Cell Research (2008) 18:711-712.

www.nature.com/cr

RESEARCH HIGHLIGHT

With a little help from my friends: modulation of phagocytosis through TLR activation

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Cell Research (2008) **18**:711-712. doi: 10.1038/cr.2008.78; published online 3 July 2008

In 1884, Elias Metschnikoff was first to report the cellular response to invading fungi in the water flea *Daphnia*. Blood cells, which he called phagocytes, surrounded and digested each invading spore. He hypothesized that this process, termed phagocytosis, was a general mechanism of host defense against an invading pathogen. One hundred years later, scientists have only begun to elucidate the complex mechanisms by which phagocytosis is regulated.

Phagocytosis begins by recognition of particles such as pathogens or apoptotic cells by phagocytes including macrophages and polymorphonuclear cells. Recognition occurs through cell surface receptors, such as scavenger or mannose receptors, which bind ligands present on the surface of the pathogen. Upon ligation, these receptors cluster on the phagocyte surface and induce rearrangement of the actin cytoskeleton through different members of the Rho GTPase family [1]. Distinct subsets of RhoGTPases are activated depending upon the ligated receptor and differentially regulate how actin is polymerized [2]. The polymerization of actin allows for the formation of pseudopodia that engulf the pathogen [1]. Once internalized, the phagosome gradually matures by increasing acidification through fusion with endosomes and finally, fusion with lysosomes, which contain hydrolytic enzymes to destroy the pathogen [3]. Furthermore, these phagosomes serve not only to deliver the pathogens to death, but also to alert the innate immune system.

Toll-like receptors (TLRs) are a family of pattern recognition receptors which recognize pathogen-associated molecular patterns (PAMPs) and activate the innate immune system. Upon ligation from PAMPs, TLR signaling through the adapter molecule MyD88 leads to activation of mitogen activated protein kinases (MAPKs) and NF-κB, which are both critical for the inflammatory response. TLR3, 7, 8 and 9 reside within endosomes, and TLR2 and TLR4 are often enriched in endosomes after activation [4]. This enrichment leads to increased TLR recognition of PAMPs from phagocytosed pathogens, enhancing activation of TLR signaling pathways and the innate immune response. The close ties between activation of TLRs and phagosomes have led researchers to investigate whether ligation of TLRs can create a feedback loop which alters the efficiency of phagocytosis.

A seminal paper by Blander and Medzitov in 2004 revealed evidence for TLR regulation of phagocytosis [5]. In this paper, bone marrow derived macrophages deficient in either TLR2/4 or MyD88 were presented with *E. coli, S. aureus*, or apoptotic cells. When compared to wild type macrophages, TLR2/4–/– and MyD88–/– macrophages exhibit normal rates of apoptotic cell clearance, but decreased rates of bacterial clearance within two hours of bacterial infection. Phagocytosis of bacteria seemed to be dependent on p38 activation downstream of TLR activation as this defect could be recapitulated by p38 inhibitors. However, Yates and Russell argued that MyD88–/– macrophages may exhibit impaired phagocytosis due to a developmental defect and designed an alternative experiment to test whether the addition of TLR ligands could enhance phagocytosis [6]. They coupled silica beads with the phagocyte receptor ligands mannosylated BSA or immunoglobulin G and analyzed the rate of phagocytosis with or without the addition of TLR ligands. While this group did not find any change in the rate of phagocyte maturation between wild type and MyD88–/– macrophages, they showed that within each genotype the TLR4 ligand lipopolysaccharide (LPS) altered the kinetics of phagosome maturation independent of MyD88.

Another possible mechanism for TLR modulation of phagocytosis is by altering gene transcription programs that affect phagocytosis at later timepoints than measured by the previous studies. Doyle *et al*. showed that activation of TLR2 or 9 results in enhanced phagocytosis of *E. coli* and *S. aureus* 24 h after ligand treatment [7]. Microarray analysis indicates that TLR stimulation upregulates the Fc recep-

tor, complement receptor, scavenger receptors MARCO and SR-A, and many other genes regulating phagocytosis. Upregulation of these genes are dependent upon p38 activation downstream of TLR signaling, and inhibition of p38 results in impaired phagocytotis. Overall, it seems that TLR ligands may modulate phagocytosis through multiple mechanisms, and the paper by Kong *et al.* [8] in *Cell Research* reveals another pathway for regulating phagocytosis.

In this report phagocytosis is enhanced by pre-treatment with TLR ligands through two distinct signaling pathways; MyD88-dependent activation of the p38 pathway and MyD88-independent activation of the Rho GTPases Cdc42 and Rac. Upon treatment with LPS, Rac/Cdc42 activation and subsequent actin polymerization occur within 30 min and return to basal levels after one hour. Inhibition of Rho GTPases by *Clostridium difficile* Toxin B or by knockdown of Rac/Cdc42 reduced actin polymerization after treatment with LPS and specific inhibition of actin polymerization (but not inhibition of microtubules) reduced Rac/Cdc42 activation, indicating that actin polymerization can regulate Rac/Cdc42 activation. Interestingly, inhibition of Rho GTPases upon initial LPS treatment results in decreased levels of phagocytosis. Furthermore, the authors show that Rac/Cdc42 activation is not a consequence of MyD88 signaling because activation was comparable between MyD88–/– and wild type macrophages.

Current data indicate that TLR ligands can activate a MyD88-independent pathway to enhance phagocytosis; however, the exact mechanism of how this pathway enhances phagocytosis needs to be clarified. Whether Rac/Cdc42 activation and actin polymerization localized near activated TLRs can directly affect the efficiency of phagocytosis mediated by distal scavenger receptors such as SR-A has not yet been addressed. The authors suggest that indirect enhancement of phagocytosis may occur through transcriptional changes by a mechanism similar to the enhancement of phagocytosis by p38 activation. Activation of TLR2 results in the recruitment of the Rac isoform 1, and Rac1 is required for complete NF-κB activation downstream of TLR2 [9]. It should be determined whether Rac1 interacts with other TLRs and if the enhanced levels of phagocytosis resulting from TLR ligand stimulation are NF-κB dependent. Another interesting question to be addressed is whether Rac/Cdc42 activation enhances phagocytosis in a general manner or is specific to bacterial phagocytosis.

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