otherwise normal ArhGAP15−/− mice. First, they saw that the numbers of circulating neutrophils and macrophages were significantly decreased. Furthermore, macrophages from ArhGAP15−/− mice showed an altered morphology but normal migration. Finally, they discovered that neutrophil motility was strongly altered, indicating an important cell-selective role of ArhGAP15 (see figure). Neutrophils from these knockout animals showed increased motility with primarily the directional migration and cell polarization affected, as was shown by time-lapse video. The investigators used agarose chemotaxis assays, where the cells have to squeeze underneath a solid agarose block to follow the chemotactic gradient, which causes strong flattening of the cells and thus more informative images. The enhancement of ArhGAP15−/− neutrophil migratory functions was accompanied by increases in phagocytosis, bacterial killing, and ROS generation. The enhanced ROS production seen in the knockout cells also depended on the stimulus employed: while ROS triggered by Fc receptors (ie, receptors of antibody–coated immune complexes) was normal in the knockout cells, the generation of oxygen species via fMLP or C5a (ie, G-protein coupled receptors) was strongly enhanced. Most importantly, the ArhGAP15−/− animals showed a clinically important phenotype because they were protected from severe polymicrobial abdominal sepsis. To this end, Costa et al had exposed wild-type and ArhGAP15−/− mice to a model of cecal ligation and perforation inducing sepsis. This resulted in a significantly increased neutrophil load in the cecum and in the peritoneum of the ArhGAP15−/− animals, while noninfected organs did not show any signs of altered neutrophil recruitment. Septic injury and mortality are strongly associated with exorbitant systemic inflammation and multiple cytokine production. Importantly, ArhGAP15−/− mice showed a dramatically reduced production of multiple cytokines during the experimental sepsis and the animals survived for extended periods of time while all control mice died within 48 hours.

Taken together these 2 papers provide novel and remarkable evidence for important negative regulatory roles of the GAP proteins ARAP3 and ArhGAP15 in neutrophil cell biology and pathophysiology. As has been shown for ArhGAP15, these findings might be exploited in the future in the clinical treatment of sepsis, for example, by the generation of specific, small molecular GAP inhibitors. Of course, there must be a reason for the evolution of such proteins, likely the maintenance of a high threshold for the onset of systemic inflammation. Thus, any future clinical application based on these factors would have to get around potentially significant side effects. Mechanistically, work needs to be done to fully understand the cell-specific actions of these GAPs. ArhGAP’s bind to other GAPs such as the ARFs, and it would be interesting to see whether these factors are involved here as well. ARAP3 can function as an ARF-GAP in vitro, although its Rho-GAP activity appears to be more required for restraining neutrophil functions, as shown by Gambardella et al. Intriguingly, the ARF–GEF/GEF-1 has been previously shown to be required for integrin-dependent leukocyte chemotaxis and, significantly, for RhoA activation. Future studies will tell whether the important discoveries presented in these 2 papers are linked more tightly at the molecular level than what is obvious now.

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REFERENCES

Comment on Meiler et al, page 1109

Switching globin, raising red cells

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In this issue of Blood, Meiler and colleagues report that Pomalidomide, an immunomodulatory drug, stimulates fetal hemoglobin (HbF) production in transgenic sickle mice to a degree similar to that of hydroxyurea, without cytotoxicity and with expanded marrow erythropoiesis, producing 2 therapeutic benefits that address the pathology of sickle cell disease: polymerization of sickle hemoglobin and hemolytic anemia. This is a highly encouraging development for a serious disease with only one approved therapeutic.

Sickle cell disease afflicts 80 000–100 000 Americans and is considered a global health burden. The pathophysiology of sickle cell disease is initiated by polymerization of deoxy sickle hemoglobin, which results in sickling of red blood cells and hemolytic anemia, reducing the red cell lifespan to an average of 16 days. The deformed red cells adhere to endothelium and cause a cascade of secondary pathology, including adhesion of other cell types, vaso-occlusion, inflammation, vascular remodeling, and widespread organ damage. Anemia contributes to cardiomegaly and exercise intolerance.

Decades of work have clearly established that fetal hemoglobin (HbF) and F-cell proportions are the major modifiers and determinants of severity in sickle cell disease (reviewed in Akinsheye et al,2 Perrine, and Bunn et al4). HbF and tetramers of αβγ inhibit the
polymerization process, and HbF levels > 20% prevent most sickle cell disease clinical events. Examples of this ameliorating effect include infants with HbSS, who are well until after completion of the fetal to sickle globin gene switch, patients with S-HPFH (who have 70% HbS and 30% HbF), or patients with the Saudi-Indian haplotype (HbF levels > 20%), who are asymptomatic or have only mild disease. The Cooperative Study of Sickle Cell Disease documented that even incremental increases in HbF reduce clinical events.

In this report, Pomalidamide treatment of sickle transgenic mice increases HbF to levels equal to those induced by treatment with hydroxyurea (HU; see figure), and comparable to the average response observed in patients on the Multi-Center Study of Hydroxyurea. There is reason to anticipate higher HbF induction by Pomalidamide in humans than in mice, because mice normally switch from embryonic to adult globin without a developmental window of fetal globin gene expression. In contrast to the cytotoxicity observed in all hematopoietic lineages in the HU-treated mice, Pomalidamide treatment resulted in a doubling of erythroid cells in the marrow. Total Hb increased by 0.5 g/dL above controls and by 1.5 g/dL above levels in HU-treated mice. These 2 beneficial effects comprise a holy grail for treatment modalities to reduce the primary pathology in sickle cell anemia, a disease for which only 1 drug has ever been approved, more than 15 years ago. Anti-inflammatory effects of Pomalidamide may add a third component. Although there was no additive activity observed with simultaneous administration of HU and Pomalidamide, sequential dosing of the 2 agents might produce higher activity, as intermittent dosing regimens have increased activity of other drugs, such as Butyrate.

