**HFE, a putative hepatocyte iron sensor?**

Hemochromatosis is now recognized to be genetically heterogeneous and caused by mutations in at least 6 genes of iron metabolism. The majority of patients presenting symptomatically with iron overload are homozygous for a founder mutation C282Y in *HFE*, the gene for hereditary or type I hemochromatosis. The finding that mice homozygous for either a null allele of *Hfe* (*Hfe<sup>−/−</sup>* or the orthologous mutation found in type I hemochromatosis (*Hfe<sup>Δ58/Δ58</sup>*) demonstrate elevated serum iron and develop hepatic iron overload in a pattern that recapitulates that found in humans confirms that this form of hemochromatosis does arise from loss of function of HFE. However, despite extensive studies it is still not known how mutations in HFE cause increased iron absorption and iron overload. The favored notion is that HFE together with transferrin receptor is part of an iron-sensing complex present on the basolateral surface of the intestinal crypt cells, programming the crypt cell with information about body iron requirements leading to appropriate expression of carrier molecules. In the case of mutated HFE, this information is faulty and leads to inappropriate absorption in relation to body iron stores. Although HFE/transferrin receptor 1 (TIR1) complexes have been detected immunologically in duodenal lysates and could theoretically modulate cellular iron uptake by influencing transferrin binding to TIR1, receptor recycling rates, or release of iron from endosomes, transfection of wild-type HFE into cultured cells has been consistently reported to induce an iron-deficient phenotype and it is difficult to reconcile the finding of iron-deficient crypt cells that demonstrate reduced uptake of transferrin iron with the presence of mutant HFE.

The development of a new model of HFE function has become necessary following the characterization of hepcidin, a novel peptide hormone synthesized by the liver hepatocytes that appears to have a determining effect on iron absorption and iron metabolism and provides a link with inflammation and body iron stores. A primary defect in hepcidin has been shown to cause iron overload in humans and in mouse models. In addition hepatic hepcidin gene expression has been found to be reduced in *HFE*-related hemochromatosis and *Hfe*-deficient mice. These findings suggest that the regulatory mechanism by which hepcidin expression is augmented in response to iron overload is HFE dependent. Hepcidin is synthesized exclusively by hepatocytes and the findings reported in this issue of *Blood* by Zhang and colleagues (page 1509) that Hfe mRNA and protein is predominantly expressed in liver hepatocytes not only supports the idea of linkage between 2 proteins on a regulatory pathway but also suggests that the liver and not the intestine has the central role in the pathogenesis of hemochromatosis. We suggest that there is a regulatory, iron-sensing pathway centered on the hepatocyte in which hepcidin lies downstream of HFE.

The mechanism by which hepcidin expression is altered in response to iron overload is HFE dependent. Hepcidin however can also be augmented by hypoxia, erythropoiesis, and inflammation via HFE-independent pathway. Recent demonstration that hypoxia increases intestinal iron uptake independent of HFE supports this notion.

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**Dimerize and die**

Adoptive immunotherapy with genetically modified T cells has many potential applications in treating viral infections and malignancy. However, this modality of therapy carries the risk of alloreactivity when donor cells are used after hematopoietic stem cell transplantation. In addition, there is a risk of autoimmune reactions if the target antigen is expressed on normal tissue and a risk of abnormal cell function induced by the transgene. An inducible suicide gene that can be activated in vivo in case of toxicity would increase the safety and broaden the application of this approach.

Initial studies focused on the herpes simplex virus 1–thymidine kinase (HSK-TK) gene, which renders transduced cells sensitive to ganciclovir. This strategy has been used in several clinical trials and has not been associated with any acute toxicity. However, this approach has been limited by immunogenicity of the TK gene product, which leads to the inadvertent destruction of TK-expressing lymphocytes. More recently, an alternate strategy relying on novel artificial death switches based on chemical inducers of dimerization (CIDs) and endogenous proapoptotic molecules has been described. In this approach, human apoptosis molecules can be linked to FK506 binding proteins that contain a binding site for a CID. Administration of this drug then results in the formation of a complex of 2 apoptosis fusion molecules, which leads to their activation and thus, apoptosis.
Transduced cells rapidly undergo apoptosis in vitro with the addition of subnanomolar concentrations of AP1903, a bivalent “dimmerizer” drug that binds FK506 binding protein and induces Fas cross-linking and that has proved safe in testing in healthy volunteers. In the current study, Berger et al evaluate this approach in a primate model and show administration of AP1903 results in elimination of autologous T cells transduced with this construct.

