players in chemokine-induced microvillar remodeling and leads to interesting ideas about how the cytoskeleton controls adhesion and extravasation. However, additional work will be needed before this process is fully understood. Brown and coworkers show that ERM dephosphorylation is inhibited by treatment with calyculin A and basal phosphorylation is decreased by treatment with staurosporine and that these drugs lead to changes in microvillar structure. However, the relevant kinases and phosphatases are yet to be identified. A second outstanding issue arises from the finding that the constitutively active moesin mutant delays but cannot prevent dissolution of microvilli in response to chemokine treatment. This finding indicates that phospho-ERM proteins are not sufficient to maintain microvilli, implicating other microvillar constituents in this process.

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1. Stein JV, Cheng G, Stockton BM, Fors BP, Butcher EC, von Andrian UH. L-selectin-mediated leukocyte adhesion in vivo: microvillous distribution determines tethering efficiency, but not rolling velocity. J Exp Med. 1999;189:37-50.
2. Alon R, Feigelson S. From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at subsecond contacts. Semin Immunol. 2002;14:93-104.
3. Bretscher A, Chambers D, Nguyen R, Reczek D. ERM-Merlin and EBP50 protein families in plasma membrane organization and function. Annu Rev Cell Dev Biol. 2000;16:113-143.

Transplantation for myelofibrosis: yes! But for whom?

Myelofibrosis is a rare disease characterized by progressive obliteration of the marrow space by fibrosis, extramedullary hematopoiesis, splenomegaly, and presence of immature hematopoietic cells in the blood. It is a clonal disorder of pluripotent hematopoietic stem cells that release fibrogenic cytokines and colonize extramedullary sites. Hence it is potentially curable if the abnormal clone can be eradicated and replaced by donor cells. Patients with myelofibrosis were long thought to be poor candidates for transplantation because of a perceived risk of graft failure related to the lack of marrow space. Several previous smaller studies and retrospective surveys confirmed that transplantation could induce durable remissions in at least some patients with myelofibrosis. The study by Deeg and colleagues (page 3912), a single-institution series of 56 patients accrued over a 22-year period, firmly establishes transplantation as a curative procedure.

But the report generates as many questions as it answers. The most important ones are for whom to recommend this treatment and when to recommend it. Myelofibrosis is a pleomorphic disorder with a highly variable clinical course. Deeg et al find that patients with lower Dupriez scores (a prognostic score based on white blood cell count and hemoglobin level), higher platelet counts, a lesser degree of marrow fibrosis, and a normal karyotype fared better than patients with more advanced disease. Younger age was also associated with a better outcome. But, as discussed by Barosi,[1] normal karyotype, low Dupriez score, and younger age also predict an indolent natural history. Younger patients in particular often do remarkably well. Cervantes et al[2] found a median survival of 128 months in a group of 121 patients younger than 55 years old. It is difficult to escape the conclusion that transplantation for myelofibrosis has the best results in the subgroup of patients with the most favorable presentation. The dilemma for such patients and their physicians will be whether to choose transplantation with a definite likelihood of cure, but also a considerable risk of treatment-related mortality, or to take a conservative approach with a considerable long-term risk of death from the disease. This dilemma is quite familiar to hematologists when discussing treatment recommendations for a more common disease, chronic myeloid leukemia. Ultimately, it can only be resolved by the patient who is well-informed about risks and benefits of all treatment options. One can only hope that more accurate risk classification schemes will make future decisions more straightforward. In this regard, the recently reported association between peripheral blood CD34 number and prognosis of myelofibrosis may be of interest.[3]

For the older patient and the patient with unfavorable prognostic features, the dilemma is different but no less difficult. At least 50% of such patients died, usually from transplantation-related complications. Transplantation as currently performed is at best marginally effective in such patients and there is a need for better-tolerated yet effective conditioning regimens. Whether the new generation of nonmyeloablative regimens will fulfill that promise can only be established through continued accrual and additional follow-up.[4]

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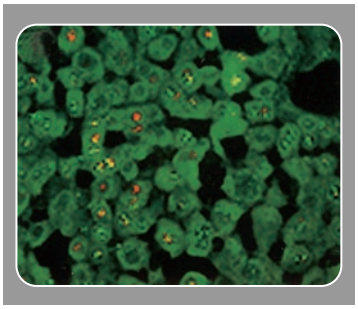
1. Barosi G. Myelofibrosis with myeloid metaplasia: diagnostic definition and prognostic classification for clinical studies and treatment guidelines. J Clin Oncol. 1999;17:2954-2970.
2. Cervantes F, Barosi G, Demory JL, et al. Myelofibrosis with myeloid metaplasia in young individuals: disease characteristics, prognostic factors and identification of risk groups. Br J Haematol. 1998;102:684-690.
3. Barosi G, Viarengo G, Pecci A, et al. Diagnostic and clinical relevance of the number of circulating CD34(+) cells in myelofibrosis with myeloid metaplasia. Blood. 2001;98:3249-3255.
4. Devine SM, Hoffman R, Verma A, et al. Allogeneic blood cell transplantation following reduced-intensity conditioning is effective therapy for older patients with myelofibrosis with myeloid metaplasia. Blood. 2002;99:2255-2258.

