

Poster #5**Laura Whitmore****PhD Candidate, College of Medicine
Pediatrics****Title of Research:** A Common SNP Mediates Human Neutrophil Priming Responses to a TLR2/1 Agonist**Other Authors:** Jessica Hook Amanda Philip Brieanna Hilkin Xinyu Bing Kelli Harmon Hector Wong Polly Ferguson Jessica G. Moreland**Introduction/Purpose:**

Neutrophils, critical mediators of the innate immune response, recognize pathogens through pattern recognition receptors including Toll-like receptors (TLRs). Our laboratory has previously demonstrated that endotoxin, a TLR4 agonist, activates neutrophil signaling pathways resulting in reactive oxygen species (ROS) generation, MAPK phosphorylation, and granule mobilization. These cellular events characterize priming, an intermediate state of neutrophil activation. Primed neutrophils have enhanced responsiveness to subsequent stimuli, which can be beneficial in eliminating microbes but can cause host tissue damage in some disease contexts including sepsis. While neutrophil priming by TLR4 agonists is well-described, there is limited data on neutrophil priming by TLR2 agonists. TLR2 forms heterodimers with TLR1 or TLR6 to recognize a diverse spectrum of ligands. We hypothesized that ligation of TLR2/1 or TLR2/6 with their respective agonists would prime neutrophils.

Experimental Design:

Freshly isolated human neutrophils were treated with FSL-1, a TLR2/6 agonist, or Pam3CSK4, a TLR2/1 agonist, and evaluated for priming responses including generation of intracellular and extracellular ROS, MAPK phosphorylation, integrin activation, secondary granule exocytosis, and cytokine secretion. Donors were also genotyped for two common SNPs in TLR1.

Results:

Surprisingly, we found that neutrophils from all donors were primed by the TLR2/6 agonist, FSL-1, but that neutrophils from only ~50% of donors were primed in response to the TLR2/1 agonist, Pam3CSK4. Genotyping studies revealed that donors whose cells were primed by Pam3CSK4 had a common SNP located in exon 4 of TLR1 that was absent in the donors whose cells were not primed. Notably, neutrophils from donors with the SNP had higher levels of TLR1 expressed on their surface.

Conclusions:

Based on these data, we conclude that neutrophil priming in response to TLR2/1 agonists is donor-dependent, whereas TLR2/6 agonists prime neutrophils from all donors. We are currently investigating whether this SNP affects the outcome of pediatric patients with sepsis.