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. 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 Sardà,* M. Jesús Barceló,* Montserrat Baiget*
*Hematology department and *Genetics department, Hospital de Sant Pau, Barcelona, Spain

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

References

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 (SCID).

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. 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 SCID (group I) we observed that in the first months, as generally happens in immune reconstitution after BMT, the striking predominance of CD4+ cells co-expressed the CD45RO 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. 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.

We compare here these data with those obtained in 9 children with SCID who received a BMT, after in vitro T-cell depletion with Campath-1M, 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 [25th-75th 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+CD45RO+ 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-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. 6-12) 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-
of CD4+CD45RA+ cells (r = 0.73; p < 0.001), 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.

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). In particular, the survival is very poor in patients with defective T-cell reconstitution, but good in those who achieve full T-cell reconstitution.

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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.

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

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 multimers.