2 non-JAK2\textsuperscript{V617F} models were used to study the contribution of thrombocytosis/MF (TPO\textsuperscript{high} retroviral RV model, in which marrow cells were retrovirally transduced with the murine TPO gene and grafted into irradiated wild-type recipient mice) and erythrocytosis (EPO-treated mice).

Analysis of these mouse models clearly revealed both direct and delayed effects of JAK2\textsuperscript{V617F} on platelet functional responses, thrombosis, and bleeding (see table). Expression of both inducible and constitutive JAK2\textsuperscript{V617F} resulted in the same hemostatic phenotype, providing additional confidence in these findings. JAK2\textsuperscript{V617F} expression resulted in early effects such as platelet hypoactivity, decreased in vitro thrombosis, and increased bleeding time. The concurrent reduction in plasma high-molecular-weight von Willebrand factor (vWF) multimers, which support thrombosis, suggested that these impairments could be caused by vWF hydrolysis. A similar reduction in vWF multimers was recently observed in the Tie2-Cre/FF1 mouse model of JAK2\textsuperscript{V617F}.

Interestingly, a recent report by Hobbs et al, using a hematopoietic-specific JAK2\textsuperscript{V617F} KI mouse model of ET (Mx1Cre/JAK2\textsuperscript{V617F}), showed opposing results with increased platelet function in response to thrombin and collagen-related peptide and enhanced in vitro thrombosis.\textsuperscript{6} These differences may be explained by the MPN phenotype of the 2 models, ET and PV/MF, respectively, or differences in gene load. Furthermore, it is unknown whether changes in vWF multimers occurred in the model used by Hobbs et al.\textsuperscript{6} Nonetheless, the decreased in vitro platelet function observed in the study of Lamrani et al is consistent with the in vitro thrombocytopathy phenotype in MPN patients. A popular explanation for this is that platelets are hyperactive in MPN patients in vivo and become preactivated and exhausted.\textsuperscript{2} However, there was no evidence of platelet preactivation in the JAK2\textsuperscript{V617F} models, arguing against this.

More delayed effects of JAK2\textsuperscript{V617F} expression included a reduction in expression of the platelet collagen receptor GPVI and vasodilation of blood vessels. The reduction in GPVI levels was also observed in platelets from TPO\textsuperscript{high} retroviral mice, which have symptoms of MF, without the hemostatic changes observed in the JAK2\textsuperscript{V617F} mice. Reduced GPVI expression is therefore unlikely to be involved in the observed reduction in in vitro thrombotic response because (1) TPO\textsuperscript{high} mice do not show these hemostatic changes, and (2) it occurs later (60 days). Vasodilation was observed in both JAK2\textsuperscript{V617F} and the EPO-treated mice and could therefore be the result of increased blood viscosity due to the higher erythrocyte count and subsequent vessel adaptation.

One of the more interesting delayed effects of JAK2\textsuperscript{V617F} expression was the accelerated formation of occlusive thrombi in a FeCl\textsubscript{3} in vivo thrombosis model. These results are consistent with the increased risk of thrombosis in MPN patients. The thrombi, however, were less stable, with increased embolization and clot lysis being observed. Interestingly, the Tie2-Cre/JAK2\textsuperscript{V617F} model also showed increased thrombus instability and embolization.\textsuperscript{3} Although thrombin generation was not determined by Lamrani et al, thromboelastometry showed that in vitro tissue factor-induced coagulation was delayed in JAK2\textsuperscript{V617F} platelets, which may contribute to thrombus instability.

Is the JAK2\textsuperscript{V617F} mutation directly responsible for the increased thrombotic risk of MPN patients? The data in the study of Lamrani et al demonstrate that it is more likely related to the progression of MPN disease than a direct result of JAK2\textsuperscript{V617F} mutation. Although the study cannot completely rule out a direct role of JAK2\textsuperscript{V617F} in the early changes in platelet function, recent work by Etheridge et al\textsuperscript{3} showed that platelet function was unaltered in mice expressing JAK2\textsuperscript{V617F} (PF4-CRE/JAK2\textsuperscript{V617F}) in megakaryocytes/platelets only. This is consistent with patient studies, where impaired in vitro platelet function in ET patients was unrelated to JAK2\textsuperscript{V617F} status.\textsuperscript{5} Thus, although JAK2\textsuperscript{V617F} in platelets may not directly affect platelet function and the prothrombotic phenotype, the possibility that it contributes at a later stage of the disease cannot be completely ruled out. These findings are also interesting in the light of recent publications describing a role for erythrocytes, leukocytes, and endothelial cells in hemostatic changes in MPN mouse models and the potential impact of JAK2\textsuperscript{V617F} on the prothrombotic potential of erythrocytes and endothelial cells.\textsuperscript{2-4} This leaves open the question as to whether JAK2\textsuperscript{V617F} can contribute directly to the prothrombotic phenotype. This, plus a more detailed understanding of the cellular mechanisms that underlie the hemostatic disorders in the complex spectrum of MPN, will form important new directions for the future in this work, building on the important contribution made here.

