Successful treatment with T depleted autologous peripheral blood stem cell transplantation of refractory chronic autoimmune thrombocytopenic purpura

Autoimmune thrombocytopenia (AITP) is a disorder due to specific platelet auto-antibodies directed against platelet surface glycoproteins. AITP in adults is usually chronic, idiopathic and frequently refractory to conventional treatments. Myelo- and immuno- suppressive chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation is an experimental approach for severe chronic refractory AITP. We report a case of a woman with AITP, refractory to the conventional therapy, submitted to T-cell-depleted autologous PBSC transplantation, which obtained long term stable response on platelet count. We deem that the positive outcome of our patient depends on T-cells depletion of the graft, which reduces autoreactive T clones.

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Introduction

Autoimmune thrombocytopenia (AITP) is a disorder due to specific platelet auto-antibodies directed against platelet surface glycoproteins, usually GPIIb/IIa, GPIb/IX or both. AITP is characterized by a peripheral destruction of the platelets (PLT), usually occurring in the reticular macrophage system splenic. AITP may have a favourable evolution within 6 months from the diagnosis (acute AITP, occurring mainly in children and frequently secondary to viral infections[1]), while the persistence of thrombocytopenia for longer than 6 months characterizes chronic AITP. This occurrence is more common in adults and usually confers refractoriness to conventional treatments[1,2]. In fact, nearly one third of patients fails to respond to standard treatment with corticosteroids, intravenous immunoglobulin (IVIg) or splenectomy[2]. Chronic refractory AITP has been reported to have a mortality rate of 4% to 16%, largely attributable to bleeding or secondary infections[3]. These patients may respond to immunosuppressive cytotoxic agents (cyclophosphamide, cyclosporine, azathioprine), androgens, vinca alkaloids but long-term results of such treatments are disappointing and relapse commonly occurs after drugs discontinuation[4]. Myelo- and immuno- suppressive chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation is an experimental approach for severe chronic refractory AITP, but in different reports its success has been variable. Lim *et al*.[5] reported complete remission of AITP in two patients treated with cyclophosphamide 200 mg/kg over four days, followed by infusion of unmanipulated autologous PBSC, mobilized with cyclophosphamide and G-CSF. Unfortunately both patients relapsed within 18 months. Recently Huhn *et al*.[6] reported the results of a pilot study on 14 patients with chronic AITP, treated with autologous PBSC depleted from lymphocytes by immunomagnetic CD34+ selection. Conditioning regimen consisted of high dose cyclophosphamide (50 mg/kg/day for 4 days) only. In this study eight patients obtained durable responses, probably due to T-cells depletion of the graft, which reduces autoreactive clones. In fact, T lymphocytes may initiate and maintain immune recognition of autologous PLT and stimulate B lymphocytes to produce anti-PLT antibodies. In this setting autoreactive T lymphocytes, collected in PBSC graft, could re-establish autoimmunity, so T-cells depletion appears as a fundamental condition for a positive outcome of transplantation in AITP, similar to that observed in other autoimmune diseases submitted to autologous transplantation. We report below the clinical history, therapy and outcome after T-cell-depleted autologous PBSC transplantation of a woman with AITP refractory to the conventional therapy.

Case report

In September 2002 a 60 years old female patient was referred to our observation with mucosal and cutaneous bleeding. In particular she presented mild epistaxis, gingival bleeding and diffused petechiae to arms and legs. Laboratory investigations showed only a platelet count less than 10.000/mm^3, without other abnormalities in the peripheral blood smear. Family history, personal anamnesis and clinical status of the patient excluded other conditions, which could be associated with autoimmune or non-immune thrombocytopenia (drugs, HIV or Hepatitis C infection, other autoimmune disorders, malignancy). Physical examination did not reveal particular features: no enlargement of lymph-nodes, liver and spleen had been detected. Clinical diagnosis of AITP had been done and confirmed with bone marrow examination. The patient received four monthly courses with high dose dexamethasone (20 mg/m^2/daily for 4 non-consecutive days each course), by intravenous infusion (i.v.) with a partial and transient response only (PLT 50.000/mm^3). The patient had been submitted to splenectomy in December 2002, after high dose of Immunoglobulin treatment (0,4 g/Kg/day for 5 days). After an initial increase in PLT count, the patient failed to obtain a stable response and PLT count returned to less than 10.000/mm^3. Immunosuppressive agents as cyclophosphamide, cyclosporine, azathioprine and bolus of vincristine (1 mg each two or three weeks for 15 infusions) did not get a stable normal PLT count. Therefore the patient had been submitted to T-cell-depleted autologous PBSC transplantation two years after the onset of the disease (September 2004).

