Targeted disruption of the HFE gene

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Iron is not a trace element. The 2 liters of erythrocytes that circulate through a 70-kg man contain 2 gm of iron, and another gram of reserve iron is stored in the liver and in macrophages throughout the body. All living organisms require iron. The same unique chemical properties that make iron metabolically useful in facilitating electron transfer make an excess of iron hazardous to health by generating activated oxygen species. Although living organisms are forced to extract iron efficiently from their environment they must also protect themselves against accumulating too much. Mammals do not excrete iron. Instead, iron is lost from the body in an essentially unregulated fashion through bleeding, pregnancy, and cell desquamation. Control of body iron content is achieved through adjustment of the amount of iron absorbed from the gastrointestinal tract. In humans either a deficiency or an excess of iron produces disease. Iron deficiency is a debilitating anemia where only a valine substitution for Glu-6 in the gene product (15), analogous perhaps to the situation in sickle cell anemia where only a valine substitution for Glu-6 in the β-globin chain can produce the sickling phenotype. Although one expects gain-of-function mutations to be dominant rather than recessive, such a mutation might have been one explanation for the singular lack of other mutations in HFE. Now, it appears that other mutations involving HFE will surely be found and these will play a role in causing hemochromatosis.

Another potentially valuable contribution that the knockout mouse may make to our understanding of hemochromatosis is in investigation of pathogenesis. How can a class I MHC molecule regulate iron absorption? Does its cleft bind a peptide? Heme? Some other compound? Or nothing at all? Does HFE act as a transport molecule or does it signal like the Fc receptor family to which it is related? A faulty signal to the gastrointestinal tract from a peripherally located iron detector in a cell such as a macrophage might result in an inappropriate increase in iron absorption. Does HFE affect the function of the transferrin receptor, to which it has recently been reported to bind (16)? What is the role of calreticulin (mobilferrin) (17), which has been shown to play a role in iron absorption and which also binds class I MHC proteins (18)? And where does Nramp2 (19, 20), a newly described iron transport protein, fit in? A further question is raised by the finding that in the small intestine, the primary site of iron absorption, the distribution of HFE seems to be mainly intracellular and perinuclear and limited to cells in deep crypts (21). In other parts of the gastrointestinal tract (21) and in tissue culture cells (22) it is exposed on the cell membrane. Does this imply that HFE may have different functions in different tissues, perhaps prevent-
ing absorption at the gastrointestinal tract level and enhancing transport elsewhere? It is notable, although Northern blot studies indicated that \textit{HFE} mRNA is broadly expressed in many tissues (3), there is so little of the protein in most cell lines that virtually all of the studies on which our current understanding is based have been performed in cells transfected with \textit{HFE} or a combination of \textit{HFE} and \textit{\beta}-2 microglobulin genes. Conclusions drawn from investigation of cells with such unphysiologic overexpression of the protein may prove to be misleading, and the simulation of the deficient state in cells derived from the knockout mouse may provide more robust data bearing on many of the questions that need to be answered.

The mouse model may also aid in understanding more clearly the pathogenesis of the diverse clinical findings in patients with hemochromatosis. The massive accumulation of iron and the fact that phlebotomy stops or even reverses most of the manifestations of hemochromatosis has focused attention on iron as the main culprit in pathogenesis. It is often not appreciated, however, that the absorption of iron and some other metals may be controlled coordinately and may even traverse a common pathway. When iron absorption is increased in iron deficiency, that of other metals such as cobalt and copper seems to be enhanced (23), and the newly described iron transporter, Nramp 2 (20), manifests activity with many different metals. In hemochromatosis the tissue levels of lead and copper are increased and the levels of aluminum are very low (24). Copper was at one time believed to play an important role in causing the cirrhosis that occurs in hemochromatosis (25). But do other metals actually play a role in the pathogenesis of some or all of the manifestations of the disease? It is notable in this regard that the arthropathy of hemochromatosis does not respond to phlebotomy. This is a question almost impossible to answer in humans, but the importance of metal other than iron might well be approached in an animal model now developed by Zhou \textit{et al.} (12).

The study of genetic disorders has often led the way in understanding normal physiology. We do not understand what regulates iron absorption. The study of hemochromatosis, the most common disorder of iron absorption, will very likely make major contributions to the unraveling of this complex system. Important new discoveries often raise more questions than they answer, and this has certainly been the case with the discovery of mutations of \textit{HFE} as a cause of hemochromatosis. The production of an \textit{HFE} knockout may well be an important tool in helping to delineate that pathway of iron absorption and its regulation.

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