough LPS (Jiang et al., 2005). In addition to the control of the TRIF-dependent pathway, CD14 owns TLR4-independent signal transduction capacities in myeloid cells, such as DCs and neutrophils (Zanoni et al., 2009). Following LPS stimulation, CD14, through the activation of src family kinases (SFKs) and PLCγ2, induces an increase of intracellular Ca^{2+} concentration and the consequent calcineurin activation which, in turn, dephosphorylates different NFATc transcription factor family members causing their nuclear translocation. The CD14 controlled Syk pathway for endosomal TLR4 relocation and the src pathway for NFAT activation are independently regulated (Zanoni et al., 2011).

Schappe et al. add a new piece to the puzzle of LPS-induced signaling pathways in myeloid cells. They started from the observation that macrophages deficient for TRPM7 do not produce inflammatory cytokines in response to LPS. TRPM7 is a member of a large group of ion channels usually expressed at the cell surface of multiple cell types. The TRPM subfamily comprises 8 members, TRPM1–TRPM8. They have six transmembrane segments, S1–S6, with the S1–S4 segments that form the sensors and the S5 and S6 segments that form the membrane pore (Venkatachalam and Montell, 2007). The TRPM7 is ubiquitous and highly expressed in macrophages. It is a channel that conducts bivalent and monovalent cations and contains a functional serine-threonine kinase located at the carboxyl terminus. Multiple roles for TRPM7 have been proposed in immune cells, including regulation of mast cell degranulation by the kinase activity, regulation of platelet function by the control of Mg^{2+} homeostasis and cytoskeletal myosin IIA heavy chain activities, activation of macrophages and monocytes, B cell proliferation and thymic T cell development.

To define whether the TIRAP-MyD88 pathway or the TRAM-TRIF pathway is affected by the absence of TRPM7, the authors analyzed the membrane activation and the endocytosis of TLR4, as well as the phosphorylation and nuclear translocation of NF-κBp65 and IRF3 in LPS-stimulated macrophages. Although the dimerization of TLR4 still occurred at the cell surface in the absence of TRPM7, its internalization was affected. Accordingly, NF-κBp65 and IRF3 phosphorylation and nuclear translocation were decreased but not IkBα degradation.

