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Endotoxin: Back to the Future

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Despite decades of research, it has been remarkably difficult to demonstrate the role of endotoxin in human disease. The dilemma in part stems from our inability to accurately measure endotoxin in the circulation and body fluids and make meaningful clinical correlations. The most commonly used method for detecting endotoxin is the *Limulus* amebocyte lysate (LAL) assay. Routinely used in the pharmaceutical industry to detect endotoxin contamination, this assay measures endotoxin's activity, not endotoxin itself. Endotoxins activate a cascade of procoagulant proteins found in the blood amebocytes of the horseshoe crab, *Limulus polyphemus*, resulting in coagulation that may be detected by clot formation, turbidity or a chromogenic readout. Several factors may interfere with the sensitivity of the assay, including plasma proteins and lipids that bind the endotoxin. Consequently, heating and dilution of samples are required for more accurate measurement. The *Limulus* lysate also reacts with β -glucans found in fungi, but most commercial assay kits now include inhibitors of this reaction.

In the current issue, Charbonney *et al* used a different assay, the Endotoxin Activity (EA) Assay, to evaluate the prevalence and kinetics of systemic endotoxemia in a cohort of 48 patients who were admitted to an ICU within 24 hr of sustaining severe trauma (1). While 46/48 of patients had no endotoxemia on admission, endotoxemia developed in 75% of them, particularly after shock or early surgery, and endotoxemia predicted organ dysfunction. Since few patients had Gram-negative bacterial infections, the authors concluded that a loss of gastrointestinal barrier integrity was the most likely source of the circulating endotoxin.

The EA assay measures neither endotoxin nor its activity, but rather the ability of a putative LPS/Mab complex to prime the generation of reactive oxygen species (ROS) by the PMN in the patient's blood. The blood sample is mixed with an IgM Mab, E5, that targets a broad range of LPS species. If LPS is present in the blood and binds to E5, the LPS/E5 immune complex activates complement, which interacts with neutrophil complement receptors to prime the cells; when opsonized zymosan is added, greater amounts of ROS are produced.

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The ROS are detected by adding luminol, which produces a chemiluminescent (CL) signal. The assay compares the CL reading in the presence of the LPS/E5 complex with the CL produced in control tubes that either lack the IgM antibody or contain maximal amounts of added LPS. The authors claim that this FDA-approved assay can be rapidly performed (<30 vs. 90–120 min for LAL) with <1 ml of whole blood and without need for cell purification or sample manipulation.

Several questions arise, however. E5 may detect the various LPSs that translocate into the circulation from the GI tract with differing degrees of sensitivity. This may explain, in part, the unsuccessful clinical trial with E5 Mab for the treatment of sepsis (2). In addition, pre-existing anti-endotoxin antibodies, or endotoxin-binding proteins such as LBP and BPI, may compete with the E5 Mab for the LPS in the blood (3). Several confounding variables, such as possible endotoxin tolerance, soluble mediators (*e.g.* other immune complexes, cytokines/chemokines, and circulating mitochondrial DAMPs present during trauma [4]) or sepsis itself may also modify the PMN CL response.

The detection of circulating endotoxin by LAL or EA alone cannot predict the impact of LPS on the septic course. Some LPSs that activate the LAL cannot stimulate human cells, while the E5 MAb may bind LPSs that are very weak agonists. Further, differences in a host's capacity to respond to LPS depends on many clinical factors that may limit the clinical utility of the LPS measurement.

Despite all the limitations of LPS measurement in biological fluids, reasonably consistent findings from several clinical studies, including the present one, suggest that high levels of plasma LPS are associated with excess risk of morbidity and mortality that is largely independent of the nature of the bacteria responsible for the septic episodes (3). During the last 25 years not one of the clinical trials in which the downstream biologic effects of endotoxin (rather than the endotoxin itself) were targeted resulted in a licensed intervention. The authors importantly conclude that therapies directed at endotoxin should be re-examined. Given the likelihood that endotoxemia also may play a role in various "leaky gut" syndromes, such therapy may be useful in conditions beyond sepsis (5, 6).

Still, there is a need to establish that specifically targeting LPS with therapeutic agents will reduce mortality in septic shock. Earlier studies documented that the level of anti-core endotoxin antibodies at the onset of sepsis correlated with outcome as did more recent studies with a commercial kit (7–9). A clinical trial that administered polyclonal antibodies directed against a highly conserved epitope of endotoxin demonstrated impressive protective efficacy (10). Although subsequent studies by other investigators failed to demonstrate similar protection, none insured that there were adequate antibody levels either before administration or during the trial (11). Failure of clinical trials with anti-endotoxin MAbs may be attributable to the inaccessibility of the lipid A target (2, 12).

