Defective Iron Homeostasis in β2-Microglobulin Knockout Mice Recapitulates Hereditary Hemochromatosis in Man

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Summary

Previously, hepatic iron overload resembling that in hereditary hemochromatosis (HH) has been found in β2-microglobulin knockout (β2m-/-) mice. We have now characterized iron metabolism in β2m-/- mice. The mutant mice fail to limit the transfer of iron from mucosal cells into the plasma. Transferrin saturation is abnormally high. Pathologic iron depositions occur predominantly in liver parenchymal cells. Reconstitution with normal hematopoietic cells redistributes the iron from parenchymal to Kupffer cells, but does not correct the mucosal defect. We conclude that (a) iron metabolism is defective in the gut mucosa as well as the liver of β2m-/- mice; and (b) a β2m-dependent gene product is involved in iron homeostasis. Recently, a novel gene of the major histocompatibility complex class I family, HLA-H, has been found to be mutated in a large proportion of HH patients. Our data provide functional support for the proposed causative role of HLA-H mutations in HH.

Hereditary hemochromatosis (HH), an autosomal recessive, HLA-linked disease, is one of the most common genetic disorders in man. It is characterized by a failure in regulation of iron absorption, an increase of transferrin saturation, and progressive iron deposition predominantly in the parenchymal cells of several organs (1). Increased iron absorption in HH homozygotes leads to accumulation of iron, with eventual tissue damage and organ dysfunction. When the disorder remains untreated, premature mortality resulting from hepatocellular carcinoma, cirrhosis, cardiomyopathy, or diabetes mellitus is common (2, 3). Removal of iron and the prevention of its reaccumulation by phlebotomy treatment dramatically improves life expectancy in these patients (4–7). The discovery of a tight linkage between HH and the HLA-A locus has led to the clarification of the genetic nature and the autosomal recessive inheritance of the disease (8, 9). A similar disease is not known in animals, and although many attempts have been made to develop an appropriate animal model by dietary application of different iron compounds, severe organ damage as seen in HH could not be reproduced in these models (10).

In a previous study, we have revealed the existence of hepatic iron overload in β2-microglobulin (β2m)-deficient mice similar to that found in HH (11, 12). This was based on a histological and quantitative study of the steady-state iron distribution and content in tissues of β2m-/- mice. These results were confirmed more recently by others (13).

In this report, we characterize the nature of the iron metabolism defect found in β2m-/- mice. β2m is required for the normal surface expression of classical and nonclassical MHC class I molecules (14, 15). Thus, mice lacking a functional β2m gene fail to express MHC class I products (16, 17). Such mice lack CD8+ lymphocytes. To analyze a possible involvement of CD8+ cells, we also analyzed iron metabolism in CD8-/- mice. Mice that are mutant in the TAP-1 peptide transporter do not express classical class I molecules on the surface of their cells (18). Some nonclassical MHC-I molecules can be expressed independently of TAP function, however (19, 20). Therefore, to extend our analysis of the nature of the β2m-dependent molecules involved in iron metabolism, we included the TAP1-/- mice in our study. β2m-/- mice failed to reduce intestinal iron absorption in the face of iron overload and had abnormally high (>80%) values of transferrin saturation. This defect

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**Abbreviations used in this paper.** β2m, β2-microglobulin; β2m-/-, β2m knockout (mice); Hb, hemoglobin; HCT, hematocrit; HH, hereditary hemochromatosis; IR, iron retention; MCV, mean corpuscular volume; MT, mucosal transfer fraction of iron; MU, mucosal uptake of iron; RE, reticuloendothelial; TBIC, total iron-binding capacity; TS, transferrin saturation.
was specific for β2m−/− mice, since the iron status of TAP1−/− mice and CD8−/− mice was comparable to that of controls. Additional results are presented that suggest that the iron metabolism in β2m−/− mice is affected at the level of the gut mucosa, as well as at the level of iron storage and the liver. Published November 1, 1996

