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KLF1 deficiency releases this repression, leading to elevated HbF.[7,8] CDA patients had a mutation in a conserved residue, E325K, in the second zinc finger of KLF1. Erythroid cells from affected CDA patients exhibited failure of terminal erythroid differentiation, with elevated fetal and embryonic globins, and absent CD44 and AQP1 expression.

A report from Sardinia revealed the rich complexity of gene regulation by KLF1 and its alteration by different mutations.[9] Erythrocytes from patients heterozygous for 1 of 2 KLF1 mutations, a nonsense mutation S270X or a missense mutation in a conserved residue K332Q, unexpectedly had only minimally elevated HbF, whereas erythrocytes from compound heterozygotes for these mutations had significantly elevated HbF and increased zinc protoporphyrin.

The pleiotrophic effects of KLF1 mutations can be attributed in part to quantitative and qualitative effects on KLF1 structure and function. KLF1 haploinsufficiency appears to be most commonly associated with the In(Lu) phenotype and with mild elevation in HbF. Missense mutations exert their influence by their location and the change they impose on KLF1 protein structure and function. Missense mutations may alter protein-protein or protein-DNA interactions. Study of KLF1 mutants in the second zinc finger revealed different mutations led to differing alterations in KLF1-DNA binding affinity.[10] Clinical severity did not correlate with alterations in binding affinities in all cases. Some variants may alter composition of KLF1-associated protein complexes, affecting transcriptional regulators, chromatin-associated factors, and other important regulatory proteins. Missense mutations could also alter KLF1 by impairing trafficking, altering sites of posttranslational modification, or producing a mutant protein acting as a dominant negative.

Detailed expression and genomics studies indicate that there are many KLF1 targets in erythroid cells, including regulators of cell metabolism, structural membrane proteins, heme synthesis enzymes, and proteins responding to oxidative stress. Different mutations may perturb interactions based on variability in the quality of associated KLF1-DNA binding sites and their interactions. Other potential sources of clinical variability in KLF1 mutant patients include inheritance of other genetic modifiers. In some but not all kindreds with mutant KLF1, variability in HbF has been attributed, in part, to genetic variability at the BCL11A locus. Genetic variability may reside in factors regulated by KLF1, or in factors that are dysregulated when there is altered KLF1 structure or function.

The influence of KLF1 on HbA_2 has not been examined in detail. Cultured erythroid cells from patients with elevated HbA_2 showed an increase in the expression of the absolute amount of δ-globin mRNA relative to β-globin mRNA as differentiation progressed, suggesting a delay in the switch from δ-globin to β-globin.[1] KLF1 or KLF1-regulated factors such as BCL11A could influence δ-globin gene interactions with the LCR and/or with local chromatin-associated proteins. The δ-globin gene could be a target of KLF1, although its promoter CACCC box is degenerate and no other KLF1 binding motifs are nearby.

The mysteries of KLF1 are now beginning to be revealed. When evaluating a patient with HPFH, atypical CDA, borderline HbA_2, In(Lu) blood group, or elevated zinc protoporphyrin, one should consider the presence of a KLF1 mutation. And now, the astute clinician will entertain defects of KLF1 when evaluating patients with otherwise unexplained erythrocyte disorders.

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Comment on Merindol et al, page 4480

Resurrecting the recalcitrant T-cell

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Delayed immune recovery after allogeneic hematopoietic stem cell transplantation (alloHSCT) is arguably the single greatest barrier to the successful use of this treatment modality.[1] In this issue of *Blood*, Merindol et al dissect the phenotypic and functional patterns of T-cell recovery in recipients of partially HLA-matched unrelated donor umbilical cord blood (UCB).[2]

While an effective treatment for selected malignant and nonmalignant disorders, alloHSCT is associated with a profound and prolonged immunodeficiency state. As a consequence, alloHSCT is associated with a high incidence of opportunistic infection. Furthermore, data suggest that a delay in immune recovery may also contribute the risk of relapse at least in some patient populations.[3] It is already known that the recovery of antigen specific T cells after alloHSCT is dependent on donor and host factors, like HLA match, T-cell depletion of the allograft, degree of tissue damage from the conditioning regimen, and the development of acute graft-versus-host disease (GVHD).[4] Based on studies in murine models, immune recovery after alloHSCT is characterized by an initial wave of thymic independent,

