genomic DNA for analysis of TNF gene SNPs. The data revealed a significant increase in the prevalence of iron deficiency and iron deficiency anemia (IDA), together with a marginal rise in the incidence of anemia of chronic disease (ACD) over the malaria season. While TNF gene polymorphisms were not significantly associated with aberrant iron status at baseline, individuals with the TNF-308A genotype had a significantly increased risk of developing iron deficiency and IDA by the end of the malaria season. Similarly, children carrying the TNF-238G genotype also had a significantly greater risk of IDA, as detailed in the figure. Of the 9 TNF haplotypes identified, only 1 (discriminated by the TNF-308A allele) was associated with increased incidences of iron deficiency and IDA. Interestingly, there was no association between TNF gene SNPs and haplotypes and the development of ACD over the malaria season.

How might TNF SNPs be related to the development of iron deficiency and IDA? Atkinson et al hypothesize that malarial infection together with TNF polymorphisms significantly increase plasma TNF levels (they did not measure circulating TNF concentration in this study). Previous work has shown that TNFα is a powerful inhibitor of iron absorption by the intestinal epithelium, iron recycling by reticuloendothelial macrophages, and erythropoiesis. These effects are likely to be greatly exacerbated by the fact that the malaria season in The Gambia coincides with the “hungry season” when dietary iron supply is limited.

Interestingly, Atkinson et al also found that children homozygous for a second haplotype distinguished by SNPs in the inhibitory kappa B-like (IkBL) and lymphotixin alpha (LTA) genes, which lie immediately upstream of TNF, were more likely to be iron replete at the end of the malaria season. Other members of the IkB family of proteins inhibit the actions of the transcription factor Nuclear Factor–kappa B (NF-κB), which is required for the transcriptional activation of the TNFα gene. The authors speculate that IkBL might also inhibit NF-κB and thereby diminish the effects of TNFα on intestinal iron absorption and macrophage iron recycling. The possible involvement of the LTA SNP in controlling homeostasis is unclear at present.

Despite being portrayed in many studies as a disease risk–associated region, Atkinson et al speculate that there might in fact be potential benefits in carrying SNPs in the TNF gene locus. They suggest that the association between TNF promoter polymorphisms, malaria, and nutritional iron deficiency and IDA may have developed as an evolutionary adaptation to limit iron availability for microorganisms and thereby offer protection against the development of infectious diseases.

**Comment on Benimetskaya et al, page 4343**

**SOS! Defibrotide to the rescue**

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Hepatic SOS, formerly referred to as veno-occlusive disease, develops in up to 10% of patients undergoing stem cell transplantation, a substantial percentage of whom succumb to this disorder. A number of therapeutic approaches have failed to significantly alter the relentless course of SOS, though recent evidence suggests that defibrotide ameliorates SOS and may improve survival. In this issue of Blood, Benimetskaya and colleagues characterize the interactions of defibrotide with endothelial cells, providing new insight into potential mechanisms underlying its efficacy in SOS.
Sinusoidal obstruction syndrome (SOS) is initiated by exposure to naturally toxic pyrrolizidine alkaloids, liver irradiation, or conventional chemotherapy. More commonly, however, SOS occurs after high-dose chemotherapy and hematopoietic stem cell infusion, especially after prior exposure to the immunoconjugate gemtuzumab ozogamicin (Mylotarg). Patients with SOS generally present with tender hepatomegaly, jaundice and ascites, or unexplained weight gain, most often within the first 3 weeks after a hematopoietic stem cell transplant. Attempts to treat SOS with vigorous supportive care, systemic anticoagulation, thrombolytic therapy, and/or surgical shunting have not proven effective. Recent reports, however, suggest that defibrotide, a mixture of porcine-derived phosphodiester oligonucleotides, has significant efficacy in the treatment of SOS. This investigational agent is now being used with in criticised frequency in the active treatment set-1

The pathogenesis of SOS appears to reflect direct insult to hepatic sinusoidal endothelial cells. In an animal model of SOS prepared by treating Sprague-Dawley rats with monocrotaline, the earliest morphologic changes included loss of fenestration of sinusoidal endothelial cells and gaps in the sinusoidal endothelial cell barrier. Subsequently, endothelial cells rounded up, red blood cells penetrated into the space of Disse beneath the damaged endothelium, and the sinusoidal lining cells (endothelium, Kupffer cells, and stellate cells) were sloughed and embolized distally, resulting in obstruction of sinusoidal flow. In the rat model, SOS is ameliorated by concomitant administration of glutathione, which prevents endothelial cell rounding and sloughing of the sinusoidal lining, possibly by inhibiting matrix metalloproteases released by endothelial cells following monocrotaline-induced depolymerization of endothelial actin.

The article by Benimetskaya et al provides new information concerning the interactions of defibrotide with endothelial cells. These investigators demonstrate that defibrotide, as well as a series of well-defined phosphodiester oligonucleotides, bind to heparin-binding proteins, in particular bFGF, but not VEGF-165. Once bound by defibrotide, bFGF retains its ability to bind FGFR1c with high affinity and stimulate endothelial cell mitogenesis. Defibrotide also mobilizes bFGF from storage sites in the endothelial matrix and protects bFGF from degradation by trypsin and chymotrypsin as well as air oxidation. Finally, defibrotide binds collagen I with nanomolar affinity and promotes endothelial tubular morphogenesis in 3-dimensional collagen I gels, perhaps through enhancing either α2 or β1 integrin interactions with collagen I. Taken together, these effects would clearly favor angiogenesis, and the authors hypothesize that the efficacy of defibrotide in SOS may be related to its ability to promote revascularization of an injured, hypoxic hepatic parenchyma.

By defining in vitro interactions of defibrotide with endothelial cells, this report provides clues to the pathophysiology of SOS as well as to the potential therapeutic mechanisms of defibrotide. However, additional work is required to validate these mechanisms in the in vivo setting, which is far more complex than a cell culture system. For example, although the authors hypothesize that the activity of defibrotide results from its proangiogenic activity and revascularization of the hepatic parenchyma after angiotoxic injury, it seems plausible that defibrotide may also directly protect sinusoidal endothelium from toxin-induced apoptosis or necrosis through activation of direct or indirect (induction of local VEGF release) prosurvival pathways. In the rat model of SOS, it is hypothesized that the protective activity of glutathione results from inhibition of matrix metalloproteases, although one might wonder whether the angiotoxic or proapoptotic effect of monocrotaline involves oxidant stress pathways, which may be counteracted by glutathione or perhaps even defibrotide. Could defibrotide also protect bFGF or other endothelial growth and survival factors from degradation by metalloproteases, which may have greater pathophysiologic importance in SOS than trypsin or chymotrypsin?

Benimetskaya et al have raised additional questions and provided important insight into the pathogenesis of SOS by describing novel interactions of defibrotide with endothelial cells. It is hoped that these studies will stimulate additional exploration in attempts to validate the authors’ observations in vitro.

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REFERENCES
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