Autoimmune hemolityc anemia after treatment of severe systemic lupus erythematosus with high-dose chemotherapy and autotransplantation of selected peripheral hematopoietic progenitors

Bone marrow transplantation is an emerging tool for treatment of immune-mediated diseases. The use of animal models have shown that these diseases can be treated by hematopoietic stem cell transplantation. These findings have recently been confirmed in humans; diseases like multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus were resolved or alleviated after allogeneic or autologous stem cell transplantation. We described here a patient with longstanding and refractory SLE in whom clinical and serological remission was achieved after a CD34⁺ cell autotransplantation. However, she relapsed 5 months later with a severe AIHA, a clinical manifestation not previously recognized in the course of the patient's disease. She was treated by prednisone and cyclophosphamide without success. Only with the administration of anti-CD20 (Rituximab) the AIHA partially resolved. This case reflects the necessity of controlled protocols to known not only the benefits of these potent therapies, but also their possible unwanted effects.

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Patients with refractory systemic lupus erythematosus (SLE) still remain a challenge for physicians who treat them. Although the introduction of pulses of intravenous cyclophosphamide and new antihypertensive drugs had reduced the morbidity of these patients, the mortality remains significant. Although the exact pathogenesis of lupus is unknown, this disorder is associated with immune-mediated abnormalities of T-cell compartment. The hyperactive helper T-cells can cause polyclonal B-cell secretion of autoantibodies that attack the target organs.

Several reports of patients with coincidental malignancy with autoimmune diseases, treated by stem cell transplantation, lead to the assumption that this type of procedure could ameliorate these processes.[1] In addition, preclinical animal models support the concept that the course of severe autoimmune disease can be altered by intensive immunoablation.[2] Moreover there are reports of longstanding remission of SLE after autologous transplantation.[3] We don't know the exact mechanism of the transplant-induced remission in this disease. It would act as a potent immunosupressor or conversely like a hard disk reset in the computer setting, destroying the aberrant immune system and initiating a new population of non autoreactive T-cells.

We report the clinical course of a patient with severe SLE treated by autotransplantation of peripheral CD34⁺ selected cells who developed a severe autoimmune hemolytic anemia (AIHA) with positive direct antiglobulin test (DAT) after a short period of postransplant clinical and biological SLE remission.

A 16-year-old white female was diagnosed of severe SLE in November 1995 with polyartralgias, Raynaud syndrome and malar erythema. In addition, she showed in May 1996 proteinuria and pathologic urinary sediment that lead to a kidney biopsy consistent with the diagnosis of focal proliferative glomerulonephritis (grade III). She was treated by steroids and azathioprine with good initial response. In November 1996 she suffered a renal relapse irresponsive to augmentation of steroids. For these reasons she was treated by monthly 1 g cyclophosphamide bolus,[6] followed by 5 bolus every 2 months. After a short response, she developed a nephrotic syndrome with proteinuria and impairment of the renal function. In February 1998 a second renal biopsy was informed of diffuse proliferative glomerulonephritis (grade IV) with extracapilar proliferation in 50% of the glomerules. Cyclosporine was added to treatment with good response in terms of lowering of proteinuria and negativization of ANA and anti-DNA. In July 2000 another reactivation of her disease led to the increment of ANA, and anti-DNA, hypocomplementemia, and proteinuria. Shortly thereafter, she suffered an ischemic optical neuritis that was treated by high-dose steroid therapy.

Table 1. Evolution of laboratory data

	Anti-DNA (titer)	ANA titer.	C3/C4 mg/dL	T4/T8 cells/μL
Pretransplant	30?	>1/160	77/5	ND
30 d post	49	1/40	ND	ND
45 d post	33	1/40	119/18	48/650
90 d post	115	>1/160	83/8	19/276
120 d post	41	>1/160	ND	222/710
relapse	43	>1/160	63/3	ND

ND: not done

The patient was remitted to our center for high-dose chemotherapy with autologous stem cell rescue. The protocol was approved by the Ethical Committee and the patient was treated by cyclophosphamide (4 mg/m^2) and G-CSF (10 mg/kg) for hematopoietic stem cell mobilization. The stem cells were collected by means of a CS 3000-Plus cell separator (Fenwal). Only 2 apheresis procedures were necessary. The CD 34⁺ cells of the first apheresis were selected by Clinimacs® system (Mylteny Biotec GmbH). The product had a purity of 95%. The second apheresis was stored as a back up. Both were cryopreserved using a mechanical method and stored at –80°C4. The conditioning regimen consisted in cyclophosphamide 200 mg/kg plus anti-thymocytic globuline (ATG) 60 mg/kg plus methylprednisolone 8 mg/kg. The selected CD34⁺ cells were thawed and reinfused. The total number of CD34⁺ and CD3⁺ cells were 5.2×10^6/kg and 3x10^5/kg, respectively. The patient engrafted early (day +11 for >0.5×10^9/L neutrophils and day +12 for platelet transfusion independence). The clinical course was uneventful with only grade 2 mucositis and fever without focal infection and without isolation of microorganisms. During the next 15 days following the discharge of the patient the steroids were tapered until the discountinuation. The relevant data are showed in Table 1.

At day 140 post-transplant the patient was admitted to the hospital because fever, dyspnea, dark urine and anemia. The clinical and biological evaluations led to diagnosis of Coombs + autoimmune hemolytic anemia. She was treated with prednisone 2 mg/kg/weight and red blood cell transfusions (20 units). Despite of this therapy the patient showed a continuous severe hemolysis and she received one bolus of 1g of cyclophosphamide. Because the patient did not respond to treatment, 4 cycles of anti-CD20 MoAb (rituximab) 375 mg/m^2 weekly, were added to treatment. The hemolysis was controlled and the patient became clinically stable. During all this period the renal function remained normal, the complement was slightly reduced and ANA antibodies

slightly increased. Now, the patient showed slight sings of hemolysis with a moderate anemia and is treated with 10 mg of prednisone and 100 mg ciclosporine/12h.

In spite of recently reported cases of improvement of patients with severe LES after PHSCT5, our case showed that this kind of therapy can produce some unwanted effects. This treatment might alter the immune system in different ways. The explosive start of the autoimmune hemolysis, had been an unexpected secondary effect. After the high-dose therapy certain clones directed against some specific antigens can survive and be selected. This could explain why the immune activity was directed only to some antigens and sparing others. We treated the AIHA of this patient with steroids and cyclophosphamide without success. Some reports about the use of an humanized antibody against B-cells bearing the CD antigen (rituximab) in immune cytopenias prompt us to treat desesperately this patient with this antibody.[6-8] The response cannot be attributed solely to infusion of rituximab but after the first infusion the anemia began to be controlled. The effect of this drug is directed against the antigen CD 20 that is bearing by the B-lymphocytes. The drug is highly effective in the disappearance of B-lymphocytes that are the producers of immunoglobulins. One of the amazing things in this case was that the other clinical manifestations of the disease, were under relative control after the transplantation and were spared on relapse.

Finally, this case showed us the necessity of prospective studies to known, not only the benefits of these potent therapies, but also their possible unwanted effects.

J. Besalduch, J. Bargay, J. Buades, A. Galmés, M. Morey, A. Sampol

Servicio de Hematología, Hospital Son Dureta, Andrea Doria, 55, 07014-Palma de Mallorca, Spain

Correspondence:Juan Besalduch, Servicio de Hematología, Hospital Son Dureta, Andrea Doria, 55, 07014 Palma de Mallorca, Spain

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