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Et al.

Characterization of neutrophils and macrophages from *ex vivo* cultured murine bone marrow for morphologic maturation and functional responses by imaging flow cytometry

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ABSTRACT
Neutrophils and macrophages differentiate from common myeloid progenitors in the bone marrow, where they undergo unique nuclear morphologic changes as they mature into fully functional phagocytes. These changes include condensation of chromatin, the most pronounced exhibited by mature neutrophils. Both myeloid cells acquire multiple functions critical to their ability to kill pathogens, including phagocytosis, the production of proteolytic enzymes and reactive oxygen species (ROS), and in the case of neutrophils, release of nuclear material known as nuclear extracellular traps (NETs). Studies on these functions often rely on the use of cells acquired from mature mouse tissues, but these tend to produce limited numbers of cells. Strategies to analyze the morphologic features and functional responses of these cells include the use of conventional brightfield or fluorescence microscopy to examine changes in nuclear structure, internalization of fluorescein-labeled bacterial or yeast particles, or release of nuclear material. Flow cytometry also is often used, especially for identifying changes in the expression of lineage-specific cell surface markers and ROS production. However, each of these techniques presents certain limitations. Here we describe methods to generate abundant populations highly enriched for neutrophils or macrophages from previously frozen, *ex vivo* cultured mouse bone marrow. We then apply state-of-the-art imaging flow cytometry, which combines the resolution of microscopy with the speed of flow cytometry, to analyze each lineage for changes in nuclear structure and expression of key cell surface markers. Different gating and masking strategies are applied to characterize phagocytosis of pH-dependent fluorescein-labeled *E. coli*, ROS production, and NET release by neutrophils. We also demonstrate that neutrophils engulfing *E. coli* bioparticles produce NETs in a process we term PhagoNETosis. Together these assays reveal the power of imaging flow cytometry for simultaneously assessing the maturation features and functional responses of these critical mediators of innate immunity.

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