individuals.[8] Therefore, there is a possibility that the detrimental effect of CMV infection in CLL patients results from the constriction of the total T-cell repertoire by the overwhelmingly increased numbers of the CMV-specific T-cell population (see figure) because of the competition for essential growth factors and/or overcrowding of tissue niches that support memory T-cell survival. Such an effect would lead to the loss of certain essential memory T-cell populations directed towards other microorganisms. The increased reactivation of latent pathogens (ie, *Varicella zoster* virus [VZV]) that occurs in CMV-seropositive CLL patients supports this possibility.[1] A similar situation may be at work during the aging of healthy CMV-seropositive humans, where accumulation of CMV-specific cells may occlude VZV-specific cells from tissue niches thus leading to the increased incidence of VZV reactivation (shingles) in older humans (see figure).[9] A crucial point that requires further investigation is whether the expanded CMV-specific T-cell populations in CLL patients may restrict the quality of the T-cell response to the leukemic cells themselves.

Thus, the study of Pourgheysari et al provides interesting insight into the dominant role that CMV infection has on shaping the T-cell repertoire in CLL patients. Although the exact cause for the negative association of CMV seropositivity and survival in these patients is not currently clear, determining whether targeting the virus itself—or the T-cell expansions that it induces—may improve the survival of both CLL patients and older humans.

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● ● ● RED CELLS & IRON

Comment on Kapitsinou et al, page 3039

Dual control: the HIF-2 regulator

Terry Lappin QUEEN'S UNIVERSITY BELFAST

Identification of the specific roles of individual members of the hypoxia-inducible factor (HIF) family of transcriptional activators provides insights into the pathogenesis of anemia and erythrocytosis that may enable the development of novel therapies for these disorders. In this issue of *Blood*, Kapitsinou and colleagues[1] use conditional ablation of Hif-2α in the murine kidney to establish that hypoxic induction of erythropoietin (Epo) is completely dependent on Hif-2α and that in the absence of renal Hif-2, hepatic Hif-2 becomes the main regulator of serum Epo.

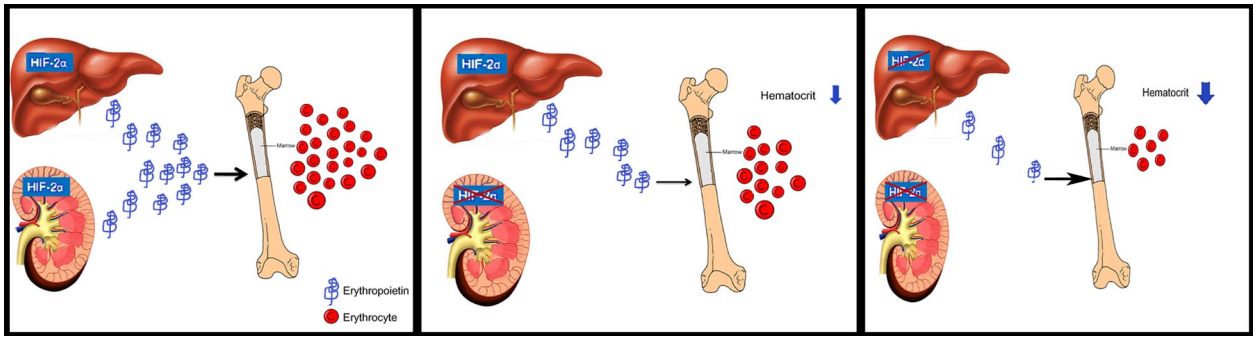
Toward the end of the 19th century, 3 Frenchmen—Paul Bert, Denis Jourdanet, and Francois-Gilbert Viault—established the relationship between reduced oxygen pressure and increased circulating red blood cells (RBCs) based on their work on altitude in Mexico and the Peruvian Andes.[2] Interest in understanding the physiologic and molecular basis of this adaptation to hypoxia led to the discovery of EPO and the HIF family of transcriptional activators.

During human fetal development EPO is produced mainly in the liver but its primary site of production changes to the kidney during late gestation. Although the adult liver does not normally produce EPO under normoxic conditions, it retains the capacity to produce EPO in the event of renal impairment.

In the 1990s, in vitro studies by Gregg Semenza and colleagues identified a novel transcription factor, HIF-1, which was widely assumed to be responsible for the hypoxia-induced increase in EPO found in Hep3B cells, based on its binding to an 18-nucleotide fragment of the oxygen-sensitive 3′ *EPO* regulatory element.[3,4] However, recent genetic studies in mice[5-7] and investigations of patients with familial erythrocytosis[8-10] have provided strong evidence that HIF-2α, not HIF-1α, is the prevalent regulator of circulating EPO levels.

