ERYTHROPOIETIN PROTECTS CELLS FROM OXIDATIVE STRESS BY REDUCING OXIDATIVE DNA DAMAGE

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ABSTRACT

Erythropoietin (EPO) was, at one time, considered only as an erythropoietic lineage-specific regulator. The production of EPO is under the influence of a complex molecular signal detection system influenced by the partial oxygen pressure. More recently, EPO has been shown to exhibit multifunctional properties and its receptor has been found not only on erythropoietic cells, but also on cells from many different tissues and organs. Erythropoietin is a proliferation factor for primitive erythropoietic cells, but a differentiation/survival factor for late erythroid cells due to its anti-apoptotic function. We now demonstrate that EPO also exhibits anti-oxidative effects. Fresh, human bone marrow cells were incubated in the absence or presence of 0.1, 1 and 10U/ml human recombinant EPO. At the time cultures were prepared and after 24h incubation, the cells were incubated in the absence or presence of 1mM methylene blue (MeB), which, when illuminated with bright light, induces oxidative DNA damage (ODD) and the production of 8-oxoguanine residues. Additional controls included the absence or presence of light. After incubation, oxidative DNA damage was detected by OxyFLOW™, a flow cytometric detection system that uses a fluorescene isothiocyanate (FITC)-conjugated 8-oxoguanine – specific binding protein to measure changes in median fluorescence intensity. The results indicate that at 0h, EPO reduced ODD, but not in a dose dependent manner. In contrast, 10U/ml EPO significantly reduced ODD by more than 40% compared to cells without EPO. These results demonstrate that EPO has an anti-oxidative function by acting to reduce oxidative DNA damage. This may occur in a number ways, e.g. iron store depletion, increase in glutathione peroxidase. The net result is protection against free radicals and oxidative stress for a large number of cell types that express the EPO receptor.

Figure 1
THE ORGANIZATION AND HIERARCHY OF THE LYMPHO-HEMATOPOIETIC SYSTEM

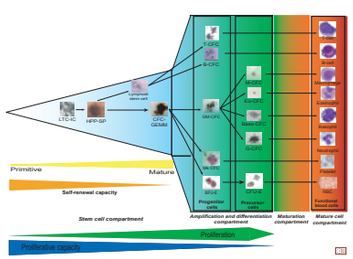
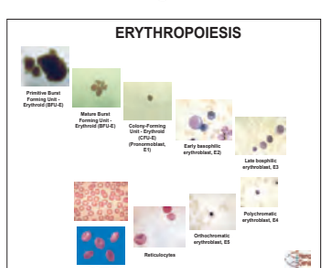


Figure 2



Methods

Figure 3.
Detecting Proliferation using HALO™-96 MeC

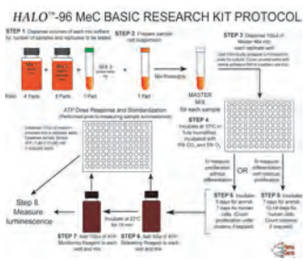


Figure 4.
Detecting Oxidative DNA Damage using OxyFLOW™

Erythropoietin Acts Bi-Functionally by Inducing Proliferation of Primitve Erythropoietic Cells, but not of Differentiating Cells
Figure 5.

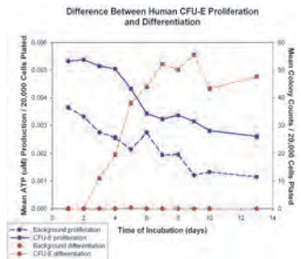


Figure 6.

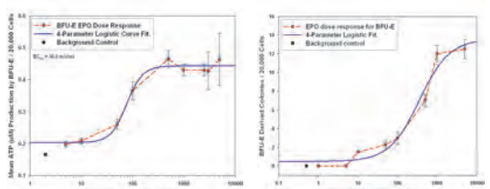
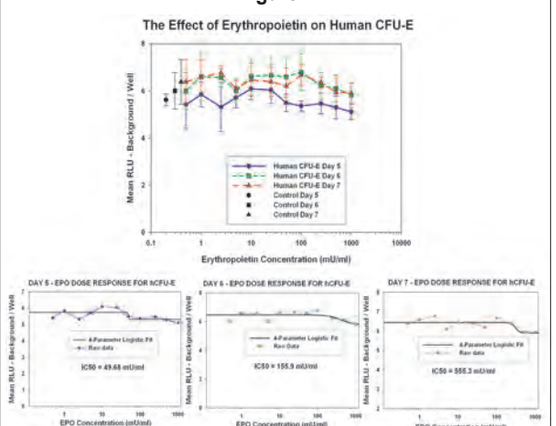


Figure 7.



