Label-Free Optical Metabolic Imaging of Neutrophils in Infection

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Abstract

Neutrophils are the most abundant leukocytes in human peripheral circulation. They are part of the innate immune system and are the first to arrive at sites of infection and inflammation. Neutrophils in circulation are quiescent, and their activation is a critical component of a robust immune response. Upon activation, neutrophils combat infiltrating pathogens through a variety of effector functions, including oxidative burst, phagocytosis, and neutrophil extracellular traps (NETs). These processes necessitate energy consumption, rendering cellular metabolism essential for neutrophil function. This work aims to study the metabolic reprogramming in neutrophils essential for performing their function and combating infection. In this study, human neutrophil metabolism is investigated using two-photon microscopy of intrinsic fluorophores reduced nicotinamide adenine dinucleotide (phosphate), NAD(P)H, and flavin adenine dinucleotide, FAD. We measure both the optical redox ratio (ORR), which is defined as NAD(P)H / (NAD(P)H + FAD) and perform fluorescence lifetime imaging microscopy (FLIM) of NAD(P)H. Single-cell metabolic profiles of neutrophils were generated using an automatic cell segmentation pipeline. We found that pharmacological activation of neutrophils shifted the metabolism towards glycolysis and Pentose Phosphate Pathway. Furthermore, pathogen infection with *Pseudomonas aeruginosa*, *Toxoplasma gondii,* or *Cryptosporidium parvum* induced a significant metabolic change and displayed single cell heterogeneity. As a step towards clinical translation, integration with an autofluorescence lifetime-based flow cytometry system, was demonstrated, which could enable rapid and high-throughput assessment of neutrophil metabolism and heterogeneity in infectious diseases.