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T cell-replete bone marrow and PBSCs as introduced by the Beijing transplant group of Huang.[8] Each patient received a graft from a family member sharing 1 HLA haplotype with the recipient, but they differed to a variable degree for the unshared haplotype. Based on their large patient number, they could identify donor characteristics that significantly impact the outcome of haploidentical transplantation with respect to the development of GVHD and the incidence of TRM and survival. First, they found that the degree of HLA matching or mismatching had no influence on TRM, acute or chronic GVHD, or survival. Younger donors (<30 years) were associated with a lower incidence of acute GVHD than older donors (>30 years), and younger male donors were associated with less TRM and better survival than older or female donors.

The degree of the donors' family relationship also had a significant impact on the outcome. Mother donors induced a higher risk of acute GVHD independent of the gender of the recipient and were associated with a higher rate of TRM and lower survival when the patient was a male recipient compared with a female recipient. The mothers' ages had no impact in this subgroup analysis, and father donors were better than mother donors especially for male recipients. In the child vs sibling donor analysis, offspring donors conferred a lower risk of acute GVHD compared with sibling donors, but donor age had a greater impact on TRM and survival than the offspring or sibling family relationship. The analysis of other subsets showed a lower risk of acute GVHD for sibling donors <30 years of age compared with older siblings (>30 years) or father donors, whereas the outcome was not different from brother donors >30 years of age compared with father donors. Older sisters (>30 years) conferred a higher TRM and lower survival, especially for male recipients. Another factor with respect to the development of acute GVHD was the noninherited maternal antigen (NIMA) and noninherited paternal antigen (NIPA) disparity, and transplantation from NIMA-mismatched siblings showed a lower incidence of acute GVHD compared with NIPA-mismatched sibling donors or paternal and maternal donors, although the NIMA or NIPA mismatch had no influence on TRM, chronic GVHD, relapse, or survival.

Based on their analysis, Wang et al propose an algorithm for donor selection focused to reduce the incidence of TRM and GVHD and, according to their analysis, young, male, and NIMA-mismatched donors are preferred over NIPA-mismatched donors or older mothers (see figure).

Despite the large numbers of patients and the uniform treatment protocol, some questions regarding haploidentical donor selection remain. Factors such as cytomegalovirus status, number of donor pregnancies, or KIR disparity were not analyzed, and it is unclear whether these factors play a role in this setting of T cell-replete haploidentical transplantation. There might be fundamental differences between T cell-replete and T cell-depleted transplantations, because, in contrast to this study, mothers were found to be better donors in T cell-depleted transplants.[7] The influence of NIMA disparity on the occurrence of GVHD in haploidentical transplantation has already been reported in non-T cell-depleted transplantation, and NIMA-mismatched siblings conferred a lower risk of acute GVHD and TRM compared with NIPA-mismatched siblings.[9]

The limitation of the study by Wang et al is that the analysis might only be applied to the setting of T cell-replete haploidentical transplantation of G-CSF-mobilized bone marrow and PBSCs mainly used in China and might not be applicable to T cell-depleted transplants or T cell-replete transplants followed by high-dose posttransplant cyclophosphamide mainly used in the western world. Based on the large Chinese patient population, it can be anticipated that more than half of the HLA haplotype-mismatched transplantations performed worldwide will follow similar protocols as described in this study. Therefore, the analysis by Wang et al and their proposed algorithm for haploidentical donor selection will have a major impact on outcome for a large number of patients.

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

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● ● ● IMMUNOBIOLOGY

Comment on Rensing-Ehl et al, page 851

Live and let die at TEMRA

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In this issue of *Blood*, Rensing-Ehl and colleagues[1] provide phenotypic and genomic data revealing a FAS-dependent, stage-specific developmental checkpoint in humans presenting with autoimmune lymphoproliferative syndrome (ALPS).

ALPS-FAS, a rare genetic disorder characterized by a benign lymphoproliferation and autoimmune cytopenia, is associated with dominant germ line,[2,3] somatic,[4] or combined germ line and somatic mutations[5] of the proapoptotic FAS gene. The hallmark of FAS deficiencies is the accumulation of a rare and unusual T-cell subset expressing neither CD4 nor CD8 coreceptors and thus called double-negative T cells (DNTs). These cells display markers of activated T cells, among which are human leukocyte antigen class II, CD69, and markers of naive T cells such as CD45RA, and they lack CD25 expression. DNTs also contain cytotoxic molecules such as granzymes and perforin.[6] Accordingly, observations made in Fas-deficient mouse models and repertoire analysis performed on ALPS-FAS DNTs supported a peripheral CD8 T-cell origin.[7] However, DNTs fail to produce interleukin-2 (IL-2) or interferon-γ or to proliferate upon T-cell receptor (TCR) stimulation but produce high amounts of IL-10 and soluble Fas-ligand, which are used as prediagnosis biomarkers.[8] Moreover, they exhibit a paradoxical high rate of spontaneous apoptosis in vitro. They are thus regarded as anergic or senescent cells accumulating as a result of a missing FAS signal and dying through a FAS-independent mechanism after extensive in vivo proliferation at a so-far unknown developmental stage. This last feature has made them very difficult to study and contributed to the lack of definitive evidence with regard to their function and origin.