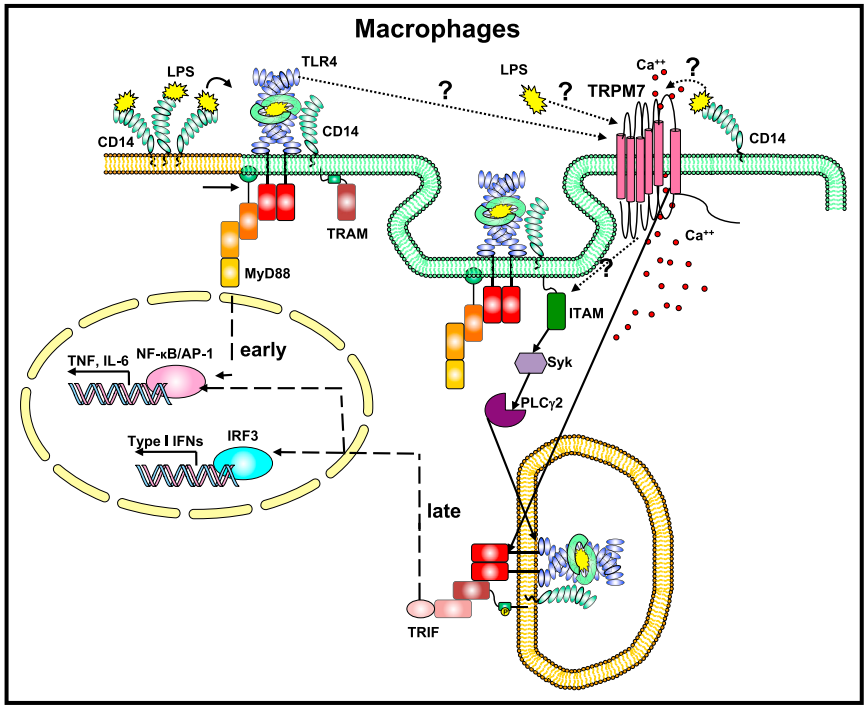

Figure 1. The LPS Multi-Receptor Complex
After LBP-dependent extraction from bacterial membrane, LPS is presented by CD14 to the TLR4-MD2 complex that dimerizes and initiates the MyD88-dependent signaling pathway from the plasma membrane. A first wave of NF-κB and AP1 nuclear translocation occurs. Subsequently CD14 promotes the entire LPS receptor complex internalization and its relocation to the endosome for the activation of the TRIF-dependent pathway. This allows the sustainment of NF-κB nuclear translocation and the activation of IRF3 for type I IFN production. TRPM7 favors the late events of LPS induced signaling by promoting TLR4 endocytosis and the activation of the TRIF-dependent pathway with a mechanism involving Ca^{2+} mobilization.

These observations indicated that TRMP7 does not influence TLR4 dimerization and the initiation of the MyD88-dependent but influences the TRIF-dependent pathway presumably controlling TLR4 endosomal relocation. Interestingly, TLR4 was similarly internalized in mutant and wild-type macrophages at early time points after LPS exposure but a quick restoration of membrane TLR4 expression was observed in mutant macrophages. The authors did not investigate how TRPM7 regulates TLR4 endocytosis. Nevertheless, the biphasic nature of TLR4 internalization kinetic suggests the interesting hypothesis that TRPM7 controls TLR4 recycling rather than internalization itself. It is, thus, possible to speculate that in the absence of TRPM7, TLR4 could be quickly recycled to the cell surface after internalization. This, in turn, would impede the stable localization of TLR4 in the endosomes and, consequently, it would reduce the activation of the TRIF pathway.

TRPM7 is a cation channels, therefore the authors investigated whether Ca^{2+} mobilization was required for TRPM7 mediated regulation of TLR4 functions. Compared to DCs (Zanoni et al., 2009), in macrophages Ca^{2+} mobilization takes place late (around 1 min and half) after LPS exposure. The authors observed that TRMP7 at least partially accounted for this late wave of Ca^{2+} rise. The absence of TRMP7 strongly decreased, although not abolished, Ca^{2+} fluxes. Moreover, intracellular Ca^{2+} chelation in LPS-stimulated macrophages reduced NF-κBp65 phosphorylation and its nuclear translocation as well as the transcription of mRNAs of inflammatory cytokines. These data indicate that Ca^{2+} mobilization is one of the mechanisms by which TRPM7 influences the TLR4 signaling pathway. Nevertheless, Ca^{2+} mobilization is not the only mechanism regulated by TRMP7. The decrease of cytokine mRNA transcription in the presence of Ca^{2+} chelators was far less dramatic than the reduction obtained in the absence of TRMP7, suggesting that TRMP7 might influence the LPS signaling machinery both as channel and with its kinase activity. Moreover, in the absence of Ca^{2+} fluxes, TLR4 internalization did not show the bimodal kinetic observed in TRPM7-deficient macrophages but was almost completely inhibited with only a minimal reduction of cell surface expression at late time points. This is an additional indication that Ca^{2+} mobilization only partially explains the mechanism by which TRMP7 influences the TLR4 signaling pathway.

In DCs, CD14 is required for an early rise of intracellular Ca^{2+} concentration that leads to NFAT signaling pathway activation upon smooth LPS recognition (Zanoni et al., 2009). To investigate whether CD14 was required also for the late wave of Ca^{2+} mobilization relevant for the initiation of the TRAM-TRIF pathway in macrophages after LPS stimulation, the authors analyzed Ca^{2+} fluxes in CD14-deficient macrophages. Remarkably, in the absence of CD14, Ca^{2+} fluxes were completely abrogated and the effect was even more dramatic than the effect on Ca^{2+} mobilization observed in the absence of TRPM7, leaving open the possibility that CD14 can regulate late Ca^{2+} fluxes in macrophages by multiple mechanisms, one of which possibly involving the chanzyme TRPM7 (Figure 1).

