lodged and will decrease the likelihood that a branch will reopen since the proximal HbS red cells will not be able to escape before becoming rigid.

In vessels larger than capillaries, blood flow is determined in part by the bulk viscosity of blood which is affected by the viscous properties of red cells in plasma and, importantly, by hematocrit. Bulk viscosity is also a function of shear, which is a function of flow rate in a vessel. In low-shear environments such as post-capillary venules and some precapillary arterioles, disoid red cells form rouleaux (ie, stacks of red cells) that make the blood much more viscous (see figure). However, in high-shear areas such as the middle meningeal artery, rouleaux are dispersed by shear forces and blood viscosity is reduced. Adding sickle red cells to a suspension of normal cells increases the viscosity at high and low shear. With this in mind, consider the findings of Alexy and colleagues who studied the viscosity of mixtures of normal and sickle red cells in plasma at various shear levels. Their results of mixtures of normal and sickle red cells in plasma, and importantly, by blood which is affected by the viscous properties to approach normal. H-types of red blood cell mixtures in sickle cell disease are affected by the viscous properties to approach normal. HVR and flow in the middle meningeal artery similar or larger-sized vessels should be improved by transfusion. Certainly, transfusion significantly reduces stroke recurrence rates in SCD patients with larger artery disease and reduces transcerebral Doppler velocity. While the clinical outcome of transfusion is consistent with predictions made from in vitro rheology, the pathophysiology is certainly more complicated than implied herein. For example, the prediction assumes, perhaps incorrectly, that silent strokes are due to sickle-related vaso-occlusion. Similar silent stroke-like lesions occur in more than 50% of thalassemia intermedia patients and in normal subjects as well. Duration of exposure to high shear results in change in endothelial function which further complicates the picture. Current information does not allow us to determine the level of HbS necessary to protect against progression of cerebrovascular disease, in part because only the average HbS values but not the time-average values before events are known. However, from a practical standpoint, there is a progression of cerebrovascular disease in spite of chronic transfusion according to best practices at centers of excellence. The results of the silent infant transfusion study will thus be of critical importance and hopefully will shed more light on these issues. Clearly, transfusion is not a panacea and more work is needed to understand how transfusion works and how to prevent the progressive vascular disease seen in sickle cell patients. 

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**MYELOID NEOPLASIA**

Comment on Guo et al, page 936

**Families get mobilized to treat AML**

Stephen Mackinnon and Ronjon Chakraverty

In this issue of *Blood*, Guo and colleagues from Beijing report that infusion of human leukocyte antigen (HLA)-mismatched peripheral blood stem cells (PBSC) improves survival in elderly patients with acute myeloid leukemia (AML) when used in combination with chemotherapy. Despite rapid advances in our understanding of the biology of AML, most patients with this disease continue to have poor outcomes with chemotherapy. This is particularly so in individuals older than 60 years of age, who constitute the majority of patients and...
where overall survival remains stubbornly low (reviewed in Estey5). Higher rates of poor-risk disease are linked to more chemotherapy resistance. Additional comorbidities, which are common in this age group, also lead to more treatment-related deaths. Thus, there continues to be an urgent need to improve treatment for elderly patients with AML.

One important advance has been the use of reduced-intensity conditioned allogeneic stem cell transplantation with the intent to exploit the graft-versus-leukemia (GVL) effect mediated by donor immune cells contained within the graft. Indeed, evidence from recent series suggests that this approach can overcome some of the therapeutic resistance of AML in older patients.6 However, individuals enrolled into such studies represent only a select group who are probably not representative of most elderly patients with AML. Furthermore, only patients with a suitably HLA-matched donor are eligible for this approach. The intention of Guo and colleagues was to devise a means of delivering immune antitumor effects without requiring that a patient undergo allogeneic stem cell transplantation.1 In their approach, patients would get standard chemotherapy followed by mobilized peripheral blood stem cells from a haploidentical-related donor. Unpublished preclinical data had suggested that this led to rapid hematopoietic recovery without durable donor engraftment and no graft-versus-host disease (GVHD).

In their study, 58 patients over 60 years of age with AML and without an HLA-identical sibling donor were randomized to receive either standard AML chemotherapy (mitoxantrone and cytarabine according to a “3 + 7” schedule) or the same treatment followed by infusion of granulocyte-colony stimulating factor (G-CSF)–mobilized PBSC from an HLA-mismatched relative.1 Patients achieving complete remission went on to receive consolidation with 2 further cycles of cytarabine with or without PBSC infusion (see figure). A single mobilized PBSC product was split into aliquots to be given during induction and consolidation. Over a third of the study population was over 70 years of age and as might be expected, there were a high proportion of patients with high-risk features including multilineage dysplasia (26%) and/or poor-risk cytogenetics (43%). Both the PBSC and control group were well balanced in terms of their risk profile. The key findings were a significantly higher complete remission rate in the PBSC group (80% vs 43%) and importantly, a higher 2-year probability of overall survival (39% vs 10%). Although hematopoietic recovery was more rapid in the PBSC group, this could not be explained by engraftment of donor hematopoietic stem cells. Associated with the lack of significant donor cell engraftment, GVHD did not occur. Although the study was small and selected for patients who were chemotherapy candidates, the randomized design was likely to have reduced the potential for bias that affects other single-arm studies of new treatments in AML. This study is therefore a potentially important advance in a clinical arena where improvements in outcome have been exceptionally difficult to achieve.

