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Case report

Refractory Evans' syndrome treated with allogeneic SCT followed by DLI. Demonstration of a graft-versus-autoimmunity effect

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Summary:

Evans' syndrome, a combination of autoimmune haemolytic anaemia and autoimmune (idiopathic) thrombocytopenic purpura, is generally harder to treat and more refractory than the single entities. In a male patient with refractory disease, predominantly thrombocytopenic, an allogeneic reduced intensity BMT from his human leukocyte antigen (HLA)-identical sister was followed by a dramatic platelet peak while he was still experiencing initial engraftment (presumably of autologous origin), but subsequently by progressive relapse associated with mixed chimerism. Five gradually incremental DLI achieved complete donor chimerism, which was associated not only with grade II graft-versus-host disease (GVHD), but also with complete clinical and biological remission for 2 years post-transplant. Long-term FU is necessary before claiming that allogeneic stem cell transplantation (SCT) is capable of curing an autoimmune blood disease. However, there is evidence for a graft-versus-autoimmunity effect in this case.
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Evans' syndrome (ES), a combination of two autoimmune diseases of the blood, haemolytic anaemia (AIHA) and thrombocytopenic purpura (AITP),[1] is still a devastating disease,[2] and appears to be more aggressive, refractory and potentially fatal than the much more frequent AITP.[3] Survival data have been collected recently totalling 75 patients: mortality at 13 years was 3/42 patients, at 8 years 4/11 and 4/12, and at 13 years 3/10, roughly equivalent to 18%.[4] Intense immunosuppression ('immuno ablation'), followed or not by autologous SCT, has also given unsatisfactory results,[5] except in a case of ES secondary to SLE.[6] In refractory AITP, the first cases failed to obtain long-term remissions.[7,8] The encouraging remissions initially reported by Lim *et al*[9] did not hold in the FU (Lim SH, personal communication). Of 12 patients collected by the International Stem Cell Project for Autoimmune Diseases, only three responded completely and recovered normal platelet counts, with an observation time exceeding 1 year in all.[10] A total of 15 patients, four of whom had ES, were treated with HDCY (200 mg/kg) followed by autologous CD34+ cell support, and an overall 57% response rate was obtained.[11] It is of interest to note that a CR was achieved following a syngeneic peripheral blood stem cell (PBSC) transplant.[12] Returning to ES, very few cases of extremely refractory disease have been treated with allogeneic SCT,[4,13] but according to the published reports, all of them achieved CR, although the patient transplanted with cord blood SC died of liver failure on day +289.[13]

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Case report

Past history

A 21-year-old male was admitted on 29 October 2000 in order to receive a BMT from his HLA-identical, 12-year-old sister. In November 1992, he had first suffered from severe, DAT (Coombs)-positive AIHA, and achieved a DAT-positive remission following HD-IgG plus corticosteroids. After 1 year, he had extensive mucocutaneous purpura with 2×10^9/l platelets (plts). He was treated again with corticosteroids. In August 1994, he again developed DAT-positive (IgG) AIHA (Hb = 8 g/dl) and thrombocytopenia (plts 57×10^9/l). He was treated with corticosteroids and CY, 1 g monthly for 6 months, and had a seronegative remission. In April 1996, he had thrombocytopenia (plts 7×10^9/l) again, was treated with corticosteroids, azathioprine, CY bolus and achieved a partial, seropositive remission. He was splenectomized in July 1996 and achieved a CR. However, in November 1998, he had thrombocytopenia (plts 4×10^9/l) again, which remitted following HD corticosteroids. In May 2000, he had thrombocytopenia (plts 7×10^9/l) again, developed a Guillain–Barré syndrome and remitted following HD-IgG plus corticosteroids. At that time he was suffering from extremely disfiguring hypercorticism and severe depression. The suggestions of an allogeneic SCT was made to patient and parents, and his only, 12-year-old sister was found to be HLA-identical, and of the same blood group (O Rh+).