Novel therapies for hemophilia A

Hemophilia A is the most common severe bleeding disorder, with an incidence of 1 in 5000 males. It is due to deficiency of factor VIII (FVIII), which is a 280-kDa protein that associates with an even larger carrier protein, von Willebrand factor. Intravenous administration of FVIII on an as-needed or prophylactic basis is inconvenient, expensive, and still carries some risk of transmission of infections. Permanent correction of the bleeding diathesis would be an important advance for patients and their families. This issue reports 2 novel approaches to treating hemophilia A. Scallan and colleagues (page 3919) used liver-directed gene

therapy with 2 different adeno-associated virus (AAV) vectors to separately express the light and heavy chains of FVIII. The 2 chains then associated intracellularly to generate a functional protein that was secreted. Yarovoi and colleagues (page 4006) engineered platelets to express FVIII, which was released from α-granules and led to hemostasis at the site of injury.

Although gene therapy has made important advances in recent years, its use for hemophilia A has had limited success.[1,2] Adenoviral vectors can result in high-level expression, but this has been transient in large animals. Intravenous injection of a retroviral vector did not achieve appreciable



levels of expression in humans, which was likely due to low levels of gene transfer in these adults. One trial that involved transplantation of genetically modified fibroblasts into the peritoneum resulted in low levels of expression in some patients.[3] However, this approach was laborious, and the effect disappeared within 1 year.

AAV vectors show promise due to their ability to stably transfer genes into hepatocytes of adults but have a limited size capacity. Since the minimal size of the FVIII cDNA is 4.1 kb after eliminating the untranslated regions and sequences encoding the unnecessary B domain, the group at Avigen headed by Linda Couto generated 2 vectors that expressed the canine light and heavy chains separately from a strong liver-specific promoter. The 2 chains associated inside hepatocytes to generate functional FVIII that was secreted into blood, resulting in plasma FVIII activity that was 16-fold that of normal human plasma and prevention of bleeding in hemophilia A mice. Expression was higher than: (1) that achieved with trans-splicing approaches; (2) use of a very short promoter to drive expression of a complete FVIII cDNA; or (3) coexpression of both chains from separate AAV vectors with weaker promoters. However, it is unclear if the excess light chains found in blood might have adverse effects, and it will be necessary to demonstrate efficacy and safety in a large animal model prior to testing this approach in humans.

The article by Yarovoi et al from the laboratory of Mortimer Poncz reports that expression of FVIII in platelets can reduce bleeding manifestations in hemophilia A. They used the glycoprotein Ibα promoter to restrict expression of FVIII to platelets of transgenic mice, where it was probably localized to the α-granules. Although FVIII was undetectable in blood, the platelets released FVIII locally at the site of bleeding. This allowed hemostasis to occur, albeit with varying efficiency in different situations. The main advantage here is that hemostasis might be feasible even in patients with inhibitors if the FVIII can function before the antibodies inhibit its activity. Although these results are encouraging, 2 obstacles need to be overcome before this approach can be used to treat humans with hemophilia. First, it has proven difficult to transduce human pluripotent hematopoietic stem cells, and appropriate expression in platelets will need to be achieved from whatever vector is used to transfer the gene. Second, there are concerns about the risks of transferring integrating vectors into hematopoietic cells. Despite these caveats, both these studies raise hopes that long-lasting gene therapy for hemophilia A may be on the horizon.

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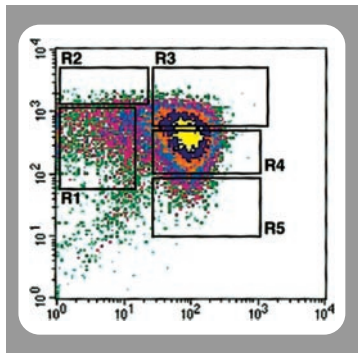
1. High KA. Gene transfer as an approach to treating hemophilia. Semin Thromb Hemost. 2003;29:107-120.
2. Walsh CE. Gene therapy progress and prospects: gene therapy for the hemophilias. Gene Ther. 2003;10:999-1003.
3. Roth DA, Tawa NE Jr, O'Brien JM, Treco DA, Selden RF. Nonviral transfer of the gene encoding coagulation factor VIII in patients with severe hemophilia A. N Engl J Med. 2001;344:1735-1742.

Ras signaling in erythropoiesis: going with the flow

The production of normal numbers of normal blood cells is a finely coordinated process requiring appropriate input from the environment (growth factors, nutrients such as iron or vitamin B_{12}, etc) and a balance of cellular differentiation and proliferation at the appropriate times. Perhaps the most complete way to evaluate this process in vitro is with assays of hematopoietic colony formation by primary cells. The development of progressively more refined assays of hematopoiesis and the insights gained through these assays (for example, in evaluating requirements for hematopoietic growth factors) are among the stellar achievements of experimental hematology. However, it may be difficult to distinguish effects on proliferation from effects on differentiation using colony-formation assays alone, and it may also be difficult to accurately identify the stages of development at which these effects occur.

In an interesting paper, Zhang and colleagues (page 3938) employ a novel means of sorting out stage-specific effects in erythropoiesis by flow cytometry to clarify the role of Ras signaling in this process. Based



on observations they had reported previously,[1] the authors used expression of erythroid-specific (TER119) and non–erythroid-specific (CD71) transferrin receptor to sort murine fetal liver cells into populations that corresponded to erythroid colony-forming units (CFU-Es) and to the various stages of erythroid precursors, from proerythroblasts