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Non-erythroid effects of erythropoietin

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Erythropoietin (EPO) regulates red blood cell production by binding to its cell surface receptor, EPO-R, expressed on erythroid progenitor cells. Although EPO was originally believed to be an erythroid-specific hematopoietic cytokine, for over a decade, a substantial body of scientific evidence has accumulated to demonstrate that the biological effects of EPO are not limited to the erythron (Figure 1). In this issue of the journal, Lifshitz and colleagues[1] report on their most recent contribution to this field of research by demonstrating that, within the hematopoietic system, EPO may exhibit modulatory effects on macrophage number and function. The authors examined *in vivo* effects of EPO on splenic macrophages and inflammatory peritoneal macrophages, as well as *in vitro* effects of EPO on bone marrow-derived macrophages in culture. The experimental data show that splenic macrophage numbers were increased in mice in response to systemic EPO treatment. In transgenic mice engineered to constitutively over-express endogenous EPO, an even more significant increase in the number of splenic macrophages was observed, possibly as part of an adaptive mechanism leading to increased erythro-phagocytosis in severely polycythemic mice.[2] Inflammatory macrophages isolated from murine peritoneum displayed enhanced activation and phagocytic function, both following exogenous EPO treatment and in association with the over-expression of endogenous EPO, but without an increase in the number of macrophages migrating into the peritoneal cavity. The *in vivo* activity of EPO observed in these studies may be associated with direct effects on macrophages, indirect effects of EPO on other cell types that modulate macrophage number and function, or a combination of direct and indirect effects. Additional experiments by the investigators using cultured murine primary bone marrow-derived macrophages revealed enhanced activation and phagocytic function of the cells following EPO treatment. These direct EPO effects were associated with increased macrophage nitric oxide and interleukin (IL)-12 secretion, whereas IL-10 production was decreased, consistent with the generation of a pro-inflammatory phenotype and classical Th1 immune response.

The investigation of non-erythroid biological effects of EPO raises the question of the role of the erythroid receptor EPO-R, which is ubiquitously expressed at relatively low levels in many non-hematopoietic tissues. Lifshitz and colleagues addressed this issue in part by demonstrating that the newly discovered effects of EPO on macrophages were associated with the expression of EPO-R mRNA in cultured murine bone marrow-derived macrophages. The investigators further demonstrated the ability of EPO to mediate the increased phosphorylation of STAT proteins, as well as the induction of AKT and ERK2 phosphorylation and the nuclear translocation of p65 NFκB in macrophages. Although the direct effects of EPO on intracellular signal transduction and the induced changes in macrophage phenotype and function are presumably mediated in part by EPO-R, further studies will be necessary to delineate the structure of the cell surface receptor that mediates the effects of EPO in macrophages. Previous studies investigating non-erythropoietic EPO activities suggested that, in some experimental models, the tissue protective activity of EPO and of some EPO derivatives without erythropoietic activity may be mediated by a heteroreceptor complex between EPO-R and the common β receptor (βC-R) – a signal-transducing component of the cellular receptors for granulocyte-macrophage colony-stimulating factor, IL-3 and IL-5.[3,4] Other studies reported, however, that the βC-R may not be required for EPO-induced signal transduction and its cellular effects in some non-hematopoietic cells.[5,6] The detection of low levels of cell surface EPO-R on non-hematopoietic cells has been made possible by using a novel radiolabeled-EPO binding assay to demonstrate as few as 50 EPO binding

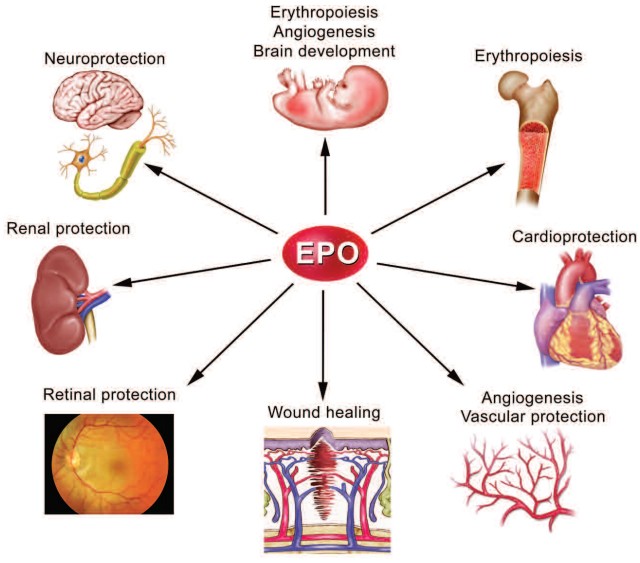

Figure 1. Schematic representation of the biological effects of erythropoietin.

sites on the cell surface, a receptor number that was nevertheless sufficient to mediate cellular effects of EPO in tumor cell lines of neural origin.[5] Whether βC-R may be involved in EPO signaling in macrophages, the mechanisms of EPO-induced effects on macrophages and the role of EPO-R remain to be determined.