At the time of transplant, serologic autoantibody reactions against RBC (DAT) and platelets (solid phase

Capture-P system, Immunocor, Norcross, USA) were strongly positive. Left ventricular ejection fraction, pulmonary function tests, renal and liver functions were normal. Conditioning was performed according to a reduced intensity regimen[13] consisting of thio-tepa (TT) 10 mg/kg plus CY 100 mg/kg. The BMT inoculum contained 4.2×10^8 cells/kg, and the CD34+ cells were 3.7×10^6 kg. The patient received 1 mg/kg CYA intravenously from day 1 to day 20, and then by mouth tapering gradually. The post-transplant course was uneventful, and only 5 plt concentrates were needed, after which an impressive plt peak occurred (700×10^9/l; Figure 1a), which gradually subsided and while there still was mixed chimerism in the marrow (cytogenetics, FISH, STR) and blood (STR). Owing to the persistence of mixed chimerism, he received four gradually incremented DLI up to 1×10^7, but the platelet level sank to 86×10^9/l on day +245, and to 33×10^9/l on day +293. On day +245, he received the fifth and last DLI (5×10^7), which was followed by gastrointestinal graft-versus-host disease (GVHD) grade II (AST 67, ALT 724, GGT 438), and also by the achievement of complete donor chimerism, normalization of the platelet level and total autoantibody negativity (Figure 1b). On day +493, the patient is in good health, there are no traces of GVHD, and he is fully independent of steroids. He still receives 80 mg of CyA/os, which is being gradually tapered with the object of final discontinuation.

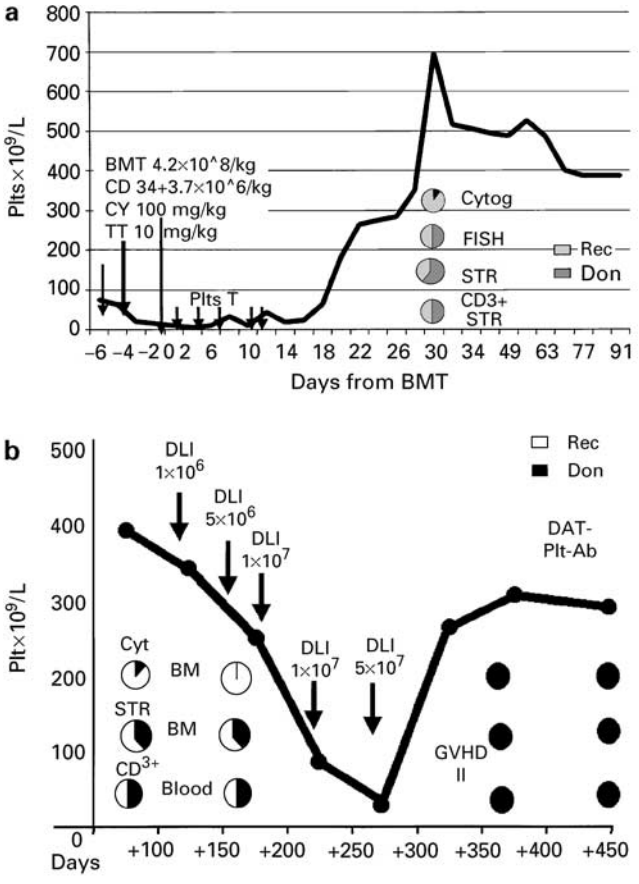

Figure 1 (a) Platelet increment following allo-BMT. A peak of 700×10^9/l has taken place on day +30, while there is still mixed chimerism of different degrees according to the procedures (cytogenetics, FISH and STR on marrow, STR also on peripheral CD3+ lymphocytes); (b) Gradual thrombocytopenic relapse in the presence of mixed chimerism. Five gradually increasing DLI are followed by grade II GVHD, platelet recovery and full donor chimerism.

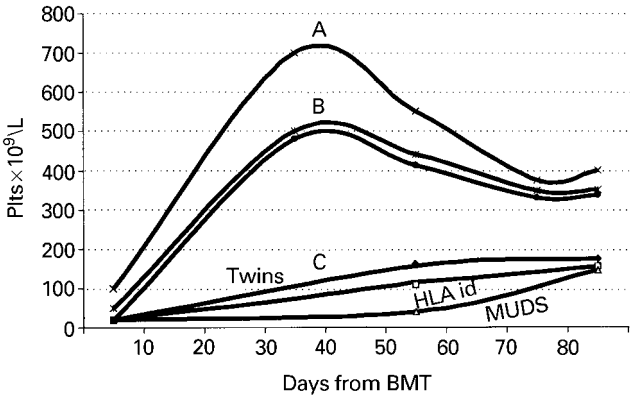

Figure 2 A comparison between platelet reconstitution in patient UP 1273 (**a**), the two patients treated with autologous ASCT by Lim *et al*[9] (**b**) and 342 allotransplanted patients in Genoa (**c**; Raetz *et al*[13]).

