[2218] Differential Requirement for Cell Division in the Development of Terminally Differentiated Neutrophils and Macrophages from Their Common Progenitors. Session Type: Poster Session 422-II

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Neutrophils and monocytes are the main population of peripheral blood nucleated cells and progenies of common progenitor cells, CFU-GM, that are derived from hematopoietic stem cells. Monocytes are non-dividing immediate precursors for macrophages. Common clinical observation that monocytes more rapidly recover than do neutrophils in myelosuppressive patients indicates the difference in regulatory mechanism of terminal differentiation between neutrophils and macrophages. In this study, we investigated the cell division profiles in the differentiation pathway from common progenitors for neutrophils and macrophages to respective post-mitotic cells in culture. We prepared lineage-negative (Lin^-) bone marrow cells from normal C57BL/6 mice by using immunomagnetic beads. The Lin^- cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), a cytoplasmic dye that is equally diluted between daughter cells, and stained with anti-Sca-1 antibody and lineage antibodies. $CFSE^{high}Sca\text{-}1^+Lin^-$ cells were sorted on a FACSVantage or FACSAria. When the $CFSE^{high}Sca\text{-}1^+Lin^-$ cells were cultured in the presence of steel factor (SF) and IL-11 for 5 days, the resulting cells could be separated into four populations based on staining with anti-fms and anti-Gr-1 antibodies: $fms^-Gr\text{-}1^-$, $fms^+Gr\text{-}1^-$, $fms^-Gr\text{-}1^+$, and $fms^+Gr\text{-}1^+$ cells. The $fms^+Gr\text{-}1^+$ cells showed morphological features typical of macrophages. The commitment of $fms^-Gr\text{-}1^+$ cells into neutrophilic lineage was evident, although they exhibited an immature morphology. CFSE labeling pattern in $fms^+Gr\text{-}1^+$ macrophages was equal to that of $fms^-Gr\text{-}1^+$ neutrophilic cells, indicating that $fms^+Gr\text{-}1^+$ macrophages and $fms^-Gr\text{-}1^+$ neutrophilic cells passed through similar successive rounds of cell division in culture of $CFSE^{high}Sca\text{-}1^+Lin^-$ cells. These $fms^+Gr\text{-}1^+$ macrophages and $fms^-Gr\text{-}1^+$ neutrophilic cells were subsequently cultured for additional 3 days in the presence of SF, IL-11, IL-3, GM-CSF, G-CSF, M-CSF, erythropoietin, and thrombopoietin. While a decline in the number of $fms^+Gr\text{-}1^+$ macrophages was observed, the number of $fms^-Gr\text{-}1^+$ neutrophilic cells increased approximately 5-fold during subsequent culture. The proliferation of $fms^-Gr\text{-}1^+$ neutrophilic cells and no cell division of $fms^+Gr\text{-}1^+$ macrophages were confirmed by Ki-67 antibody staining, BrdU incorporation, and DNA staining. The cultured $fms^-Gr\text{-}1^+$ neutrophilic cells also predominantly acquired a mature morphology. These results suggest distinct cell division histories between terminally differentiated neutrophils and macrophages, possibly explaining the early appearance of monocytes in the blood after myeloablative therapy, as compared to neutrophils.

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