To the editor:

The role of duodenal cytchrome b in intestinal iron absorption remains unclear

Duodenal cytchrome b (Dcytb; encoded by the Cybrd1 gene), a ferric reductase expressed at the brush border of duodenal enterocytes,1 has been proposed to reduce dietary ferric iron, thereby facilitating its transport into the mucosal cells by the ferrous iron transporter divalent metal transporter 1. To define the role of Dcytb in intestinal iron absorption, Gunshin et al2 have taken the facilitating its transport into the mucosal cells by the ferrous iron against an absorption defect. The authors show that direct measurements of iron absorption were made and the reliance however, this conclusion should be interpreted with caution, as no analysis of the hepatic iron levels of these animals, they concluded that Dcytb is not necessary for dietary iron absorption in mice. However, this conclusion should be interpreted with caution, as no direct measurements of iron absorption were made and the reliance on liver iron levels does not provide unequivocal evidence for or against an absorption defect. The authors show that Cybrd1−/− mice maintained on standard rodent chow have hepatic iron levels similar to wild-type mice, and at face value this would suggest that Dcytb plays no role in iron absorption under normal conditions. However, the diet used for these studies (Prolab RMH 3000 LabDiet; PMI Richmond, Richmond, IN) contains a large amount of iron (380 mg/kg), including added ferrous iron, and thus both wild-type and Cybrd1−/− mice would likely absorb comparable quantities of iron as the ferric iron reduction step is bypassed. Thus the results obtained are not unexpected. To adequately define the role of Dcytb, direct iron absorption studies should be carried out or the mice should be maintained on a diet containing ferric iron only. The former approach is preferable.

In the second part of the study, animals were maintained on an iron-deficient diet to determine whether Dcytb plays a role in iron absorption when iron is limiting. Liver iron content was measured and found to be similar in both Cybrd1−/− mice and controls. As iron absorption was not measured, this experiment gives no information about the role of Dcytb in absorption and simply shows that liver iron stores in both strains of mice decrease when the amount of iron in the diet is low. To demonstrate this point, we present comparable data from the sla mouse. These animals carry a deletion in the Heph gene (which encodes the ferroxidase hephaestin)3 and have defective basolateral export of iron from intestinal enterocytes.4 When sla mice and control animals are placed on an iron-deficient diet for 6 weeks there is no difference in their liver iron content (Figure 1), similar to the results presented for the Cybrd1−/− mouse. However, when intestinal iron absorption was measured in these animals we found that sla mice had a significantly lower absorption than control mice (Figure 1). This clearly shows that liver iron levels cannot be used as a surrogate marker of iron absorption under these conditions. We believe that the question of whether Dcytb plays a role in iron absorption remains unresolved. Only direct measurements of iron absorption using radioactive ferric iron in knockout and control animals, maintained on both standard and iron-deficient diets, will answer this question definitively.

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References

Response:

**Cybrd1 is not essential in mice**

We appreciate the interest that Frazer and colleagues have shown in our work. In contrast to their interpretation, the purpose of our initial paper was not to define the possible role of Cybrd1 (duodenal cytochrome b [Dcytb]) in intestinal iron absorption but rather to determine whether it is essential for the procurement of iron for utilization and storage in vivo. Our results clearly showed that it is not essential in 129S6/SvEvTac mice, whether they were fed a standard lab diet or an iron-deficient diet.

Frazer and his coworkers are correct that our standard chow contains ferrous iron, possibly eliminating the need for an enzymatic ferric reductase. However, as we reported, animals lacking Cybrd1 maintained iron stores comparable to wild-type mice after 8 weeks on an iron-deficient diet. In unpublished studies, we concluded Cybrd1−/− mice on the iron-deficient diet for 6 months.

Even under these conditions, their tissue iron stores and hematologic parameters were indistinguishable from wild type. Clearly, Cybrd1 is not essential for viability, for erythroid iron assimilation, or for maintenance of liver iron stores. While our data do not rule out a defect in intestinal iron absorption in Cybrd1−/− mice, they argue against a major role for Cybrd1 in vivo. For comparison, mice lacking the iron transporter Slc11a2 (divalent metal transporter 1 [DMT1]) in the intestine show a very severe reduction in liver iron stores and profound anemia that is undoubtedly due to a failure of intestinal iron absorption.

We have not yet attempted to address whether Cybrd1 facilitates intestinal iron absorption. Other ferric reductases have been identified recently, raising the possibility that there may be functional redundancy in dietary iron reduction. Future experiments should answer these questions.

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**References**


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To the editor:

**Activating FLT3 mutations in CD4+CD8− pediatric T-cell acute lymphoblastic leukemias**

Activating mutations in the FMS-like tyrosine kinase 3 gene (FLT3), including internal tandem duplications (ITDs) in the juxtamembrane (JM) domain or point mutations (PMs) in the activation loop, are the most common genetic aberration in acute myeloid leukemia (AML). Recently, Paietta et al. investigated the presence of FLT3 mutations in 69 adult T-cell acute lymphoblastic leukemia (T-ALL) patients. Three positive cases (2 ITDs and 1 PM) were identified sharing a similar early prothymocytic T-cell developmental state exclusively expressing cKIT/CD117, and a trial to test the efficacy of FLT3 inhibitors for this T-ALL subset was suggested.

To validate the incidence of FLT3 mutations and to investigate a relation to outcome and other parameters, we screened 72 diagnostic pediatric T-ALL samples for FLT3 mutations, as previously described. We identified FLT3/ITD mutations in 2 pediatric T-ALLs (Figure 1A), whereas no point mutations in the kinase domain were detected. Sequence analysis confirmed a 51-base pair insertion in patient 2112 and a 57-base pair insertion in patient 1179 (Figure 1B). Moreover, no wild-type FLT3 was identified in patient 2112, suggesting loss of the wild-type allele.

Immunophenotypic analyses revealed a similar profile for both FLT3-mutated patient samples (ie, TdT+, CD2+, CD5+, CD7+, CD4+/CD8−, cytoplasmic CD3+, surface CD3−, and CD10−). CD34 expression was detected in 24% and 21% of the leukemic blasts in patients 2112 and 1179, respectively. Only patient 2112 weakly expressed CD13 (24%) but not CD33. Although representing early T-cell differentiation stages for both patient samples, the maturation stage seems more advanced compared with FLT3-mutated T-ALL cases (CD34+, CD4−/CD8−). Since no additional patient material was left for flow cytometry, cKIT/CD117 expression was determined by real-time quantitative polymerase chain reaction (RQ-PCR) on isolated blasts (> 90% leukemic cells) from all pediatric T-ALL samples (Figure 1D). Whereas only the 3 FLT3-mutated adult T-ALL patients highly expressed cKIT, most pediatric T-ALL samples expressed cKIT mRNA to some extent. Patient 2112 highly expressed cKIT, whereas patient 1179 showed a weak cKIT expression that was about 26-fold lower. Since various non–FLT3-mutated T-ALL samples highly expressed cKIT/CD117 at levels comparable to patient 2112, we conclude that cKIT/CD117 expression is not exclusively associated with FLT3 mutations. Nevertheless, transcript levels do not necessarily correlate with protein expression levels. In line with previous observations, leukemic blasts of FLT3-mutated samples highly expressed LYL1 and LMO2. Both pediatric samples carried a HOX11L2 translocation in contrast to the FLT3-mutated adult T-ALL cases.
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