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Comment on Zhu et al, page 1146

A step forward back to (induced) fetal

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In this issue of Blood, Zhu et al examine signal transduction of hydroxyurea-induced γ-globin, implicating nuclear factor kB (NF-κB)–mediated activation of the secretion-associated and ras-related (SARI) signaling protein, which in turn activates the G-protein-associated c-Jun N-terminal kinase (JNK)/Jun pathway.
Hydroxyurea has become a central therapy for hemoglobinopathies, in large part through its stimulation of fetal hemoglobin (HbF) production. For sickle cell disease, its use has transformed many patients’ lives through amelioration of disease symptoms and improved survival.^{3} Despite widespread clinical use, the mechanisms by which hydroxyurea induces HbF in developing erythroid progenitors have remained mysterious. A ribonucleotide reductase inhibitor, hydroxyurea depletes the deoxyribonucleotide pool and inhibits DNA replication and repair. Repetitive hydroxyurea exposure from daily use shifts erythroid development toward enhanced HbF production.

Using a differential display approach in human erythroid cells, Rodgers and colleagues had previously observed that hydroxyurea stimulates transcription of SAR1,^{4} a guanosine triphosphate binding protein. SAR1 expression is also amplified in erythropoietin-stimulated cultured erythroid cells.^{5} In yeast, SAR1 influences the protein secretory pathway within the endoplasmic reticulum–Golgi system. Human SAR1 is involved in the secretory pathway and also seems to play a role in transcriptional signaling, presumably through its guanosine triphosphatase (GTPase) function.^{1} Previously, this group reported that SAR1 overexpression in cultured and primary human cells caused many of the same effects seen with hydroxyurea: decreased cell growth, cell-cycle arrest, apoptosis, and differentially enhanced γ-globin transcription.^{1}

Now Rodgers and colleagues have strengthened the link between hydroxyurea and SAR1 and substantially extended their previous observations. Silencing of SAR1 in erythroid cells reversed the effects of hydroxyurea on HbF induction and cell-cycle arrest. Interestingly, SAR1 silencing also reduced baseline HbF expression, suggesting a role in HbF expression in the absence of pharmacologic stimulation. Through phosphorylation of the gatekeeper protein, ATM (“ataxia telangiectasia mutated”), hydroxyurea treatment of erythroid cells led to nuclear localization and binding of NF-κB to the SAR1 promoter (see figure). Promoter binding assays demonstrated that hydroxyurea stimulates NF-κB-induced SAR1 transcription, probably in concert with facilitated promoter binding of the proteins Ets-like transcription factor-1 (Elk-1) and/or erythroid Krüppel-like factor. Downstream, SAR1 signaling appears to be linked to the phosphorylation and activation of the JNK/Jun transcription regulator complex. A role of SAR1 in this activation pathway had been suspected, as hydroxyurea previously has been found to stimulate c-Jun expression.^{6} In the current report, the path to SAR1-associated JNK/Jun activation of γ-globin required signaling of Gox, possibly in physical association with SAR1.

What do these elegant cell-signaling data reveal about the mechanism of hydroxyurea induction of γ-globin? Hydroxyurea causes DNA damage, which triggers a watchdog of DNA damage, phosphorylated ATM, to migrate to the cytoplasm. Cytoplasmic ATM in turn activates NF-κB through IkB kinase-mediated liberation (see figure). Nuclear migration of derepressed NF-κB is a major signal for transcription. Signal transduction commonly involves altered phosphorylation of signaling proteins, often through the GTPase function of G proteins. Similarly, JNK/Jun is a generic stress activation pathway. DNA damage is a nonspecific trigger of the cell stress pathway under conditions where the extent of insult is mild or intermittent to avoid widespread cell death. Hence, response to hydroxyurea-induced DNA damage appears to induce HbF through generic signaling mechanisms, perhaps directed toward γ-globin through SAR1 in erythroid progenitors. Of note, other genotoxic HbF–inducing agents, 5-azacytidine and cytarabine, also induced SAR1 in this experimental system, providing evidence of a more generalizable pathway. Accessory proteins such as Elk-1 or EKLF and others may also provide specificity for the SAR1 promoter or the signaling response.