Materials and Methods

**Mice.** CS7BL/6 (B6) female mice aged 6–8 wk were purchased from IfaCredo (Brussels, Belgium), and used as controls. The β2-microglobulin “knockout” (β2m−/−) mice (16) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and further bred in our animal facility; CD8−/− mice (21) were obtained from Dr. T. Mak (University of Toronto, Toronto, Canada), and further bred in our animal facility. Both mutant mice had been back-crossed onto the C57BL/6 background. Liver samples from TAP-/-mice (18) were obtained from Dr. S. Tonegawa (Massachusetts Institute of Technology, Cambridge). All animals were given a commercial diet (RMH-B®; Hope Farms, Woerden, The Netherlands) or, when indicated, an iron-enriched diet containing 0.4% (wt/wt) ferrous citrate (1,000 mg Fe/kg diet). The samples were obtained by orbital puncture under diethylether anesthesia. Hemoglobin (Hb), hematocrit (HCT), and mean corpuscular volume (MCV) were determined by a Coulter-Micro Plus counter (Coulter Electronics, Hialeah, FL). Plasma iron and total iron-binding capacity (TIBC) were determined by the Ferrozine method (Iron FZ Test; Roche, Basel, Switzerland). The mucosal transfer fraction of iron (MT) was determined as the mucosal uptake of iron (MU) and plasma iron values. Histology. Samples of liver, spleen, kidney, heart, and pancreas were fixed in 4% buffered formaldehyde. After routine histology processing, the paraffin sections were stained with hematoxylin and eosin. For the detection of ferric iron (Fe3+), the Prussian blue stain was used (22).

**Transferrin Saturation and Hematological Measurements.** Heparinized blood was obtained by orbital puncture under diethylether anesthesia. Hemoglobin (Hb), hematocrit (HCT), and mean corpuscular volume (MCV) were determined using a Coulter-S counter (Coulter Electronics, Hialeah, FL). Plasma iron and total iron-binding capacity (TIBC) were determined by the Ferrozine method for iron absorption tests, the mucosal transfer fraction of iron (MT) was calculated from the TIBC and plasma iron values.

**Statistics.** Results are presented as the mean ± SD. Student’s t test was used for comparison between the control and knockout mouse groups. For individual comparisons between two measurements, the paired t test was used. The level of significance was preset at P < 0.05.

**Results.** Increased Plasma Iron and TS in β2m−/− Mice. Under normal circumstances, the vast majority of plasma iron is bound to transferrin. Transferrin-bound iron is transported to various sites of utilization, where it is delivered to cells via transferrin receptors (24). In HH homozygotes, the earliest laboratory abnormalities are elevated plasma iron concentrations and increased TS (25, 26). Therefore, we measured plasma iron and TIBC, and calculated transferrin saturation from that in 2- and 12-mo-old mice. At both ages, β2m−/− mice had persistently higher plasma iron concentrations (Fig. 1A) and TS (Fig. 1B) than either B6 or C58−/− control mice.
Increased Iron Concentrations in Livers from β2m<sup>−/−</sup> Mice.

Diagnosis of iron storage disease normally requires a biopsy of the liver, the major organ for excess iron storage (1). Further quantitative determination of the hepatic iron concentration, histochemical visualization of the cellular distribution of iron, and pathological examination of the extent of injury provide essential information about the type and degree of iron loading.

Confirming the finding reported earlier (11, 13), 8-wk-old β2m<sup>−/−</sup> mice had hepatic iron concentrations that were more than twice as high as in B6 control mice. In contrast, iron concentration in the livers of CD8<sup>−/−</sup> mice...
Figure 2. Storage of excess iron in hepatic cells of mice fed with different diets (Prussian blue staining). (A and B) Light micrograph of a liver section from B6 (indicated as $\beta 2m^{+/+}$, A) and $\beta 2m^{-/-}$ (B) mouse aged 2 mo and fed with a standard diet. In the liver of the $\beta 2m^{-/-}$ mouse, liver parenchymal cells with an increased iron content are visible around portal spaces. (C and D) Light micrograph of a liver section from B6 (indicated as $\beta 2m^{+/+}$, C) and $\beta 2m^{-/-}$ (D) mouse aged 2 mo at the start of the experiment and fed with an iron-enriched diet (2.5% wt/wt carbonyl iron) for 2 wk. Beside parenchymal cells, heavily iron-loaded Kupffer cells are visible in the $\beta 2m^{+/+}$ liver. Kupffer cells are indicated with arrows. Original magnification ×300.

and TAP1$^{-/-}$ mice were comparable to B6 control mice (Fig. 1 C). Similar observations were made at 12 mo of age, when $\beta 2m^{-/-}$ mice had accumulated about three times more iron in their livers than age-matched B6, CD8$^{-/-}$, or TAP1$^{-/-}$ mice (not shown).

