725] Nicotinamide Modulates Ex-Vivo Expansion of Cord Blood Derived CD34+ Cells Cultured with Cytokines and Promotes Their Homing and Engraftment in SCID Mice.
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Nicotinamide (NA) is a non-competitive inhibitor of NAD(+)-dependent ADP-ribosyl transferases, of CD38 NADase (a major regulator of cellular NAD levels) and of Sir2 histone-deacetylase. These enzymes are playing a pivotal role in regulation of signal transduction pathways and gene expression. In the present study, we evaluated the effect of NA on the ex-vivo expansion of cord-blood (CB) derived CD34+ cells and their bone-marrow (BM) homing and engraftment potential.

Culturing of CD34+ cells for three weeks in the presence of cytokines (SCF, TPO, IL-6, FLT3-ligand) only or cytokines + NA (5mM) resulted in similar expansion of CD34+ cells (40-fold relative to input). However, a remarkable increase in the fraction of CD34+ cells displaying an early progenitor cell phenotype (CD34+Lin-) was observed in the NA-treated cultures as compared with cytokines-only treated cultures (18.6±3% and 0.7±0.06%, n=6, $p<0.05$, respectively). Tracking the cell-cycle history by PKH2 staining showed fewer division cycles of CD34+ cells cultured with NA. These results may suggest a direct correlation between the rate of proliferation and expansion of CD34+Lin- cells.

NA-treated CD34+ cells express similar levels of CXCR4 but display increased migratory activity in response to CXCL12 over CD34+ cells treated with cytokines only (36±19% and 11±4%, n=4, $p<0.05$, respectively).

In order to test their homing potential, similar number of mononuclear cells (MNC), before or following expansion with or without NA, were labeled with CFSE and transplanted into irradiated NOD/SCID mice. Twenty-four hours later the numbers of human cells (CD45+CFSE+) and human progenitor cells (CD34+CFSE+) in the BM were counted. Homing of CD45+CFSE+ cells was comparable in the three groups tested. However, CD34+CFSE+ cells with BM homing potential were 3-fold more numerous in NA-treated cultures relative to cytokines-treated cultures, and 6-fold more than in non-cultured CB cells (n=14, $p<0.05$).

To evaluate engraftment, SCID mice were transplanted with $3x10^3$, $6x10^3$ and $12x10^3$ non-cultured CD34+ cells or their entire progeny

following 3-week expansion with cytokines only or cytokines + NA (n = 63). The frequency of SCID repopulating cells (SRC) was estimated by limiting dilution analysis as 1/ 36,756 (non-cultured), 1/19,982 (cytokines), 1/ 2,620 (NA) (SCID engraftment was considered as ≥0.5% human CD45+ cells). We found that, in correlation with homing, NA-treated cells have a 14- and 7.6-fold more SRC than non-cultured cells or cytokine-treated cells, respectively. The marked increase in SCID engraftment potential following culturing with NA may be attributed to both improved homing of CD34+ cells as well as higher proportion of early progenitor cells within the CD34+ cell compartment.
Despite their numerical expansion, progenitor cells generated in cytokine-supplemented cultures have reduced homing and engraftment capacity. Our study demonstrates that NA modulates in-vitro expansion and augments the in-vivo homing and engraftment of CB-derived CD34+ cells cultured with cytokines.
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