aPC have become the focus of investigation by numerous groups. Indeed, the role of aPC and the EPCR in the context of cancer biology, innate immunity, and malaria pathogenesis remains a source of ongoing investigation.[2,3]

In keeping with these observations, aPC has previously been shown by Isermann et al[4] to prevent endothelial and podocyte apoptosis and thus plays a protective role against the development of DKD. DKD is a major cause of morbidity and mortality in patients with diabetes and represents the leading cause of end-stage renal failure globally.[5] Importantly, one of the earliest signs of DKD is the loss of the specialized epithelial cells (podocytes) in the renal glomerulus.[6] Therefore, greater understanding of the molecular mechanisms underpinning the premature loss of podocytes seen in diabetic nephropathy may hold the key to developing novel therapeutic strategies. Intriguingly, in addition to aPC, insulin has also been demonstrated to be a key regulator of podocyte function because mice specifically lacking the insulin receptor on podocytes develop a phenotype similar to DKD in the absence of hyperglycemia.[7]

In their study, by using multiple transgenic mouse lines, Madhusudhan et al link these 2 seminal observations by demonstrating that aPC initiates cytoprotective signaling in podocytes in a pattern that is very similar to that described for insulin. Indeed, by using a streptozotocin mouse model of diabetes (considered to be a model of type 1 diabetes), the authors show that mice with impaired aPC activation displayed exacerbated DKD, which was associated with a more prominent maladaptive renal unfolded protein response (UPR), predominantly characterized by impaired nuclear translocation of spliced-X-box binding protein-1 (sXBP1). In contrast, these effects could be rescued by restoration of aPC levels, whereas high-expressing aPC mice were protected. Therefore, to test the effect of aPC on the development of diabetic nephropathy in the absence of insulin signaling, mice lacking the insulin receptor specifically on podocytes were used. Insulin receptor–deficient mice developed overt DKD but, strikingly, transgenic overexpression or exogenous administration of aPC protected these mice from developing DKD and restored nuclear levels of sXBP1. These findings were recapitulated in a model of type 2 diabetes (db/db mice), thus confirming that aPC can confer potent nephroprotective effects and restore the UPR in the setting of insulin deficiency or resistance. Complementary in vitro assays demonstrated that aPC or insulin promote the nuclear translocation of sXBP1 via heterodimerization with p85α/p85β in podocytes. Consistent with this postulate, the renoprotective effect of high aPC expression was ameliorated in the context of XBP1, p85α, or p85β deficiency. Thus, despite having distinct receptors, podocytes share common downstream signaling events in response to either stimulus, which act to regulate the cytoprotective ER transcription factor sXBP1 (see figure).

These findings have broad-ranging implications. Much enthusiasm remains for the development of novel anticoagulants. Foremost among these are the inhibitors of FXII and FXI in preclinical and early-phase development. These approaches do not seem to cause bleeding and have been shown to confer benefit in preclinical models in a range of inflammatory conditions such as multiple sclerosis and atherosclerosis.[8] Likewise, there is now a plethora of new agents in development for the treatment of hemophilia, which include inhibitors of aPC and small interfering RNAs that knock down the expression of antithrombin.[9] Whether such approaches are associated with any unforeseen metabolic effects remains to be explored. In this context, it is intriguing that coagulation signaling, via tissue factor–FVIIa and the PAR2 receptor, promotes weight gain, insulin resistance, and adipose inflammation.[10] Conversely, given the global diabetes and obesity epidemic, the ability to potentially bypass the effects of insulin resistance or deficiency by targeting common downstream signaling pathways may open new therapeutic avenues that could represent novel approaches to reduce the huge burden of diabetes complications seen globally, and in particular, a major cause of morbidity and premature mortality, DKD.

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● ● ● TRANSPLANTATION

Comment on Miot et al, page 1456

Transplant for NEMO: this and much, much more

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In this issue of *Blood*, Miot et al report encouraging results in a retrospective analysis of hematopoietic stem cell transplantation (HSCT) in 29 patients with hypomorphic mutations in the *IKBKG* gene encoding nuclear factor κB essential modulator (NEMO).[1]

Several important nuggets can be mined from the results in this heterogeneous cohort of patients. First, the overall survival was a respectful 74%. Second, preexisting mycobacterial infections and colitis were associated with poor clinical outcome. Third, there was a trend toward better disease-free survival with a nonmyeloablative conditioning regimen. Thus, this report contributes to the important question of when and how to transplant these patients.