Cellular Distribution of Storage Iron in Livers. One of the distinguishing features of the pathology in HH in humans is that the initial deposition of iron is predominantly in the parenchymal cells of affected tissues, with insignificant early involvement of macrophages of the reticuloendothelial system (27). No detectable iron was seen histologically in B6 (Fig. 2 A), CD8$^{-/-}$, and TAP1$^{-/-}$ mice (not shown) of various ages that were fed with a standard diet. Prussian blue staining of liver sections from >50 $\beta 2m^{-/-}$ mice revealed a predominant presence of iron in parenchymal cells (Fig. 2 B), confirming the finding reported earlier (11, 13). At early stages, the iron-loaded hepatocytes were concentrated in periportal areas, but later they were distributed equally throughout all areas of the liver lobule. In older animals, iron deposits were also present in the pancreas, kidneys, and heart (not shown). Two out of seven 1-yr-old $\beta 2m^{-/-}$ mice had a sevenfold increase in liver iron concentrations (Fig. 1 C) and had developed hepatic fibrosis (not shown), which was never seen in control mice of the same age.

Examination of livers from $\beta 2m^{-/-}$ mice by electron microscopy revealed electron-dense lysosomal structures in hepatic parenchymal cells located in periportal areas. These lysosomes had a granular substructure (Fig. 3 A) and, in unstained sections, they were composed of more or less densely packed ferritin granules. Significant amounts of ferritin granules were also present in the cytoplasm of hepatic parenchymal cells (Fig. 3 B). Of interest, lysosomal deposits of ferritin and hemosiderin are prominent in the hepatocytes of patients with HH.

To study the effects of iron overloading on the iron storage in the liver, animals were fed a diet supplemented with 2.5% (wt/wt) of carbonyl-iron for 14 d. In B6 and CD8$^{-/-}$ mice, iron deposition was present in hepatocytes, but was particularly prominent in Kupffer cells (Fig. 2 C). In contrast, in dietary iron-loaded $\beta 2m^{-/-}$ mice, the iron continued to be found predominantly in parenchymal cells (Fig. 2 D).

Erythroid Parameters. Excess storage iron can occur in a large group of hematological diseases, collectively termed iron-loading anemias, in which ineffective erythropoiesis or abnormalities of hemoglobin synthesis are prominent (1). They are characterized by anemia and a subsequent increase in iron absorption. Ultimately, the inefficient use of the iron leads to iron overload. To rule out the possibility that anemia could account for the abnormal iron storage defect demonstrated in $\beta 2m^{-/-}$ mice, we determined several erythroid parameters. The results demonstrated that RBC counts, Hb, HCT, and MCV values were even
higher in the β2m−/− mice than in the other animal groups (Table 1). Thus, the excess storage iron found in β2m−/− mice could not be related to defective erythropoiesis or Hb synthesis.

**Failure to Regulate Iron Absorption in β2m−/− Mice.** One of the hallmarks of iron homeostasis is its exclusive regulation at the sites of absorption (28, 29). The capacity to reduce or increase absorption of iron in response to increased or reduced iron stores is well documented. In HH, patients fail to decrease their iron absorption despite the accumulation of iron in the body (30–32).

Iron retention, here defined as the percentage of 59Fe found in the body 7 d after the administration of an oral radioactive test dose, was plotted against iron concentration in livers (Fig. 4 A). Despite the fact that β2m−/− mice have increased amounts of iron in livers when fed a standard diet, they did not downregulate their IR as compared to control B6 mice that were fed the same standard diet. Importantly, B6 mice that were fed an iron-enriched diet promptly downregulated IR as a response to the modest increase of iron in their livers.

To test whether β2m−/− mice can downregulate iron absorption as a response to a similar overload induced by diet, iron retention was measured before and after feeding the animals with an iron-enriched diet for 14 d. Again, the downregulation of IR in response to moderately increased iron stores could readily be demonstrated in B6 and CD8−/− mice. In marked contrast to the results observed in these
Table 1. Erythroid Parameters

