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Cell surface expression of HFE protein in epithelial cells, macrophages, and monocytes

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ABSTRACT

Background and Objectives. **Most patients with hereditary hemochromatosis are homozygous for a Cys282→Tyr mutation in the HFE gene. This mutation has been shown to impair the association of the HFE gene product with β_2-microglobulin and to prevent its cell surface presentation in transfected COS-7 and 293 cells. This study was performed to examine the expression of HFE protein in epithelial cells, macrophages, and circulating leukocytes obtained from normal subjects and patients with hereditary hemochromatosis.**

Design and Methods. **Antisera against two different peptides of the HFE protein were used to immunostain tissue sections and isolate granulocytes, lymphocytes and monocytes.**

Results. **Immunocytochemical staining showed that the HFE protein is expressed in gastric epithelial cells, tissue macrophages, and circulating monocytes and granulocytes. The cell surface associated signal, which was seen in normal gastric epithelial cells, monocytes and macrophages, was also present in C282Y mutant cells from patients with hereditary hemochromatosis, although at apparently reduced amounts in these cells.**

Interpretation and Conclusions**. From these studies, it is clear that the C282Y mutation reduces but does not completely prevent presentation of the HFE protein on the cell surface of human monocytes, tissue macrophages, and gastric epithelial cells.**

Key words: hereditary hemochromatosis, HFE, iron, leukocytes, plasma membrane

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Hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in populations of Northern European origin, affecting approximately 1:250 individuals. Its pathogenesis involves a defective regulation of intestinal iron absorption, which leads to iron overload of parenchymal cells in many organs.[1] Clinical consequences of iron accumulation include cirrhosis of the liver, hepatocellular carcinoma, diabetes, heart failure, arthritis, and hypogonadism. One characteristic feature of iron overload in HH is the relative paucity of iron in Kupffer cells and macrophages.[2,3] This contrasts with transfusional iron overload and other forms of secondary iron overload, which are characterized by progressive iron accumulation in these cells. The paradox of modest reticuloendothelial iron stores in HH has led some investigators to suggest that pathogenic mechanisms in HH involve a defective function of reticuloendothelial cells.

A novel candidate gene (HFE), which is responsible for most cases of HH, was cloned by Feder *et al.*[4] Eighty-three percent of 178 HH patients were found to be homozygous for the same missense mutation (Cys282→Tyr) in this gene. The high frequency of the C282Y mutation in HH patients has been confirmed by other investigators.[5-12] The human HFE protein predicted from the cDNA sequence is an integral membrane protein which shares structural homology with major histocompatibility complex class I molecules. The C282Y mutation has been suggested to disrupt a critical disulfide bond in the α3 domain of the HFE protein and abrogate binding of the mutant HFE protein to β_2-microglobulin (β_2M).[4] Feder *et al.*[13] and Waheed *et al.*[14] showed that the C282Y mutant HFE protein does not associate with β_2M in human embryonic kidney cells (293 cells) or in COS-7 cells transfected with the mutant cDNA. Waheed *et al.*[14] also demonstrated that much of the C282Y mutant protein remains in high molecular weight aggregates, fails to undergo late Golgi processing, and is rapidly degraded. While these studies provided a mechanism whereby the C282Y mutation impairs cell surface expression and Golgi processing of the HFE protein, they did not reveal how the HFE protein is involved in iron homeostasis or how the C282Y mutation might produce HH. Definite proof that HFE is the major gene that causes HH was pro-

vided by recent observations in HFE gene knockout mice.[15,16] These mice have abnormally high transferrin saturation and increased iron storage in hepatocytes, indicating that disruption of the HFE gene results in excessive accumulation of iron. It is also notable that these mice showed a similar pattern of hepatic iron storage to that seen in HH patients with relatively little iron in Kupffer cells, suggesting that functionally abnormal HFE protein is most likely involved in this defect in HH.