Interventions that should enhance the clearance of endotoxins from the circulation, such as vaccines and MAbs against GNB, are in development. Clinical trials have examined whether polymyxin B hemoperfusion to remove circulating endotoxin may benefit patients with severe sepsis (13). Bactericidal-permeability increasing (BPI) protein, a neutrophilic cationic

peptide, both neutralizes the biologic activity of LPS and is cidal for GNB. A clinical trial with a recombinant 22 kDa segment of BPI failed to reduce the mortality from meningococcal sepsis in children, but did reduce the number of complications in survivors (14). With the dramatic increase in MDR GNB, BPI is getting a “second look”. Enthusiasm for these interventions should be tempered by knowledge that LPS neutralization might be harmful, as was suggested in two previous studies (2, 12) and by multiple preclinical studies showing that mice unable to respond to LPS are highly susceptible to lethal infection (15). Given these considerations, investigators should consider monitoring endotoxemia at the onset and during studies of endotoxin-directed interventions, albeit with an awareness of the limitations.

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References

1. Charbonney E, Tsang JY, Li Y, et al. Endotoxemia following multiple trauma: risk factors and prognostic implications. *Crit Care Med*. 2016 in press.
2. Greenman RL, Schein RM, Martin MA, et al. A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. The XOMA Sepsis Study Group. *JAMA*. 1991; 266:1097–1102. [PubMed: 1865542]
3. Opal SM. The clinical relevance of endotoxin in human sepsis: a critical analysis. *J Endotox Res*. 2002; 8:473–476.
4. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010; 464(7285):104–107. [PubMed: 20203610]
5. Hughes SM, Amadi B, Mwiya M, et al. Dendritic cell anergy results from endotoxemia in severe malnutrition. *J Immunol*. 2009; 183:2818–2826. [PubMed: 19625645]
6. Bukh AR, Meclhjorsen J, Offersen, et al. Endotoxemia is associated with altered innate and adaptive immune responses in untreated HIV-1 infected individuals. *PLoS ONE*. 2011; 6:e21275. <http://dx.doi.org/10.1371/journal.pone.0021275>. [PubMed: 21731690]
7. Pollack M, Huang AI, Prescott RK, et al. Enhanced survival in *Pseudomonas aeruginosa* septicemia associated with high levels of circulating antibody to *Escherichia coli* endotoxin core. *J Clin Invest*. 1983; 72:1874–1881. [PubMed: 6358257]
8. Nys M, Damas P, Joassin L, et al. Sequential anti-core glycolipid immunoglobulin antibody activities in patients with and without septic shock and their relation to outcome. *Ann Surg*. 1993; 217:300–306. [PubMed: 8452409]
9. Moretti EW, Newman MF, Muhlbaier LH, et al. Effects of decreased preoperative endotoxin core antibody levelson long-term mortality after coronary artery bypass graft surgery. *Arch Surg*. 2006; 141:637–641. [PubMed: 16847232]
10. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med*. 1982; 307:1225–1230. [PubMed: 6752708]
11. Cross AS, Opal SM, Bhattacharjee AK, et al. Immunotherapy of sepsis: Flawed concept or faulty implementation? *Vaccine*. 1999; 17:S13–S21. [PubMed: 10506404]
12. Ziegler EJ, Fisher CJ Jr, Sprung CL, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N Engl J Med*. 1991; 324(7):429–436. [PubMed: 1988827]

13. Cruz DN, Antonelli M, Fumagalli R, et al. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized control trial. *JAMA*. 2009; 301:2445–2452. [PubMed: 19531784]
14. Levin M, Quint PA, Goldstein B, et al. Recombinant bactericidal/permeability-increasing protein (rBPI21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial. rBPI21 Meningococcal Sepsis Study Group. *Lancet*. 2000; 16:961–967. 356. [PubMed: 11041396]
15. Cross AS, Sadoff JC, Kelly N, et al. Pretreatment with recombinant murine tumor necrosis factor/cachectin and murine interleukin-1 alpha protects mice from lethal bacterial infection. *J Exp Med*. 1989; 169:2021–2027. [PubMed: 2659724]