<table>
<thead>
<tr>
<th>Mice</th>
<th>Age</th>
<th>n</th>
<th>RBC 10^12/liter</th>
<th>Hb mmol/liter</th>
<th>HCT %</th>
<th>MCV fl</th>
</tr>
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<tbody>
<tr>
<td>B6</td>
<td>2</td>
<td>19</td>
<td>9.4 ± 0.3</td>
<td>8.9 ± 0.3</td>
<td>45 ± 1</td>
<td>48 ± 1</td>
</tr>
<tr>
<td>B6</td>
<td>12</td>
<td>6</td>
<td>8.7 ± 0.5</td>
<td>7.9 ± 0.5</td>
<td>40 ± 2</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>β2m−/−</td>
<td>2</td>
<td>11</td>
<td>10.0 ± 0.4</td>
<td>10.2 ± 0.5</td>
<td>53 ± 2</td>
<td>53 ± 1</td>
</tr>
<tr>
<td>β2m−/−</td>
<td>12</td>
<td>6</td>
<td>10.4 ± 0.3</td>
<td>10.2 ± 0.3</td>
<td>51 ± 1</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>CD8−/−</td>
<td>2</td>
<td>13</td>
<td>9.0 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>44 ± 1</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>CD8−/−</td>
<td>12</td>
<td>6</td>
<td>8.7 ± 0.7</td>
<td>8.0 ± 0.5</td>
<td>40 ± 1</td>
<td>46 ± 1</td>
</tr>
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</table>

Data are presented as mean ± SD. n, number of animals.

mice, β2m−/− mice, even when challenged with high dietary iron, were unable to reduce their iron retention (Fig. 4 B).

A more specific indicator of the regulation of iron absorption is the so-called MT, here defined as the ratio of the iron ultimately retained in the body vs. the iron initially taken up by the gut mucosa. This is believed to be the critical step that is defective in HH patients (33). We used a double-isotope technique (32; Santos, M., K.J.H. Wienk, M.W. Schilham, H. Clevers, M. DeSousa, and J.J.M. Marx, manuscript in preparation) to discriminate between initial MU and subsequent iron retention, and hence calculate MT of iron. The MT in β2m−/− mice that were maintained on a standard diet was consistently higher than MT in normal or CD8−/− mice (Table 2). After experimental iron overload, MT in B6 and CD8−/− mice was strongly reduced, whereas MT in β2m−/− mice was only mildly decreased (Fig. 4 C).

Taken together, the capacity of β2m−/− mice to regulate iron absorption in response to changes in iron stores was strikingly affected at the level of the MT of iron to the plasma.

Fetal Liver Cell Transfer. As an alternative site for the primary iron handling defect in HH patients, the reticuloendothelial (RE) system has been proposed. Specifically, it has been shown that the RE cells in HH patients are impaired in their capacity to store iron (34–36). A possible implication of these studies is that an abnormality in RE iron metabolism might affect the control of iron absorption by the intestinal mucosa (37). We attempted to address this issue by reconstituting lethally irradiated β2m−/− mice with hematopoietic cells from normal mice. It was expected that if RE cells play a role in regulating iron absorption, β2m−/−-reconstituted mice should be able to correct their abnormalities in iron homeostasis. All β2m−/− mice reconstituted with β2m−/−-derived cells had stainable iron in parenchymal cells, and they were indistinguishable from untreated β2m−/− mice (Figs. 2 B and 5, A and C). No stainable iron was detected in B6-reconstituted B6 mice (not shown). All attempts to reconstitute B6 mice with β2m−/− fetal liver cells failed, most likely because of rejection of the transplanted hematopoietic cells by host NK cells (38). A striking change in hepatocellular iron distribu-

![Figure 4. Impaired capacity to downregulate iron absorption in β2m−/− mice. All animals were 2 mo old at the start of the experiment. (A) IR vs. hepatic iron concentration in B6 control (□), B6 dietary iron overload (△), and β2m−/− (○) mice. B6 iron-overload mice were fed an iron-enriched diet containing 2.5% wt/wt carbonyl iron for 14 d. The remaining groups were maintained on a standard diet. IR was determined at day 7 after the administration of the test dose. Liver samples were analyzed by flame atomic absorption spectrometry for quantitative determination of iron. Individual values for each mouse are shown. (B) IR and (C) MT in the same mice before [□] and after iron loading [○] with carbonyl iron (2.5% wt/wt) for 14 d (six mice per group). Control B6 mice were maintained on a standard diet. IR was measured at day 7 after the administration of the test dose. Mucosal transfer of iron was calculated from the ratio IR/MU. Individual values for each mouse are shown.](https://example.com/iron-metabolism-in-β2m−/−-mice)
Table 2. Increased Intestinal MT in β2m⁻⁻ Mice