Previous immunohistochemical studies have demonstrated the localization of the HFE protein in the normal human gastrointestinal tract[17] and placenta.[18] The HFE protein in placenta is expressed on the apical surface of the syncytiotrophoblast cells, where transferrin-bound iron is normally transported to the fetus via transferrin receptor (TfR)-mediated endocytosis. In fact, we found placental HFE protein to be physically associated with TfR as well as with β_2M. These studies together with recent observations of Feder *et al.*[19] and Lebrón *et al.*,[20] showing the association of HFE protein with TfR, suggest that the HFE protein may regulate TfR-mediated iron uptake.

We previously reported that surface epithelial cells of the gastric mucosa and some subepithelial leukocytes express the HFE protein.[17] In the present study, we compare the staining of the HFE protein in gastric epithelium from HH patients and controls. We also compare HFE protein expression in tissue macrophages, circulating monocytes, and granulocytes from HH patients and controls. The difference in the expression pattern of the HFE protein between normal and C282Y mutant subjects may be linked to the paucity of iron deposition observed in reticuloendothelial cells and mononuclear phagocytes of HH patients.

Design and Methods

Antibodies

Based on the structure of the HFE protein derived from cDNA sequencing, antisera against two different peptides of the HFE protein were produced in rabbits. The production and characterization of the CT16 antibody raised against a polypeptide of 16 C-terminal amino acids have previously been described.[17] The second polyclonal antibody against a polypeptide predicted for the amino acids 76 to 91 was raised similarly in rabbits as described elsewhere,[17] and we refer to this antibody as ECTO16. Using corresponding peptide-Affigel 10 affinity resins, affinity-pure and peptide-specific IgGs were isolated and stored in 50% glycerol at –20°C. Mouse anti-human macrophage antibody (Dakopatts, Glostrup, Denmark) was used to identify macrophages in paraffin-embedded tissue sections. Mouse anti-human CD14 antibody (Zymed Laboratories, South San Francisco, CA, USA) was used to identify the circulating monocytes. The following secondary antibodies were used for the immunocytochemistry studies: biotinylated goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO, USA), biotinylated swine anti-rabbit IgG (Dakopatts), tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG (Sigma Chemical Co.), and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma Chemical Co.).

Isolation of leukocytes

Granulocytes were isolated by Ficoll-Paque density gradient centrifugation (Pharmacia Biotech, Piscataway, NJ, USA), after which the cell pellet was hemolyzed with a lysing solution containing 154 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, and 0.1 mmol/L EDTA. The cells were washed twice with a balanced salt solution containing 0.01% D-glucose, 5 µmol/L $CaCl_2$, 98 µmol/L $MgCl_2$, 0.54 mmol/L KCl, 14.5 mmol/L Tris base, and 126 mmol/L NaCl, supplemented with a mixture of protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L benzamidine, and 1 mmol/L *o*-phenanthroline). After washing, the cells were frozen in liquid nitrogen. Giemsa staining indicated that >95% of these cells were granulocytes.

Control mononuclear cells were isolated from blood of a genotyped normal (wild type) individual by Ficoll-Paque density gradient centrifugation according to the protocol of Pharmacia Biotech. Viability on isolation was >95% by the trypan blue exclusion test. Monocytes were enriched by culturing them as described previously.[21,22] Morphologically, more than 95% of non-adherent cells were lymphocytes as identified by Giemsa staining. The adherent cells were predominantly (>90%) monocytes.

Peripheral blood cells were also purified using a fluorescence-activated cell sorter (FACSCalibur, Becton Dickinson Immunocytometry Systems, San José, CA, USA) on the basis of their light scattering properties. Based on the size and granularity of the cells, leukocytes from a genotyped normal subject were separated into three distinct morphologic types: granulocytes, lymphocytes, and monocytes. For each of the groups 60,000 cells were separated, which were then centrifuged 100 x g for 10 min at room temperature. Cell preparations were placed on microscope slides, fixed in methanol, and stained with Giemsa stain. The purity of each cell fraction was >80%.

