puncture model of sepsis. However, intriguingly in the same model, T cells undergo intense apoptosis in a caspase-dependent manner and this effect can be blocked by anti-C5a antibody therapy. These data are at odds with those by Lalii et al and require further investigation. Guo and colleagues speculated that the contrasting effects on neutrophil and T-cell apoptosis in the sepsis model may be related to differences in C5aR density on neutrophils versus T cells, where T cells rapidly up-regulate C5aR. On the other hand, the differences may be due to variances in strength of the T-cell receptor (TCR) stimulation in these models, cytokine milieu, or due to utilization of distinct signaling pathways by neutrophils and T cells. Perhaps as a result, glucocorticoids have differential effects and inhibit apoptosis in neutrophils but promote thymocyte apoptosis.

Many inflammatory, autoimmune, neurodegenerative, and infectious diseases are thought to be caused, or at least perpetuated, by excessive complement activity. Therefore, inhibition or modulation of complement activity appears to be a promising novel strategy for their treatment and a number of clinical trials are already underway. Complement has also been shown to mediate graft rejection through increase in proinflammatory cytokine secretion by vessel walls and recruitment of effector T cells. Importantly, this immune-cell intrinsic phenomenon is independent of serum complement, indicating that local production and activation of complement makes a significant contribution to local tissue inflammatory injury and potentiation of adaptive immune response in many pathological conditions. For instance, in transplantation, C3 produced by a donor kidney allograft is a key mediator of experimental transplant rejection and markedly different kidney transplant outcomes are observed in humans based on kidney donor C3 polymorphisms. These findings and recent advances in understanding the novel role of complement in modulation of adaptive immunity and control of T-cell immune responses further support the need for continued efforts at better understanding the complex functions of complement. We can hope for the development of new therapies targeting specific pathways of the complement system in many immune-mediated diseases.

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Comment on Farrar et al, page 1582

Diamond-Blackfan anemia: a new facet

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In this issue of Blood, Farrar and colleagues report the first mutations affecting the 60S large ribosomal subunit in DBA, providing further support for the concept of a central role for ribosome biogenesis in marrow failure.

Mutations in an increasing number of genes encoding 40S small ribosomal subunit proteins have been associated with marrow failure and leukemogenesis. RPS19 mutations have been identified in 25% of patients with Diamond-Blackfan anemia (DBA), with resulting impairment of 18S rRNA processing and 40S ribosomal subunit formation. The subsequent association between DBA and mutations in 2 additional 40S ribosomal subunit genes, RPS24 and RPS17, further supported the notion that the small ribosomal subunit plays an important role in DBA. Acquired defects in another 40S ribosomal protein gene, RPS14, were recently identified in patients with the 5q-myelodysplastic syndrome, suggesting that 40S ribosomal abnormalities constitute a general pathway underlying hematopoietic disorders. Nonetheless, the caveat remained that additional extra-ribosomal functions have been described for many ribosomal proteins and the possibility that these 40S subunit proteins might serve additional cellular functions could not be excluded. Hence, the relative contribution of ribosomal dysfunction to marrow disorders was unclear.

Now, Farrar et al have mapped a fourth DBA gene encoding a 60S large ribosomal subunit protein, RPL35A, in 5 out of a cohort of 150 patients with DBA (3.3%). They also confirmed that RPL35A sequence changes were not found in 180 healthy controls. The authors went further to test the functional consequences of RPL35A loss. They demonstrated that reduction of RPL35A expression by shRNA resulted in decreased proliferation and viability of human hematopoietic cell lines. Impaired ribosomal 60S subunit biogenesis was observed following RPL35A knockdown and in DBA patient samples. These comprehensive findings together with prior studies of RPS19 convincingly demonstrate that disruption of either 40S or 60S ribosomal subunit biogenesis is associated with DBA. This new evidence lends considerable support for the role of ribosomal disruption as a fundamental factor in DBA.

Since mutations in the 4 known DBA genes are identified in only one-third of DBA patients, it is possible that additional 40S and 60S ribosomal protein genes might also contribute to DBA. In support of this hypothesis, Farrar et al also identified aberrant processing of the 60S subunit rRNAs in DBA patients for whom RPL35A mutations were not identified. Since the majority of DBA patients lack known mutations, it would be of considerable interest to determine whether impaired ribosome biogenesis constitutes a characteristic feature of all DBA patients. If true, functional assays for DBA-specific abnormalities in ribosome biogenesis might be useful diagnostically.

The molecular mechanisms whereby disruption of ribosome biogenesis results in marrow failure and malignant transformation remain to be defined. Several hypotheses have
be proposed. One possibility is that incomplete ribosome assembly directly contributes to these processes, for example, through the activation of cellular stress responses or through direct cellular toxicity. Another possibility is that disruption of ribosome biogenesis exerts downstream effects on protein translation that, in turn, are responsible for the DBA phenotype. Impairment of ribosome biogenesis has also been described in other phenotypically diverse marrow failure syndromes, such as Shwachman-Diamond syndrome, X-linked dyskeratosis congenita, and cartilage-hair hypoplasia. Elucidating the molecular similarities and differences between these ribosomal disorders stand to advance our understanding of how inherited and acquired abnormalities in ribosomal pathways contribute to hematopoietic failure and malignancy.

