advances in our understanding of the immune pathophysiology of the disease over the years have led to improvements in the immunosuppressive regimens used for its treatment and, in some cases, improved outcomes and survival.1,2

Over the past 3 decades, extensive work has established that cytokines play key roles in the suppression of hematopoiesis seen in aplastic anemia.2 Observations made first in the 1980s suggested a disease model in which overproduction of myelosuppressive cytokines by activated cytotoxic T cells results in immune-mediated hematopoietic destruction.3,4 The principles established by such original observations remain essentially unchanged, but subsequent work has expanded on them and defined the mechanisms of immune deregulation seen in aplastic patients. An important observation in recent years was the demonstration that the T-bet transcription factor, which binds to the promoter of the IFNγ gene, is up-regulated in T cells from aplastic anemia patients, resulting in enhanced gene expression and overproduction of myelosuppressive cytokines.5 Other recent studies have shown that regulatory T cells (Treg) are decreased in the peripheral blood of aplastic anemia patients at diagnosis,6 suggesting a mechanism of escape of autoreactive T cells during the development of the disease.

Th17 immune responses play important roles in the pathogenesis of several autoimmune disorders and syndromes,7 but their roles in the pathogenesis and pathophysiology of aplastic anemia have been unclear and undefined. There had been some clues that Th17 cells may have been involved in the pathogenesis of aplastic anemia. From previous work we know that Tregs are suppressed in aplastic anemia,8 and a dichotomy in the development of pathogenic Th17 cells and regulatory (Foxp3+) Treg cells has been shown.9 In addition, there has been some recent evidence for increased expression of IL17A mRNA in bone marrow and peripheral blood mononuclear cells of aplastic anemia patients.9

In this issue, Peffault de Latour et al examined the patterns of expression of Th17 cells in patients with aplastic anemia and compared them to those seen in normal controls.10 Increased numbers of IL-17+ cells were found in aplastic bone marrows, while the percentage and overall absolute numbers of CD3+CD8−IL-17+ T cells were elevated in the peripheral blood of 21 newly diagnosed patients with aplastic anemia, compared with 10 healthy donors. Although the numbers of Th17 cells were not predictive of response to immunosuppression, patients in complete remission after treatment had lower numbers and percentages of Th17 cells than newly diagnosed patients and an inverse relationship with Treg cells in such patients was documented. To further define the role of Th17 cells in the pathophysiology of aplastic, the authors used an experimental mouse model of bone marrow failure involving infusion of allogenic lymph node cells into sublethally irradiated recipients. Their data demonstrate that, in addition to the classical Th1 response, CD4+ and CD8+ IL-17–producing T cells were present—albeit to a lesser extent—in such mice. Remarkably, early treatment of such mice with an anti–IL-17 antibody resulted in reduced areas of hemorrhage in the marrow and improved overall bone marrow cellularity.

Altogether, the findings of this study provide evidence for an important role of Th17-mediated responses in the development and/or progression of early stages of aplastic anemia. They also suggest a synchronized Th1/Th17 response during development of marrow failure, associated with Treg deficiency. Beyond advancing our understanding of the immune pathophysiology of marrow failure, this work raises the prospect of future approaches to optimize immunosuppression regimens for the treatment of aplastic anemia patients, by targeting the Th17 response. There are already clinical trials aiming to block the Th17 response using anti–IL-17 monoclonal antibodies for autoimmune diseases. Efforts to incorporate monoclonal anti–IL-17 antibodies or other means to target Th17 T cells in current immunosuppressive regimens, such as thymoglobulin and cyclosporine A, may provide a nonoverlapping approach to enhance responses and optimize immunosuppression.

Such an approach may be particularly relevant for the treatment of patients with moderate aplastic anemia and efforts in that direction are warranted.

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Comment on Sangokoya et al, page 4338

Micro-mismanaging sickle cell stress

Don M. Wojchowski Maine Medical Center Research Institute

SCD (or “HbSS”) can vary markedly in its clinical manifestations:1 in HbSS cells, regulatory factors that skew in association with disease severity may present new prognostic and/or therapeutic opportunities. In this issue of Blood, Sangokoya et al have applied unsupervised miRNA profiling to reveal elevated microRNA–144 levels in a severe anemia subset of SCD patients (despite an essential lack of mRNA transcripts, erythrocytes can retain miRNAs).1,2,3 Evidence further is provided that the CNC-bZip transcription factor NRF2 is a target for decay by miR-144. NRF2 is known to activate the expression of several antioxidant encoding genes (eg, SOD1, CAT, GCL2) in part via antioxidant response elements. Increased miR-144 levels (and consequently decreased NRF2 levels) therefore...
may elevate oxidative species (see figure, panel A). Oxidative events can compromise erythrocyte integrity, oxygen-carrying capacity, and red cell half-life—and erythrocytes from sickle cell disease (SCD) patients are known to display heightened sensitivity to oxidative stress.

In 2009, Pase et al used morpholino knockdown as well as *Meunier* mutant approaches to provide novel evidence in zebrafish for miR-144/451 roles in erythroid maturation (miR-144 and miR-451 are expressed from a conserved single locus). This year, 3 laboratories reported on miR-144/451 gene disruption investigations in mice. Rasmussen et al reported on mild anemia among miR-144/451 null animals, and described miR-144/451 tuning of the expression of a diverse gene set. Patrick et al and Yu et al further characterized miR-144/451 protection of erythroid cells from oxidative stress, in part via miR-451 targeting of 14-3-3 zeta. As a phospho-S/T–binding protein, 14-3-3 zeta can sequester phospho-FoxO3 and restrict nuclear entry of this group-O forkhead box transcription factor. In this system, one target of FoxO3 proved to be the antioxidant enzyme, catalase. Thus, strong genetic underpinnings exist to implicate miR-144/451 in late erythropoiesis and oxidative stress contexts. Interestingly, recent investigations by Godlewski et al in glioma cells similarly have related elevations in miR-451 levels to worsened stress consequences, but in a context of sensitization to glucose deprivation.

