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.

Angel F. Remacha, Marina Carrasco,* M. Pilar Sardá,* M. Jesús Barceló,# Montserrat Baiget#*

**Hematology department and #Genetics department, Hospital de Sant Pau, Barcelona, Spain*

Key words

HFE mutation, HCV infection, iron overload

Correspondence

Angel F. Remacha, M.D., Hospital de Sant Pau, Hematology Department, Avda. Padre Claret 167, Barcelona 08025, Spain. Phone: international +34-3-2919290 – Fax: international +34-3-2919192 – E-mail: 2107@hsp.santpau.es

References

1. Farinati F, Carin R, De Maria N, et al. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 1995; 22: 449-56.
2. Press RD, Flora K, Gross C, Rabkin JM, Cerless CL. Hepatic iron overload: direct HFE (HLA-H) mutation analysis vs quantitative iron assays for the diagnosis of hereditary hemochromatosis. Am J Clin Pathol 1998; 109:577-84.
3. Merryweather-Clarke A, Pointon JJ, Shearman JD, Robson KJH. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997; 34:275-8.
4. Baiget M, Barceló MJ, Gimferrer E. Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain. J Med Genetics 1998; 35:701.
5. Aguilar Martínez P, Biron C, et al. Compound heterozygotes for hemochromatosis gene mutations: may they help to understand the pathophysiology of the disease? Blood Cells Mol Dis 1997; 23:269-76.
6. Sampietro M, Piperno A, Lupica L, et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. Hepatology 1998; 27: 181-4.
7. Piperno A. Classification and diagnosis of iron overload. Haematologica 1998; 83: 447-55.
8. Riggio O, Montagnese F, Fiore P, et al. Iron overload in patients with chronic viral hepatitis: how common is it? Am J Gastroenterol 1997; 92:1298-301.

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-

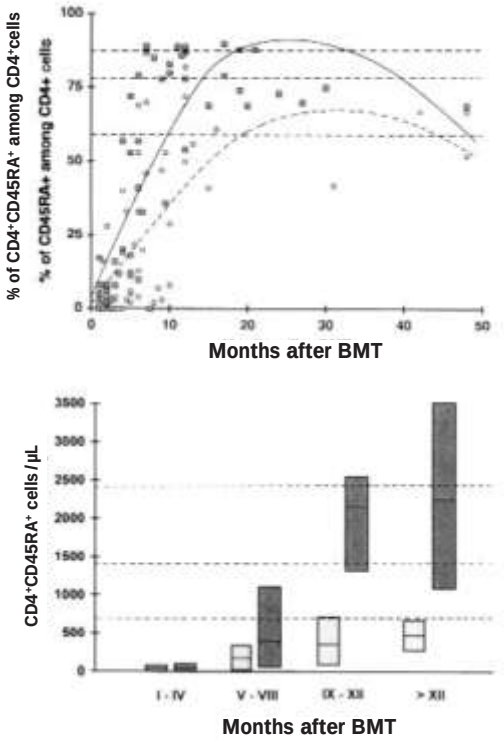

Figure 1.
Top; progressive appearance of CD45RA$^+$ cells among CD4$^+$ lymphocytes after BMT from an HLA-haploidentical family member (circles; dashed line) or from an HLA-identical unrelated donor (squares; continuous line). Lines represent the result of regression analyses.
Bottom; the rise of absolute number of CD4$^+$CD45RA$^+$ cells is impaired after BMT from an HLA-haploidentical family member (white) and slower than after BMT from HLA-identical unrelated donor (grey). Boxes represent 25th-75th percentiles and horizontal lines within boxes represent the median. Dashed horizontal lines represent the median and the 25th-75th percentiles of healthy controls of comparable age.

tion of CD4$^+$CD45RA$^+$ (r = 0.73; p <0.001) and CD8$^+$CD45RA$^+$ cells (r = 0.52; p 0.01) and inversely with that of CD4$^+$CD45R0$^+$ (r = –0.46; p = 0.01) and CD3$^+$HLA-DR$^+$ cells (r = –0.50; p <0.01) but not with that of CD8$^+$CD45R0$^+$ cells (r = –0.29; p = NS).