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

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Flow cytometric comparison of Stat5α/β<sup>+/+<sub>TC</sub></sup> and control reticulocytes revealed a 50% decrease in Tfr1 protein on the surface of mutant cells. Parallel analyses of Tfr1 mRNA expression in fetal liver cells, marrow and spleen using microarray, and qPCR all pointed to reduced transcription in Stat5α/β<sup>+/+<sub>TC</sub></sup> cells by 30% to 80% when compared with control cells. Zhu et al then tackled the question of whether STAT5 directly regulates transcription of the Tfr1 gene. They first demonstrated that expression of a constitutively active STAT5A construct in the murine erythrocytopenia (MEL) cell line resulted in a 2-fold increase in Tfr1 expression. Prior analyses of transcriptional control of Tfr1 had defined a promoter region upstream of the first exon that includes a hypoxia response element and binding sites for API1, CREB/ATF, and Ets family transcription factors. A search for Stat5 binding sites–GAS motifs (for interferon Gamma–Activated Sequence)–identified 3 consensus elements within the first intron of Tfr1. Chromatin immunoprecipitation experiments in MEL cells using a constitutively active STAT5A protein demonstrated specific binding to the intrinsic GAS elements, as displayed in the figure. Zhu et al conclude that Stat5α/β controls erythropoiesis in part through direct regulation of Tfr1 transcription.

The microcytosis observed in Stat5α/β<sup>+/+<sub>TC</sub></sup> mice is similar to that seen in mice haplo-insufficient for Tfr1 in agreement with the approximately 50% decrease in Tfr1 expression reported by Zhu et al. However, the inability of Stat5α/β<sup>+/+<sub>TC</sub></sup> hematopoietic cells to mount a hyperplastic erythroid response distinguishes between these lesions and points to additional functions of STAT5A/B in erythroid development. Previous work had demonstrated that erythroid progenitors from a separately derived line of Stat5α/β<sup>+/+<sub>TC</sub></sup> null mice had impaired survival, in part because of reduced expression of the antiapoptotic protein, Bcl-x<sub>L</sub>.<sup>1</sup> In the current study, there is evidence of increased apoptosis in cells lacking STAT5A/B (but little change in Bcl-x<sub>L</sub> levels). This suggests that ineffective erythropoiesis and impaired iron delivery are both important in the development of anemia in Stat5α/β<sup>+/+<sub>TC</sub></sup> mice.

The finding of STAT5 responsive GAS elements in the first intron of Tfr1 has implications beyond erythropoiesis. Iron delivery is critical to maintain active cell division, and

Comment on Zhu et al, page 2071

GASing up iron delivery via STAT5

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Ramping up and maintaining a high rate of iron delivery to support heme biosynthesis is both unique and fundamental to erythroid maturation. In this issue, Zhu and colleagues reveal a previously unappreciated role of the cytokine-activated transcription factor STAT5A/B as a regulator of iron delivery through transcriptional activation of the Tfr1 gene.

The transferrin receptor gene (Tfr1) has previously been shown to be essential for iron delivery during erythroid development, with knockout mice dying at midgestation with severe anemia, and haplo-insufficient mice displaying a compensated microcytosis secondary to iron-deficient erythropoiesis. Similarly, the importance of STAT5A/B in erythropoiesis was demonstrated by whole animal knockout studies that generated severely anemic animals with perinatal lethality. However, revealing a direct role for STAT5A/B in iron metabolism through regulation of Tfr1 required a refinement of technique.

Zhu et al employed a conditional knockout strategy using a Tie2-Cre transgene (known to be active in hematopoietic stem cells) to produce a hematopoietic cell–specific knockout of both Stat5 isoforms. The resultant animals (designated Stat5α/β<sup>+/+<sub>TC</sub></sup>) were born at the expected Mendelian ratio with a hematocrit of approximately 25% compared with 47% in control animals, with the anemia persisting into adulthood. Red-cell morphology in Stat5α/β<sup>+/+<sub>TC</sub></sup> mice is shown in the figure. Zhu et al then tackled the question of whether STAT5 directly regulates transcription of the Tfr1 gene. They first demonstrated that expression of a constitutively active STAT5A construct in the murine erythroleukemia (MEL) cell line resulted in a 2-fold increase in Tfr1 expression. Prior analyses of transcriptional control of Tfr1 had defined a promoter region upstream of the first exon that includes a hypoxia response element and binding sites for API1, CREB/ATF, and Ets family transcription factors. A search for Stat5 binding sites–GAS motifs (for interferon Gamma–Activated Sequence)–identified 3 consensus elements within the first intron of Tfr1. Chromatin immunoprecipitation experiments in MEL cells using a constitutively active STAT5A protein demonstrated specific binding to the intrinsic GAS elements, as displayed in the figure. Zhu et al conclude that Stat5α/β controls erythropoiesis in part through direct regulation of Tfr1 transcription.

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Akiko Shimamura

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