

## Endotoxin: MD-2 complex

with

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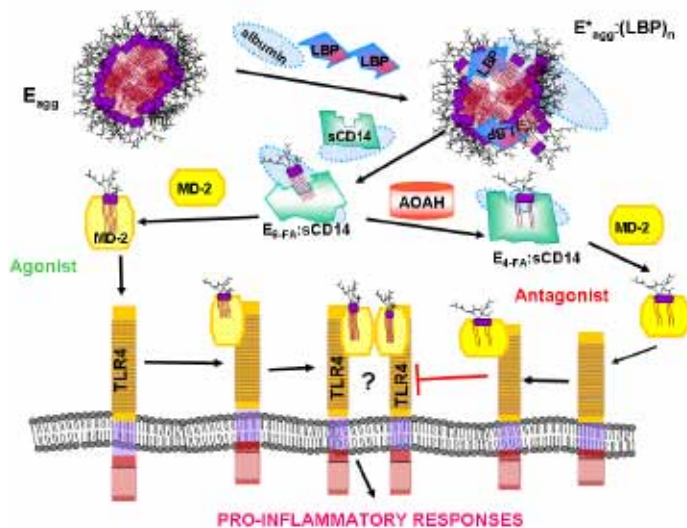


### What is an endotoxin:MD-2 complex?

Theresa L. Gioannini: Endotoxin (E):MD-2 is a 1:1 complex ( $M_r \sim 25,000$ ) of Gram-negative bacterial (GNB) E and the host protein, MD-2 (1) formed by sequential interactions of E with E binding proteins: lipopolysaccharide binding protein (LBP), soluble or membrane-bound CD14, and MD-2, either soluble or associated with Toll-like receptor 4 (TLR4) (Fig. 1). LBP and CD14, in an albumin dependent process, extract E from GNB outer membranes or aggregates of purified E to yield monomeric E:CD14, the preferred form of E for albumin-dependent transfer to MD-2 (2). For potentially active E, E:MD-2 is the apparent ligand for activation of MyD88-dependent (see below) signaling by TLR4 (2-4), with half-maximal activation induced by 30 pM E:MD-2 (2).

Lipid A—diphosphorylated  $\beta$ -1,6-diglycosamine disaccharide linked via amide and ester linkages to 3-hydroxy fatty acids substituted with nonhydroxylated fatty acids—is highly conserved

and is the major determinant of TLR4-dependent E activity (5). While E with 4, 5, or 6 fatty acids has similar reactivity with LBP, CD14, and MD-2 (6), hexaacylated E:MD-2 is a much more potent agonist of TLR4 activation. Both penta- and tetraacylated E:MD-2 inhibit TLR4 activation by hexaacylated E:MD-2 suggesting they interact with TLR4 but do not efficiently activate TLR4 (6-8). E:MD-2 complexes are stable, water-soluble monomers. This suggests sequestration of lipid A in a hydrophobic pocket of MD-2, thus affecting MD-2 and consequently TLR4 configuration, oligomerization, and activation. Stability, potency, solubility, and compact size of E:MD-2 complexes make them attractive candidates for pharmaceutical development. Depending on E structure, they can act as TLR4 agonists to prime host defenses or antagonists to blunt excessive inflammatory responses that can lead to sepsis, shock, and death.



### What is the result of Toll-like receptor 4 dependent cell activation?

Theresa L. Gioannini: Activation of TLR4 leads to intracellular signaling triggered by reactions of the cytosolic TIR domain of TLR4 with intracellular adaptor proteins (i.e., MyD88 +/- TIRAP or TRIF +/- TRAM) (4,9). These interactions include: 1) MyD88-dependent activation

of expression of pro-inflammatory cytokines important for mobilization of innate immune effectors; 2) MyD88-independent up-regulation (in antigen presenting cells) of co-stimulatory molecules needed for induction of adaptive immunity and/or initiation of type 1 IFN response.

### Is there any special role of sCD14?

Theresa L. Gioannini: One important role of CD14 is to bind GNB outer membranes or purified E aggregates modified by substoichiometric concentrations of LBP and extract monomers to form E:sCD14. Thus, one GNB outer membrane (aggregate) containing  $10^6$  E molecules is amplified into  $10^6$  E monomeric complexes that can efficiently transfer E to MD-2 and subsequently activate TLR4 (Fig. 1) (1).

Soluble (s) CD14 in extracellular fluids permits this reaction pathway to proceed in cells that do not synthesize membrane CD14. E is also easily transferred between soluble and membrane CD14 (8) facilitating extracellular scavenging of E (e.g., by lipoproteins) or delivery to cell surface MD-2/TLR4. CD14 is essential for activation of MyD88-independent signaling processes (10).

E:CD14 complex requires albumin to remain water-soluble, suggesting partial exposure of acyl chains of lipid A: consistent with the wide and shallow hydrophobic pocket seen in the crystal structure of mouse sCD14 (11). E:sCD14, but not E:MD-2, facilitates deacylation of E by acyl oxidase (AOAH) (Gioannini et al. unpubl. observations). AOAH deacylates secondary fatty acids from E, transforming potent hexaacylated TLR4 agonists into tetraacylated antagonists after transfer from CD14 to MD-2 ((8); Gioannini et al. unpubl. observations). CD14 transfer of E to MD-2 and slower partial deacylation of E by AOAH is consistent with both the need to induce prompt TLR4-dependent mobilization of host defenses in response to invading GNB yet constrain E-induced inflammation.

### How useful are studies with underacylated endotoxins?

Theresa L. Gioannini: The fatty acid composition of lipid A, including the length, number, degree of saturation, and stereochemistry of attached fatty acids, plays a major role in determining the potency of E-elicited TLR4-dependent responses (5). The weak agonist or partial antagonist properties of underacylated E derive from the inability of

underacylated E:MD-2 to productively interact with TLR4 (6,7,12). Comparison of hexaacylated E:MD-2, potent TLR4 agonists, with underacylated E:MD-2 complexes provides a tool to better understand molecular events involved in TLR4 activation and a potential pharmacologic approach to down-regulate E-provoked inflammation.

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