types of DAA-based regimens, most commonly including sofosbuvir.

As anticipated, DAA induced sustained virologic responses at 12 weeks in all but 1 patient, with very little reported toxicity. With a median follow-up of only 8 months (range, 2-30 months), and a median duration of DAAs of 12 weeks (range, 6-24 weeks), all patients received the entire intended course of antiviral therapy with the exception of 1 patient with advanced cirrhosis who had NHL progression on DAAs.

In terms of lymphoma responses, ~2 of 3 patients responded to DAAs. One of the most interesting findings of this study is that 11 of 12 complete responses occurred in patients with MZL, whereas there were no responses in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma. This suggests that the biology of the NHL and its interaction with chronic HCV infection play an important role in lymphomagenesis and therapeutics. It also cautions that effective HCV eradication may not necessarily treat non-MZL histologies, and that these patients may still require standard therapies such as chemoimmunotherapy. However, there were only 9 patients with non-MZL histologies, and this observation will require further prospective investigation.

In the entire study cohort, 12 patients had received prior IFN-based HCV therapy, and 10 patients had received prior systemic therapy for indolent NHL. Neither of these factors was associated with a lack of response to DAAs, strengthening the idea that cessation of chronic HCV antigenic stimulation is the key biologic event leading to lymphoma regression. This important assumption has therapeutic implications: HCV therapy appears to be effective as lymphoma therapy whether it is used in the first line or relapsed settings. HCV therapy makes most sense in patients with indolent NHL who do not have an immediate need for systemic therapy, and who can safely wait several weeks for the immunologic effects of DAAs to take place. At the same time, HCV therapy may also be equally appropriate following standard chemoimmunotherapy for advanced, symptomatic NHL.

Within the short length of follow-up, 6 patients experienced lymphoma progression (1 during DAA, 5 after DAA), all with MZL. It is possible that their lymphomas progressed because the initial virologic response was not sustained; however, this information is not available. It is also possible that successful HCV eradication did not sufficiently reverse the complex immunologic processes that gave rise to the NHL, particularly in the patients whose MZL progressed within 3 months of DAAs. The latter hypothesis is consistent with the observation that in other NHLs associated with chronic infections such as gastric and ocular adnexal MZL, eradication of the putative microorganism does not consistently eradicate the lymphoma.

Although the data presented by Arcaini and colleagues advance our understanding of the relationship between HCV infection, HCV therapy, and B-cell NHL, there are a number of outstanding issues. First, longer follow-up is required to determine whether sustained virologic responses and their ensuing sustained lymphoma responses will translate into favorable long-term outcomes for these patients. Second, prospective studies involving homogeneous treatments and assessments, together with biologic correlates, are necessary to identify the most effective strategy for the subgroup of patients most likely to benefit. Third, pharmacoeconomic analyses should be built into these studies because DAA-based therapy is expected to incur a significant economic burden for the payer, public or private, responsible for the cost of HCV therapy. However, this may turn out to be a cost-effective investment in the long run if these 2 chronic diseases are simultaneously successfully managed and even possibly cured.

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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Urrutia et al, page 2550

Pericytes: new EPO-producing cells in the brain

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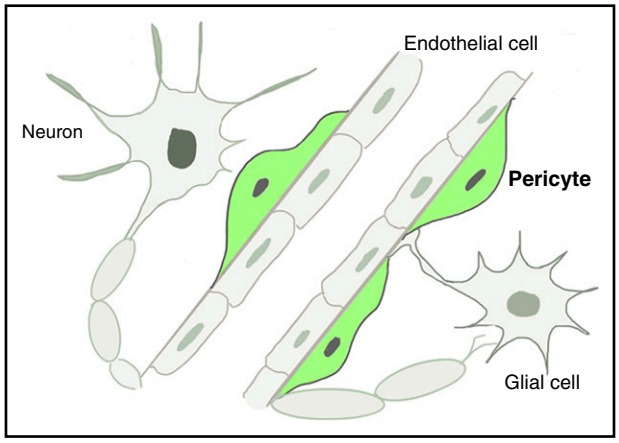
In this issue of *Blood*, Urrutia et al identified pericyte as a novel erythropoietin (EPO)-producing cell type in the brain. The brain pericytes function as oxygen sensors and respond to hypoxia. They are regulated by hypoxia-inducible factor (HIF)-2 and prolyl-4-hydroxylase (PHD) 2 and 3.[1]

EPO is the principle cytokine for the production of red blood cells, especially under hypoxic conditions. During mammalian fetal developmental, EPO is mainly generated in the liver, which is also one of the major organs for fetal erythropoiesis. After birth, the majority of EPO is produced in the kidneys. Main renal EPO-producing cells (EPCs) are peritubular interstitial cells that express markers



Schematic illustration of brain EPO-producing cells. These cells comprise neurons, glial cells (including astrocytes and oligodendrocytes), endothelial cells, and pericytes. The pericytes, which are highlighted in green, are revealed by Urrutia et al to be the main EPCs in the brain under hypoxic conditions.

including platelet-derived growth factor receptor-β (PDGFRB) and neuro-glial antigen 2 (NG2). These markers are characteristically expressed in pericytes and neuronal cells.[2] The developmental association between kidney EPCs and neuronal cells has led to the discovery of EPCs in the brain >2 decades ago.[3] To date, brain EPCs are found to include neurons, glial cells, and endothelial cells. Using elegant mouse genetics and RNA fluorescence in situ hybridization (FISH) approaches, Urrutia et al now identify pericyte as another, and the major, type of EPC in the brain, especially under the hypoxic conditions (see figure).

