Title: Proinflammatory Mediators as Determinants of Erythroid Differentiation

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Background: Anemia is a multifactorial condition that affects >10% of the elderly population and is a predictor of morbidity and frailty in the elderly. Inflammatory cytokines, particularly IL-6, TNF alpha, and MIF are up-regulated in the elderly and may influence the development of the anemia of inflammation/chronic disease (AI) and unexplained anemia. We have established semi-solid and liquid-based culture systems in which to 1) promote erythroid differentiation of CD34+ hematopoietic progenitors, 2) test the ability of TNF, IL-6 and MIF to suppress erythroid differentiation, and 3) determine whether pharmacologic doses of dexamethasone, lenalidomide, Remicade, and high-dose erythropoietin (Epo) overcome inflammatory-mediated colony suppression. The goal is to establish a culture system to evaluate the effect of inflammatory cytokines on human erythropoiesis that would lend itself to high-throughput drug library screens and identification of novel therapeutic compounds with which to treat AI in the elderly.

Specific Aim: 1) Establish parameters necessary for erythroid differentiation of human CD34+ progenitors in methyl-cellulose and liquid culture systems, 2) assay effects of TNF alpha, IL-6 and MIF on differentiation, 3) assess ability of known drugs to overcome such inhibition.

Hypothesis: Overexpression of IL-6, TNF alpha and MIF will directly suppress erythroid differentiation. This effect may be reversed by the addition of known pharmacologic agents.

Methods: Peripheral blood human CD34+ progenitors from healthy adult donors were grown in methyl-cellulose-based media containing FBS, IL-3, Epo and SCF. Erythroid colony morphology, numbers of erythroid blast-forming units (BFU-Es), and hemoglobin expression were assessed at Days 7 and 14 in the presence and absence of IL-6, TNF or MIF. In liquid cultures, CD34+ progenitors were grown in serum-free media supplemented with IL-6, TNF, or MIF on Days 0 or 4. Cell numbers and surface expression of erythroid differentiation markers (CD36, CD45, CD71, and CD235a/GPA) were determined at Days 7 and 14 by FACS analysis.

Results: In the presence of 10ng/mL IL-3, 1unit/mL Epo, and 50ng/mL SCF, 86.3 BFU-E’s were identified per 1000 CD34+ cells. Colonies stained positively for benzidine and 49.5% of cells expressed CD71 and 45% GPA. In the presence of 25 and 100ng/mL TNF alpha, the numbers of BFU-E’s were reduced 22% and 28% respectively. In liquid cultures, GPA expression was down-regulated by 50%. The addition of lenalidomide (1-10uM) or dexamethasone failed to rescue erythroid expansion in the presence of TNF. Addition of 500ng/mL IL-6 resulted in a significant decrease of 18% in numbers of BFU-E’s but addition of 100ng/mL MIF had no effect. The effect of IL-6 and MIF on CD71 and GPA expression was not significant.

Conclusions: IL-3, Epo and SCF are sufficient to promote erythroid differentiation of peripheral blood CD34+ progenitor cells. TNF alpha directly suppresses erythroid expansion in both semi-solid media and liquid media. The results for high-dose IL-6 are mixed between different culture systems. These findings suggest that TNF may act directly on erythroid precursors, whereas the effects of IL-6 may be mediated through other cells (e.g. macrophages and stromal cells) or via signaling pathways (e.g. hepcidin). Lenalidomide and dexamethasone were unable to overcome TNF or IL-6-mediated colony suppression, a finding which corroborates their limited utility in treating AI. Gene expression analysis of erythroid progenitors in the presence and absence of TNF and Remicade is presently underway. These gene signatures will be used to interrogate a small molecule library screen to identify novel compounds that may reverse the suppressive effects of TNF on erythropoiesis.