Nicotinamide, a Specific Inhibitor of NAD(+)-Dependent ADP-ribosyl Transferases Promotes Ex-Vivo Expansion of Early Subsets of Hemopoietic CD34+ Cells

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Ex vivo amplification of hematopoietic early progenitors without loss of self-renewing potential represents an important target for transplantation, cellular and gene therapies.

ADP-ribosylation is an epigenetic protein modification frequently implicated in chromatin remodeling and gene silencing. Nicotinamide (NA) is a specific inhibitor of NAD(+) depended ADP-ribosyltransferases and was also suggested as the physiological inhibitor of class-III histone deacetylases (Sir2). In the present study we demonstrated that three-week treatment of cord-blood-derived CD34+ cells with NA (5-mM) and early-acting cytokines resulted in substantial enrichment of the CD34+ cell compartment with cell subsets displaying phenotypes of early progenitors, CD34+CD38- and CD34+Lin-CD38-, (48±3.7%, n=9 and 18.6±3%, n=8, $p<0.0001$, respectively) compared to control cultures (2.8±0.7%, n=9 and 0.7±0.06%, n=8, respectively). This resulted in a markedly enhanced extended-long-term expansion potential of CFC and CD34+ cells over control cultures. When CD34 cells were cultured for three weeks in the presence of early-acting cytokines and the late-acting IL-3, differentiation was accelerated and the cells exhibited reduced engraftment in SCID mice (6 out of 13 mice engrafted). Addition of NA inhibited in vitro differentiation and enhanced in vivo engraftment (13 out of 13 mice engrafted). Tracking of the cell-cycle history of cultured cells by PKH2 staining demonstrated that NA slow-down the proliferation rate of all cultured cells. This effect was more incredible within the early progenitor cell compartment. After 7 days the median fluorescence intensity of CD34+CD38- cells was two-fold higher, indicating fewer division cycles, in NA-treated than in control cultures; yet, their number was 1.5-3-fold higher than in control. Phenotype reversion of sorted CD34+CD38+ cells toward the more primitive phenotype, CD34+CD38-Lin-, was demonstrated to occur in the

presence of NA, however at relatively low frequency. Therefore, inhibition of differentiation followed by self-replication may mainly support NA mechanism leading in accumulation of early progenitor cells following ex vivo expansion.