

Case Report

Development of common variable immunodeficiency in a patient with Evans syndrome treated by autologous stem cell transplantation

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We describe a case report of a patient who developed common variable immunodeficiency (CVID) after autologous haematopoietic stem cell transplantation (SCT) for recurrent Evans syndrome. The disease manifested as attacks of haemolytic anaemia, thrombocytopenia and neutropenia from the age of 12 years. Presence of autoantibodies to blood elements was confirmed together with C4 deficiency. The patient also suffered from dermatitis herpetiformis Dühring without signs of coeliac disease. Autologous T cell-depleted peripheral blood stem cell (PBSC) transplant following conditioning regimen was performed at the age of 20 years. Immunological reconstitution was incomplete and 2 years after SCT he fulfilled laboratory criteria for common variable immunodeficiency (CVID). The patient was found to be a carrier of a risk haplotype for development of CVID DRB1*03/DQB1*0201. We conclude that T cell-depleted SCT here performed for autoimmune manifestations can hasten development of CVID in genetically predisposed patients.

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Evans syndrome is an uncommon haematological disorder characterized by simultaneous or sequential development of immune thrombocytopenic purpura (ITP), autoimmune haemolytic anaemia (AIHA) and less frequent neutropenia. The course of disease is chronic, recurrent and is associated with significant morbidity and mortality (1). The response to the common immunosuppressive therapy used in ITP and AIHA (steroids and intravenous globulin-IVIG) is often slow and incomplete. Splenectomy brings only transient effect. The consensus on best treatment strategy is still lacking. High-dose immunoablative therapy with stem-cell rescue might therefore be a reasonable alternative. Evans syndrome is on the list of autoimmune disorders in which autologous stem-cell transplantation (SCT) could bring significant clinical benefit (2), but the number

of patients with Evans syndrome who underwent SCT is small and the results are still controversial (3).

Common variable immunodeficiency (CVID) is a genetically determined complex immunological disorder characterized by marked reduction in serum levels of both IgG (< 3 g/l) and IgA (< 0.05 g/l) (4). Apart from hypogammaglobulinaemia, many autoimmune complications (including Evans syndrome) occur in approximately 10% of patients (5). CVID represents a complex genetic defect most likely involving immunoglobulin class switching. The predisposing genetic locus maps to the MHC class II or III region on chromosome 6p21 (6). The triggering events leading to the arrest of immunoglobulin synthesis include environmental factors such as infections and drugs, but mostly unknown intrinsic factors are involved.

Here we describe a case of Evans syndrome in a patient who developed CVID within 2 years of autologous T cell-depleted (TCD) SCT.

Case report

In June 1989 at the age of 12 years, our patient presented with severe Coombs-positive autoimmune haemolytic anaemia (AIHA), followed by the development of dermatitis herpetiformis Duhring. Coeliac disease was not confirmed. Two years later the patient suffered from his first attack of ITP and a diagnosis of Evans syndrome was established. During the next 6 years, he suffered from repeated relapses of ITP (seven times) and/or immune pancytopenia. The number of platelets decreased below $20 \times 10^9/l$ in all relapses. The longest haematological remission lasted 20 months. Coombs' test remained positive and platelet antibodies were continually detected. Dermatitis herpetiformis resolved on continuing immunosuppressive therapy. The intervals between relapses of ITP became shorter. Front-line therapy of relapses consisted of steroids and IVIG with unpredictable response. Neither cyclosporin, a course of intermediate-dose cyclophosphamide (CY) nor rabbit antithymocyte globulin (ATG) induced long-lasting remission or serological negativity. Other drugs used during the interim period were vincristine, Danazol and dexamethasone (pulses). A 5-day course of fludarabine ($20 \text{ mg/m}^2/\text{day}$) in combination with prednisolone 1.5 mg/kg/day was given in April 1997, and negativity in Coombs' test and platelet antibodies was achieved. Autologous SCT was then performed as a consolidation therapy to maintain that remission.