Several molecular routes to chromatin derepression of the γ-globin locus have been identified, as have specific transcriptional modulators of γ-globin such as B-cell lymphoma/leukemia 11A (BCL11A) and KLF1.^{7,8} Some naturally occurring KLF1

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A model of a hydroxyurea-induced SAR1-mediated pathway for activating transcription in erythroid cells. Hydroxyurea binds to an unknown cellular receptor, leading to ATM-mediated activation of NF-κB, which in turn activates transcription of the SAR1 promoter, possibly facilitated by cobinding of Elk-1 or EKLF. Increased SAR1 activates JNK/Jun in association with the G protein Gox, affecting the cell cycle and enhancing γ-globin expression. Some of the moderators have yet to be identified. Professional illustration by Patrick Lane, ScEYEnce Studios.
mutations are associated with high HbF levels.\textsuperscript{9,10} The role of BCL11A in SAR1-mediated HbF expression remains unclear, as BCL11A mRNA but not protein levels was affected by experimental manipulation of SAR1. Relationships between these and other known \(\gamma\)-globin modifiers may be sensitive to cellular context, requiring animal-based rather than cell-based assays to decipher.

This report suggests that hydroxyurea induces HbF through cellular pathways that overlap with those of other HbF-inducing agents, especially if DNA damage or modification is involved. Zhu et al bring the signaling mechanisms of hydroxyurea-mediated induction of HbF into sharper focus. These findings suggest that SAR1 and/or its signaling partners may provide targets for designing clinically useful HbF-stimulating agents. Identification of specific components of a pathway to \(\gamma\)-globin induction also raises the possibility that screening for such agents may even be adaptable for in vitro assays.

\textbf{Conflict-of-interest disclosure:} The author declares no competing financial interests. 

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\section*{TRANSPLANTATION}

\textbf{Comment on Taur et al, page 1174}

\textbf{Less (bacterial diversity) is more (deaths)}

\textbf{John E. Levine \textit{University of Michigan}}

In this issue of \textit{Blood}, Taur et al demonstrate that a lack of intestinal microbial diversity independently predicts nonrelapse mortality (NRM) in allogeneic hematopoietic cell transplant recipients.\textsuperscript{1} At the time of engraftment, patients with low microbial diversity were at fivefold higher risk for NRM than patients with high microbial diversity, primarily because of graft-versus-host disease (GVHD).

GVHD remains the major cause of morbidity and mortality following allogeneic hematopoietic cell transplant and limits its use as a curative therapy for malignant and nonmalignant hematologic diseases. The gastrointestinal (GI) tract is affected in nearly all cases of severe GVHD,\textsuperscript{2} and thus better prevention and control of GI GVHD is essential to reducing NRM. Recent studies show that the GI microbiome plays a role in orchestrating the immune responses that culminate in both experimental and human GVHD.\textsuperscript{3,4} The detection of specific microbes by Toll-like receptors on intestinal epithelial cells triggers an inflammatory response that leads to the recruitment of T cells to the GI tract and activation of the antigen-presenting cells that drive the adaptive immune response.\textsuperscript{5} Damage to the intestinal epithelial barrier, either by pathogens or the pretransplant conditioning regimen, results in translocation of bacteria and their byproducts that can further damage to the intestinal epithelium.\textsuperscript{6} The intestinal mucosal barrier protects itself from such damage by the secretion of antimicrobial peptides, which are primarily produced by Paneth cells and which regulate the composition of the microbiome and maintain intestinal health. When these peptides are in short supply, intestinal homeostasis is disrupted and pathogenic bacterial species can predominate. The role of these antimicrobial peptides in clinical GVHD was confirmed when Reg3\(\alpha\) was validated as a GI GVHD biomarker.\textsuperscript{7} The important role played by regulators of bacterial composition in GVHD is further supported by the observation that Paneth cell loss correlates with higher NRM in both experimental and clinical allogeneic hematopoietic cell transplantation.\textsuperscript{3,8}

If regulation of commensal bacteria is important to GI GVHD control, then one would predict that the loss of regulation and the subsequent bacterial species overgrowth and loss of diversity would increase the risk of lethal GVHD. It is in this context that the present

\begin{figure}[h]
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\includegraphics[width=\textwidth]{hypothetical_model.png}
\caption{Hypothetical model of the relationship between intestinal bacterial diversity and GVHD. (A) When bacterial diversity is high, antimicrobial peptides such as Reg3\(\alpha\) can protect intestinal epithelial cells from damage and GVHD is averted. (B) When bacterial diversity is low, Reg3\(\alpha\) is insufficient to counter the overwhelming number of bacteria resulting in intestinal epithelial cell apoptosis and GVHD.}
\end{figure}
A step forward back to (induced) fetal

Nancy S. Green