Eythropoietin Inhibits Oxidative DNA Damge During Differentiation and Maturation of Human Bone Marrow Cells into Mature Red Blood Cells

Controls - Figure 8.

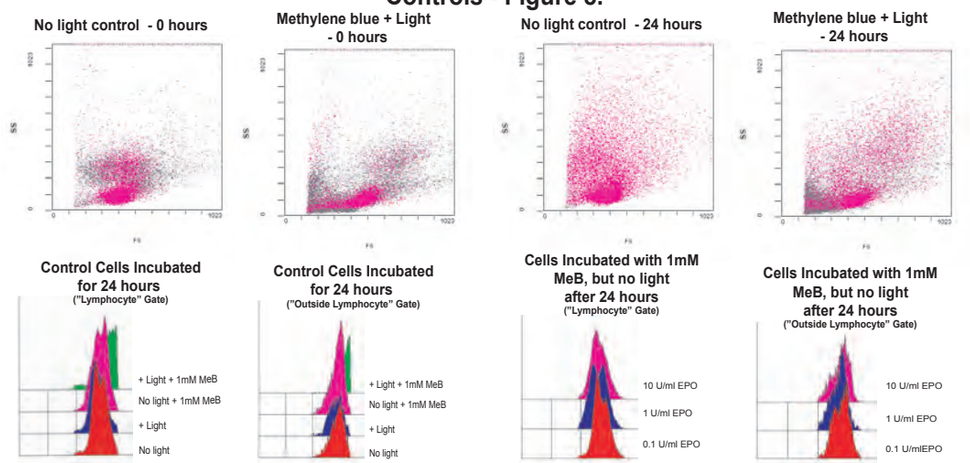
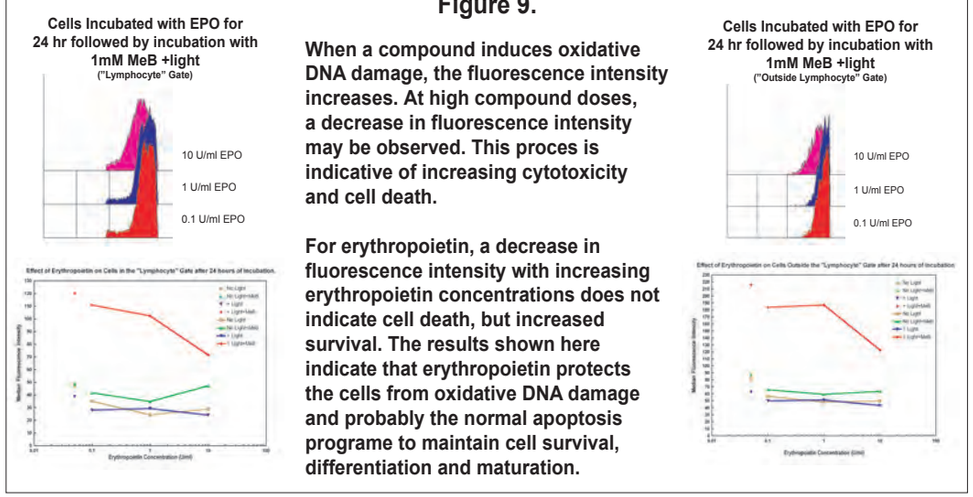


Figure 9.



When a compound induces oxidative DNA damage, the fluorescence intensity increases. At high compound doses, a decrease in fluorescence intensity may be observed. This proces is indicative of increasing cytotoxicity and cell death.

For erythropoietin, a decrease in fluorescence intensity with increasing erythropoietin concentrations does not indicate cell death, but increased survival. The results shown here indicate that erythropoietin protects the cells from oxidative DNA damage and probably the normal apoptosis programe to maintain cell survival, differentiation and maturation.