In the present contexts of erythropoiesis, SCD, NRF2, and oxidative stress, the study by Sangokoya et al initially employs a K562 cell line model to map a 3′ untranslated region target site in *NRF2* for degradation by miR-144. Ectopic expression of miR-144 then is demonstrated to decrease NRF2 levels in both K562 and primary erythroblasts, and to also potentiate peroxide–induced cell death. In K562 cells, enforced expression of miR-144 decreased superoxide dismutase (SOD1) levels, as well as glutathione synthesis enzyme subunits GCL-M plus GCL-C. Among HbSS cells, erythrocytes with high-level miR-144 similarly exhibited decreased SOD activity, and decreased GCL-M and GCL-C. In addition, NRF2 levels varied inversely with miR-144 levels, and in primary (pro)erythroblasts NRF2 was observed to decay at a developmental stage when miR-144 levels increased. Because erythrocytes have no nuclei, the demonstrated skewing of miR-144 plus NRF2 in primary HbSS (pro)erythroblasts fills a mechanistic gap.

Observed miR-144 increases in HbSS (pro)erythroblasts also suggest that miRNA half-lives can be sharply regulated. In mouse models, and in a miR-144 vs miR-451 context, it is also interesting to note that miR-451 per se can phenocopy erythropoietic effects of miR-144/451. In addition, miR-451 processing has been shown to depend on nucleolytic activity of an Ago-2 Argonaute factor, and to be atypically Dicer-independent. In future studies, the extent to which miR-144 might modulate 14-3-3 zeta within HbSS cells also should be interesting to assess.

Sangokoya et al further raise the clinical prospect of using erythrocyte miRNA profiles as co-correlates for predicting SCD progression and/or susceptibility to stroke. Extended studies with larger sets of variant SCD samples will be needed to establish such possible uses as related to SCD’s variable severity. Additional attention also may need to be paid to the potential younger ontogenic age of HbSS erythrocytes (and possibly, HbSS erythrocyte progenitors) due to accelerated turnover of the SCD erythron. For SCD, one established clinical correlate of severity relates to HbF levels. In closing, it is therefore noteworthy to at least cite recent advances toward understanding of gamma-globin gene regulators. These include the discoveries of BCL11A as a gamma-globin gene repressor and Klf1 as a regulator of *Bcl11a*. By speculation, potential roles of these factors (together with GATA1) in modulating miR-144/451 expression may also prove productive for consideration.

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phosphates exert differential effects on blood clotting depending on polymer size. Longer polymers (1000 mer, 250 mer, 60-250 mer, 10-60 mer, and very short phosphates (mono-, pyro-, and triphosphates). The green arrows direct to the point in the clotting cascade that is shown to be affected maximally by the specific range of polymer size. (Professional illustration by Paulette Dennis.)

Invading microorganisms release longer polyP polymers whereas activated platelets release shorter polymers (60-200 mer) or very short phosphates (pyrophosphate). Longer polymers (1000+ mer) are very effective in activating the contact pathway of coagulation whereas shorter polymers (60-200 mer) are more effective in activating FV; ≥ 250 mer support maximal fibrin clot turbidity. Pyrophosphate released by platelets can block polyP-induced fibrin clot turbidity. Very short polymers (10-80 mer) can inhibit the contact pathway activation initiated by long-chain polyP. The polymers shown in the figure represent ~ 1000 mer, 250+ mer, 60-250 mer, 10-60 mer, and very short phosphates (mono-, pyro-, and triphosphates). The green arrows direct to the point in the clotting cascade that is shown to be affected maximally by the specific range of polymer size. (Professional illustration by Paulette Dennis.)

Inorganic polyphosphates (polyP), linear polymers of orthophosphate units linked by phosphoanhydride bonds, exist in all living organisms. In bacteria, polyP functions in basic metabolism and is important for growth and survival. However, the physiologic relevance of polyP in higher eukaryotes is unclear as has not been investigated to a great extent. Recent studies from Morrissey and colleagues show that polyP acts at 3 different points in the blood clotting cascade: (1) triggering the contact pathway by activating factor XII; (2) accelerating the activation of factor V by thrombin and factor Xa; and (3) increasing the stability of fibrin clot by enhancing the thickness of fibrin fibers. In addition to modulating hemostasis, polyP is also shown to play a role in inflammation through activation of factor XII, which triggers the release of the inflammatory mediator bradykinin by plasma kallikrein-mediated kininogen processing. All previous studies on polyP were conducted with heterodisperse synthetic polyP or polyP purified from activated platelets. In the present study, Smith et al fractionated polyP preparations carefully to defined polymer lengths, from 30 mer to 1700 mer, and used them to investigate the effects of polyP on the blood-clotting system. Their data show that 30 mer does not activate the contact pathway. The procoagulant activity of polyP was detectable with a 53 mer, and thereafter the procoagulant activity increased as the length of polyP polymer increased. The strongest procoagulant activity was obtained with polyP1000+ mer. An excess of short-chain polyP (30 mer to 80 mer), but not very short phosphates, inhibited plasma clotting initiated by long-chain polyP via the contact pathway. In contrast, maximal specific activities in FXa-initiated clotting were achieved with polyP polymers that were approximately 125 to

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