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Clear resolution of different hsc and mk-p bm populations and their subsets in normal and disease states enabled by high definition flow cytometry

Varda R Deutsch[1], Sigi Kay[2], Shoshana Baron[2] and Elizabeth Naparstek[1]

[1]Dept. of Hematology, Tel Aviv Medical Center, Israel
[2]Hematology Institute, Tel Aviv Medical Center, Israel

Thrombopoiesis, which is regulated by physiological demand, requires several developmental steps beginning with hematopoietic stem cell (HSC) commitment to Mk progenitors (MK-P) followed by proliferation, terminal Mk maturation and platelet production. While each step in this complex pathway is crucial for platelet production, the Mk-p pool provides the platform for platelet development. Studying Mk-p populations under normal and disease states may provide new insights into the molecular and cellular mechanisms that regulate normal and aberrant thrombopoiesis. The aim of the study was to assess the utility of high definition flow cytometry to provide a high resolution tool for detecting HSC and MK-p and their subpopulations in normal and thrombopoietic disease states. The populations studied were from normal bone marrow-BM, cord blood-CB, mobilized peripheral blood, and BM from Immune Thrombocytopenia Purpura-ITP, chronic myeloid leukemia-CML and essential thrombocythemia-ET. Studying Mk-p populations under different physiological conditions and in disease states has now provided us with new information regarding the populations that contribute to thrombopoiesis. We demonstrate for the first time that the relative proportion of Mk-p and early Mk-p which still maintain CD34 and CD34+ HSPC that have acquired high levels of CD41 are not the same under different physiological conditions. $CD34^+$ and $CD34^+/CD41^+$ cells were increased in PBSC and decreased in CB, correlating with the known shorter platelet nadir in patients transplanted with PBSC and the prolonged thrombocytopenia following CB transplant. In CML and ET, where platelet counts are higher than normal, $CD34^+/CD41^+$ cells were reduced, implying forced maturation of MK-p and facilitated thrombopoiesis. No significant differences were found in the proportion of CD41+ megakaryocyte progenitors ($CD41+/SSC^{low/CD45dim/neg}$) in PBSC and CB compared to normal BM, 0.2%, when analyzed by our recently established method (1). The proportion of Mk-p was 0.2% in ITP, 0.1% in CML with a ten fold increase observed in ET. This population newly defined MK-p population from normal BM and ET was sorted and cloned in full cytokine methocult with the addition of thrombopoietin for CFU-meg and results confirmed these findings. Further analysis of the progenitors in normal BM and PBSC resolved that 1-2% of the MK-p remained early progenitors and maintained CD34. In CB the CD41+ Mk-p population contains no detectable CD34+ cells, once again pointing to lower numbers of transplantable early Mk-p in CB. We further resolved the subpopulations of Mk-p that expressed CD34 in ITP, CML and ET. In ITP and CML a higher proportion of early Mk-p that maintained CD34+ were noted. In ET which is characterized by MK maturation and increase platelet production, the BM contains a relatively larger proportion of Mk-p (which were cloneable), than normal BM (1% vs 0.2%). However, these cells contain only low numbers of CD34+ cells implying accelerated maturation and loss of CD34 on their surface. This notion is supported by the reduced CD34+ CD41+ HSPC mentioned above. Not surprisingly, most normal BM Mk-p are CD33+ (76%). However, this is not the case in PBSC (16%) and CB Mk-p which express lower levels of CD33 (3%). ITP and CML Mk-p also express lower levels of CD33. This study which utilizes the power of high resolution flow cytometry and sorting demonstrates for the first time that the relative proportion of Mk-p and early Mk-p differ under different physiological conditions. Discovering the markers of HSC and Mk-p subsets that vary in normal and disease states may contribute to our understanding of normal and aberrant thrombopoiesis.