Immunocytochemistry

The specimens from pyloric antrum (three normal controls and three HH patients) were obtained after informed consent, together with routine histopathologic specimens taken during a surgical operation or endoscopy. All HH patients were C282Y homozygotes, and all had their body iron stores reduced to normal by phlebotomy before the endoscopy. They were all Caucasian males, and their ages at endoscopy were 49, 49 and 53 years. The samples were processed and immunostained as described elsewhere.[17]

Isolated leukocytes were spread onto the microscope slides and fixed in 4% paraformaldehyde in PBS for 20 min. Saponin (0.05%) was used to permeabilize the cells. The steps in the immunostaining were essentially the same as described by Waheed *et al.*[14]

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

All the reagents for SDS-PAGE were from Bio-Rad Laboratories (Richmond, CA, USA) or Sigma Chemical Co. SDS-PAGE was performed using a Mini-Protean electrophoresis unit (Bio-Rad Laboratories) under reducing conditions according to Laemmli.[23]

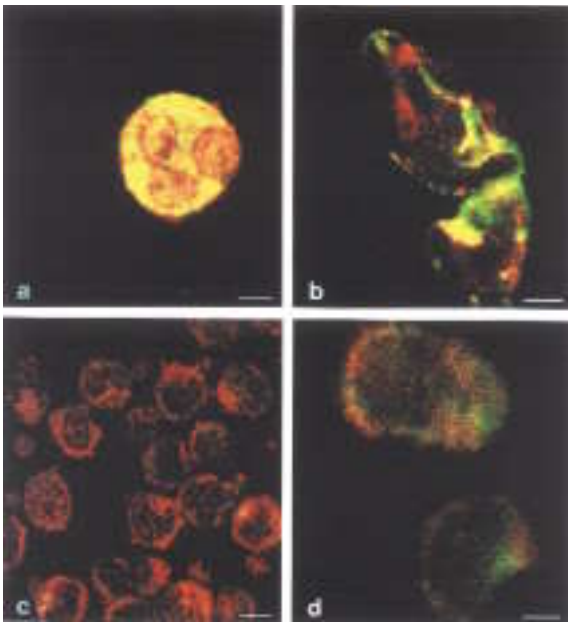

Figure 1. Immunocytochemical staining of HFE protein in FACS-purified leukocytes (a-c) and cultured monocytes (d) obtained from a genotyped normal subject. Granulocyte (a), monocyte (b,d), and lymphocyte (c) fractions were immunostained using CT16 antibody. A granulocyte shows a strong, granular, intracellular reaction (a); the monocytes show a predominantly plasma membrane-associated signal (b). No positive staining is seen in lymphocytes (c). After overnight culture, monocytes lose much of their cell surface-associated staining and retain only a faint intracellular immunoreaction (d). The green color is the FITC-fluorescence from HFE-immunostaining, the red color is produced by reflected light, and yellow color is produced where green and red colors overlap. Bars a: 3 µm, b-d: 5 µm.

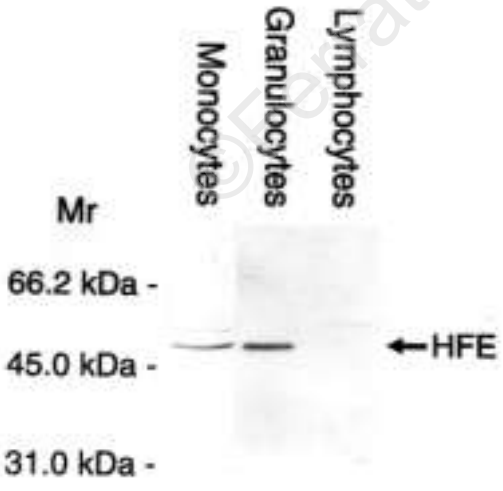

Figure 2. Western blots of proteins from normal granulocytes, monocytes, and lymphocytes. Proteins (20 µg) from granulocyte, monocyte, and lymphocyte total cell homogenates were separated by SDS-PAGE, transferred, and analyzed by Western blot using anti-HFE (CT16) antibody. A 48-kDa polypeptide corresponding to HFE protein is prominent in granulocytes and monocytes.

