SAND2014-4344P

Lipopolysaccharide subtypes differentially affect TLR4 membrane reorganization and signaling

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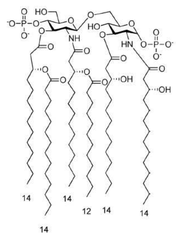
Motivation & Background

- Toll like receptors (TLRs) reside in plasma and endosomal membranes and function to recognize and respond to pathogen associated molecular patterns (PAMPs). TLR4 is responsible for immune response to bacterial lipopolysaccharide (LPS)
- Bacteria modify the Lipid A portion of their LPS to evade immune recognition. Structures for interaction between LPS and their associated immune binding partners (LPS Binding Protein, CD14, MD2 and TLR4) have been solved, and the consequences of Lipid A modifications to immune stimulation have become better understood.
- Changes in TLR4 localization within the membrane are essential to activation and signaling.
- Cutting edge microscopy techniques (TIRF: Total Internal Reflection Fluorescence and STORM: Stochastic Optical Reconstruction Microscopy) allow nanoscale resolution of cellular membrane dynamics.

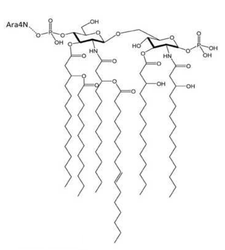
Lipid A

E. coli Lipid A stimulatory



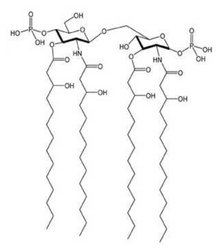
Structure = C.R.H. Raetz et al., J. Lipid Res. 2009

Y. pestis Lipid A (21C) stimulatory



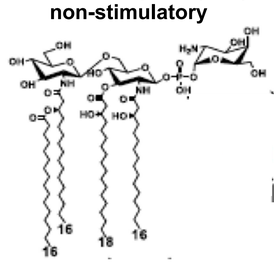
Structure = N. Maeshima et. al., Front. Cell Infect. Microbiol. 2013

Y. pestis Lipid A (37C) less stimulatory



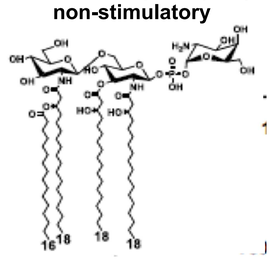
Structure = N. Maeshima et. al., Front. Cell Infect. Microbiol. 2013

F. novicida Lipid A (18C) non-stimulatory



Structure = Y. Li et.al., PNAS 2012

F. novicida Lipid A (37C) non-stimulatory



Structure = Y. Li et.al., PNAS 2012

Figure 1: Structures of different subtypes of Lipid A.

TLR4 and LPS localization

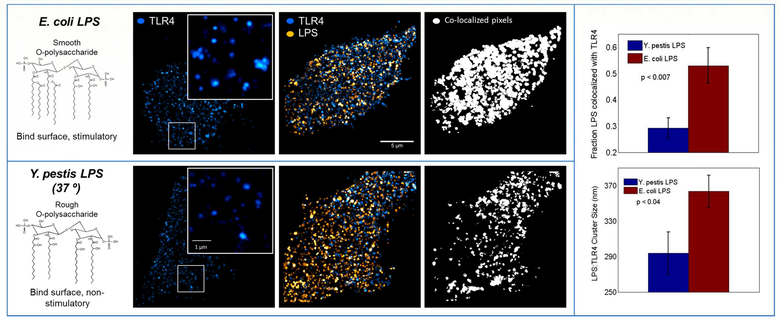


Figure 2: STORM imaging of nanoscale organization of TLR4 and LPS within mouse macrophage (P388D1) plasma membranes. Differential responses in TLR4-LPS colocalization and cluster size are observed when cells are exposed to LPS from *E. coli* (top row, left three panels) versus from *Y. pestis* grown at 37C (bottom row, left three panels). The differential responses are quantified using a Ripley's K-function algorithm (right panel).