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>MT</th>
<th>n</th>
<th>MT</th>
<th>n</th>
<th>MT</th>
<th>vs. B6</th>
<th>vs. CD8⁻⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>0.74 ± 0.06</td>
<td>6</td>
<td>0.72 ± 0.06</td>
<td>6</td>
<td>0.89 ± 0.04</td>
<td>&lt;0.0001</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.74 ± 0.04</td>
<td>5</td>
<td>0.64 ± 0.04</td>
<td>7</td>
<td>0.86 ± 0.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>0.70 ± 0.04</td>
<td>6</td>
<td>0.72 ± 0.05</td>
<td>6</td>
<td>0.82 ± 0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>0.64 ± 0.05</td>
<td>6</td>
<td>0.65 ± 0.05</td>
<td>6</td>
<td>0.86 ± 0.06</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

MT was determined as the ratio of IR/MU. Each mouse received orally a double isotope test dose containing ⁵⁹Fe and ⁵¹Cr. MU was calculated 1 d later. ⁵⁹Fe retention was determined by whole-body counting 7 d after administration of the test dose. Data are presented as mean ± SD. n = number of animals; P = Student's t test for comparison of β2m⁻⁻ mice with B6 control and CD8⁻⁻ mice.

Figure 5. Liver iron in radiation chimeras (Prussian blue staining). All animals were 6 wk old at the start of the experiment. (A and C) Liver sections from β2m⁻⁻ mice 1 mo (A) and 3 mo (C) after reconstitution with fetal liver cells from β2m⁻⁻ donor mice. (B and D) Liver sections from β2m⁻⁻ mice 1 mo (B) and 3 mo (D) after reconstitution with fetal liver cells from B6 donor mice. Heavily iron-loaded Kupffer cells (arrows) are visible 1 mo after reconstitution. After 3 mo, no iron depositions are present. Original magnification ×300.
heteropoietic cells was higher (MT = 0.86 ± 0.04; n = 5 and MT = 0.84 ± 0.05; n = 5, respectively) than in B6 mice reconstituted with B6 hematopoietic cells (MT = 0.67 ± 0.05; n = 5). These results suggest that hematopoietic cells have no influence on iron absorption at the level of the gut mucosa. They indicate, however, that such cells affect the pattern of cellular iron storage in the liver.

Discussion

Previously, we have reported the existence of hepatic iron overload in B2m−/− mice similar to that found in HH (11). This was based on a histological and quantitative studies of the steady-state iron distribution and content in tissues of B2m−/− mice. These results were confirmed more recently by others (13).

Now we report a detailed analysis of the nature of the iron metabolism defect of B2m−/− mice. These mice recapitulate all central features of HH; i.e., increased TS, visible iron depositions, specifically in parenchymal cells of the liver, and a dysregulation of intestinal iron absorption. The defect appears to be caused by the lack of a class I-like, B2m-dependent molecule, and not by the lack of CD8+ T cells or TAP-dependent MHC molecules. Moreover, although the storage abnormalities in the liver of B2m−/− mice can be compensated by cell transfer, the increased intestinal iron absorption is not reduced by substitution of the hematopoietic compartment.

Intestinal Iron Absorption. The capacity of B2m−/− mice to regulate intestinal iron absorption in the face of increased iron stores seems to be manifestly impaired. Similarly, in HH, patients fail to decrease their iron absorption despite the accumulation of iron in the body (30–32).

Iron retention is only the ultimate result of the iron absorption process, which can be divided into two steps: MU from the intestinal lumen and the subsequent transfer of iron through mucosal cells into the blood. Under normal conditions, not all the iron is transferred into the circulation, but remains inside the mucosal cell and is rapidly lost after desquamation of the epithelial cells. MU is influenced by many intraepithelial factors: the state of the iron in the test dose (ferric or ferrous, heme or nonheme), the amount of iron, the composition of the test dose, gastric and intestinal secretions, and the state of the brush border of the mucosal cells. The next step, MT, is less dependent on these factors and will better reflect the iron status of the body. In B2m−/− mice, MT of iron into the plasma is invariably increased, even under normal dietary conditions. Importantly, MT is believed to be the critical step that is defective in HH patients (33). Under increased iron loading, the differences between normal and B2m−/− mice become accentuated, as the B2m−/− mice fail to downregulate MT and iron retention.