Western blotting was carried out using the CT16 antibody as described earlier.[14]

Results

HFE protein in circulating leukocytes

FACS-purification of normal leukocytes allowed us to immunostain enriched fractions of granulocytes, monocytes, and lymphocytes. Figure 1a-c shows typical immunostaining patterns of the HFE protein in granulocyte (a), monocyte (b), and lymphocyte (c) fractions. The granulocytes showed a granular, intracellular reaction. The monocytes showed predominantly a plasma membrane-associated immunoreaction. No positive signal for the HFE protein was detected in lymphocytes. Figure 1d shows that the cultured monocytes also expressed the HFE protein, but the signal was weaker than in fresh cell preparations, suggesting that the expression level of the HFE protein on the surface of monocytes depends on the culture conditions and/or cell differentiation.

Western blotting

The specificity of the HFE immunostaining in circulating monocytes and granulocytes was confirmed by Western blotting. Figure 2 shows that under reducing conditions, the major polypeptide identified by this antibody in each cell preparation was the 48-kDa monomeric HFE protein. The 48-kDa signal was prominent in granulocytes and monocytes, but was absent from lymphocytes.

Comparison of the protein expression in normal and C282Y mutant leukocytes

The staining patterns of normal and C282Y mutant HFE proteins in leukocytes were compared using double-immunostaining of cells from fresh peripheral blood. Monocytes were identified using a monoclonal anti-CD14 antibody and the expression of HFE protein was examined using the polyclonal CT16 antibody. Figure 3a shows that much of the wild-type HFE protein was expressed on the cell surface of a monocyte. Figure 3b shows the immunolocalization of the HFE protein in a monocyte from a genotyped C282Y homozygous HH patient. The cell surface-associated signal was lower. There was also a slight decrease in the intensity of the intracellular signal in the monocytes from C282Y homozygous patients when compared to the intensity of signal from normal monocytes. This difference might be due to faster intracellular degradation of the C282Y-mutant HFE protein that has been described by Waheed *et al.*[14] The granulocytes from C282Y homozygous HH patients showed similar immunostaining to that seen in normal granulocytes (data not shown).

Comparison of the HFE protein expression in normal and C282Y mutant gastric epithelial cells and tissue macrophages

Normal human gastric mucosa (Figure 4a) showed a positive signal for the HFE protein on the basolateral surface of the epithelial cells. It also showed a positive immunoreaction in the tissue macrophages, where the staining was predominantly in the plasma membrane. A similar distribution pattern was

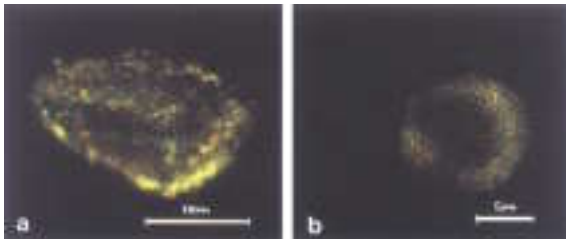

Figure 3. Comparison of immunostaining for HFE protein in normal (a) and C282Y mutant (b) monocytes. CD14-immunostaining (TRITC) was used to identify the monocytes, and HFE protein-immunostaining (FITC) was performed using the CT16 antibody. The cell surface-associated signal is quite prominent in the normal monocyte (a). The cell surface-associated immunoreaction is below the detection limit in the C282Y mutant monocyte, although a weak intracellular signal is evident (b).

