Killing without collateral damage: New hope for sepsis therapy

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Sepsis is a common condition, and more than 19 million cases of severe sepsis (i.e. cases where organ dysfunction has occurred as a result of sepsis) occur each year\(^1\). Despite many decades of research, the mortality rate remains approximately 30%, and survivors are often left with a significant morbidity burden, including neurocognitive impairment, neuromuscular weakness and persistent organ dysfunction\(^2\). End-organ dysfunction and injury ensues in part as a result of innate immune cells (including neutrophils) failing to control pathogen invasion, which leads to microbial proliferation, release of inflammatory mediators, endothelial dysfunction, and tissue injury. On page xxx of this issue, Ruchaud-Sparagano and colleagues present novel data investigating the hypothesis that monophosphoryl lipid A (MPLA), a derivative of lipopolysaccharide (LPS), can ameliorate the release of cytotoxic mediators from neutrophils without impairing bacterial clearance\(^3\). Maintenance of efficient neutrophil phagocytic and bactericidal function in the absence of significant release of cytotoxic mediators would be an attractive therapeutic strategy for septic patients.

Using clinically relevant *in vitro* models, the authors show that exposing neutrophils to MPLA, in contrast to LPS, does not result in the release of reactive oxygen species and myeloperoxidase. In contrast to LPS, pre-exposure of MPLA treated neutrophils to a blocking antibody for TLR4 augmented myeloperoxidase release, suggesting that LPS and MPLA differentially modulates TLR4. Despite ameliorated production of superoxide and myeloperoxidase, MPLA exposed neutrophils retained *P. aeruginosa* killing abilities, implying that bacterial killing may not be related to the amount of enzymes released by neutrophils, and intriguingly that superoxide independent killing mechanisms may exist, at least in MPLA exposed neutrophils. Ruchaud-Sparagano and colleagues also observed no difference in the release of TNF\(\alpha\), IL-1\(\beta\) and IL-10 from MPLA and LPS treated neutrophils. However, there was a significant reduction in the release of the potent neutrophil chemoattractant IL-8
from MPLA treated cells, mediated via the PI3 kinase pathway. LPS exposure was also found to increase neutrophil cell surface TLR4 expression, a phenomenon that did not occur with MPLA. The contrasting effects of MPLA and LPS are summarized in Table One.

The observations that administration of MPLA after LPS exposure reduced superoxide and myeloperoxidase release, and that MPLA abrogated increased TLR4 cell surface expression in LPS treated neutrophils whilst preserving efficient bacterial killing, support the notion that MPLA may hold promise as adjunctive therapy for Gram negative sepsis. Persistent activation of TLR pathways, as occurs in the early stages of sepsis, has been associated with excessive release of inflammatory cytokines and the development of tissue injury in sepsis\textsuperscript{4,5}. In contrast to the current paper, a study of 20 patients treated with a synthetic lipid A analogue prior to intravenous endotoxin, demonstrated decreased serum concentrations of the pro-inflammatory cytokines TNF\textsubscript{α}, IL-1\textbeta, IL-8 and IL-6, in the presence of increased circulating neutrophil numbers. It would seem reasonable to speculate that reduced IL-8 release, coupled with preserved chemotaxis in MPLA treated cells, to other chemoattractants such as LTB\textsubscript{4}, may serve to optimize neutrophil recruitment to sites of pathogen invasion without the sequellae of exuberant, mediator-induced tissue injury.

The current work also seeks to determine the mechanistic basis of the contrasting responses of neutrophils to LPS and MPLA, demonstrating the differential signaling responses of LPS and MPLA downstream of TLR4. TLR4 activation can lead to activation of the MyD88 and TRIF pathways. IL-8 production requires recruitment of MyD88 to TLR4; based on the ameliorated concentrations of IL-8 produced by MPLA exposed neutrophils, it is plausible that the differential TLR4 effects of LPS and MPLA may be mediated via differential activation of pathways downstream of TLR4.
LPS, but not MPLA, triggered IkB kinase (IKK) phosphorylation, which results from MyD88 activation, whereas both LPS and MPLA induced interferon regulatory factor 3 (IRF3) activation, which is downstream of TRIF. Both LPS and MPLA were able to trigger p38 MAPK phosphorylation (an event downstream from both MyD88 and TRIF), and LPS, but not MPLA induced JNK phosphorylation, which occurs downstream from MyD88, and to a less extent TRIF. Thus this differential activation of MyD88 and TRIF likely explains the different effects of LPS and MPLA on TLR4 up-regulation.

Sepsis is a heterogeneous and poorly defined clinical syndrome. The continued absence of sepsis therapies suggests that our knowledge of the underlying biology of sepsis remains limited, and a paucity of mechanistic and preclinical data to support the interventions trialled, continues to hamper the rational development of therapeutic interventions for sepsis (e.g.\textsuperscript{7,8,9}). Attempts to target (and antagonise) single molecules implicated in sepsis have proved futile in clinical trials, reinforcing the notion that therapies that modulate the body’s innate immune response, such as MPLA, may be more likely to demonstrate clinical benefit as adjuncts to standard therapy (antibiotics). We should therefore welcome preclinical human data such as these that inform the field of neutrophil biology and may lead to the development of new therapies for this widespread and often devastating condition.
### Table one – effects of LPS and MPLA on human neutrophils

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<thead>
<tr>
<th></th>
<th>LPS</th>
<th>MPLA</th>
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<tbody>
<tr>
<td>Superoxide anion generation</td>
<td>Increased</td>
<td>Not increased</td>
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<tr>
<td>Myeloperoxidase release</td>
<td>Increased</td>
<td>Not increased</td>
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<tr>
<td>Phagocytosis</td>
<td>Increased</td>
<td>Trending towards increased</td>
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<td>Gram negative bacterial killing</td>
<td>Preserved</td>
<td>Preserved</td>
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<td>Acidification of phagolysosomes</td>
<td>Preserved</td>
<td>Preserved</td>
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<tr>
<td>IL-8 release</td>
<td>Increased</td>
<td>Not increased</td>
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<td>TLR4 cell surface expression</td>
<td>Increased</td>
<td>Not increased</td>
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<td>Chemotaxis towards fMLP</td>
<td>Decreased</td>
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References