One potential drawback is that a small number of transduced cells were unresponsive to CIDs and survived. While it is unclear what the clinical significance of this will be, the authors make a compelling argument that the surviving cells are resting T cells with a low level of transgene expression and are as such less likely to be alloreactive. Alternatively, it may reflect an effect of cellular inhibitors of apoptosis and could potentially be overcome by using a downstream-acting molecule in the caspase family.

A second issue is that a transgene-specific immune response was generated, but epitope mapping showed that it was directed at sequences in the Fas suicide construct that differed between human and macaque, so it is unlikely to be a problem in human clinical studies. The results described in Berger et al are therefore encouraging that CIDs can be used to induce death if necessary in adoptively transferred T cells transduced with human apoptosis molecules.

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**Anti-CD40L: biology and therapy in ITP**

Immune thrombocytopenic purpura (ITP) is an autoantibody-mediated disease. Platelet-reactive T cells have been found in the blood of patients with this disorder, with the major target antigen being platelet membrane glycoprotein Iib/IIIa (GPIib-IIIa); autoreactive CD4+ T cells are found in lesser numbers in the blood of healthy controls. Kuwana, Ikeda, et al in a series of studies have demonstrated that B-cell production of antiplatelet antibody requires antigen-specific CD4+ T-cell help. Thus, T cells are related to the pathogenic process in chronic ITP.

CD40L (CD154, gp39), a transmembrane protein and member of the tumor necrosis factor (TNF) family, is expressed on activated CD4+ T cells, mast cells, basophils, eosinophils, natural killer (NK) cells, and activated platelets. CD40L is important for T-cell-dependent B-cell responses; a prominent function of CD40L, isotype switching, is demonstrated by the hyper-immunoglobulin M (IgM) syndrome in which CD40L is congenitally deficient. The interaction of CD40L-CD40 (on antigen-presenting cells such as dendritic cells) is essential for T-cell priming and the T-cell-dependent humoral immune response.

Therefore, interruption of the CD40-CD40L interaction with an anti-CD40L monoclonal antibody (mAb) has been considered to be a possible therapeutic strategy in human autoimmune disease, based upon the above information and on studies in animals.

In this issue, Kuwana and colleagues report on a phase 1 study of anti-CD40L humanized mAb (IDEC-131/E6040) in patients with refractory ITP that allows them to link pathobiology with treatment effect. The investigators explored the in vivo effects of a cohort dose escalation (1 to 10 mg/kg) single infusion study of the effects of anti-CD40L mAb on 3 types of autoreactive T- and B-cell responses to GPIIb/IIIa at 3 time points (before treatment at day 0; after treatment at days 7 and 42) and compared them with the platelet responses. A platelet response was achieved only in 3 of the 5 patients treated at the highest dose (10 mg/kg), even though all 5 patients at this dose level had decreased numbers of B cells producing anti-GPIIb/IIIa antibodies by enzyme-linked immunospot (ELISPOT), reduced GPIIb/IIIa-dependent T-cell proliferation, and decreased anti-GPIIb/IIIa antibody in vitro. No platelet increases were seen at the lower doses, even though patients treated at both the 5 and 10 mg/kg doses showed these autimmune responses to be decreased. T-cell responses to an irrelevant antigen (to which there was no ongoing stimulation) was not affected by anti-CD40L. The authors speculate that failure to achieve a platelet effect at 5 mg/kg, despite suppression of the autoimmune responses, may have been related to the shorter duration of the effect. It is also likely that extravascular effects (ie, in the spleen or other parts of the reticuloendothelial system) are critical and require a higher dose. In addition, there may be a heterogeneity in the pathophysiology of ITP since the responders and nonresponders at 10 mg/kg could not be distinguished on the basis of their in vitro responses.

As the authors indicate, there are 3 reports on clinical trials using another anti-CD40L mAb (hu5c8) in patients whose ITP was substantially more refractory than those included in this study (only 7 of 20 reported here had undergone splenectomy). From these reports and unpublished data (J. B. B., January 2004) for IDEC-131/E6040, it appears that 25% to 50% of patients with refractory ITP will have platelet responses to doses of 10

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Dimerize and die

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