When Erythropoietin Meddles in Immune Affairs

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A century ago, two French investigators reported that when injected into normal animals, small amounts of plasma from anemic rabbits caused an increase in red blood cell production. They referred to this activity as hemopoietine. Over time, erythropoietin (Epo) was identified as the erythropoietic factor, which was then purified, and the molecular cloning of the Epo gene led to high-level production of recombinant human erythropoietin (rhEpo) in sufficient purity and quantity for the development of therapy. In 1987, clinical trials were conducted to test the safety and effectiveness of rhEpo for treating anemia in patients with kidney failure. The results were dramatic, and since this time, millions of patients worldwide have benefited from Epo therapy.

In response to decreased systemic and local oxygen tension, Epo is produced by a subset of peritubular fibroblasts in the cortex close to the boundary with the kidney medulla as well as by interstitial cells and hepatocytes in the liver. The transcription of the gene encoding Epo is under the control of the transcription factor hypoxia-inducible factor-2α and, during hypoxia, its hydroxylation by prolyl hydroxylase domain hydroxylases, ubiquitination, and degradation by the proteasome are reduced.

Epo is a heavily glycosylated cytokine, and its concentration in the blood is low in the absence of anemia; however, hypoxic stress can enhance the concentration of Epo by a factor of 1000.

Importantly, glycosylation is of paramount importance for controlling its naturally short (5–8 hours) half-life. Epo circulates in the plasma and binds to receptors abundantly expressed on erythroid progenitor cells, thereby promoting the viability, proliferation, and terminal differentiation of erythroid precursors and causing an increase in red blood cell mass. The oxygen-carrying capacity of the blood is thereby enhanced, increasing tissue oxygen tension and thus completing the feedback loop and suppressing further expression of Epo.

Epo signaling occurs through the activation of its membrane receptor, EpoR, which is expressed at high levels on the surface of erythroid progenitors as a homodimer. Upon binding to Epo, the receptor undergoes a conformational change that brings its intracellular domains into close apposition. As a result, Janus kinase 2 and several subsequent signal transduction pathways are activated, including the phosphatidylinositol-3 kinase/Akt axis, signal transducer and activator of transcription 5, and extracellular signal-related kinases, which are implicated in cell proliferation and survival. This signaling subsequently leads to the activation of cell survival factors, such as the B-cell lymphoma 2 family members, resulting in protection against programmed cell death.

In addition to the hematopoietic properties of rhEpo, EpoR-mediated signaling activates antiapoptotic and proliferative pathways and confers clinically relevant tissue-protective effects to rhEpo in cases of nonhematologic experimental disorders, such as stroke, AKI and CKD, retinal degeneration, and ischemia-reperfusion injuries (e.g., cardiac, liver, kidney). Unexpectedly, several clinical trials failed to confirm in clinical settings the encouraging preclinical findings, particularly in the prevention of acute ischemic injury.

In contrast to erythroid precursor cells, the expression of EpoR on nonerythroid cells is low and is unlikely to be sufficient for EpoR activation to occur. In addition, the protective activity of Epo may be due to the presence of a low-affinity heterodimeric receptor made with another partner, perhaps CD131, the cytokine receptor common β subunit. Consequently, the tissue-protective properties of rhEpo are reached at higher doses of rhEpo, which may promote an increase in cardiovascular and thromboembolic events. Nonerythropoietic erythropoietin derivatives have been developed by chemically modifying or mutating Epo. As an example, carbamylated Epo and ARA290 lack erythropoietic activity but maintain the tissue-protective effect of Epo and protect the kidneys from ischemic injury.

The nonhematopoietic functions of rhEpo constitute an exciting research avenue, and numerous questions remain to be answered, one of the most important being the discrepancies between the encouraging results of preclinical studies and the lack of efficacy observed in clinical trials. In this context, a new study from a team led by Peter Heeger addressed the intriguing question of the immunomodulatory properties of Epo. On the basis of the observations that rhEpo protects against chronic allograft injury in a rat kidney transplant model.
independent of anemia correction\(^1\) and that rhEpo reduces the clinical expression of murine experimental autoimmune encephalomyelitis,\(^2\) they tested whether rhEpo could shape T-cell functions.