PBSC transplantation

In September 1997, the mobilization of PBSC (CY $4 \text{ g/m}^2 + \text{G-CSF } 10 \mu\text{g/kg}$) was performed in haematological and serological remission followed by leukaphereses, starting at day +13 after CY (separator Cobe Spectra; Cobe, München, Germany). CD34^+ -positive cells selected by Cell-Pro (Ceprate LC, Bothell, WA, USA) were then T cell-depleted by E-rosetting using donor red blood cells coated with tetrameric complexes of CD2 and CD3 antibodies. The final graft contained $7.1 \times 10^6/\text{kg}$ CD34^+ cells and $2.2 \times 10^4/\text{kg}$ CD3^+ cells. In October 1997, 8 years after the first manifestation of Evans syndrome, the patient underwent autologous SCT after a preparative regimen consisting of ATG (horse) $15 \text{ mg/kg/day} \times 4$ (days -9 to -6) and CY $50 \text{ mg/kg/day} \times 4$ (days -5 to -2). The post-transplant course was

complicated by *Escherichia coli* sepsis. White cell engraftment ($\text{ANC} > 0.5 \times 10^9/l$) was achieved on day +11 and platelet engraftment (platelets $> 20 \times 10^9/l$) on day +9. Platelets increased to over $100 \times 10^9/l$ on day +12 and the patient was discharged from the hospital 18 days after SCT on acyclovir (ACV) prophylaxis and an intermittent dose of prednisolone (20 mg every other day, later on 10 mg every other day). ACV was finished on day +50. Five months after transplantation the patient developed herpes zoster (VZV) infection. Steroids were discontinued 6 months after SCT; 1 month later (May 1998) relapse of ITP occurred, responding quickly to steroid therapy. Coombs' test and platelet antibodies were negative until 2 years after SCT, when for the first time an immunofluorescence test for free platelets antibodies showed weak positivity. At that time mild and temporary decrease of platelets to $87 \times 10^9/l$ appeared followed by spontaneous normalization. In November 2001, 4 years after SCT, he suffered from an attack of severe neutropenia ($\text{ANC } 0.02 \times 10^9/l$), responding well to steroids. Coombs' test remained negative and platelets were maintained in the lower range of normal value. From a haematological point of view SCT was effective. The frequency of the autoimmune cytopenias was much lower than before SCT.

Effect of TCD SCT on patient's immune system

The reconstitution of the cellular immunity post-SCT is presented in Table 1. Total lymphocyte count of $> 1000/\mu\text{l}$ was achieved on day +25. Table 2 demonstrates gradual decline of humoral immunity from normal levels before the transplant to severe hypogammaglobulinaemia. All subclasses of IgG had decreased, most distinctly IgG2. Mitogen response to phytohemagglutinin was less than 50% of the control value prior to transplant and even 3 years after PBSC $\geq 50\%$ of the control value was not achieved. The patient's response to

Table 1. Reconstitution of the cellular immunity after SCT

		Day +30	Day +90	Day +180	12 months	24 months	36 months	48 months
CD3	%	69	82	75	81	80	73	83
	$\times 10^9/l$	0.58	1.30	1.07	1.40	1.35	1.48	1.44
CD4	%	7	6	26	28	41	42	51
	$\times 10^9/l$	0.06	0.10	0.37	0.49	0.69	0.85	0.88
CD8	%	70	69	24	52	37	29	35
	$\times 10^9/l$	0.59	1.10	0.34	0.90	0.62	0.59	0.61
CD19	%	1	6	10	8	11	14	8
	$\times 10^9/l$	0.01	0.10	0.14	0.14	0.19	0.28	0.14

Table 2. Humoral immunity before and after SCT

	Before SCT*	Day +30	Day +90	Day +180	12 months	24 months	48 months	Normal ranges
IgG (g/l)	8–11.5	8.8	4.24	7.56	3.06	3.03	2.9	9–16
IgA (g/l)	1.0–1.2	0.17	<0.08	0.12	<0.08	<0.08	<0.058	1.0–3.5
IgM (g/l)	1.2–2.2	0.77	0.41	1.9	1.07	0.58	0.4	0.7–2.9