PBSC were mobilized with cyclophosphamide (2,5 g/m2) and G-CSF (10 μg/Kg/day) i.v., and collected with a single leukapheresis. CD34+ positive immunomagnetic selection (CliniMACS, Miltenyi) allowed to select stem cells but not T-cells, which were eliminated from the graft7. This strategy combined *in vivo* and *in vitro* immuno-suppression, and in the successive conditioning regimen a prevalent myeloablative approach was required. Therefore the patient had been treated with Melphalan 100 mg/m^2, administered 2 days before PBSC infusion. The final dose of CD34+ cells was 7.2x10^6/Kg, and the residual dose of CD3+ cells in the graft was 1,43x10^3/kg only (CD3+ total dose infused: 0,1x10^6). Hematopoietic recovery was supported with G-CSF at 5 μg/Kg/die daily i.v. until the absolute neutrophil count (ANC) exceeded 500/mm3 for 3 successive days. No significant and persistent improvement on PLT count had been noted between PBSC mobilization and transplantation.

Prophylactic antimicrobical therapy (levofloxacin 500 mg orally once a day and fluconazole 100 mg orally twice a day) had been administered until the engraftment. Trimethoprim/Sulfamethoxazole 160/800 mg orally twice a day on 2 days weekly and acyclovir 400 mg orally 3 times a day had been given for six months after transplantation. ANC recovery (>500/mm^3), supported with G-CSF, was observed from day +9 and PLT recovery (>20000/mm^3) from day +18 after transplanta-

tion.

Neither major adverse events nor infections were observed during and following the transplant phase. Mild epistaxis and mouth bleeding were controlled by PLT transfusions during pre-engraftment phase. Asymptomatic CMV reactivation was documented from day + 22 (CMV antigenemia: 10/200.000 cells) to day +36 (CMV antigenemia: 12/200.000 cells) only and not confirmed during the following weekly controls. Therefore no specific CMV treatment has been performed and no steroid treatment has been administered after transplantation.

After a follow-up of two years the patient shows a good response with a stable PLT count around 120.000/mm^3, despite an initial reduction immediately after the engraftment, probably secondary to CMV reactivation (Figure 1). No treatment is required at present.

Discussion

we found that autologous lymphocyte-depleted PBSC transplantation was feasible in patients with severe refractory chronic AITP. The procedure of mobilization of hematopoietic progenitors cells with cyclophosphamide and G-CSF is well tolerated. In our patient PBSC were collected during one single apheresis session. We did not found any life-threatening or significantly morbid hemorragic event related to transplantation, neither several infections have been documented. The patient showed one single episode of asymptomatic CMV reactivation, but the possible role of T cell depleted autograft in this event is controversial. After 24 months of observation, the patient shows a good sustained PLT response without need of other treatments. We deem that the positive long term outcome of our patient could depend on T-cells depletion of the graft, which reduces autoreactive T clones. In fact, according to Huhn *et al.*6, we suppose that autoreactive T cells collected in PBSC graft could re-establish autoimmunity, via initiating and maintaining immune recognition of autologous PLT and stimulate B cells to produce anti-PLT antibodies.

Conclusion

T cell-depleted autologous PBSC transplantation may be considered a feasible and valid therapeutic option in refractory severe AITP, so as for other refractory autoimmune disorders. Deeper studies and prolonged follow-up need to demonstrate the percentage and the durability of the remission and to determine the real risk/benefit profile.

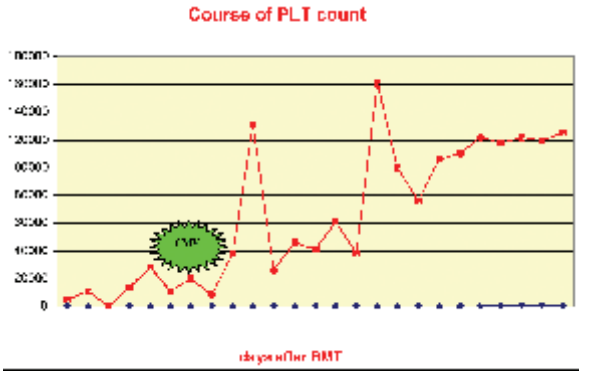

Figure 1: Course of PLT count after autologous PBSC transplantation; day – 4: PLT level pre-transplant; day 0: PLT level at transplant

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