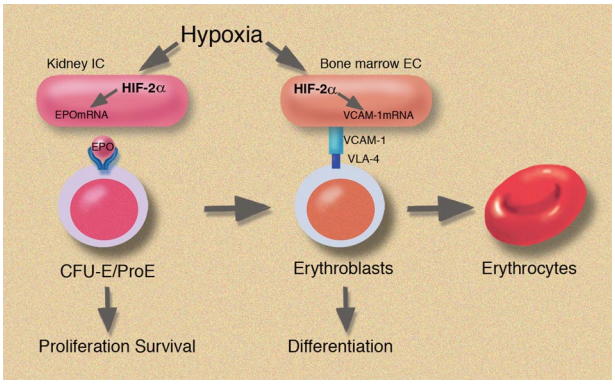

Hypoxia stimulates EPO production mainly in the adult kidneys and cell-cell contacts of erythroblasts and endothelial cells (ECs) in the bone marrow through hypoxia-induced accumulation of HIF-2α. Professional illustration by Marie Dauenheimer.

incomplete deletion of the transcription factor subunit are sufficient for EPO expression. On the other hand, a recent report by Percy et al[5] indicates that EPO synthesis is very sensitive to increased HIF-2α. In a family with a genetic mutation that reduces the degradation of HIF-2α under high oxygen tension, constitutively high levels of HIF-2α were made responsible for familial erythrocytosis.[5] Although erythroid progenitor maturation was not specifically studied, increased HIF-2α levels may also have had an effect on the bone marrow microenvironment.

The HIF pathway is a potential therapeutic target, as evidenced by recent clinical trials in which attempts at pharmacologic inhibition of HIF degradation have been undertaken to increase EPO synthesis in patients.[6] From the widespread importance of HIF-dependent regulation of gene expression, multiple effects would have been expected. So far, preliminary data indicate that erythropoiesis was preferentially induced through increased EPO synthesis and effects on enzymes involved in iron metabolism that are also under the control of HIF.[6] The present study by Yamashita et al may explain why erythropoiesis is particularly sensitive to modulation of HIF-αs: because increased EPO falls on "fertile soil," a bone marrow microenvironment that has been optimized by endothelial-specific, HIF-2α–driven expression of VCAM-1 to provide cell-cell contacts between stromal and hematopoietic progenitors.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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TRANSPLANTATION

Comment on Gatza et al, page 1515

GVHD therapy: let there be light!

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In this issue of *Blood*, Gatza and colleagues demonstrate in a murine model of GVHD that ECP induces increases in circulating endogenous Tregs.

Graft-versus-host disease (GVHD) remains one of the most frequent and challenging complications faced by allogeneic stem cell transplantation clinicians.[1] While glucocorticoids remain the mainstay of therapy for patients who develop GVHD following the failure of prophylactic immunosuppression, treatment failures are all too common, as are complications associated with the immunologic and systemic effects of steroids. Sadly, the identification of the best course of action for patients with steroid-refractory GVHD has proven even more vexing. Prompted in part by the failure of pharmacologic therapies that specifically inhibit alloreactive T cells (while sparing critical pathogen-specific and cancer-specific T cells), a great deal of interest has been focused on the therapeutic potential of $CD4^+$ T regulatory cells (Tregs), which have been shown convincingly to inhibit GVHD following adoptive transfer in murine models.[2]

The therapeutic potential of the combination of photosensitizers (including psoralen compounds) and sunlight was recognized in both ancient Egypt and India, inspiring the development of modern therapies combining ingested psoralen derivatives and ultraviolet light exposure.[3] In extracorporeal photopheresis (ECP), blood is circulated mechanically outside the body, where peripheral blood mononuclear cells are first exposed to the photosensitizer 8-methoxypsoralen (8-MOP), and then to long-wavelength ultraviolet light (UVA). All of this is accomplished ex vivo in a machine resembling those used for hemodialysis, with the treated blood fraction similarly circulated back to the patient. Although ECP has primarily been applied in the treatment of malignant diseases of the skin, including subsets of cutaneous T-cell lymphoma, the application of ECP has yielded promising results in studies of steroid-refractory GVHD.[4] Multiple putative mechanisms have been proposed to explain the therapeutic effects of ECP in the setting of GVHD, but only indirect evidence to support such mechanisms has been available, in part due to the lack of available animal models for ECP.[5]

To examine the mechanisms by which ECP exerts its therapeutic effects, the authors first approximated ECP in the mouse; this alone was an accomplishment, since Lilliputian ECP machines suitable for mice do not yet exist. They exposed splenocytes, necessary to induce GVHD in allogeneic murine models, to 8-MOP and UVA ex vivo, and transferred them into murine recipients of both minor antigen-mismatched and haploidentical grafts. The transfer of ECP-treated splenocytes convincingly reduced GVHD-associated mortality; this was associated with significant decreases in GVHD scores in the liver, gut, and skin. The authors next demonstrated that numbers of IFNγ-producing effector $CD8^+$ T cells were reduced following the transfer of ECP-treated splenocytes. Most critically, they then demonstrated that donor-derived Tregs

were induced by the transfer of ECP-treated splenocytes, and that in vivo purging of Tregs eliminated the benefits of ECP.

Some limitations of this study deserve mention. First, the models used primarily recapitulate acute GVHD, while most of our clinical information about the utility of ECP is derived from studies of patients with chronic GVHD, which is much more difficult to model in mice. Although the authors demonstrate clearly that Tregs were induced by ECP, they do little to demonstrate the mechanism of Treg induction. IFNγ production was shown to be attenuated, yet the authors did not examine TNFα, shown in their own prior studies to be critical in GVHD-associated inflammation, nor did they examine whether ECP induced tolerogenic cytokines, including IL-10 and TGF-β. Finally, while it was shown that ECP-induced apoptosis indirectly induced Tregs, the direct role of cellular intermediaries (especially antigen-presenting cells including dendritic cells) was not examined.

Despite these limitations, this study provides the most convincing evidence to date that the induction of lymphocyte apoptosis by ECP exerts its therapeutic effects through induction of Tregs. This study suggests that altering the ratio of Tregs to alloreactive effectors may effectively reduce GVHD, and provides impetus for other approaches that augment natural levels of Tregs. Better longitudinal studies of GVHD patients, examined before and after ECP therapy, will be required to confirm whether Tregs are similarly induced in humans, and whether other immune functions (eg, virus- and cancer-specific T cells) are relatively spared. It will also be important to utilize this valuable model of ECP therapy to better define the mechanisms of Treg induction. Such knowledge will surely lead us to more effective and/or less invasive approaches to treat GVHD and other human diseases mediated by T-cell dysregulation.

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