STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Identifying Regulators of Monocyte Phagocytosis

Background: Monocytes are key cells that regulate innate immune responses, and are capable of a phagocytosis, which can be enhanced or impaired in response to acute and chronic infection, and allergic stimulation.[1] For example, IgE-mediated stimulation of monocytes inhibits bacterial phagocytosis, however the mechanisms involved are unknown.[2] We recently identified a molecule that regulates IgE-mediated phenotypes in monocytes, the SH-2 domain containing inositol-phosphatase, SHIP-1.[3] SHIP-1 has also been shown to regulate macrophage phagocytosis.[4] In order to better understand phagocytic mechanisms, modalities have been developed for no-lyse protocols to detect phagocytosis.[5] Using fluorescent bioparticles derived from inactivated, unopsonized *Staphylococcus aureus,* increased fluorescence of acid-activated fluorogenic dye can be observed. We utilized this methodology to investigate how various stimuli, including inhibition of SHIP-1, impacted phagocytosis after *Staphylococcus aureus* exposure.

Hypothesis: We hypothesized that IgE-mediated regulation of SHIP-1 regulates monocyte phagocytosis.

Objectives:

- Develop a flow-cytometry based assay to measure phagocytosis of *S. aureus* in human monocytes.
- Determine how various stimuli, including IgE-mediated stimulation and SHIP-1 inhibition, affect *S. aureus* phagocytosis by human monocytes.

Methods:
We utilized flow cytometry to measure the activity of pHrodo™ Green *S. aureus* BioParticles™, an inactivated, unopsonized *S. aureus* with increased fluorescence in low-pH conditions, as a methodology to detect phagocytotic activity. The THP-1 monocyte cell line or primary human monocytes were treated with *S.aureus* bioparticles, incubated at 37^{o}C, fixed, and analyzed by flow cytometry analysis. We first performed a time-course at various concentrations. To measure the role of SHIP-1, cells were pre-treated with the SHIP-1 inhibitor, 3AC at 10uM for 1h. To determine effects of cellular differentiation, THP-1 cells were treated for 3 days with either phorbol myristate acetate (PMA) and macrophage colony-stimulating factor (M-CSF), followed by phagocytosis assay. THP-1 cells were also stimulated with R848 (Resiquimod), IL-10, and 2'3'-cyclic GMP-AMP (cGAMP) for 6 hours to simulate cellular activation. Primary human monocytes were purified from human PBMCs and then treated with IgE-crosslinking (anti-human IgE) antibody, media, or IgG isotype controls. Fluorescence measured by flow cytometry and data analyzed by FCS Express. Fold change in mean fluorescence intensity (MFI) and % positive cells were calculated and compared using one-sample t-tests in Excel.

Results:
Fold change MFI of *S.aureus* bioparticle treated THP-1 cells was determined to be most sensitive at a 1:20 dilution (1.58±.13) as compared to a 1:10 dilution (1.11±.22) and 1:5 dilution (1.22±0.19). A time course experiment of THP-1 cells exposed to a 1:20 dilution of *S.aureus* bioparticles indicated a maximal fluorescence at the 90-120 minutes time range, with a percent over a 2000 gated MFI of 8.32% vs. 4.76% nonincubated. SHIP-1 inhibition with 3AC at 6 hours indicated an increased 0-hour binding of *S.aureus* to THP-1 cells ($p=0.0043$), however did not display statistically significant increased phagocytosis via fold change after two hours compared to untreated ($p=0.463$). M-CSF differentiation of THP-1 cells similarly showed increased baseline (0h) binding of 10.02% (+M-CSF) compared to 7.79% binding

(undifferentiated) with exposure to SHIP-1 inhibition, however, did not result in increased phagocytosis (13.56% vs. 10.02%). PMA differentiation also resulted in iSHIP-1 enhanced baseline bacterial fluorescence (8.71% for iSHIP-1 treated vs 7.60% untreated). No significant difference in phagocytosis of *S.aureus* bioparticles in THP-1 cells were observed following 6-hour stimulation with cGAMP (1.62±0.22, p=0.503), IL-10 (1.87±0.05, p=0.249), and R848 (1.83±0.10, p=0.521) compared to a media control (1.75±0.09). In preliminary data using primary human monocytes, IgE-mediated stimulation showed possible enhancement of phagocytosis of *S.aureus* bioparticles.

Conclusion:

We optimized a protocol for investigating phagocytosis in human monocytes that can be controlled in a dose-and-time dependent manner. Using this functional assay of gram positive phagocytosis (*S.aureus*), we did not identify significant changes in phagocytosis with a variety of cellular stimuli. However, we did observe increased baseline (0-hour) binding of *S.aureus* bioparticles on the cell surface in the setting of SHIP-1 inhibition, suggesting that SHIP-1 may regulate surface binding of gram positive bacteria. Future studies will further investigate whether SHIP-1 plays a role in IgE-mediated regulation of bacterial binding and phagocytosis.

References:

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