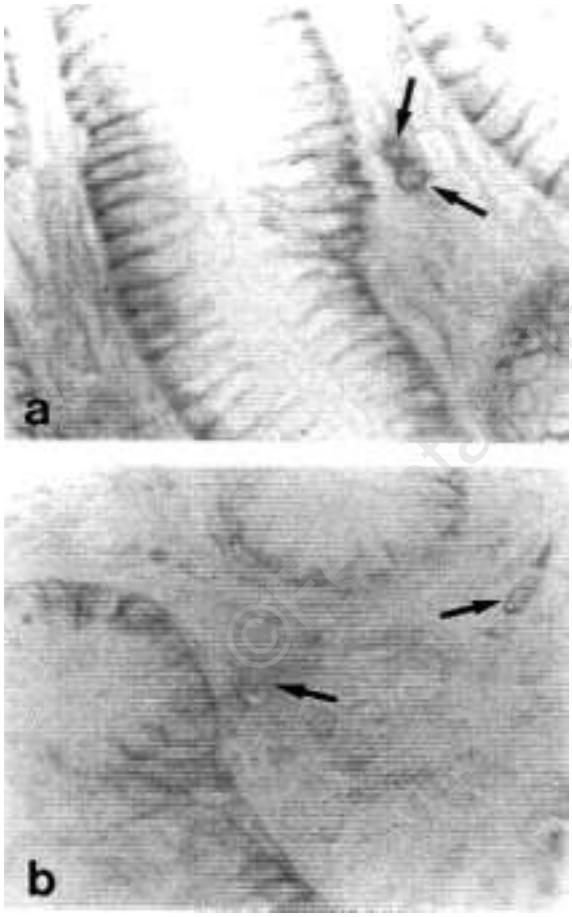

Figure 4. High magnification views of immunostaining for HFE protein in specimens of pyloric antrum obtained from a normal subject (a) and a C282Y mutant HH patient (b). In the normal individual, the HFE protein is strongly immunostained on the basolateral plasma membrane of the epithelial cells. The tissue macrophages also show cell surface-associated immunoreaction (a; arrows). The cell surface-associated immunoreaction is reduced in both C282Y mutant epithelial cells and macrophages (b; arrows) when compared to the normal cells. Bars 20 µm.

obtained using the ECTO16 antibody (data not shown). Positive staining for the HFE protein was also seen in the gastric mucosa from C282Y homozygous HH patients (Figure 4b), but the intensity of the reaction was reduced, both in the surface epithelium and in the tissue macrophages. The positive immunostaining of the HFE protein was blocked in the presence of added CT16 peptide, and no staining was seen with non-immune serum (data not shown).

Discussion

Kupffer cells and macrophages have been suggested to be important in iron metabolism. Macrophages are numerous in the cellular lamina propria of intestinal villi, where absorbed iron is transported from epithelial cells to the circulation.[24] Kupffer cells and splenic macrophages also play important roles in the destruction of aged, abnormal, or damaged erythrocytes. The iron from hemoglobin is normally freed and then released by the Kupffer cells and macrophages. In transfusional iron overload, iron is stored as ferritin and hemosiderin in these cells. In contrast, the excess iron in HH accumulates mainly in hepatocytes with relative sparing of reticuloendothelial cells until late in the disease.[2,3]

The paradox of modest reticuloendothelial iron stores in the face of extensive iron overload in parenchymal cells in HH has even led some investigators to suggest that the primary pathogenic mechanism in HH involves a defect in iron metabolism in Kupffer cells, monocytes, and macrophages. It has been observed that in HH, the lamina propria macrophages of the gut are located in the lower portions of the villi, whereas in secondary iron overload they are confined to the tips of the villi.[24,25] The reason for this difference is not clear. Björn-Rasmussen *et al.*[26] demonstrated that it cannot be explained by defective chemotactic properties of monocytes. Other approaches to search for the basic defect in HH in reticuloendothelial cells included studies of the uptake of transferrin-bound iron, of expression of TfR, and of metabolism of ferritin in monocytes of HH patients.[27-32] Despite these studies, conclusive evidence for a specific defect has not been found. Recently, Cairo *et al.*[33] showed that the binding activity of iron regulatory protein (IRP) was inappropriately high in monocytes of HH patients, which would be consistent with impaired reticuloendothelial iron storage of ferritin in macrophages. The mechanism of the reduced iron storage in C282Y mutant reticuloendothelial cells is most likely linked to defective function of the mutant HFE protein in these cells.