In an elegant study, Kapitsinou and colleagues inactivate Hif-2α specifically in the kidney by Cre-loxP recombination enabling the contribution of renal Hif-2 signaling to Epo homeostasis to be determined directly (see figure). Loss of renal Hif-2α resulted in hypoproliferative anemia with RBCs and hematocrit (HCT) reduced to half of the normal levels, due to significantly lower circulating serum Epo levels in the mutant mice. The mutant mice also had increased levels of renal Hif-1α protein and increased expression of the Hif target genes *Glut1, Pkf, Ldha, and Phd3*, but not Epo production, providing further evidence that hypoxic regulation of Epo in the kidney is not dependent on Hif-1α.

To investigate the role of hepatocyte-derived Hif-2, the authors generated double mutants lacking or substantially reducing Hif-2α in the kidney and liver (see figure). These animals had significantly reduced serum Epo levels compared with their renal Hif-2–deficient, single mutant littermates indicating that hepatic Hif-2 plays a role in Epo regulation of the single mutants under baseline conditions. When further challenged by phlebotomy the double mutants had a 70% reduction in serum Epo levels compared with their single mutant counterparts. This suggests that in this scenario, at least 70% of serum



Erythropoietin (Epo) regulates red cell production in response to tissue oxygenation. Hypoxia, the main physiological stimulus of enhanced Epo gene transcription, can induce several 100-fold increases in circulating serum Epo levels mediated by hypoxia-inducible factor (Hif). Kapitsinou and colleagues ablated Hif-2α in the kidney and partially in the liver. They found that hypoxic induction of Epo is completely Hif-2–dependent and that in the absence of renal Hif-2, hepatic Hif-2 takes over as the main regulator of the serum Epo response to hypoxia. Their report corroborates recent genetic studies in mice and investigations of patients with familial erythrocytosis which have provided strong evidence that HIF-2α, not HIF-1α, is the prevalent regulator of circulating Epo levels. The image provided is a composite of the liver, kidney, and bone and RBC images as follows: liver image,[11] kidney image,[12] and bone and RBC image.[13]

Epo is produced via hepatocyte Hif-2 signaling, taking into account that Hif-2 is not produced in all of the hepatocytes due to limitations in recombination efficiency.

Hypothesizing that Hif-2 may coordinate Epo production with iron metabolism, the authors generated 2 different models of Hif activation based on cell-type–specific von Hippel-Lindau protein (pVHL) inactivation in hepatocytes, and on stabilization of systemic hypoxia in all liver cells. The experimental data provide convincing evidence that Hif-2 is involved in the transcriptional regulation of several genes implicated in iron metabolism, such as *Dmt1*, *ceruloplasmin*, and *hephaestin*, but its contribution to the coordination of Epo synthesis with iron homeostasis requires further investigation.

Although the present work confirms that Hif-2 responds to the body's demand for oxygen and is the predominant regulator of the erythropoiesis, its role in the supply of iron vis-à-vis Hif-1 remains to be established. As Kapitsinou et al indicate, stabilization of human HIF has the potential to stimulate latent hepatic human EPO synthesis in patients with renal impairment, through prolyl-4-hydroxylase domain (PHD) inhibition with 2-oxoglutarate analogs. Because inhibition of PHD causes stimulation of multiple HIF-regulated biologic processes, a superior strategy would entail the design of a drug that preferentially enhances HIF-2 activity, but such an approach is likely to be extremely challenging.

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Comellas-Kirkerup et al, page 3058

Blind men and the APS

Keith R. McCrae CLEVELAND CLINIC

In this issue of *Blood*, Comellas-Kirkerup and colleagues describe the course of 55 patients with laboratory criteria of the antiphospholipid syndrome (APS) who displayed concurrent thrombocytopenia and/or autoimmune hemolytic anemia (AIHA).[1] None of these individuals had serologic or clinical features of lupus. This manuscript raises important questions concerning the relationship of cytopenias to the clinical events that comprise the Sapporo criteria for APS.[2]

Defining the clinical manifestations of APS is somewhat reminiscent of the story of the blind men and the elephant, in which 5 blind men are asked to touch and then describe an elephant. Each man touches a different part of the elephant, ranging from the trunk to the tail, and reaches a different conclusion of what an elephant looks like (see figure). One interpretation of this parable is that people sometimes understand only a small

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