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Quantifying effects of neutrophil memory on migration patterns using microfluidic platforms and ODE modeling of the mechanistic molecular pathways

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During sepsis, the current leading cause of death in hospitals, neutrophils migrate and accumulate in healthy organs instead of migrating toward the infection. Previous work from us described a dysfunctional phenotype, including oscillatory and spontaneous migration, in neutrophils isolated from septic burn patients [1]. In this study, we present a microfluidic platform to measure neutrophil chemotaxis in an opposing chemoattractant gradient to quantify neutrophil decision-making, with single-cell resolution. We use two chemoattractants: a pro-resolution (fMLP) and pro-inflammatory (LTB_4) chemoattractant to model how a neutrophil makes a decision to move toward a bacterial infection versus an inflammatory signal. Our hypothesis, is that pro-inflammatory programming or training signals, such as lipopolysaccharide, have a central role in determining the final neutrophil phenotype and in the development of sepsis. Furthermore, the concentration of the LPS exposure is critical in determining the impending neutrophil phenotype. Leukocyte memory in relation to primary low-levels of LPS may lead to a dysfunctional immune response to a secondary bacterial infection. On the other hand, exposure to high-levels of LPS lead to insufficient and dysfunctional migration and neutrophils are unable to contain infection. Despite tremendous advances in the understanding of signaling molecules and pathways acting inside neutrophils, our understanding of the directional decision-making process of neutrophils is limited, and consequently, our abilities to modulate the activity of neutrophils restricted.

In our microfluidic platform, opposing linear chemoattractant gradients are formed along migration channels, and neutrophils (HL-60s) in the central loading chamber must make the decision to migrate toward a preferred chemoattractant. Time-lapse images were taken every 2 min. and cell mazes were incorporated to measure directional migration and capture oscillatory behavior of the cells. To test the importance of leukocyte memory, we stimulate the cells overnight with a pro-inflammatory mediator (Lipopolysaccharide (LPS)) at both high (100 ng/mL) and low (1ng/mL) doses to determine if LPS plays a role in neutrophil decision-making. We show that unstimulated cells migrate toward fMLP over LTB4 in a 2:1 ratio. Cells stimulated with high-dose LPS show migration in a similar ratio, but with a higher total percentage of migrating cells. Surprisingly, cells stimulated with a low dose of LPS migrate toward fMLP and LTB4 in a 1:1 ratio, showing an increase in migration toward LTB_4, as well as higher migration. We see higher velocity toward LTB_4 and lower velocity toward fMLP compared to unstimulated cells. This study suggests that low-dose LPS stimulation can alter the decision-making properties of the neutrophil to migrate toward an inflammatory signal over a bacterial infection.

To understand the molecular mechanism of this cell memory, we combined our wet-bench work with an ODE-based dynamical framework to model the interaction of the mutually inhibitory GRK2 and GRK5 proteins and its role in neutrophil decision-making. GRK2 and GRK5 were of interest due to importance as drug targets as well as their interactions with the chemoattractant receptors and LPS. Our computational model results show a bimodal switch between high and low levels of GRK2. We are currently using open microfluidics to extract neutrophils on-chip after migration, to measure receptor levels to determine the underlying molecular mechanism and determine biologically accurate parameter values for our computational model. In the future, this platform can be used for early diagnosis of sepsis or to test the effect of pro-resolving mediators on neutrophil function.

Reference: [1] Jones CN, PLoS One, 2014, 9(12), e114509