Rensing-Ehl et al used 3 different approaches and took elegant advantage of a recently described family in which patients carry a germ line mutation of the FAS initiation codon as well as a somatic loss of heterozygosity (sLOH) of the wild-type allele (ALPS-FAS-sLOH).[1] Indeed, mutations affecting the extracellular domain of FAS are most often associated with haploinsufficiency because the FAS mutant is not expressed at the cell surface. Such mutations display a partial clinical penetrance because healthy carriers of the germ line mutation are frequently observed among patients' relatives. In addition to the germ line haploinsufficient mutations, somatic events affecting the second FAS allele have been identified in patients only.[5] These somatic LOHs take place in hematopoietic progenitors (or at an earlier developmental stage during embryogenesis); they can be found in several lineages such as lymphocytes, monocytes, or even granulocytes, but usually remain undetectable in epithelial cells. In the present situation, the germ line initiation-codon mutation and the somatic LOH are leading to a complete FAS defect, both at the protein and RNA levels. This ALPS-FAS-sLOH case is, so far, the first example of human lymphocytes completely devoid of FAS molecules. This model offers the possibility to trace the disease-related T cells, notably the DNTs and their precursors, at both the cell-surface and genetic levels.

The first original observation of the present work comes from DNTs' phenotypic analysis. Rensing-Ehl and collaborators found that these cells express a combination of surface markers usually observed on terminally differentiated effector memory CD45RA$^+$ T cells (TEMRA cells). Nevertheless, DNTs can be distinguished from TEMRA cells by a high expression of CD27 and CD28 as well as by a lack of expression of KLRG1, an inhibitory receptor normally coexpressed with CD57 on TEMRA cells. Because the differentiation of TEMRA cells is governed by the presence of transcription factors such as Eomes and T-bet, the assessment of their expression was the next step of the study. Surprisingly, whereas Eomes is normally expressed, a complete lack of T-bet expression is observed in DNTs. These last observations correlate nicely with the absence of KLRG1 expression.

Moreover, this abnormal pattern of differentiation is also seen in ALPS-CD4$^+$ or CD8$^+$ single positive T cells, indicating that a proportion of single positive T cells has already acquired this "ALPS DNT-like" phenotype, pointing out DNT progenitors in both single positive T-cell subsets. This new concept is supported by the genetic approach in ALPS-FAS-sLOH patients because FAS-null cells accumulate both in CD4$^+$ and CD8$^+$ central memory cells. This is finally demonstrated by the identification of common TCR β CDR3 sequences (the variable sequence of the TCR that defines a clone identity) between CD4$^+$ TEMRA cells and DNTs. The accumulation of DNT precursors at the TEMRA stage may reflect at least 2 nonexclusive differentiation pathways. On the one hand, the TEMRA stage can be a FAS-dependent checkpoint contributing to the elimination of highly proliferating or even autoreactive T cells. On the other hand, the DNT-like phenotype found in the TEMRA subset may reflect an aberrant proliferation of T cells that should have been eliminated through FAS at an earlier stage, possibly as early as the recent thymic emigrant stage or even in thymocytes. Previous studies on mice[9] and our unpublished data showing a low content of recombination circles in patients' DNTs do not support this last hypothesis, but it cannot be firmly ruled out. Further analysis of mouse models in light of the present findings might help answer these differentiation questions.

Previous studies supported the notion that DNTs mainly stemmed from CD8$^+$ single positive T cells.[6,7] Thus the present observation indicates that the relative contribution of CD4$^+$ and CD8$^+$ T cells to the generation of the DNT subset may vary among ALPS patients or even in response to various stimuli. The nature of the stimuli leading to the generalized lymphoproliferation in ALPS-FAS patients is not clearly defined. Given that two-thirds of the patients developed autoimmune cytopenia, one may assume that self-antigens might be a main source of stimuli and that DNTs and their TEMRA precursors are highly enriched in self-reactive T cells. The anergic status of the DNTs precluded functional analysis, but now such studies can be contemplated, with the TEMRA cells or their immediate precursors most likely being easier to manipulate in vitro. Demonstrating the self-reactive nature of these cells is undoubtedly a future challenge. Therefore, if the TEMRA stage turns out to be a crucial checkpoint of self-reactive T cells, they can be of great help to clinicians who monitor treatment efficiency and potentially to define new therapeutic targets.