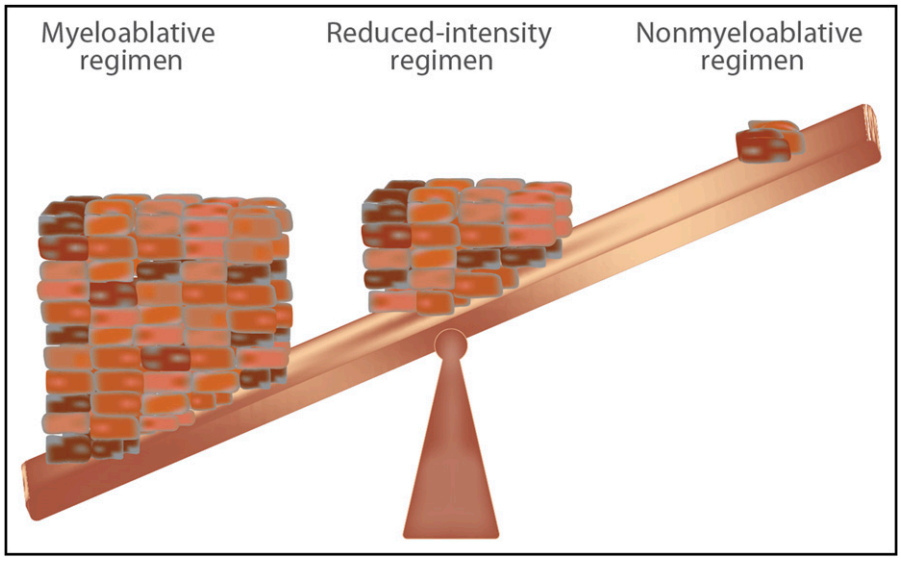
Perhaps equally important, this study brings up all the questions that confront the issue of HSCT for patients with primary immunodeficiency diseases (PIDs): the patient's genotypes were quite heterogeneous, with 23 different mutations in the 29 patients; the conditioning regimen and donor sources were variable; and the extent of donor chimerism required to reverse the phenotype was not clear. Finally, a significant proportion of patients experienced serious complications, including graft failure, graft-versus host-disease (GVHD), persistence or even de novo development of severe inflammatory bowel disease, serious infections, and conditioning regimen–associated toxicities.

Distilling the key ingredients responsible for successful HSCT for PIDs represents a challenging task. Nevertheless, for the field to progress, some attempt must be made to identify and formulate the rules of engagement for HSCT in this setting. First, the natural history of the disease should determine whether and when to proceed to HSCT. For instance, X-linked severe combined immunodeficiency disease (X-SCID) is typically fatal in the first years of life, and the best results are achieved with HSCT in the first 3 months of life.[2] In contrast, patients with mutations in GATA2 may remain asymptomatic into old age; thus, HSCT can be deferred until patients develop symptoms or signs of disease.[3] In the study by Miot et al, age at transplantation had no impact on survival, indicating that other variables play a more important role. In the case of NEMO deficiency, HSCT has often been reserved for patients with serious clinical complications, and yet this study demonstrates that the presence of mycobacterial disease and colitis at the time of transplantation may represent negative risk factors for a successful HSCT outcome of the procedure. This observation should set the benchmarks for future studies aimed at better defining how to control infections and inflammation pretransplant. It will also be important to assess whether the use of more aggressive regimens to dampen gut inflammation before transplant may be beneficial. The role of abnormalities in the microbiome should also be investigated in greater detail, and interventions aimed at improving the composition of the gut flora should be considered.

The second issue in HSCT for PIDs revolves around the level of chimerism and the leukocyte subset–specific chimerism necessary to reverse the disease phenotype. In some PIDs, this is known, and in some PIDs, this remains unclear. For instance, in canine models of leukocyte adhesion deficiency type 1 (LAD-1), even 5% of $CD18^+$ neutrophils results in reversal of the phenotype.[4] If the donor cells have a competitive advantage, such as normal donor T cells in X-SCID, these cells should accumulate to normal levels over time resulting in reversal of the phenotype.[2] Skewed X-chromosome inactivation has been reported in multiple blood lineages from most carrier females of NEMO deficiency suggesting selective advantage for cells with normal NEMO function.[5] However, random X-inactivation has been reported in a few carrier females.[6]

Possibly no question divides the field of HSCT for PIDs more than the third issue: the type of pretransplant conditioning to use. The intensity of conditioning for PIDs prior to HSCT ranges from immunosuppressive only to nonmyeloablative, to reduced intensity, to myeloablative. The level of conditioning is influenced by whether the T-cell arm of the immune system is intact. If the T-cell compartment is either depleted (SCID) or functionally defective, no conditioning or a reduced-intensity regimen may be effective in enabling engraftment. However, if the T-cell compartment is intact (eg, chronic granulomatous disease[7] or GATA2 deficiency[8]), some degree of myeloablation is required for engraftment. Many studies are now addressing the optimal level of myeloablation and immunoablation in HSCT for different PIDs (see figure). Although statistical significance was not reached in the study by Miot et al, there was a clear trend toward improved survival among recipients of reduced-intensity conditioning. This may be a better choice, also considering that the vast majority of patients attained more than 90% donor chimerism.