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peripherally expanded T cells that has a limited and skewed T-cell repertoire, with a second wave of thymic-educated donor HSC-derived T cells occurring months afterward, ultimately leading to the redevelopment of adaptive immunity in the transplant recipient.[5,6] Studies in the human have proven vastly more problematic. Variables in the transplant recipient, such as differences in patient age, graft source, HLA match, tissue damage, viral infections, and severity of GVHD, and the absence of validated assays of immune reconstitution have hindered progress in the field. The one thing that is clear is the presence of lymphocytes or any specific T-cell subset after transplant is itself not equivalent to restoration of T-cell immunity.[5]

Recent technologic advances have led to a better understanding of the T-cell response to pathogens and cancer. Detection of low frequency antigen-specific T cells is now possible using major histocompatibility complex (MHC) tetramer technology, intracellular cytokine staining of epitope-stimulated T cells, and IFN-γ ELISPOT assay for peptide-stimulated cells. In this study by Merindol et al the extent of $CD8^+$ T-cell reconstitution was interrogated in 26 recipients of HLA-$A2^+$ UCB at 1,2, 3, 6, 12, 18, 24, and 36 months after transplantation.[2] Using the HLA-A2–restricted Melan-A_{26-35} A27L peptide (A2/Melan-A), which is one of the few preimmune T-cell repertoires that can be studied in humans,[7] the authors observed a decline in the clonal diversity of Melan-A_{26-35}–specific $CD8^+$ T cells reaching its nadir at 3 months after transplantation. At 6 months, however, naive T cells emerged with increasing clonal diversity and polyfunctionality as measured in sorted tetramer-specific $CD8^+$ T cells. Expectedly during the period of acute homeostatic proliferation after transplant, high frequencies of programmed death-1 (PD-1)–expressing $CD8^+$ T cells were observed as clonal diversity declined. Interestingly, a higher frequency of PD-1^+CD8^+ T cells was associated with a higher risk of relapse.

However, in addition to providing us with a detailed characterization of the decline and restoration of T-cell subsets after umbilical cord blood transplantation, the current study by Merindol et al also unveiled a unique opportunity for analyzing and repairing the immune system. In this setting where 50% to 75% of patients receive UCB grafts that are mismatched at 2 HLA antigens,[8,9] it follows that we can potentially "select for" a specific, preferred HLA antigen, such as HLA-A0201, regardless of whether it is matched or mismatched with the recipient. Perhaps this is a new raison d'etre for UCB! First, we already know that we can select UCB units with a "desired" HLA antigen—we have already done this to track UCB-derived T regulatory cells.[10] Second, HLA-A0201 occurs with relatively high frequency in the general population (29.6% of Europeans, 12.4% African-Americans, 9.5% Asian-Pacific Islanders, and 19.4% of Hispanic haplotypes).[11] Considering the myriad of HLA-A0201 based reagents for dissecting the immune response after alloHSCT, one could envision a new roadmap for designing and testing novel interventions for enhancing T-cell recovery—proof-of-concept studies initially in HLA-A2 transgenic mice followed by clinical testing of the most promising candidate strategies specifically in recipients of HLA-$A0201^+$ UCB units that could occur at an unprecedented frequency. Studies could include the use of ex vivo–expanded T-progenitor cells, agents to enhance thymic function or protect/preserve thymus and lymph node architecture, in vitro priming to generate antigen-specific T cells specific for problematic viral pathogens like CMV, HHV6, BK, EBV, and adenovirus, or tolerance-inducing agents like T regulatory cells. The possibility of parallel studies in mouse and man and the capacity to enhance the proportion of patients transplanted with HLA $A2^+$ alloHSC could dramatically alter the pace of discovery and allow us to overcome this last great barrier that limits the successful use of alloHSCT.

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