The studies by Lifshitz and colleagues are likely to pave the way to new avenues of research investigating the role of EPO in the biology of macrophages – key effector cells of the immune system which influence inflammatory responses, microbial defenses, wound healing, angiogenesis, tumor biology, as well as physiological erythropoiesis within erythroblastic islands in the bone marrow.[7-9] EPO derivatives without erythropoietic activity have been explored recently in pre-clinical studies of wound healing.[10] Another study investigated EPO expression and function in macrophages in the context of atherosclerosis and the inhibitory effect of EPO on the formation of foam cells – the hallmark of early-stage atherosclerosis due to uptake of modified low-density lipoprotein (LDL) leading to cholesterol accumulation in the cells.[11] Treatment of macrophages with exogenous EPO and oxidized LDL increased cholesterol efflux from cells by EPO-induced upregulation of major transporters of cholesterol efflux from foam cells to mitigate lipid accumulation. Bone marrow-derived macrophages from transgenic mice that overexpressed EPO exhibited decreased lipid accumulation in response to oxidized LDL, an effect that was abolished by treatment with EPO antibody. Furthermore, endogenous EPO protein was found to be elevated in the aortic atherosclerotic lesions of apoE$^{-/-}$ mice and treatment of macrophages with oxidized LDL led to increased EPO expression and secretion from cells suggesting an autocrine involvement of EPO in modulating this process.[11] In view of the possibility that macrophages may produce functional EPO, further work will be required to delineate the effects of EPO as a paracrine factor in influencing the various physiological and pathological processes that macrophages are involved in, including regulation of erythropoiesis in bone marrow erythroblastic islands.[9]

Several previous studies investigated the role of EPO as an immunomodulatory cytokine, showing that EPO may attenuate inflammatory responses in some experimental models. For instance, EPO treatment was reported to improve neurological recovery in a mouse model of autoimmune encephalomyelitis, an effect that was associated with significant reduction of inflammatory glial cell and macrophage infiltration in the spinal cord, delayed appearance of tumor necrosis factor and decreased levels of IL-6.[12] In the ischemic brain in a rodent stroke model, EPO treatment was reported to reduce astrocyte activation and the recruitment of leukocytes and microglia in the infarct site, associated with a reduction of levels of inflammatory cytokines such as tumor necrosis factor and IL-6, although EPO did not directly inhibit cytokine release by astrocytes in culture.[13] In another example, anti-inflammatory effects were observed in an experimental rat model of autoimmune myocarditis in which EPO treatment resulted in a reduction in the area of myocarditis associated with decreased expression of the inflammatory cytokines tumor necrosis factor and IL-6.[14] Among other reported immunomodulatory effects, in a rodent model of multiple myeloma the administration of EPO resulted in an anti-tumor effect that was dependent on a T-cell mediated mechanism[15] and in patients with multiple myeloma, EPO therapy was associated with decreased levels of serum IL-6 and normalization of the CD4:CD8 T-lymphocyte ratio.[16] The mechanisms by which EPO exerts its observed immunomodulatory effects, whether EPO may elicit pro- *versus* anti-inflammatory responses in different organs and the discovery of macrophages as a target for EPO will undoubtedly constitute subjects for future investigation to better understand the direct and/or indirect role that the action of EPO on macrophages might play in modulating EPO-regulated functions including erythropoiesis, as well as the various non-hematopoietic activities of EPO.

The data reported by Lifshitz and colleagues contribute to previous work by many investigators indicating that EPO exerts biological effects in non-erythroid cells. More than two decades following the cloning of EPO-R and the availability of recombinant human EPO for the treatment of patients with anemia associated with chronic kidney disease, there is still much to learn about the full spectrum of EPO effects and mechanisms of EPO-R signaling.[17] The adverse effects of EPO therapy observed in randomized clinical trials involving patients with chronic kidney disease and cancer, such as increased thromboembolic complications, cardiovascular mortality, tumor progression and impaired survival may potentially be related to non-erythropoietic actions of EPO.[18] The biological consequences of EPO signaling in non-erythroid cells and organs is an important area of research that will contribute to the optimization of the current, safe use of recombinant EPO in the clinic and to better understanding of the risks involved in potential tissue-protective applications of EPO and novel EPO derivatives without erythropoietic activity.[19,20]

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Autoimmune lymphoproliferative syndrome: a multifactorial disorder

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Homeostasis of peripheral lymphocytes is maintained by numerous mechanisms including anergy, suppression and apoptosis. The last can be triggered by specialized receptors belonging to a subgroup of the tumor necrosis factor receptor superfamily called death receptors.[1] Fas, also termed tumor necrosis factor receptor superfamily 6 (TNFRSF 6), CD95 or Apo-1, is the prototypic member of the death receptor family. Its intracellular domain of 60 to 80 amino acid residues called the death domain allows homotypic interactions with a cytoplasmic death domain-containing protein named the Fas-associated death domain (FADD). FADD contains another domain called the death-effector domain (DED) mediating the recruitment of DED-containing cystein proteases such as pro-caspase-8 and pro-caspase-10 (also called Flice and Flice-2, respectively) in humans. These pro-enzymes are processed in their active form into a death-inducing signaling complex which, in turn, triggers a biochemical cascade composed of other pro-caspases and culminating in apoptosis. Initiation of this process can be inhibited by recruitment of an inactive caspase analog, the Flice inhibitory protein (FLIP). Finely tuned stoichiometry of FLIP, caspase-8 and caspase-10 regulates the stability and pro-apoptotic activity of the death-inducing signaling complex. This Fas-induced cell death can be triggered by cell-cell contact between a Fas-ligand-positive effector cell and a Fas-positive target, or following chronic stimulation through the antigen T-cell receptor (TCR). This activation-induced cell death is mainly controlled by Fas and Fas-ligand.[2]

The role of Fas in human lymphocyte homeostasis was illuminated by the discovery of Fas mutations in patients with autoimmune lymphoproliferative syndrome (ALPS).[3] ALPS was first described in 1967 by Canale and Smith and is now recognized as a chronic or recurrent, non-malignant lymphoproliferative condition frequently accompanied by autoimmune manifestations (mostly autoimmune cytopenias). Manifestations usually appear in the first 5 years of life (median onset at 3.5 years). The most frequent presentation of ALPS is a benign lymphoproliferation limited to lymphoid organs. Enlargement of spleen