Discussion

This 21-year-old male with refractory-relapsing ES, which had gradually evolved to AITP, was treated with a reduced intensity conditioned allogeneic BMT from his HLA-identical 12-year-old sister. He had an initial dramatic platelet peak, but while still evidencing mixed chimerism he again became progressively thrombocytopenic, and finally remitted following five DLI. Some significant points will be discussed.

Therapeutic value of allo-SCT

As of spring 2001, about 500 autologous SCT had been performed worldwide for an extensive spectrum of severe autoimmune diseases.[10] The procedure does induce long-lasting remissions in some categories of patients, while in others a more realistic, short-term goal may fall somewhere between broad immune suppression and total tolerance.[14] An advantage of the allogeneic *vs* the autologous procedure was reported in a case of refractory AIHA,[15] but there is not (yet?) much evidence that a new immune system will confer an advantage over the original one, although reports of successful syngeneic transplants from nonconcordant twin donors[12,16] are intriguing. Here we discuss the possibility of a graft-versus-autoimmunity (GVA) effect.

Immune identity of early platelet peak

There was a peak of 700×10^9/l platelets on day +30, when donor marrow engraftment was 10% by cytogenetics, 50% by FISH and 60% by STR. Measurement of chimerism was in line with recent recommendations.[17] Platelet recovery following allogeneic unmanipulated SCT was recently analysed in 342 patients with haematological malignan-

cies.[18] On day +50, twin grafts reached a median of 149×10^9/l, while an identical sibling graft had a median of 108×10^9/l. Contrarily, in the two autologous AITP cases reported by Lim *et al*[9], there were platelet peaks of 500×10^9/l on day +35 (Figure 2). Considering that the 700×10^9/l peak in our case appeared on day +30 with minimal donor engraftment, we feel that the available evidence strongly favours an initial autologous reconstitution. Such platelet responses, especially in splenectomized patients, reflect, in our opinion, the highly cycling and productive status of autologous megakaryocytes, and are reminiscent of platelet responses following successful splenectomies.

Mechanism of action of allo-SCT

The first effect is clearly mediated by cytotoxic suppression of the autoimmune clones and their autoantibody products, some of which are now being shown to be clonal.[19] However, an additional effect may be hypothesized, consisting of the abrogation of the original autoantigens/epitopes, known to reside preferentially in the GPIIb/IIIa or GPIb/IX regions, in view of the ablation of the original marrow. A new, healthy marrow should be devoid of epitopes challenging an equally new immune system. Conversely, if relapses should occur with long FU notwithstanding persisting complete donor lymphohaematopoietic chimerism, the main pathogenic mechanism would have to be shifted towards other contributing factors.

GVA

Evidence is accumulating that a GVA effect exists, consisting most probably in the substitution of normal T, B and lymphoid progenitor cells for the autoimmune clones of the patient's immune system.[20] Experimentally, it has been shown that mixed chimerism utilizing a sublethal irradiation conditioning regimen followed by allogeneic BMT can prevent the onset of diabetes and even reverse pre-existing autoimmune insulitis in nonobese diabetic (NOD) mice, whereas the same radiation protocol without allogeneic SCT is insufficient.[21] A similar effect has been demonstrated using sublethal conditioning and an anti-CD54 monoclonal antibody.[22] Some clinical evidence is also appearing. In a similar case of ES who had received an allogeneic STC, CR was achieved only after grade IV GVHD supervened following discontinuation of immunosuppressive therapy because of intolerance.[4] Another case is that of a patient with CML in the first chronic phase, who was also suffering from severe psoriasis and psoriatic polyarthritis. He was given a nonmyeloablative allogeneic PBSCT, and enjoyed a CR of both diseases. However, relapse occurred coincident with an increase of host DNA, but finally discontinuation of CYA was followed by complete chimerism and remission of both diseases.[23] The protective antiautoimmune relapse effect of GVHD has been confirmed in a meta-analysis of individual patient data.[24]

The use of nonmyeloablative conditioning in allogeneic SCT for severe autoimmune diseases (SADs), followed by the combination of CYA discontinuation and, if necessary, gradually incremental DLI has been strongly recommended by Slavin *et al*,[23] and was undertaken in this patient because of his poor general condition and the necessity for a (hopefully) decisive treatment. There are conflicting immune reactions following allogeneic SCT for SADs. GVH and GVL show the usual mix that has been observed for decades in malignancies, although separation of GVH and GVL is now being envisaged.[25] Separation of GVH from GVA may be even more complex, taking into consideration the much rarer cases of patients with SADs for which allo-SCT may become an attractive option. Whether long-term remission and tolerance[26] will be achieved in this, and in similar cases, remains to be established.

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