These data suggest that the defective proliferative response observed in the first months after BMT is linked to the presence of primed/activated T-cells and recovers in parallel with the regeneration of naive T-cells. The T-cell hyperactivated status, causing down-regulation of Bcl-2, and high level of CD95/Fas expression might account for their susceptibility to apoptosis and impaired ability to mount proliferative responses.[9]

Hyperactivation of T-cells, probably resulting from greater donor/recipient diversity, is more pronounced and prolonged in group II and may contribute to the impairment of T-cell reconstitution in this setting. However, patients given BMT from haploidentical donors, differently from patients given BMT from HLA-identical MUD, received a T-cell depleted marrow and the different number of mature T-cells infused in the two groups of patients could also have played a major role in determining the different patterns of T-cell regeneration after transplantation.

These observations are reflected by clinical data: overall survival in children with primary immunodeficiencies transplanted from HLA-mismatched family donors (irrespective of whether T-cell depleted or not) is worse than after transplantation from MUD (which approaches that obtained with an HLA-identical sibling donor).[10] In particular, the survival is very poor in patients with defective T-cell reconstitution, but good in those who achieve full T-cell reconstitution.[10]

Paolo Airò, Duilio Brugnoni, Alberto Malagoli, Marta Pennacchio, Fulvio Porta,* Roberto Cattaneo*

*Servizio di Immunologia Clinica; *Clinica Pediatrica; Spedali Civili, Università di Brescia, Italy*

Key words

Bone marrow transplantation, severe combined immunodeficiency, marrow unrelated donors, HLA, CD4$^+$ CD45RA$^+$ cells.

Funding

AM is the recipient of a grant from the Associazione Donatori Midollo Osseo.

Correspondence

Paolo Airò, M.D., Immunologia Clinica, Spedali Civili Brescia, piazza Spedali Civili 1, 25123 Brescia, Italy. Phone: international +39-030-3995487 – Fax: international + 39-030-382796 or +39-030-3995085 – E-mail: cattaneo@master.cci.unibs.it

References

1. Locatelli F, Burgio GR. Transplant of haematopoietic stem cells in childhood: where we are and where we are going. Haematologica 1998; 83:550-63.
2. Fisher A, Landais P, Freidrich W, et al. European experience of bone marrow transplantation for SCID. Lancet 1990; 336:850-4.
3. Filipovich AH, Shapiro RS, Ramsay NK, et al. Unrelated donor bone marrow transplantation for correction of lethal congenital immunodeficiencies. Blood 1992; 80:270-6.
4. Haddad E, Landais P, Friedreich W, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. Blood 1998; 91:3646-53.
5. Airò P, Gorla R, Cattaneo R. Immune reconstitution after bone marrow transplantation: expansion of primed/memory T-cells rather than recapitulation of ontogeny. Haematologica 1993; 78:137.
6. Brugnoni D, Airò P, Malagoli A, Cattaneo R, Pennac-

chio M, Porta F. Rapid regeneration of normally functional naive CD4+ T-cells after bone marrow transplantation from unrelated donors for combined immunodeficiency. Blood 1998; 92:3484-6.
7. Mackall CL, Hakim FT, Gress RE. T-cell regeneration: all repertoires are not created equal. Immunol Today 1997; 18:245-51.
8. Dickinson AM, Reid MM, Abinum M, et al. In vitro T cell depletion using Campath 1M for mismatched BMT for severe combined immunodeficiency (SCID). Bone Marrow Transplant 1997; 19:323-9.
9. Brugnoni D, Airò P, Pennacchio M, et al. Immune reconstitution after bone marrow transplantation for combined immunodeficiencies: down-modulation of Bcl-2 and high expression of CD95/Fas account for increased susceptibility to spontaneous and activation-induced lymphocyte cell death. Bone Marrow Transplant (in press).
10. Porta F, Mazzolari E, Lanfranchi A, Pennacchio M, Fischer A. Immunological reconstitution after bone marrow transplantation. Bone Marrow Transplant 1996; 18 (Suppl. 2):148-50.