At least half of adult patients in most practices are considered responsive to HU, the only approved therapeutic available, and higher responses occur in children. This is a significant degree of benefit for any drug, as differences in drug metabolism alone render most therapeutics effective in only 25%-60% of patients. A recent 17.5-year follow-up study has shown there is still high mortality in sickle cell patients (43%), and prolonged HU therapy (at least 5 years) is needed for a survival benefit. Costs of supportive therapy for sickle cell disease are still high: analgesia, hospitalizations, transfusions, chelation, orthopedic surgeries, and diagnostic studies consume billions of health care dollars annually, and these costs do not include the disabilities and compromised lives caused by the disease. Some patients who still suffer some vaso-occlusive crises despite taking HU are wary of long-term use of a drug with a black-box warning regarding carcinogenicity. New HbF-inducing agents, particularly those with distinct mechanisms of action such as Pomalidomide, could make a great impact on the treatment of sickle cell disease as single agents or in a combination chemotherapy approach.

Trials of therapeutics for sickle cell disease have been challenging, at least partially due to the use of unrealistic end points. No single drug can effectively reduce all disease manifestations in a condition as pathophysiologically complex as sickle cell disease. Furthermore, the phenotypic heterogeneity of this global disease, affected by genetic modifiers only now being elucidated, make study populations diverse (reviewed in Akinsheye et al). Trial end points that are meaningful (such as total Hb and HbF) and achievable within a reasonable time duration, and in patient numbers feasible to enroll for an orphan disease that is frequently confounded by serious clinical events, should be accepted for drug approval. Trial end points that are more relevant to the molecular actions or target of the drug should be considered. For example, drugs that are not designed to affect hemoglobin polymerization and consequent sickling (such as IGA17403 [Senicapoc] or Flocar) should not be held to an end point of reduction in secondary events such as pain crises for approval, when other meaningful, targeted end points were met. Indeed, drugs targeting aspects of sickle disease other than pain crises could be highly beneficial additions to our limited therapeutic repertoire. Surrogate biochemical markers have been accepted by the FDA for other diseases for many years (eg, cholesterol lowering for atherosclerosis), and elevations in HbF should be considered an acceptable surrogate end point for certain sickle cell therapeutic candidates.

Pomalidomide joins a short, sorely needed pipeline of new sickle cell therapeutic candidates, which target HbF, HbS, inflammatory or adhesion molecules. As patients continue to suffer the pain and ravages of this complex condition, approval of new therapeutics cannot come soon enough.
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REFERENCES


VASCULAR BIOLOGY

Comment on Zheng et al, page 1154

To sprout or “Notch” to sprout?

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In this issue of Blood, Zheng and colleagues demonstrate that the Notch signaling pathway is active in lymphatic endothelial cells and that inhibition of Notch signaling, in concert with vascular endothelial growth factor (VEGF) stimulation, promotes lymphatic endothelial cell sprouting.1 This work suggests that Notch signaling plays a key role in regulating the switch between lymphatic endothelial cell quiescence and sprouting, akin to the role played by Notch signaling during angiogenic sprouting of the blood vasculature, and has important implications for therapeutic targeting of the Notch pathway as a treatment for vascular disorders and cancer.

Notch signaling plays a variety of key roles in vascular development and physiologic and pathologic angiogenesis (the sprouting growth of blood vessels).2,3 The role of Notch signaling in lymphangiogenesis is just beginning to be explored; conflicting results in various model systems have made it difficult to ascribe a definitive role to Notch pathway activity in lymphatic endothelial cells. Recent work in zebrafish suggested that Notch signaling is important for the initial sprouting of lymphangioblasts from the axial vein; abating Notch activity via a number of approaches interrupted formation of the thoracic duct.4 Moreover, work performed in that study suggested that after lymphatic endothelial cell fate specification, Notch signaling was important for guiding the navigation of lymphatic vessels along the intersomitic arteries.5

In contrast to the study by Guedens et al,4 work in a mouse model demonstrated that Notch signaling is not required for the specification of Prox1-positive lymphatic endothelial progenitor cells in the embryonic cardinal veins.6 Srinivasan and colleagues inactivated RhoC, a major transcriptional effector of Notch signaling, specifically in endothelial cells. While few embryos survived to embryonic day 10.5, reflecting the fact that Notch is required for early aspects of vascular development, the specification of lymphatic endothelial cells in the embryonic cardinal veins was not impeded. In this model, embryonic lethality precluded the analysis of the role of Notch signaling in later stages of lymphatic vascular development.

In the article by Zheng et al, the use of novel human lymphatic endothelial cell sprouting assays, together with a mouse model of in vivo sprouting lymphangiogenesis, has revealed a role for Notch signaling in maintaining quiescence of the lymphatic vasculature by suppressing lymphatic endothelial cell sprouting.1 Inhibition of Notch signaling using a soluble Dll4-Fc protein, or a chemical inhibitor of Notch cleavage and activation, promoted lymphatic vascular sprouting induced by VEGF in vitro and in vivo (see figure). Interestingly, in an in vitro sprouting assay, Notch inhibition selectively promoted sprouting induced by VEGF and low doses of VEGF-C, but at high doses of VEGF-C, lymphatic vascular sprouting was not further increased by Notch pathway inhibition. This observation goes some way toward explaining the enhanced effects of VEGF-C compared with VEGF in the promotion of lymphangiogenesis, although additional factors such as cell surface VEGFR-3 levels on lymphatic endothelial cells, together with the levels of other factors involved in Notch, VEGF and VEGF-C signal transduction may contribute to this effect. An intriguing finding of this work is that VEGFR-2 inhibition in vitro,
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