*Ranges depending on the actual immunosuppressive therapy.

pokeweed mitogen was also less than 50% of the control value. Relative and absolute numbers of circulating B cells expressing CD19 were normal post-transplantation. Antibody response to diphtheria, tetanus, poliomyelitis, pneumococcus, *Hemophilus influenzae B* and meningococcus A and C vaccines, given at 12–24 months post-transplant, was characterized by low increment and rapid decline (data not shown). Proliferation of lymphocytes to tuberculin and tetanus toxoid was unmeasurable. VZV infection 5 months after transplant failed to induce sustained immunological response with negative VZV IgG 2 years after SCT. Autoantibodies (nonorgan specific) were repeatedly negative before and after the transplant. An intestinal biopsy did not show any abnormalities. C4 complement was deficient before and after SCT (serum concentration <0.05 g/l). The HLA tissue type was A2, B14, B18, Cw5 (serology) and DRB1*03/03-DQA1*05/05-DQB1*0201/0201 (PCR-SSP).

Discussion

Evans syndrome in our patient was associated with another autoimmune disorder, dermatitis herpetiformis Duhring (without coeliac disease). Long-lasting immunosuppressive therapy before SCT achieved clearance of the typical skin exanthema, and 4 years after SCT the patient shows no signs of this disorder. C4 deficiency was confirmed in this patient, known to be a predisposing factor in several autoimmune diseases (7). Autologous SCT with log 4 reduction of T cells was performed in the clinical and laboratory remission of Evans syndrome as rescue therapy. Engraftment was prompt and the stay of the patient at the transplant unit was short. Reconstitution of the cellular and humoral immunity was initially in the range seen after TCD autologous SCT (8), with the only exception being in profound IgA deficiency. Five months after SCT the patient suffered from herpes zoster infection, which is not unusual at this time after TCD SCT. Shortly after this infection relapse of ITP occurred, possibly due to viral herpetic infection or completion of steroids 1 month prior to development of ITP. Complete remission lasted

for 7 months after SCT and for 15 months after the last relapse of ITP before SCT.

Immunological reconstitution 2 years after SCT was poor, e.g. serum concentration of IgG and IgM was low and severe IgA deficiency was confirmed. Lymphocyte proliferative response to mitogens was impaired. All in all, the patient fulfilled laboratory criteria for the diagnosis of CVID but, clinically, he did not suffer from recurrent infections (5). He was placed on regular supportive therapy with IVIG 2 years after SCT. Four years after SCT the hypogammaglobulinaemia persists (Table 2 shows the mean preinfusion concentration of immunoglobulins).

Immunoglobulin levels were in the range of normal values before SCT, but lymphocyte response to mitogens was already impaired. The explanation for this could be the long-lasting immunosuppressive therapy or slowly developing CVID. Patient has C4-complement deficiency. The genetic susceptibility to IgA deficiency is shared with CVID, and the susceptibility locus of both disorders maps to the region on chromosome 6 *p21.3* containing the HLA complex, as well as genes encoding for C4 complement. The patient's haplotype represents another risk factor as DRB1*03-DQA1*05-DQB1*0201 is associated with dermatitis herpetiformis, CVID and other autoimmune diseases (9).

Evans syndrome is a heterogeneous disorder that can represent a stage of a generalized immune dysregulation. Defects of humoral and cellular immunity are not uncommon in patients with Evans syndrome (1). Immune cytopenias are particularly associated with CVID and can precede the manifestation of CVID for years (5, 10). SCT with T-cell depletion complicated by bacterial and herpetic viral infections, and further immunosuppressive therapy for relapse of ITP, probably hastened the development of CVID in our patient, which otherwise would manifest after much longer period. Having a high-risk haplotype for CVID and C4 complement deficiency probably heralded the risk of development of this disorder.

In conclusion, we recommend careful immunological investigation before autologous SCT with T cell depletion in patients with autoim-

mune disorders. Great caution should be exercised when considering such therapeutic modality in patients genetically predisposed to the development of CVID, in whom immune dysregulation after TCD SCT can hasten development of primary immunodeficiency.

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