Abnormalities of plasma von Willebrand factor multimeric structure induced by extracorporeal circulation

Sir,

Using an immunoblotting technique, we analyzed the multimeric structure of plasma von Willebrand factor (vWF) after extracorporeal circulation (ECC) in patients undergoing cardiovascular surgery intervention. An abnormal vWf structure, similar to that observed in type 2A von Willebrand disease, was demonstrated in 7 out of 12 patients. This finding induces us to evaluate vWF function further in these patients.

Extracorporeal circulation is a prerequisite for cardiovascular surgery. However, repeated passage of the patient's blood through the cardiopulmonary bypass results in contact activation of blood, which leads to the initiation of the clotting cascade, complement activation, fibrinolytic mechanisms and kinin release; moreover, platelet function is impaired and leukocyte activation occurs (including neutrophil degranulation and protease release, oxygen radical production, and the synthesis of cytokines by mononuclear cells).[1,2]

To assess the validity of blood recovery after ECC in pediatric cardiovascular surgery, we studied the multimeric structure of plasma vWF in 12 patients (4 to 26 yrs, mean 11.6) with congenital heart disease undergoing cardiovascular surgery. ACD-anticoagulated blood was withdrawn before ECC. No blood transfusions were given during ECC, and none of the patients had significant hemorrhagic or thrombotic diathesis after ECC. Immediately after ECC we collected the residual blood in the circuit to study vWF. vWF multimeric analysis was performed as described elsewhere.[3] Briefly, plasma samples were electrophoresed on a mini-gel system (1.1% and 2.6% LGT-agarose for low and high resolution analysis, respectively), then gels were transferred onto nitrocellulose filters by electroblotting. Filters were incubated with anti-vWF antibody, then with an alkaline phosphatase-labeled secondary antibody; finally, vWF multimers were visualized using a chromogenic substrate, and filters were scanned by a densitometer.

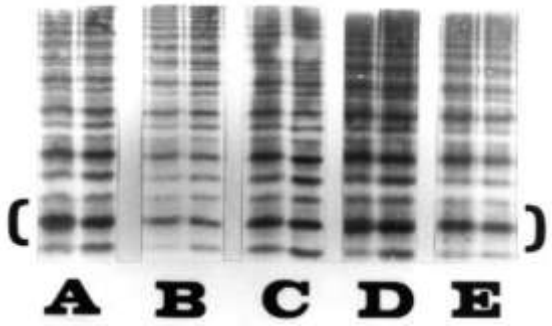

Figure 1. High resolution vWF multimeric analysis of plasma of 5 patients is shown, before and after ECC (left and right lane of each pair, respectively). The increased prominence of the faster satellite band of each multimer is evident. Brackets indicate the lowest molecular weight multimer.

An identical spectrum of vWF multimers was assessed before and after ECC in all patients by low resolution analysis. Before ECC, high resolution analysis demonstrated a normal composition of individual vWF multimers in all patients; on the other hand, an abnormal multimeric structure was detected in 7 patients after ECC: the abnormal pattern always consisted of increased prominence of the faster sub-band of each multimer (Figure 1), closely resembling that occurring in type 2A von Willebrand disease (vWD).[4] vWF is synthesized by megakaryocytes and endothelial cells in the form of very large polymers of 225-kDa subunits. However, circulating vWF undergoes proteolysis under physiologic conditions in order to prevent pathological formation of platelet thrombi.[5] An abnormal susceptibility to proteolysis leads to abnormal vWF multimeric structure in some patients affected by type 2 vWD.[6,7]

In our experience, the finding of a normal spectrum of vWF multimers after ECC excluded significant loss of the largest multimers caused by adsorption to the ECC circuit surface.

Tsai *et al.*[8] demonstrated that shear stress in capillary tubings may increase vWF proteolysis by enhancing the proteolytic activity in plasma, and by causing conformational changes in the vWF molecule itself, which render the cleavage site more accessible to plasma proteases. It is probable that both shear stress in the ECC circuit and an increase of the overall proteolytic activity of plasma could be responsible for the