Recent studies have demonstrated an association between HFE protein and TfR, suggesting that the HFE protein may play a role in TfR-mediated iron transport.[18-20] Comparisons of previous immunohistochemical results on the distribution of the HFE protein[17,18,34] and TfR[34,35] reveal that these proteins may be co-expressed in many tissues including esophageal, gastric and intestinal mucosa, and the placenta. So far, the physical association between these proteins has been demonstrated only in placental syncytiotrophoblasts,[18] duodenal crypt cells[34] and mammalian cell lines.[19,20] Since the HFE protein and TfR[30,36,37] are

both expressed in monocytes and macrophages, the physical association of the normal HFE protein and TfR may exist in these cells as well.

The abundance of HFE protein in granulocytes and its localization predominantly in intracellular granules or vesicles are provocative observations. Cytochemical iron staining has shown that neutrophils contain large amounts of intragranular iron,[38] which participates in bacterial killing.[39] This is similar to the localization of β_2M, which is present as an intragranular protein in neutrophils.[40] It seems likely that HFE protein and β_2M are associated in these cells as they are in transfected cells[13,14,19,20] and placental syncytiotrophoblasts.[18] An iron-binding protein, lactoferrin, has also been demonstrated in the cytoplasmic granules of neutrophils with a staining pattern that resembles the localization of the HFE protein.[41] It will be of interest to determine whether the HFE protein in neutrophils associates with lactoferrin or lactoferrin receptor.

Although immunocytochemistry is, by nature, a semiquantitative method, the present data suggest that cell surface expression of HFE protein is reduced in the epithelial cells, macrophages, and circulating monocytes of HH patients. Nonetheless, these results indicate that some C282Y mutant HFE protein is expressed on the cell surface of these cells. This contrasts with earlier results in human Kupffer cells and transfected 293 and COS-7 cells in which cell surface expression of C282Y mutant HFE was not evident.[13,14,42] Some studies have suggested that normal transportation of major histocompatibility complex class I proteins to the cell surface requires association with β_2M.[43-45] This association is clearly impaired between the C282Y mutant HFE protein and β_2M.[13,14] Nonetheless, there is good evidence that limited expression of human leukocyte antigens can occur on the cell surface even in the absence of β_2M.[46,47] This evidence is in agreement with our results showing a weak but definite expression of the C282Y mutant HFE protein on the cell surface of epithelial cells and macrophages. An interesting question is whether the C282Y mutation produces the HFE phenotype by reducing the amount of functional HFE protein at the cell surface, or by producing a dysfunctional protein that disrupts the regulation of iron absorption. The recently developed knockout mouse models of HH[15,16] may be helpful in addressing this and other important questions related to the mechanism of iron overload in HH.

Contributions and Acknowledgments

SP designed and performed the experiments. SP, RSB and WSS wrote the manuscript. AKP helped with cell cultures and immunocytochemistry. AW prepared antibodies. RSB and BRB were responsible for patient referral to our laboratory. XYZ, REF and ST contributed to the review of the paper. All authors were equally responsible for conception and design of the study and approved the final version of the paper.

The order of names takes into account the time, work and scientific contribution given by all authors. BRB and WSS, as senior authors, are cited last. We thank Dr. Lynn Dustin for the help with FACS purification and acknowledge editorial assistance of Elizabeth Torno.

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Disclosures

Conflict of interest: none.

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Potential Implications for clinical practice

- The reduced cell-surface expression of HFE protein in C282Y-homozygous HH patients may contribute to the paucity of iron deposition observed in reticuloendothelial cells and mononuclear phagocytes in these patients.