Smooth LPS was used throughout this study. Because a mixture of smooth and rough LPS is produced and released by Gram-negative bacteria and both can account for the toxic effects of LPS *in vivo*, and given that the relevance of CD14 for Ca^{2+} mobilization has been clearly demonstrated only for smooth LPS (Zanoni et al., 2012; Zanoni et al., 2009), it would be interesting to examine whether TRPM7 is required also for rough LPS responses. Moreover, many mechanistic aspects remain to be determined: is TRPM7 activated downstream of CD14, TLR4, or directly by LPS? How precisely does this chanzyme intervene in the LPS-induced TLR4 signaling pathways? What is the role of the kinase domain? Is TRPM7 chanzyme also important in human macrophages and in mouse and human $CD14^+$ DCs?

Beyond the mechanism of action, Schappe et al. showed that TRMP7 is fundamental for the responses of

macrophages to LPS *in vivo*. Mice conditionally deficient for TRMP7 expression in myeloid cells were significantly less sensitive to LPS. They showed less toxic symptoms compared to wild-type animals induced by intra-peritoneal LPS administration over 24 hr after challenge, showed a significant decrease of inflammatory cytokine production and a decrease of overall inflammatory cell recruitment at the site of injection. Therefore, mice conditionally deficient for TRPM7 in myeloid cells show a significant resistance to LPS-induced peritonitis.

The striking effect of TRPM7 deletion in the responses of macrophages to LPS *in vitro* and *in vivo* opens new roads for the comprehension of the complex process of inflammation. A detailed characterization of the role of TRPM7 in innate immune myeloid cells in LPS- and, possibly, other TLR agonist-mediated inflammatory conditions could offer new potential therapeutic targets for modulating immune system activation during sepsis, bacterial infections and inflammatory diseases that chronically expose the organism to LPS and other TLR agonists.

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moDCs, Less Problems

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Type 1 conventional dendritic cells are necessary for the development of anti-tumor immunity. In this issue of *Immunity*, Sharma et al. (2018) identify a phenotypically similar monocyte-derived population within inflamed tumors that promotes T cell responses during therapy.

Conventional dendritic cells (cDCs) develop from pre-DCs and a common DC progenitor (CDP) into populations expressing CD11b (cDC2) or either CD8α or CD103 (cDC1) (Merad et al., 2013). Of these, the cDC1 population has attracted considerable interest as the inducer of $CD8^+$ T cell immunity during infection and cancer, and mice lacking lineage-defining transcription factors for this subset (*Batf3*, *Irf8*) fail to develop spontaneous immunity against tumors or to respond to immune-based therapies (Gardner and Ruffell, 2016). This is thought to be due to the superior ability of cDC1s to transport antigen from tissues and cross-present to $CD8^+$ T cells in the draining lymph nodes (Roberts et al., 2016; Salmon et al., 2016) but may also be due to recruitment and enhancement of effector function through chemokine and interleukin-12 (IL-12) expression, respectively (Ruffell et al., 2014; Spranger et al., 2017). However, cDC1s are rare within tumors, a potentially limiting factor in the ability of tumors to respond to immunotherapy (Salmon et al., 2016). In this issue of *Immunity*, Sharma et al. (2018) identify the development of $CD103^+$ monocyte-derived DCs (moDCs) during chemoimmunotherapy and show that this population is necessary for tumor regression.

Sharma et al. (2018) begin their study by phenotyping DCs within inflamed, regressing B16 melanoma tumors, using mice with PTEN-deficient regulatory T cells or treatment with the PTEN inhibitor VO-OHpic to relieve immune suppression, and combining this with cyclophosphamide chemotherapy, presumably to promote the release of damage-associated molecular patterns and enhance DC maturation (Ma et al., 2013). In both scenarios they identified the emergence of a $CD11c^+Ly6C^+CD103^+$ subset that was not found within control tumors. In addition to CD103, this population expressed a number of other markers normally associated with the cDC1 subset, including XCR1, CD24, IL-12, and the transcription factors BATF3 and IRF8.