Cellular Iron Storage. The parenchymal iron accumulation in the livers of B2m−/− mice resembles that of HH patients and contrasts with the histopathological findings in other iron storage disorders in man. The amount of demonstrable iron in macrophages in HH is minimal until the late stages of the disease (27, 39–42). By contrast, iron accumulation in the overload diseases Bantu siderosis (in Africa) and Kaschinen-Beck (in Asia) is prominent both in mononuclear phagocyte system cells and in hepatic parenchymal cells (43–45). The organ damage that occurs in HH patients subsequent to the iron accumulation (e.g., liver fibrosis, cirrhosis, hepatocellular carcinomas, and diabetic hyperglycemia caused by pancreatic islet destruction) also occurs in old B2m−/− mice (13, 46).

The relevance of the observed iron storage abnormalities in B2m−/− mice emerges from numerous studies performed in animals in an attempt to mimic HH (47–51). In these studies, excess dietary iron alone failed to produce the typical parenchymal iron accumulation and the resulting organ damage, even when iron concentrations in the liver were three to four times higher than the critical iron level associated with fibrosis in HH (52). In accordance with these findings, we observed heavily iron-loaded macrophages in livers from control B6 mice after dietary manipulation. Apparently, surplus iron is redistributed from parenchymal cells to the mononuclear phagocyte system in liver of dietary iron–loaded animals (53). This notion is consistent with previous studies that show a decreased iron storage capacity of macrophages in HH (35, 36, 54). Similarly, it would appear that the Kupffer cells of B2m−/− mice fail to store excess iron, even when surrounded by iron-loaded parenchymal cells. The apparent redistribution of stored iron from parenchymal to Kupffer cells upon transfer of hematopoietic cells might suggest that B2m−/− Kupffer cells, like HH macrophages, carry an intrinsic defect in iron storage.

Molecular Nature of the Defect. B2m is required for the normal surface expression of classical and nonclassical MHC class I molecules (14, 15). Thus, mice lacking a functional B2m gene fail to express MHC class I products (16, 17), and consequently they lack CD8+ lymphocytes.

The TAP-1 and TAP-2 proteins are required for transport of cytosolic peptides into the endoplasmatic reticulum for association with classical class I molecules (55–57). TAP-1 mutant mice are deficient in peptide transport, and consequently do not express classical class I molecules on the surface of their cells (18). Recently, it was shown that some nonclassical MHC I molecules may be expressed independently of TAP function (19, 20). Livers from TAP1−/− mice had normal iron concentration values, implying that classical MHC class I molecules are not responsible for the observed iron storage phenotype in B2m−/− mice. Rather, it would appear that a "nonclassical," B2m–associated molecule performs a function in iron metabolism. Such a molecule would be dependent on the presence of B2m, but independent of TAP function.

The gene responsible for HH has been located on the short arm of chromosome 6, in linkage disequilibrium with the HLA-A locus (8, 58), where several nonclassical MHC-I genes have been identified (59–61). While this paper was under submission, a novel MHC class I-like gene, termed HLA-H, was reported to be mutated in a large majority of HH patients (62). Two missense variants were found in 87% of HH patients. Importantly, the most preva-
lent mutation was inferred to disrupt the putative \( \beta_2m \)-binding site on the HLA-H molecule. The current data lend independent, functional support for a causative role of HLA-H mutations in HH.

Iron Homeostasis. One of the hallmarks of iron homeostasis is its exclusive regulation at the sites of absorption (28, 29). It is not clear yet what mechanism regulates iron absorption from the gut, but two main hypotheses have been suggested from observations made in HH patients. One assumes a generalized defect in iron storage in the RE system and in the feedback regulation of iron absorption (37). The other hypothesis assumes a direct role of the intestinal mucosal cells in regulating iron absorption (63–68).

Our results in the reconstituted \( \beta_2m^{−/−} \) mice support the view that the primary iron metabolism defect is expressed both at the level of the gut epithelium and of Kupffer cells in the liver. The histopathological analysis indicated that the liver iron storage pattern was normalized, indicating the involvement of hematopoietic-derived cells, Kupffer cells or others, in the regulation of cellular iron storage in the liver. However, the iron absorption defect of the mucosal intestinal cells could not be corrected by the transferred cells. The defect appears not to reside in the liver parenchyma. Consistent with this view is the observation that inadvertently transplanted livers from HH patients into recipients with otherwise normal iron metabolism returned to normal iron concentrations (69–71). In these documented cases, a diseased liver was transplanted into recipients with normal hematopoietic compartment and gut mucosa. We propose that HLA-H is responsible for both primary metabolic defects in HH, namely the pattern of iron accumulation in the liver and the failure to regulate iron absorption.

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