References

1. Cartwright GE, Edwards CQ, Kravitz K, et al. Hereditary hemochromatosis. Phenotypic expression of the disease. N Engl J Med 1979; 301:175-9.
2. Ross CE, Muir WA, Alan BP, Graham RC, Kellermeyer RW. Hemochromatosis. Pathophysiologic and genetic considerations. Am J Clin Pathol 1975; 63: 179-91.
3. McLaren GD. Reticuloendothelial iron stores and hereditary hemochromatosis: a paradox. J Lab Clin Med 1989; 113:137-8.
4. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
5. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996; 22:187-94.
6. Jouanolle AM, Gandon G, Jézéquel P, et al. Haemochromatosis and HLA-H. Nat Genet 1996; 14:251-2.
7. Jazwinska EC, Cullen LM, Busfield F, et al. Haemochromatosis and HLA-H. Nat Genet 1996; 14:249-51.
8. Carella M, D'Ambrosio L, Totaro A, et al. Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet 1997; 60:828-32.
9. Borot N, Roth M-P, Malfroy L, et al. Mutations in the MHC class I-like candidate gene for hemochromatosis in French patients. Immunogenetics 1997; 45:320-4.
10. Datz C, Lalloz MR, Vogel W, et al. Predominance of the HLA-H Cys282Tyr mutation in Austrian patients with genetic haemochromatosis. J Hepatol 1997; 27: 773-9.
11. Sham RL, Ou C-Y, Cappuccio J, Braggins C, Dunnigan K, Phatak PD. Correlation between genotype and phenotype in hereditary hemochromatosis: analysis of 61 cases. Blood Cells Mol Dis 1997; 23:314-20.
12. Beckman LE, Saha N, Spitsyn V, Van Landeghem G, Beckman L. Ethnic differences in the HFE codon 282 (Cys/Tyr) polymorphism. Hum Hered 1997; 47:263-7.
13. Feder JN, Tsuchihashi Z, Irrinki A, et al. The hemochromatosis founder mutation in HLA-H disrupts β2-