In the kidneys, the hypoxic induction of EPO is controlled by HIF-2, which is composed of an oxygen-sensitive α-subunit and a constitutively expressed β-subunit. HIF-α is regulated by oxygen, iron, and PHD domain-containing proteins 1, 2, and 3. PHD proteins hydroxylate HIF-α, which leads to its binding of von Hippel-Lindau (VHL) E3 ubiquitin ligase and subsequent degradation by the proteasome. As a proof-of-concept, Urrutia et al first investigated the PHD/VHL-HIF-EPO pathway in a mouse model with VHL knockout specifically in NG2-expressing cells (NG2-$Vhl^{-/-}$). These mice developed polycythemia with increased plasma EPO concentration. Although NG2 is expressed in various cell types in addition to pericytes, statistically significant *Epo* transcripts were only observed in the brain, kidneys, and bone. Among these tissues, brain showed the most pronounced fold increase. In addition, the authors performed high-resolution RNA FISH and convincingly demonstrated that the pericyte is the main source of EPO in the brain in NG2-$Vhl^{-/-}$ mice by showing that a majority (60%) of *Epo* transcript-positive cells are also positive for *Pdgfrb* transcript in these mice.

These intriguing data reveal the pericyte as a major type of EPC in the brain. However, the relative contribution of EPO produced by the brain pericytes to circulating EPO level remains unclear. Although this may not be easy to investigate in normoxic conditions given the impermeable nature of the blood–brain barrier to EPO, Urrutia et al show that, under hypoxic situations, pericytes that coexpress EPO and PDGFRB represent the major EPCs in the brain. They demonstrate that when the wild-type brain pericytes are exposed to 8% oxygen, the *Epo* and *Pdgfrb* double-positive cells represent 25% to 45% of the total number of *Epo*-positive cells. When they induce the wild-type mice to anemic hypoxia through phlebotomy, this number rises to 70%. The rest of the *Epo*-positive cells are presumably other types of EPCs in the brain as mentioned above.

EPO production in the kidneys is negatively regulated by PHD2, whereas combined inactivation of all 3 PHD proteins is needed to induce EPO in hepatocytes. In the brain pericytes, PHD2 and PHD3 are now shown to be required to reduce HIF-2. Using a similar genetic approach, the authors show that NG2-$Phd2^{-/-}Phd3^{-/-}$ mice exhibit polycythemia that can be reverted in NG2-$Phd2^{-/-}Phd3^{-/-}Hif2a^{-/-}$ mice. The involvement of various PHD proteins in different tissues indicates cell type–specific regulation of HIF activity and EPO production. It is also possible that the PHD proteins play a distinct function in addition to EPO production. In the brain, this could be that the combined activities of PHD proteins facilitate the delivery of the pericyte-produced EPO to the systemic circulation through their influences on blood–brain barrier permeability. Consistent with this hypothesis, NG2-$Phd1^{-/-}Phd2^{-/-}Phd3^{-/-}$ triple knockout mice were found to have increased plasma EPO than NG2-$Phd2^{-/-}Phd3^{-/-}$ mice. However, the brain *Epo* mRNA or EPO protein levels showed no differences between these 2 groups of mice.

EPO production by the brain pericytes has perhaps more significant implications than the contribution to circulating EPO during hypoxia. The EPO receptor (EPO-R) is expressed in neural progenitor cells, neurons, glial cells, and endothelial cells. EPO has a well-documented role in neuroprotection, especially in ischemic brain injury.[4] Targeted knockout of EPO-R in the mouse brain reduces neural cell proliferation and impairs poststroke neurogenesis.[5] EPO injection also protects brain injury in vivo.[6] The most compelling evidence of EPO in neuroprotection comes from the fact that EPO derivatives lacking erythropoiesis stimulating activity still conserve the neuroprotective effects in animal models of brain injury.[7] In addition to EPO production, endothelial cells, one of the critical components of neutral stem cell niche, are responsive to EPO stimulation to contribute to neurogenesis and neuroprotection.[4] EPO promotes endothelial cell migration, proliferation, production of nitric oxide, and angiogenesis.[8] In this respect, the direct interaction of pericytes with endothelial cells could provide immediate EPO stimulation to endothelial cells for their neuroprotective function. This could also circumvent the difficulty of circulating EPO to reach the injury sites in the brain given the robust EPO generation by the brain pericytes under hypoxic conditions.