They observed that Epo, at the concentrations typically used for \textit{in vitro} experiments (between 500 and 2000 IU/ml), decreased Th1 cell differentiation and proliferation but did not modify their viability. These effects applied to CD4\(^+\) and CD8\(^+\) subsets and to naïve and memory cells (these last cells required higher rhEpo concentrations). Conversely, rhEpo did not interfere with regulatory T-cell homeostasis or the allostimulatory capacity of dendritic cells. The effects of rhEpo were mediated by the EpoR homodimer, and the authors demonstrated that CD131, albeit expressed by T cells, did not transduce Epo signaling. rhEpo reduced T-cell proliferation by interfering with IL-2R signaling and reducing Akt signaling, but it did not affect signal transducer and activator of transcription 5 phosphorylation. These results are counterintuitive because Epo classically activates, rather than inhibits, Akt signaling, but the reasons for this specificity in T cells remain to be determined. Clearly, the molecular biology of the EpoR/IL-2R signaling inhibitory cross-talk must be deciphered. Lastly, rhEPO reduced the proliferation of T cells and IFN-\(\gamma\) production in response to murine xenoantigens \textit{in vivo}. Whether these findings translate to relevant experimental models of allo- or autoimmune diseases remains to be determined.

This is a landmark study because it demonstrates for the first time an original biologic concept and paves the way for further studies, which will refer to it as a starting point to confirm, refute, or apply the concept to other settings. These observations could be integrated into the expanding field of the metabolic control of T-cell plasticity. Indeed, the control of T-cell differentiation and proliferation by hypoxia-driven cytokines such as Epo is reminiscent of the profound effect that nutritional microenvironmental changes have on the differentiation profile of T cells, by acting at the epigenetic, transcriptional, and post-translational levels.\(^2\) A metabolically restrictive microenvironment can shape T-cell function and fate. For example, decreased local oxygen tension at sites of inflammation and in the tumor microenvironment is likely to stabilize hypoxia-inducible factor-1\(\alpha\) to drive a proinflammatory Th17-cell phenotype,\(^2\) whereas the scarcity of available glucose can limit the ability of effector T cells to produce cytokines, such as IFN-\(\gamma\).\(^2\) Therefore, there is great interest in understanding how metabolic responses to hypoxia influence immune responses and how Epo signaling in the immune system is integrated in this complex biochemical network.

There is no doubt that this work will fuel an interesting debate, and arguments supporting or challenging the concept will emerge. Supporters of the concept will use the arguments developed by the authors of the study: the immunomodulatory effects of Epo could explain the benefits of rhEpo treatment in allotraft survival,\(^19,24\) the amelioration of rheumatoid arthritis symptoms,\(^15\) and the worst outcomes in patients with cancer treated with rhEpo.\(^26\) Skeptics will argue that if rhEPO behaves as an immunosuppressive agent, it cannot explain the immunodeficient state of patients with CKD who lack Epo or why infections and cancers have not been reported as adverse effects of long-term rhEpo treatment in CKD. The arguments used to foster the controversy will remain speculative until solid experimental and clinical evidence becomes available.

In addition to this debate, this study raises some appealing questions to be tested in further studies: are the immunoregulatory effects of rhEpo local or systemic? Do nonerythropoietic derivatives share the same immunomodulatory effects as rhEpo? Are the immunoregulatory effects of rhEpo affected by the duration of treatment? How does rhEpo interfere with the immune control of the atherosclerotic process? The story is only beginning to be unraveled.

**DISCLOSURES**

None.

**REFERENCES**


### Urinary Exosomes Join the Fight against Infection

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Urine is a complex fluid containing proteins, cells, nucleic acids, and a variety of extracellular vesicles. In the current issue of JASN, Hiemstra et al. demonstrate that exosomes, a specific type of extracellular vesicle, have antibacterial activity that could mediate host resistance to urinary tract infection (UTI). Given that UTI is the most common human bacterial infection (around 150 million cases annually), this new mechanism of urinary bacterial killing is an important discovery.1 However, researchers need new experimental tools to be able to move forward and understand the functional importance of exosomes in vivo.

Cells release lipid membrane–bound vesicles that can be broadly classified into two types: cell membrane–derived “microparticles” and exosomes, which originate from the cell's endosomal system. A panel of physicochemical properties are measured to identify exosomes: they characteristically contain proteins involved in their intracellular formation (such as TSG101, ALIX, and LAMP1) and are approximately 20–100 nm in size, smaller than particles derived directly from the cell membrane.2 Exosomes that originate from the glomerulus and all regions of the nephron are present in urine.3 They contain protein, mRNA, microRNA (miRNA), and mitochondrial DNA from their kidney cell of origin. Therefore, urinary exosomes promise to be a productive reservoir for biomarker discovery, even a noninvasive replacement for the renal biopsy; however, this promise has not yet been translated into a biomarker with clinical utility (and this seems a long way off). The roadblock to clinical translation is partly caused by inadequate techniques for exosome isolation, which are too time-consuming.

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