ESPERITE advances in understanding of septic shock, the mortality related to this syndrome remains high.\(^1\) Sepsis is an infection associated with a systemic inflammatory response. A better understanding of signaling pathways triggered by pathogens in the host immune cells may help to identify targets for novel therapeutic approaches.\(^1\) In this issue of *Anesthesiology*, Gong et al. now identify a novel mechanism by which bacterial components affect mitochondrial function in host’s peritoneal leukocytes by engaging an innate pattern recognition receptor called Toll-like receptor 2 (TLR2).\(^2\) By unraveling an axis between pattern recognition receptor and mitochondrial function, they paved the road for new options in the management of patients with septic shock.

The presence of invading microbes is initially recognized by innate pattern recognition receptors among which there are TLRs (table 1). Engagement of pattern recognition receptors leads to the intracellular activation of kinases such as IkB kinases and mitogen-activated protein kinases that activate nuclear factor kappa-light-chain enhancer of activated B cells and activator protein 1 transcription factors, respectively. These induce a battery of genes encoding inflammatory molecules involved in the elimination of pathogens and infected cells. However, aberrant activation of this system may lead to multiorgan failure and death.

Gong et al.\(^2\) show first that *ex vivo* activation of TLR1/2, but not TLR2/6, TLR3, TLR4, or TLR9, induces a significant intracellular and mitochondrial reactive oxygen species (ROS) production in murine peritoneal leukocytes. Next, they used a murine model of cecum ligation and puncture (CLP), which mimics septic shock related to intraabdominal infection in humans. The strength of this study was to perform the experiments in a context of polymicrobial infection, which is present in 80% of severe postoperative abdominal sepsis. The blockade of TLR4, which selectively recognizes lipopolysaccharide,\(^3\) a major component of the outer membrane of Gram-negative bacteria failed to improve the survival of patients with severe sepsis.\(^4\) In the first hours of severe sepsis, as the clinicians do not know the nature of pathogens, they need an nonspecific drug acting on a large panel of pathogens. In this setting, TLR2 signaling pathway offers a new option for therapeutic interventions. However, this model has several weaknesses. Mice are around 8- to 12-week old, corresponding to human aged from 25 to 40 yr old. As septic patients are around 65 yr of age, this difference is a limitation of most experimental models. In these young and healthy animals, the innate immune response is characterized by a proinflammatory response, whereas our patients with septic shock tend to be immunosuppressed.\(^5\)

Gong et al. found that peritoneal leukocytes freshly harvested from mice with CLP exhibit increased intracellular and mitochondrial ROS as well as features of impaired mitochondrial functions such as decreased adenosine triphosphate production.\(^2\) Interestingly, peritoneal leukocytes from TLR2 knockout mice with CLP have decreased intracellular and mitochondrial ROS production and increased adenosine triphosphate production. These findings highlight the role of TLR2 activation on the mitochondrial function in peritoneal leukocytes.

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*Corresponding article on page 1236.*

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TLRs Localization Ligand Origin of the Ligand

<table>
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<tr>
<th>TLR1/TLR2</th>
<th>Plasma membrane</th>
<th>Triacyl lipopeptides</th>
<th>Bacteria, mycoplasma</th>
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<td>TLR13†</td>
<td>Endolysosome</td>
<td>23S ribosomal RNA</td>
<td>Bacteria</td>
</tr>
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</table>

*TLRs may also recognize endogenous (i.e., host) molecules. †Expressed in mice but not humans. TLR = Toll-like receptor.

septic shock. However, the study cannot determine whether or not this dysfunction is the driving force of multiorgan failure in septic shock.

During bacterial infection, the innate phagocyte response involves the production of ROS via the phagosomal nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst. This response is required for the clearance of intracellular pathogens. The mitochondrial oxidative phosphorylation is a major site of ROS production in most cells. However, until recently, mitochondrial ROS were seen as by-products of oxidative respiration. Therefore, their synthesis was thought to be unregulated. It is now known whether mitochondrial ROS production is regulated in response to the stimulation of innate immune cells with some TLR ligands. In mouse leukemic monocyte macrophage cell lines, the production of mitochondrial ROS is stimulated by the engagement of cell surface TLRs (TLR1, TLR2, and TLR4), whereas stimulation of endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) does not increase mitochondrial ROS. In mouse bone marrow-derived macrophages, stimulation of TLR1, TLR2, or TLR4 also increased mitochondrial ROS. This effect was not observed after TLR9 ligation. A major and original finding of Gong et al. is that unlike cultured innate cells (i.e., mouse leukemic monocyte macrophage cell lines, bone marrow-derived macrophages), which exhibit increased mitochondrial ROS production in response to most cell surface TLRs, leukocytes freshly isolated from the site of infection increase mitochondrial ROS induced by TLR2 engagement but not following stimulation of other cell surface TLRs. These results suggest that TLR2 could be a target for novel approaches aiming to manipulate selectively ROS production in leukocytes that are present at the site of infection.

In mice infected via the peritoneal route with Salmonella typhimurium, the stimulation of mitochondrial ROS production in innate immune cells plays a critical role in the reduction of bacterial burden in liver and spleen. This finding clearly shows that ROS produced by macrophage mitochondria play a crucial role in the S. typhimurium clearance. The recognition of these bacteria by innate immune cells is largely mediated by TLR4, TLR2, and TLR5. Gong et al. show that TLR2 engagement is a key mechanism for the in vivo production of mitochondrial ROS in peritoneal leukocytes. This suggests that TLR2-mediated mitochondrial ROS may have beneficial effects in contributing to the clearance of S. typhimurium. This hypothesis leads necessarily to the question of whether TLR2-induced mitochondrial ROS is involved in the host resistance to CLP-induced polymicrobial sepsis. Unfortunately, Gong et al. did not address this question. They did not measure bacterial burden at different sites in this model of CLP.

TLR4 stimulation via nicotinamide adenine dinucleotide phosphate oxidase-derived ROS engages an intracellular signaling cascade resulting in the mitogen-activated protein kinase p38 activation and subsequent overproduction of pro-inflammatory cytokines, tissue damage, and death. By analogy, one can hypothesize that TLR2-mediated mitochondrial ROS production could result in excessive proinflammatory response, which would be detrimental for the host. However, Gong et al. did not examine the potential role the TLR2 mitochondria axis in the induction of innate proinflammatory molecules in this model. In addition, they looked at a single time point, but immune response is time-dependent. Another limitation is that the authors did not compare survival following CLP in wild-type and TLR2 knockout mice.

In septic shock, all potential magic bullets that were active on a single pattern resulted in clinical failure. This suggests the need for multifaceted interventions at different levels of signaling pathways. In the future, we can hypothesize that the solution would be to inhibit or stimulate several pathways at the same time. The results by Gong et al. should be interpreted along these lines. They reveal the existence of...
the TLR2-mitochondria axis as a new player in the intracellular signaling triggered by bacterial components in innate immune cells. This underlines the importance of experimental models to decipher the complexity of the innate immune response to pathogen cues. Future studies are needed to elucidate the role of the TLR2-mitochondria axis in bacterial clearance, the induction of the inflammatory response, the development of tissue damage, and the outcome. As prerequisite to any clinical application, these studies should help us to address whether TLR2 signaling should be stimulated or conversely inhibited in experimental models of sepsis.

Competing Interests
The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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References