Although the significance of the brain pericytes in EPO production under normoxic and hypoxic conditions in human remains to be investigated, this study advances the field and establishes the basis for future studies on the collaborative roles of various brain EPCs in oxygen sensing, EPO production, and neuroprotection.

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THROMBOSIS AND HEMOSTASIS

Comment on Verbij et al, page e51

Glycans of plasma ADAMTS13

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In this issue of *Blood*, Verbij et al identified the sites of glycosylation in plasma ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) and determined the composition of the glycan structures at these sites.[1]

ADAMTS13 is a blood enzyme that controls the multimer size of the hemostatic protein von Willebrand factor.[2] After synthesis, ultralarge, hyperreactive von Willebrand factor multimers (up to 20 000 kDa) are secreted into the flowing blood and are immediately cleaved by ADAMTS13 into smaller more quiescent multimers (<10 000 kDa). When ADAMTS13 is deficient, patients suffer from the devastating thrombotic thrombocytopenic purpura (TTP) disorder.[3] In TTP patients, ultralarge von Willebrand factor multimers spontaneously bind platelets, and microthrombi are formed that block arterioles and capillaries. This results in severe organ failure, thrombocytopenia, and hemolytic anemia. TTP can be caused by mutations in the ADAMTS13 gene (congenital TTP) or by the development of autoantibodies against ADAMTS13 (acquired TTP).

ADAMTS13 is a multidomain enzyme consisting of 1427 amino acids. Plasma ADAMTS13 is heavily glycosylated (20%) and has an apparent molecular weight of 180 to 190 kDa.[2] It is well known that glycosylation plays an important role in many processes such as immune recognition of proteins and protein folding, final structure, secretion, function, and eventual clearance. Only a few studies have investigated the role of glycosylation in ADAMTS13 folding and secretion and in ADAMTS13 function. It has been shown that recombinant ADAMTS13 contains *N*-linked glycosylation and *O*-fucosylation sites.[4,5] Both *N*-linked glycosylation and *O*-fucosylation seemed to be crucial for proper folding in the heterologous cells and for efficient secretion of recombinant ADAMTS13.[4,5] However, when *N*-linked glycans were removed from recombinant ADAMTS13, the proteolytic activity of ADAMTS13 was not altered.[5]

The glycan profile of recombinant ADAMTS13 might to some extent differ from the glycan structures of plasma ADAMTS13. Hence, to understand the role of glycans in ADAMTS13 biology and pathophysiology, it is crucial to unravel the glycosylation profile of plasma ADAMTS13. Verbij et al used the elegant approach of tandem mass spectrometry with higher-energy collision dissociation and electron transfer dissociation to complete this challenging task. Importantly, they were able to identify or confirm the amino acids that carry ADAMTS13 glycans, and they were also able to unravel the composition of each glycan chain. By using this knowledge, the complete structure of all ADAMTS13 glycosylation chains could be deduced. This work led to 3 categories of glycan structures on plasma ADAMTS13: complex *N*-linked carbohydrate structures, less complex *O*-(GalNAc)-linked glycan structures, and simple *O*-linked fucose and C-linked mannose glycans. Nine of the 10 *N*-linked glycans are composed of 11 to 13 monosaccharides, including a terminal sialic acid. However, 1 *N*-linked glycan (8 monosaccharides), situated in the spacer domain, is not sialylated but contains a high mannose structure. The 6 *O*-(GalNAc)-linked glycans consist of 4 to 7 monosaccharides, again including a terminal sialic acid. Typical for thrombospondin type 1 (TSP) repeats, and with the exception of TSP4, all 7 remaining TSP domains are *O*-fucosylated with disaccharide structures. Unexpectedly, another *O*-fucosylation site was identified in the disintegrin domain. Finally, the TSP1, -4, and -7 domains are each C-mannosylated with a single mannose residue.

Knowledge of the glycosylation profile of proteins allows better understanding of the role of glycans in the biology and pathophysiology of proteins. For example, glycans could control the structure of ADAMTS13. The crystal structure of only the disintegrin-like domain/first TSP repeat/cysteine-rich domain/spacer domain fragment of ADAMTS13 is known.[6] However, it was recently shown that the ADAMTS13 spacer domain interacts with its CUB1-2 domains, suggesting that ADAMTS13 adopts a folded conformation.[7,8] Thus, it will be interesting to unravel whether the glycans in these domains contribute to the stabilization of this overall folded structure of ADAMTS13. In addition, if glycans stabilize the structure of ADAMTS13, then changes in glycosylation patterns, which could occur spontaneously as a consequence of pathological processes,[9] might lead to different ADAMTS13 conformations. These different conformations might be more prone to proteolysis that renders ADAMTS13 inactive, which could be an explanation of why lower levels of ADAMTS13 activity are detected in certain diseases.[10] In addition, alterations in protein glycosylation may modify or create novel B-cell epitopes.[9] It has been suggested that changes in glycosylation of ADAMTS13 could expose neo-epitopes which could explain the formation of autoantibodies in acquired TTP. Verbij et al also hypothesized that high-mannose glycans identified in plasma

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