deletions in the ALAS2 gene lead to gain of function and cause X-linked dominant protoporphyria without anemia or iron overload. *Am J Hum Genet.* 2008;83(3):408–414.

6. Leitch CC, Zaghoul NA, Davis EE, et al. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nature Genet*. 2008;40(4):443–448.

7. Kan YW, Nathan DG. Mild thalassemia: the result of interactions of alpha and beta thalassemia genes. *J Clin Invest*. 1970;49(4):635–642.

8. Musumeci S, Schiuro G, Pizzarelli G, Fischer A, Russo G. Thalassaemia of intermediate severity resulting from the interaction between alpha- and beta-thalassaemia. *J Med Genet*. 1978;15(6):448–451.

**Comment on Luft et al, page 1685**

**ANG2/VEGF in steroid-refractory GVHD**

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Clinicians typically assume that steroid–refractory acute GVHD can be overcome only by increasing the intensity of immunosuppressive treatment, but in this issue of *Blood* Luft et al suggest that more effective approaches might emerge from an unexpected direction.1

| Biomarker            | Interpretation            | Change after Immunosuppressive Treatment |
|----------------------|---------------------------|------------------------------------------|
| Soluble Fas          | T cell attack             | Decreased-Decreased                      |
| Cytokeratin-18       | Epithelial injury         | Decreased-Increased                      |
| fragment             |                           |                                          |
| Soluble thrombomodulin| Endothelial injury        | No change-Increased                      |
| VEGF                 | Endothelial protection    | No change-Decreased                      |

**Biomarker changes after immunosuppressive treatment in steroid-sensitive and steroid-refractory acute GVHD.**

Steroid–refractory acute GVHD poses one of the most vexing clinical problems faced by physicians involved in hematopoietic cell transplantation. We have very little information regarding the pathogenesis of this problem, and clinical experience indicates that intensified immunosuppression does not usually provide an effective remedy.2 In a recent case at our center, for example, a patient was treated sequentially with infliximab, mycophenolate mofetil, extracorporeal photopheresis, sirolimus, and both equine and rabbit antithymocyte globulin in rapid succession within a period of 8 weeks, all to no avail.

Luft and colleagues now offer a novel perspective and some thought-provoking ideas regarding the pathogenesis of steroid–refractory GVHD. They analyzed biomarkers indicating T-cell attack, epithelial injury, and endothelial injury in serum samples collected serially from 23 patients with steroid–sensitive acute GVHD and 25 patients with steroid–refractory GVHD, using the initial immunosuppressive treatment of GVHD as the time anchor (see figure). The concentration of biomarkers indicating T-cell attack, including soluble Fas ligand, among others, decreased in both groups after treatment was started. Epithelial injury measured according to the concentration of cytokeratin-18 fragments3 decreased after immunosuppressive treatment in the steroid–sensitive group but increased in the steroid–refractory group. Endothelial injury measured according to the concentration of soluble thrombomodulin-1 also increased after immunosuppressive treatment in the steroid–refractory group but not in the steroid–sensitive group.

Taken together, these observations support previous results indicating that GVHD involves an attack on endothelial cells as well as epithelial cells.4,5 but earlier studies could not explain how endothelial injury emerges while T-cell attack is subsiding during immunosuppressive treatment. Luft and colleagues showed that the steroid–responsive and steroid–refractory groups differed in 2 key ways. First, concentrations of angiopoietin–2 (ANG2) were higher in the steroid–refractory group before transplantation, at the beginning of immunosuppressive treatment, and during subsequent follow-up. ANG2 makes endothelial cells more sensitive to inflammatory cytokines produced by alloactivated donor T cells.6 These cytokines cause endothelial cells to lose expression of thrombomodulin, a molecule that protects cells by catalyzing activation of protein C and by inhibiting apoptosis.7 Endothelial cells with reduced expression of thrombomodulin are therefore more vulnerable to damage after stress. The second key observation is that vascular endothelial-derived growth factor (VEGF) concentrations decreased after immunosuppressive treatment in the steroid–refractory group but not in the steroid–sensitive group. VEGF inhibits ANG2, and in the absence of VEGF, ANG2 causes endothelial cell death.8

At first glance, it might seem plausible that the increased concentrations of thrombomodulin–1 and ANG2 in the serum represent results of steroid–refractory GVHD, and not the cause. As noted by Luft et al, however, the observation that ANG2 concentrations were higher in the steroid–refractory group even before transplantation supports their hypothesis that baseline endothelial vulnerability has a causal role in the pathogenesis of steroid–refractory acute GVHD. If correct, this hypothesis would imply that efforts to control steroid refractory GVHD by increasing the intensity of immunosuppressive treatment are aimed at the wrong target.

