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HFE mutation analysis in patients with hepatitis C virus with positive screening for iron overload

Sir,

Patients with hepatitis C virus (HCV) infection frequently have increased levels of serum ferritin and transferrin saturation. Determination of the HFE mutations for hereditary hemochromatosis was performed in 37 patients with positive iron overload screening and HCV infection. The C282Y mutation was observed only in few cases (allele frequency 6.7%). However, the H63D mutation was more frequent than in the control group (28.4% vs 15.7%). The role of this mutation in the iron abnormalities of these patients needs to be clarified.

It has been reported that patients with liver damage due to HCV have higher levels of serum ferritin and transferrin saturation than hepatitis B virus-positive patients. The levels of serum ferritin and transferrin saturation have been correlated with liver iron concentration in HCV patients.[1] Moreover, some authors have postulated that determination of HFE mutations is the best diagnostic test for hereditary hemochromatosis (HH).[2] The aim of this work was to ascertain the role of HFE mutations in HCV patients with high levels of transferrin saturation and serum ferritin (a positive iron overload screening).

Accordingly, 37 outpatients with HCV infection and positive screening for iron overload (serum ferritin higher than 450 µg/L and transferrin saturation exceeding 45%) were anlayzed by PCR reactions and enzymatic digestions of amplified products for the two known HFE gene mutations related to HH (C282Y, in which cysteine is replaced by tyrosine at position 282, and H63D, in which aspartic acid replaces histidine).[3]

Only 1 case out of 37 was homozygous for the C282Y mutation of the HFE gene and 3 were heterozygous (allele frequency 6.7%). As for the second mutation, two cases were homozygous and 17 were heterozygous for the H63D mutation of the HFE gene (allele frequency 28.4%).

When these results were compared with those of a group of blood donors who were studied by our group, no difference in the C282Y mutation was observed (Fisher's exact test, p=0.21). However, the prevalence of the second mutation (H63D) was significantly higher than that in the anonymous voluntary donors living in our area (Chi square, p=0.016)[4] (Table 1).

Our data do not confirm an association of iron abnormalities in HCV infected patients with the C282Y mutation of HFE; however, it seems that a larger group of patients will be necessary to establish the frequency of the C282Ymutation in HCV patients. On the other hand, the high frequency of the H63D mutation in these patients suggests that this mutation plays a role. However, the significance of this mutation remains obscure and some authors have suggested that in these cases the HFE protein has an abnormal function.[5] Similar results have been published regarding patients with porphyria cutanea tarda in an Italian study.[6,7]

Table 1. Genotype frequencies for the HFE mutations in HCV infected patients compared with those in a group of blood donors.

Genotypes	*HCV (n=37)*	*Blood donors (n=108)*
CC/HH	14	70
CY/HH	3	6
YY/HH	1	–
CC/HD	17	28
CC/DD	2	2
CY/HD	–	2
C282Y*	6.7%	3.7%
H63D*	28.4%	15.7%#

*Genotypes are given for amino acid 282 (C282Y)/amino acid 63 (H63D) of the HFE protein. CC/HH is the wild type. *Allele frequencies. #Statistical difference (p=0.012, Chi square test).*

Indeed, when the iron content of liver biopsies from HCV-infected patients was measured, there was only a real increase in hepatic iron in 10% of the cases, despite a rise in serum ferritin and transferrin saturation.[8] It should be pointed out that cytolysis could have led to the increase in both serum ferritin and transferrin saturation.

In conclusion, it is unlikely that the C282Y mutation of the HFE gene accounts for the iron alterations related to HCV infection. Nevertheless, the role of the H63D mutation in the iron abnormalities warrants further studies.

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Key words

HFE mutation, HCV infection, iron overload

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Relevance of donor source to T-cell regeneration after bone marrow transplantation for severe combined immunodeficiency

Sir,

The regeneration of naive $CD4^+CD45RA^+$ T-cells and of proliferative response in children with severe combined immunodeficiencies after bone marrow transplantation from HLA-partially mismatched family donors is slower than after bone marrow transplantation from HLA-identical unrelated donors and is still impaired 12 months after.

Bone marrow transplantation (BMT) is the only curative treatment for children with (severe) combined immunodeficiency ((S)CID).[1,2] However, only 25-30% of patients have HLA-identical siblings suitable as donors. Thus, alternative sources have been used, such as HLA-identical marrow unrelated donors (MUD) or HLA-haploidentical family members.[3,4] The source of donor might influence the T-cell regeneration after BMT, but data on this topic are still lacking in the literature.

Evaluating the reconstitution of T-cell number and function after BMT from MUD in 8 children affected by (S)CID (group I) we observed that in the first months, as generally happens in immune reconstitution after BMT,[5] the striking predominance of $CD4^+$ cells co-expressed the CD45R0 molecule, associated with a primed/activated phenotype, whereas naive $CD4^+CD45RA^+$ cells were, at first, rare. However, fast regeneration of normally functioning naive $CD4^+$ T-cells occurred in these patients, leading to full T-cell reconstitution (including proliferative response) within 8 months.[6] This observation confirmed that the ability to regenerate naive $CD4^+$ T-cell after BMT (being inversely correlated with age) is optimal in children because of the essential role of the thymic-dependent pathway, which is still operating in the first years of postnatal life but which becomes limited with advancing age, in the process of T-cell regeneration.[7]

We compare here these data with those obtained in 9 children with (S)CID who received a BMT, after *in vitro* T-cell depletion with Campath-1M,[8] from HLA-haploidentical parents (group II). Typically, both groups received conditioning therapy with busulfan and cyclophosphamide and prophylaxis for GVHD with cyclosporin A. In group II, the generation of naive $CD4^+CD45RA^+$ cells was slow and impaired, not reaching normal levels by even more than 1 year after BMT (Figure 1). Moreover, the number of $CD8^+CD45RA^+$ cells was still low 12 months after BMT (median: 255/µL [25^{th}-75^{th} percentile: 220-509] versus 765/mL [510-1279] in healthy controls; $p = 0.04$; Mann-Whitney test). Conversely, in the first months after BMT, the absolute number of $CD4^+CD45R0^+$ cells was higher in group II than in group I (months 1-4: 378 cells/µL [131-843] vs. 172 [78-310]; $p < 0.05$), and the proportion of activated T-cells ($CD3^+HLA\text{-}DR^+$) was raised (months 1-4: 48% [31-66] vs. 30 [12-42] $p < 0.05$; months 5-8: 22% [12-26] vs. 6 [1-11] $p < 0.01$).

The proliferative response to PHA (evaluated at mo. +8-+12) was lower in group II than in group I (39,000 [31,175-57,975] c.p.m. vs. 94,050 [60,650-158,300]; $p < 0.03$). Similar data were observed in CD3-stimulated cultures. Proliferative response in group II was positively correlated with the propor-