microglobulin interaction and cell surface expression. J Biol Chem 1997; 272:14025-8.
14. Waheed A, Parkkila S, Zhou XY, et al. Hereditary hemochromatosis: Effects of C282Y and H63D mutations on association with β2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci USA 1997; 94:12384-9.
15. Zhou XY, Tomatsu S, Fleming RE, et al. HFE gene knockout produces mouse model of hereditary hemochromatosis. Proc Natl Acad Sci USA 1998; 95:2492-7.
16. Levy JE, Montross LK, Cohen DE, Fleming MD, Andrews NC. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. Blood 1999; 94:9-11.
17. Parkkila S, Waheed A, Britton RS, et al. Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. Proc Natl Acad Sci USA 1997; 94:2534-9.
18. Parkkila S, Waheed A, Britton RS, et al. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci USA 1997; 94:13198-202.
19. Feder JN, Penny DM, Irrinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci USA 1998; 95:1475-7.
20. Lebrón JA, Bennett MJ, Vaughn DE, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 1998; 93:111-23.
21. Shah PK, Falk E, Badimon JJ, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. Circulation 1995; 95:1565-9.
22. Leb L, Crusberg T, Fortier N, Snyder LM. Evaluation of methods using adherence to substrate and density gradient for the isolation of human monocytes. J Immunol Methods 1983; 58:309-21.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680-5.
24. Cattan D. Iron and mucosal intestinal macrophages. Lancet 1983; 12:106.
25. Astaldi G, Meardi G, Lisino T. The iron content of jejunal mucosa obtained by Crosby's biopsy in hemochromatosis and hemosiderosis. Blood 1966; 28:70-82.
26. Björn-Rasmussen E, Nielsen H, Hageman J, van den Dungen P. Monocyte chemotactic properties in hereditary hemochromatosis. Acta Med Scand 1986; 219: 309-13.
27. Jacobs A, Summers MR. Iron uptake and ferritin synthesis by peripheral blood leucocytes in patients with primary idiopathic haemochromatosis. Br J Haematol 1981; 49:649-52.
28. Bassett ML, Halliday JW, Powell LW. Ferritin synthesis in peripheral blood monocytes in idiopathic hemochromatosis. J Lab Clin Med 1982; 100:137-45.
29. Sizemore DJ, Bassett ML. Monocyte transferrin-iron uptake in hereditary hemochromatosis. Am J Hematol 1984; 16:347-54.
30. Björn-Rasmussen E, Hageman J, van den Dungen P, Prowit-Ksiazek A, Biberfeld P. Transferrin receptors on circulating monocytes in hereditary haemochromatosis. Scand J Haematol 1985; 34:308-11.
31. Flanagan PR, Lam D, Banerjee D, Valberg LS. Ferritin release by mononuclear cells in hereditary hemochromatosis. J Lab Clin Med 1989; 113:145-50.
32. Adams PC, Chau LA, White M, Lazarovits A. Expression of transferrin receptors on monocytes in hemochromatosis. Am J Hematol 1991; 37:247-52.
33. Cairo G, Recalcati S, Montosi G, Castrusini E, Conte D, Pietrangelo A. Inappropriately high iron regulatory protein activity in monocytes of patients with genetic hemochromatosis. Blood 1997; 89:2546-53.
34. Waheed A, Parkkila S, Saarnio J, et al. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. Proc Natl Acad Sci USA 1999; 96:1579-84.
35. Gatter KC, Brown G, Trowbridge IS, Woolston R-E, Mason DY. Transferrin receptors in human tissues: their distribution and possible clinical relevance. J Clin Pathol 1983; 36:539-45.
36. Weiel JE, Adams DO, Hamilton TA. Murine monocytes express transferrin receptors: Evidence for similarity to inflammatory macrophages. Cell Immunol 1984; 88:343-9.
37. Taetle R, Honeysett JM. γ-interferon modulates human monocyte/macrophage transferrin receptor expression. Blood 1988; 71:1590-5.
38. Parmley RT, Gilbert CS, White DA, Barton JC. Ultrastructural silver enhancement of Prussian blue-reactive iron in hematopoietic and intestinal cells. J Histochem Cytochem 1988; 36:433-40.
39. Murakawa H, Bland CE, Willis WT, Dallman PR. Iron deficiency and neutrophil function: different rates of correction of the depressions in oxidative burst and myeloperoxidase activity after iron treatment. Blood 1987; 69:1464-8.
40. Bjerrum OW, Bjerrum OJ, Borregaard N. β2-microglobulin in neutrophils: an intragranular protein. J Immunol 1987; 138:3913-7.
41. Afeltra A, Caccavo D, Ferri GM, et al. Expression of lactoferrin on human granulocytes: analysis with polyclonal and monoclonal antibodies. Clin Exp Immunol 1997; 109:279-85.
42. Bastin JM, Jones M, O'Callaghan CA, Schimanski L, Mason DY, Townsend ARM. Kupffer cell staining by an HFE-specific monoclonal antibody: implications for hereditary haemochromatosis. Br J Haematol 1998; 103:931-41.
43. Koller BH, Marrack P, Kappler JW, Smithies O. Normal development of mice deficient in β2M, MHC class I proteins, and CD8+ T cells. Science 1990; 248:1227-30.
44. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. β2-microglobulin deficient mice lack CD4-8+ cytolytic T cells. Nature 1990; 344:742-6.
45. Rock KL, Gamble S, Rothstein L, Gramm C, Benacerraf B. Dissociation of β2-microglobulin leads to the accumulation of a substantial pool of inactive class I MHC heavy chains on the cell surface. Cell 1991; 65:611-20.
46. Ljunggren H-G, Stam NJ, Öhlén C, et al. Empty MHC class I molecules come out in the cold. Nature 1990; 346:476-80.
47. Raulet DH. MHC class I-deficient mice. Adv Immunol 1994; 55:381-421.