Over the past decade, several other groups have also performed early-phase studies involving transfer of donor leukocytes to induce graft-versus-tumor responses in patients with solid or hematologic malignancies without prior allogeneic stem cell transplantation.1,6 The protocols involved have varied with the cells transferred derived from steady-state or mobilized aphereses, irradiated or nonirradiated, from HLA-identical or mismatched donors and administered with no or some level of immunosuppressive treatment. In several of these studies, objective tumor responses were observed, often in association with engraftment of transferred cells and the development of GVHD or, unfortunately, in some cases, aplasia as is seen in transfusion-associated GVHD.1 Engraftment of donor cells and GVHD is more likely in patients who have had extensive prior treatment, particularly prior autologous transplantation.3 A striking difference between these studies and the Beijing trial is that in the latter case, engraftment of donor cells was minimal and no GVHD was observed.1 This is likely because patients undergoing anthracycline-based chemotherapy for AML still retain substantial host cellular immunity sufficient to rapidly reject the majority of infused cells.7

This of course then leaves the question of what mechanisms underlie the accelerated hematopoietic recovery and improved leukemia responsiveness even though the infused cells fail to survive. Are mobilized PBSC required for the effect or would cells derived from steady-state aphereses be just as effective? What are the cellular constituents within the infused product that mediate the positive effects? Dissection of potential mechanisms will require detailed preclinical experiments, but the failure to observe T-cell engraftment makes it unlikely that the increased responses observed in this study relate to a “classical” GVL response. It is of interest that in other contexts, rejection of donor hematopoietic cells has been linked to reduced rates of relapse.8,9 For example, after dual cord transplantation, where only 1 cord unit engrafts while the other is rejected, rates of acute leukemia relapse are significantly lower than after single-cord transplantation.4 Durable responses in patients with previously refractory disease have also been reported after allogeneic transplantation despite early rejection of the donor graft.2 Together, these findings suggest the possibility that the rejection response itself is important in mediating antileukemia effects. Indeed, animal experiments deliberately simulating the process of rejection of donor hematopoietic cells have demonstrated enhancement of specific antitumor responses that involve interferon-γ and both host (eg, CD4 and invariant natural killer T cells) and donor (eg, CD8 T cells) immune cells.10,11

It will be important for future studies to evaluate this novel approach in larger numbers of patients, including in other age groups. Caution, however, should be exercised in patients at risk of transfusion-associated GVHD, for example, those treated with purine analogues or those who have received prior autologous stem cell transplantation, where the risks of GVHD might be greater. For the same reason, this approach would also be inadvisable where the donor is homozygous for an HLA haplotype that is shared by the recipient. These concerns aside, the study of the Beijing
group could ultimately help to open another front in the battle to cure AML.

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Comment on Ulyanova et al, page 975

Anchoring at an island to relieve stress

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In this issue of Blood, Ulyanova and colleagues present a systematic analysis of the role of β1 integrins in erythropoiesis and demonstrate that αvβ1 integrin is a critical requirement for mounting successful stress erythropoiesis.1

Stress erythropoiesis is increasingly recognized as more than simply an expansion of steady-state erythropoiesis. Rather, it is a process arising from unique progenitor cells with distinct quantitative and qualitative cytokine requirements. Increased erythropoietin (Epo) levels resulting from kidney hypoxia due to anemia, initiate the response of stress erythropoiesis. A progenitor, named stress burst-forming unit-erythroid (BFU-E) and found in the spleen but not in bone marrow of mice subjected to erythropoietic stress, has been identified as able to produce erythroid colonies when exposed to high Epo concentrations with no additional in vitro requirement for stem cell factor (SCF) or interleukin-3, in contrast to bone marrow BFU-E.2 Stress BFU-E are stimulated to expand by bone morphogenetic protein 4 (BMP4) produced in the spleen in response to hypoxia. Erythropoietic stress leads to mobilization of bone marrow progenitors which home to the spleen and differentiate into stress BFU-E, under the influence of BMP4 and Hedgehog signaling.3 Nevertheless, homeostatic erythropoietic pathways like SCF–c-kit and glucocorticoid receptor–mediated signaling are also vital for successful stress erythropoietic response in vivo.4,5 Stress erythropoiesis has been shown to be impaired in gene-targeted mouse models where steady-state erythropoiesis is fairly normal, like in mice with deficiency of growth arrest–specific gene 6 (Gas6)6 or conditional deletion of focal adhesion kinase (FAK).7 On the other hand, stress erythropoiesis in the splenic microenvironment can be successful in cases where homeostatic erythropoiesis in the bone marrow is pathologic, like in deficiency of Rac1 and Rac2 GTPases.8

The interactions of erythroid progenitors and precursors with macrophages and extracellular matrix components such as fibronectin and laminin within erythroblastic islands are critical for optimal proliferation, survival, differentiation, and terminal maturation into red blood cells.9 αvβ1 and αvβ3 integrins are the main erythroid cell receptors that interact with fibronectin. Whereas αvβ1 mediates adhesion to several sites on fibronectin, αvβ3 binds predominantly to the Arg-Gly-Asp (RGD) domain. αv expression is noted to be higher in early erythroblasts, whereas α3 is widely expressed throughout nucleated erythroid cells. VCAM-1 represents the major and preferred ligand for αvβ1 in stroma cells. The role of these integrins and their ligands in erythropoiesis has been extensively studied by in vitro and in vivo assays, frequently with contradictory results. In this issue, Ulyanova and colleagues offer a resolution to these conflicts, using a systematic in vivo genetic approach to analyze the erythropoietic phenotype after conditional deletion of β1, αv, or VCAM-1 in hematopoietic tissues of adult mice.1 β1 deletion produces β1Δ/Δ mice which demonstrate deficiency of αvβ3 and compensatory over-expression of αvβ1 in erythroid cells. αvβ3Δ mice are deficient in αvβ1 and αvβ3; (but not
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