Labeling of internalized TLR4

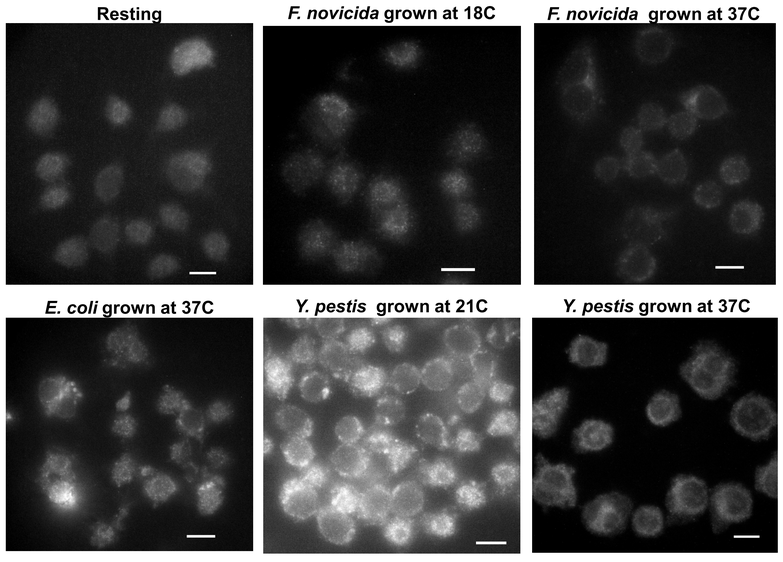


Figure 3: RAW264.7 macrophage-like cells were incubated with different subtypes of Lipid A for 30 min at 37C, 5% CO_2. Cells were then chilled and treated with a permeabilization agent and Alexa633 labeled α-TLR4 for 10 min on ice followed by paraformaldehyde fixation. Widefield images were adjusted to the same brightness and contrast scale for direct comparison of TLR4 label. Scale bars are 10 μm.

Tracking TLR4 on live cells

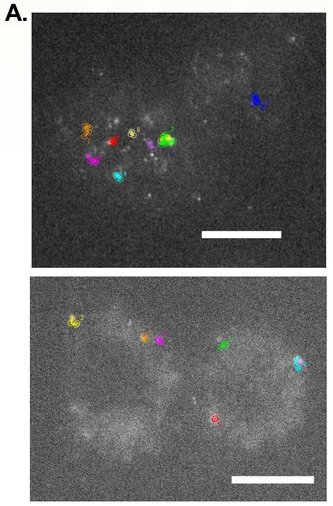


Figure 4: A) TLR4 movement (colored tracks) manually plotted with ImageJ MTrackJ (www.imagescience.org/meijering/software/**mtrackj**/) **from a 30 second image series taken of live RAW264.7 macrophages labeled with cleaved Alexa488 α-TLR4. Top panel: resting cells, Middle panel: 10 min after stimulation with 100ng/mL *E. coli* LPS, Bottom panel: 20 min after stimulation with *E. coli* LPS. Scale bars are 10 μm.**

B) Pictorial representation of cleaved α-TLR4 used for live cell tracking binding to TLR4-MD2-LPS complex. Use of the cleaved antibody allows for visualization of receptor without resulting in receptor crosslinking in the absence of LPS.

Summary & Future Directions

- **Development of a fluorescently tagged, cleaved α-TLR4 antibody allows for live cell imaging as well as characterization of TLR4 internalization.**
- **Internalization studies will measure internalization of TLR4 over a time course in response to addition of different subtypes of LipidA/LPS**
- **Single particle tracking and dSTORM will be performed on live cells to characterize dynamics of human or mouse TLR4 in response to different subtypes of LipidA/LPS**
- **Fluorescently labeled LipidA analogs will be used in conjunction with the cleaved α-TLR4 probe to elucidate interactions between Lipid A and TLR4 within the macrophage membrane.**

National Nuclear Security Administration

U.S. DEPARTMENT OF ENERGY

This work was supported by the Laboratory Directed Research and Development (LDRD) program at Sandia National Laboratories, the U.S. Department of Homeland Security, and the National Institutes of Health through the NIH Director's New Innovator Award Program, 1-DP2-OD006673-01.
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

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