The best type of donor and donor graft source for HSCT for PIDs represents the fourth challenge. Matched related donors are clearly the first choice. However, haploidentical related donors are currently neck and neck with matched unrelated donors in many HSCT scenarios.[9] In the 29 NEMO patients, 7 were matched related donors, 12 were matched unrelated donors, 8 were mismatched unrelated donors (mostly umbilical cord blood), and only 2 were haploidentical related donors.[1] Moreover, 4 of the 5 patients who received HSCT from a related carrier female donor developed complications post-HSCT, including



Intensity of pretransplant conditioning chemotherapy regimens required to facilitate engraftment but minimize toxicity for HSCT for PIDs. Professional illustration by Somersault18:24.

recurrent infections and persistence or de novo development of colitis. Additional studies are needed to establish whether HSCT from carrier females (and especially from symptomatic females) should be avoided in this disease.

Lastly, strategies to prevent GVHD are paramount in HSCT for PIDs because there is no advantage to GVHD where there is no preexisting malignancy, GATA2 deficiency representing an exception to this rule. NEMO patients had a 50% incidence of GVHD in this study. The latest development in GVHD prophylaxis uses posttransplant cyclophosphamide in matched related and unrelated donors, as well as haploidentical related donors.

The field of HSCT, and especially HSCT for PIDs, has come a long way in 50 years. Studies such as the report by Miot et al are critical for the field to advance.

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● ● ● TRANSPLANTATION

Comment on Krämer et al, page 1477

Are outcomes of allografts for CLL still relevant?

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In this issue of *Blood*, Krämer et al have provided a long-term update on the outcomes of patients enrolled in the German CLL Study Group CLL3X trial who underwent a matched related or unrelated allogeneic hematopoietic cell transplantation (allo-HCT) with a reduced-intensity fludarabine/alkylator-based approach for high-risk chronic lymphocytic leukemia (HR-CLL).[1]

Although it is inherently less aggressive than acute leukemia and is frequently associated with an indolent course, this common adult leukemia comprises a wide spectrum of disease activity. As demonstrated in the initial report of Dreger et al,[2] the strongest negative predictors for both progression-free survival (PFS) and overall survival (OS) in the study by Krämer et al were the use of alemtuzumab in the conditioning regimen and in refractory disease at the time of transplantation. Their results indicate a 20% non–relapse mortality (NRM) rate (9% for those without refractory disease or alemtuzumab treatment) and a 34% disease-free survival rate for all patients at 10 years. A landmark analysis of 32 patients who were alive and progression free at 6 years revealed a low rate of late relapse (18%), very low NRM (3%), and very high PFS (79%) for this population. These data indicate that long-term remissions with a low incidence of late relapse and low NRM are possible for HR-CLL patients, including those with TP53 abnormalities who did not seem to have adverse overall outcomes compared with the remainder of the group.

Additional analyses examined the important effect of minimal residual disease (MRD) and immune modulation on the eventual outcomes of these patients. Not surprisingly, persistent MRD at 1 year after transplantation was associated with poor outcomes, but patients who had MRD that was eradicated after withdrawal of immunosuppression actually did significantly better than those who became MRD negative immediately after transplantation and remained so at 1 year (see figure). This indicates the potency of the graft-versus-leukemia and immunotherapeutic effect of the donor graft and its critical role in long-term disease control. Unfortunately, this also correlated with a 73% incidence of some degree of chronic graft-versus-host disease (cGVHD), but the authors state that of those who remained in remission and alive at 6 years, 50% were not receiving immunosuppression therapy within 1 year of transplantation, indicating an extended period during which they remained in remission and free of cGVHD.

Perhaps the most important questions raised by the Krämer et al study relate to the current use of agents such as the B-cell receptor (bcr)/kinase inhibitor ibrutinib.[3] These agents have clearly demonstrated dramatic and sustained responses in both standard-risk and high-risk patients without the short-term cytopenias or secondary hematologic malignancies associated with conventional chemotherapy combinations such as fludarabine, cyclophosphamide, and rituximab.[4] The initial results with ibrutinib are promising, with 3-year PFS estimates of more than 90%, but complete remission remains the exception rather than the rule. The study also raises a question about long-term duration of response and the possibility of cure with these agents, even if they are administered continuously.[5] Over time, mutations in the bcr pathway lead to the development of ibrutinib resistance and clinical relapse which, in many cases, is associated with generalized drug resistance and rapid progression.[6] In addition