As always, unanswered questions remain, and more work in this new and potentially exciting direction is needed. What causes the increased ANG2 concentration at baseline in patients at risk of steroid–refractory GVHD? How is endothelial injury linked to continued epithelial injury in the absence of sustained attack by T cells in patients with steroid–refractory GVHD? What causes VEGF concentrations to decrease during immunosuppressive treatment in patients with steroid–refractory GVHD? What other factors influence the vulnerability of endothelial cells to injury after hematopoietic cell transplantation? The novel observations by Luft and colleagues, together with eagerly awaited answers to these questions, provide hope that insights...
from vascular biology might yield long-awaited improvements in treatment for steroid-refractory GVHD.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
1. Laft T, Dietrich S, Falk C, et al. Steroid-refractory GVHD: T-cell attack within a vulnerable endothelial system. Blood. 2011;118(6):1685-1692.
2. Deeg HJ. How I treat refractory acute GVHD. Blood. 2007;109(10):4119-4126.
3. Laft T, Conzelmann M, Benner A, et al. Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. Blood. 2007;110(13):4535-4542.
4. Dumler JS, Beschorner WE, Farmer ER, DiGennaro KA, Saral R, Santos GW. Endothelial-cell injury in cutaneous graft-versus-host disease. Am J Path. 1989;135(6):1097-1103.
5. Holler E, Kolb HJ, Hiller E, et al. Microangiopathy in patients on cyclosporine prophylaxis who developed acute graft-versus-host disease after HLA-identical bone marrow transplantation. Blood. 1989;73(7):2018-2024.
6. Fiedler U, Reit Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med. 2006;12(2):235-239.
7. Isermann B, Sood R, Pawlinski R, et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. Nat Med. 2003;9(3):331-337.
8. Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. Proc Natl Acad Sci U S A. 2002;99(17):11205-11210.

Comment on Daikeler et al, page 1693

HSCT for AID: entering prime time?

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How would immune reconstitution gone wrong as a consequence of treatment be detected when the patient received a transplant to treat an autoimmune disorder? The article by Daikeler et al in this issue of Blood demonstrates how complicated this question might be.1

Autoimmune diseases (AIDs) affect about 5% of the population. As systemic therapies are rarely curative for patients with severe, life-threatening forms of AIDs, there is a need for better treatment. During the past 15 years, HSCT has been developed as a potential clinical therapy. Durable, progression-free survivals of approximately 50% after autologous HSCT are now reported.2 Clinical studies of posttransplantation immunologic recovery have demonstrated a renewed T- and B-cell repertoire consistent with “resetting” of the immune system, supporting the fundamental hypothesis for the use of HSCT.3,4 Since 1996, international transplant registries, which capture a large portion of world transplantation activity, have registered patients receiving transplants for AIDs. More than 1300 patients by the European Blood and Marrow Transplant Group (EBMT) and almost 500 by the Center for International Blood and Marrow Transplant Research (CIBMTR) have been reported.3,4 Most of these transplants (~ 90%) are autologous procedures. The major indications were for multiple sclerosis, systemic sclerosis, or systemic lupus, and less commonly for other indications such as Crohn disease, type I diabetes, and juvenile idiopathic arthritis. Results have demonstrated a significant increase in safety and a decrease in transplant-related mortality over time, largely driven by improved supportive care, better patient selection, and increasing transplant center experience.2 While allogeneic transplantations for these indications remain rare, several randomized trials of autologous HSCT are in progress or being planned in Europe and the United States. The reports of the outcomes for autologous HSCT compared with conventional therapy are eagerly awaited.7 Over the past few years, more biologic therapies for AIDs have been developed, but these may lack the curative potential of transplantation. In addition, the use of biologic therapies raises issues of cumulative costs and morbidity and mortality related to prolonged therapy.8 Thus the prospect of having the therapeutic option of a one-time intervention with the potential for curative or durable, treatment-free survival is very attractive. In fact, this is a reasonable time to ask, “Is autologous HSCT for severe autoimmune disease entering prime time?” The relatively small numbers of such transplants performed, ongoing debates over the short-term risk/benefit ratio in the setting of nonmalignant disease, and the availability of competing nontransplantation therapies dictate that this procedure should be limited to controlled clinical trials.

This report by Daikeler et al illustrates the need for careful and well-planned development in this field.1 The authors report the largest study to date describing the incidence and risk factors for new, secondary autoimmune disease after autologous HSCT for AIDs. They retrospectively analyzed 347 patients with AIDs reported to the EBMT from 1995 to 2009 for whom the follow-up information was available. Twenty-nine patients developed at least 1 secondary AID at a median of 21.9 (0.6-49) months after HSCT, with a cumulative incidence of 9.8% at 5 years. Consistent with previous case reports, the majority of these secondary AIDs are organ-specific involving thyroid (n = 15), cytopenias (n = 6), acquired hemophilia (n = 3), and others. Of interest, 3 of 16 patients developed secondary AIDs after allogeneic transplantation. Risk factors for the development of a secondary AID after autologous HSCT included an initial diagnosis of systemic lupus erythematosus (hazard ratio [HR] 3.21), the association of...
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