

[3652] Antagonists to Retinoid Receptors Down-Regulate CD38 Expression and Inhibit In Vitro Differentiation of Cord Blood Derived CD34+ Cells. Session Type: Poster Session, Board #881-III, ASH December 11, 2006

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CD38, originally described as a differentiation marker, has emerged as an important multifunctional protein. Its most well-characterized function is the ability to catalyze the synthesis of cyclic ADP-ribose (cADPR) from NAD. However, its major enzymatic activity is the hydrolysis of NAD (NADase) implicating it as the major regulator of cellular NAD levels. CD38 expression increases with commitment and differentiation. It is not clear, however, whether such changes in CD38 are merely phenotypic, or reflect an active role for CD38 in the regulation of cell differentiation. The regulation of CD38 gene expression is under the direct control of retinoid receptors (RAR). Antagonists to RAR abolish up-regulation of CD38 gene expression as well as RA induction of granulocytic differentiation down-stream of the myeloid compartment.

In the present study we evaluated the involvement of CD38 in the regulation of HPC differentiation by treatment of ex-vivo cultures with LMW antagonists, targeted to either CD38 expression or to its biological activities.

CB derived CD34+ cells were cultured with cytokines (S,T,F,6). Treatment of these cultures with an RAR-antagonist (AGN194310) abolished the expression of surface CD38. After 3 weeks in culture, the content of CFC was 3 ± 1.1 -fold higher, the content of CD34+ cells was 2.4 ± 0.24 -fold higher and percentage CD34+ cells displaying CD34+Lin- phenotype was by 35 ± 10 -fold higher ($p < 0.05$, $n = 14$) in RAR-antagonist (10^{-6} M) compared to cytokines-only treated cultures. Colonies derived from RAR-antagonist treated cultures sustained high re-plating capacity, a property that was lost during the first 3-weeks of expansion with cytokines only. In long-term cultures, the peak of CFUc and CD34+ cell expansion of RAR-antagonist treated cultures was 6-10 weeks later than control cultures. At the peak of expansion, cumulative numbers of CD34+ and CFUc were by 130- and 512-fold higher ($p < 0.05$, $n = 4$), respectively, in treated than in control cultures. CFU-MIX colonies were exclusively observed in RAR-antagonist treated cultures (between weeks 7-10). Interestingly, limited (1 week) exposure to the RAR-antagonist was sufficient for this long-term effect. Similarly, we tested the effect of an RXR antagonist (LGN 100754) (10^{-9} - 10^{-5} M) on short- and long-term cultures. Treatment with the RXR-antagonist did not down-regulate CD38 expression and only slightly improved ex-vivo expansion parameters over cytokines-only treated cultures.

We next evaluated whether inhibition of CD38 enzymatic activities will also modulate in-vitro differentiation of cultured cells. To this end, CD34+ cell cultures were treated with nicotinamide (NA), a non-competitive inhibitor of CD38 NADase, previously demonstrated to abolish its enzymatic activities. 3-week treatment with NA (5mM) resulted in a marked decrease in CD38 expression and a marked increase in the fraction of CD34+Lin- cells as compared to cytokines-only treated cultures ($48.0 \pm 3.7\%$ vs. $2.8 \pm 0.7\%$ and $18.6 \pm 3\%$ vs. $0.7 \pm 0.06\%$, $n = 6$, $p < 0.05$, respectively). As with the RAR-antagonist, long-term expansion potential, as determined by CFC and CD34+ cell content, was significantly higher in cultures treated with NA relative to cytokines-only treated cultures.

These results demonstrate that both a pan-RAR antagonist and NA inhibit differentiation and promote ex-vivo expansion of progenitor cells, suggesting the possible involvement of CD38 protein in these processes.

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