Toll-like receptors and phagosome maturation

To the editor:

In a recent Perspective in *Nature Immunology*[1], Blander and Medzhitov explored the function of Toll-like receptor (TLR) signaling in controlling the maturation of phagosomes in macrophages and dendritic cells. As the phagosome is the key organelle for the degradation of microbes and for the generation of bacterial peptides to be presented to lymphocytes, this issue is fundamental for understanding the innate-acquired immune interface.

In their Perspective, Blander and Medzhitov discuss data from their published studies that found a key function for TLR signaling in the maturation of phagosomes. However, Blander and Medzhitov also revisit our paper in which we reported no influence of TLR signaling on phagosome maturation in macrophages[2]. Their comments, offering an alternative interpretation of our data, are based on the idea that some phagocytic receptors are 'more equal than others'.

In our paper, we used real-time, quantitative assays to measure the rates of acidification and phagosome-lysosome fusion phagosomes formed around silica beads bearing ligands (immunoglobulin G or mannosylated BSA), both with and without TLR agonists[2,3]. Blander and Medzhitov speculate that the beads we used, internalized by Fc receptors and mannose receptors, 'maxed out' those receptors and thus the rapid phagosome maturation program so that possible effects of additional TLR agonists added to those particles could not be discerned. That interpretation is proposed alongside descriptions of their own studies on phagosome cargos, including *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*, which they state are more relevant than immunoglobulin G or mannosylated BSA for the 'nonopsonic' uptake pathways they studied[4].

In their Perspective, Blander and Medzhitov did not mention that we studied real-time kinetics of maturation and that to exclude the possibility that Fc receptors and mannose receptors provided too 'dominant' a signal, we examined beads in the context of two conditions beyond use of immunoglobulin G and mannosylated BSA. We supplied wild-type and TLR2-deficient macrophages with fixed *S. aureus*, with and without lipopolysaccharide (LPS)[2] and, as a 'surrogate' for apoptotic cells, we assessed phosphatidylserine-mediated uptake with lipid-coated beads in the presence and absence of the TLR agonists LPS and Pam_3Cys (tripalmitoyl cysteinyl lipopeptide)[2]. In neither of those series of experiments did we find any effect on the rate of phagosome maturation, despite manipulation of both the TLR agonist and the TLR receptor and verification of appropriate TLR activation. In summary, we found no effect of TLR signaling on the phagosomes formed around particles internalized through four distinct phagocytic routes.

In contrast, we believe that the complexity of evaluating phagocytosis of *E. coli*, *S. typhimurium* and *S. aureus*, as used by Blander and Medzhitov, challenges any effort to establish the 'receptor hierarchy' proposed to explain the dichotomy between their results and ours. Moreover, rather than manipulating the TLR agonist makeup of their particles, Blander and Medzhitov relied soley on TLR-deficient phagocytes or phagocytes deficient in the MyD88 signaling adapter[4].

Two other results emerged from our study. First, we found that MyD88-deficient macrophages have less phagosome-lysosome fusion regardless of the identity of the internalized particles (which is perhaps a more likely explanation of the discrepancy between the results of our laboratories). Second, LPS-laden beads affect phagosome-lysosome fusion similarly in both wild-type and MyD88-deficient macrophages[2]. That finding, we believe, should raise concerns regarding the proposal by Blander and Medzhitov that LPS modulates dendritic cell phagosomes directly through TLR4 stimulation[5], a proposal, we further believe, that would have benefited from verification by experiments with MyD88- or TLR4-deficient cells. We therefore favor our conclusion that stimulation of TLRs by agonists present on the internalized particle does not affect the rate of phagosome maturation.

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Blander and Medzhitov reply:

Two years ago we published our observations that engagement of TLRs expressed by macrophages can positively regulate macrophage phagocytosis of bacteria and phagosome maturation[1]. The next year, Yates and Russell published a paper[2] that arrived at a different conclusion based on experiments that did not show TLR maturation in several conditions. Although there are many possible explanations for the discrepancy between their data and ours, the most likely is the experimental conditions used.

The experimental system used by Yates and Russell differed from ours, which we think explains why they failed to observe the same phenomena. In but one part of our discussion in the Perspective[3] mentioned above, we offered an interpretation of data published by Yates and Russell[2]. They have now written supporting their conclusions and making critical remarks about our published work[1,4], to which we will now respond.

We are confident of our initial results and conclusions[1], which are supported by our follow-up study[4]; those conclusions are further supported by independent studies reported by other laboratories[5,6]. All our results are consistent with each other and were obtained using different experimental systems and different 'readouts'[1,4]. In particular, we have now