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

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● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Helton et al, page 891

Understanding sickle cell brain drain

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In this issue of *Blood*, Helton et al highlight the neuroradiological findings of children enrolled in the Strokes With Transfusions Changing to Hydroxyurea (SWiTCH) trial.[1] This study, which screened 161 children with sickle cell anemia (SCA) receiving chronic transfusion therapy for prevention of recurrent strokes, is the largest SCA cohort followed prospectively with magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) published to date, giving additional insights into the relationships between strokes and cerebral vasculopathy in SCA.

Cerebral vasculopathy in children with SCA is a significant predictor of first[2] and subsequent[3] strokes and progresses despite chronic transfusion therapy.[3] The SWiTCH participants had a significant burden of vasculopathy: 69% of children demonstrated arterial stenosis at study entry.[1] Children with more severe vasculopathy were more likely to have low or uninterpretable transcranial Doppler ultrasound (TCD) velocities in the cerebral arteries, reinforcing that cerebral blood flow through these arterial territories is likely severely compromised. Not surprisingly, children with more severe arterial stenosis had a greater likelihood of cortical and subcortical infarctions. Furthermore, during the study period, 16% of the participants had evidence of brain ischemia: 7 children experienced recurrent overt strokes, 1 had a silent infarct, and 17 had transient ischemic attacks.

SWiTCH's primary aim was to compare the efficacy of substituting hydroxyurea therapy plus serial phlebotomy vs standard therapy of transfusions plus chelation on a composite end point of preventing strokes plus reducing iron overload. The study was terminated early due to predicted futility of the intervention on the composite end point, but the neuroimaging data shed light on the severity of cerebrovascular disease in children with SCA and strokes. Most importantly, how can clinicians use this information to target more effective therapies to high-risk children?

Children with SCA receiving transfusions for stroke prevention are typically followed with serial MRI/MRA and may not undergo conventional angiography due to the potential risks of anesthesia and intravenous contrast agents. Helton et al developed an MRA-based vasculopathy grading scale[1] that they plan to validate in the ongoing TCD With Transfusions Changing to Hydroxyurea (TWiTCH) study. A standardized, validated SCA-specific grading scale would give hematologists a common language with which to describe vasculopathy in our patients. Although TCD is validated for detecting children at high risk for a first stroke, no tool currently exists for predicting strokes while receiving transfusion therapy, so validation of this scale is clearly needed.

For a deeper understanding of stroke pathophysiology in SCA, however, we should look beyond TCD blood flow velocities and vascular stenosis seen on angiography to tissue-level cerebral blood flow (CBF) and regional oxygen metabolism. The cerebral metabolic rate of oxygen consumption ($CMRO_2$) is determined by the product of CBF, the fraction of oxygen extracted from blood (OEF), and blood oxygen content (CaO_2). There is scant published literature about cerebral oxygen metabolism in SCA, but parallels may be drawn between children with SCA vasculopathy and adults with carotid atherosclerosis and ischemic stroke, about whom much more is known. Positron emission tomography (PET) studies in these adults demonstrate that the initial compensatory response to perfusion pressure limitation is vasodilation of cerebral arterioles to preserve CBF, termed autoregulation. If perfusion pressure decreases beyond maximal compensatory vasodilation, OEF increases as a second compensatory mechanism to maintain constant $CMRO_2$. If perfusion pressure continues to fall, both compensatory mechanisms can no longer maintain the $CMRO_2$ required for tissue survival, and cerebral infarction ensues[4] (panel A).

Although the pathophysiological mechanisms described above likely apply to children with cerebral vasculopathy, SCA imposes additional physiological challenges. First, CaO_2 is reduced due to anemia, inducing compensatory increases in baseline CBF.[5] Second, individuals with SCA have limited autoregulatory cerebral vasodilation because of their baseline CBF elevation.[5] Third, the increased viscosity of hemoglobin S-containing blood under low-shear conditions further limits tissue oxygen delivery and persists despite transfusion therapy for stroke prevention.[6] Additional physiologic stressors, such as decreased CaO_2 due to acute worsening of anemia or hypoxemia, decreased CBF due to acute hypotension, or increased tissue oxygen demand due to febrile illness, may lead to inadequate $CMRO_2$ and thus a stroke (panel B). Thus, children with SCA and vasculopathy are exposed to the double jeopardy of flow-limiting stenosis and underlying SCA physiology.

Only 2 studies in children and adults with SCA have used PET plus MRI to characterize cerebral infarcts. Both found areas of decreased blood flow and glucose metabolism on PET that correlated with, and in some cases were more extensive than, MRI-identified infarcts.[7,8] While demonstration of existing brain injury is important, a means of predicting future stroke risk would be even more beneficial for patients. PET studies

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