

# Biopterin Synthesis in Mouse Spleen during Bone Marrow Transplantation Correlates with Unimpaired Hemopoietic Engraftment

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*Dedicated to Professor Helmut Simon on the occasion of his 60th birthday*

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Normal engraftment of bone marrow transplants depends on histocompatibility between donor and recipient. In this case reconstitution of hemopoiesis and lymphopoiesis in the mouse spleen is correlated with transient biopterin synthesis in these cells. Allogeneic bone marrow progeny cells undergo donor cell proliferation in the spleen prior to the incidence of graft-versus-host reaction. These cells are not committed to biopterin synthesis. Thy-1 monoclonal antibody fully repairs engraftment of allogeneic transplants together with biopterin synthesis.

Bone marrow transplantation (BMT) has become a curative therapy for severe marrow aplasia, congenital immune deficiency and for certain leukemias [1]. Its general application however, is limited by complications due to histoincompatibility between the marrow graft and the patient. Since the patient is usually immunosuppressed by high-dose radiation or chemotherapy in order to prevent graft rejection, the major immunological complication in BMT results from immunocompetent donor T lymphocytes in the hemopoietic graft which attack the patient's tissue in the form of graft-versus-host reactions (GVH). Inhibition of GVH induced by donor T lymphocytes without damaging hemopoietic stem cells [2], which are necessary for bone marrow transplantation, requires cell-specific immunotherapy. This was originally studied with a GVH model in mice where the donor marrow was initially preincubated *in vitro*, with polyclonal, and later with monoclonal anti-pan T cell antibodies [3, 4]. Recently a group of monoclonal T cell antibodies with Thy-1 specificity was identified which suppressed murine GVH when injected *in vivo*, i.e. the marrow recipient [5].

Less clear is the interrelation between immunobiological findings in GVH and biochemical changes. In addition to known functions, such as aromatic amino acid hydroxylation cofactor or as part of molybdo-cofactor (for review see ref. [6]), pteridine synthesis proved to be intimately associ-

ated with (i) hemopoietic cell proliferation and (ii) with immunoproliferation of lymphocytes. Conclusion (i) was first reached as a result of pteridine analysis during the course of autologous BMT in beagle dogs [7], a situation which excludes immunological reactions due to histoincompatibility. In patients who had received BM infusion three episodes of varying pteridine levels were observed. These changes affected both peripheral blood cell biopterin and urinary neopterin levels. Initial radiation or cyclophosphamide-induced aplasia caused their decline. A few minutes after infusion, blood cell biopterin rose transiently due to the presence of immature donor cells in the peripheral circulation; subsequent reconstitution of hemopoiesis was furthermore indicated by increased blood cell biopterin and urinary neopterin. Incidence of GVH entailed a third episode of pteridine synthesis which takes place in the immunoproliferating lymphocytes and which is manifested by increased urinary neopterin (for review see ref. [8]).

The potential value of monitoring those parameters is considerable, because so far the diagnosis of GVH has to rely on less specific symptoms such as skin involvement, diarrhoea or liver malfunction.

The following study of *in vivo* changes of biopterin levels uses a well standardized inbred mouse BMT model, in which we compare syngeneic and allogeneic BMT. Allogeneic BMT resulting in donor T cell-induced GVH was also compared to allogeneic BMT in which GVH was prevented by anti-Thy-1 monoclonal antibodies. The question was raised whether the

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three types of BMT are characterized by altered biopterin levels in the spleen and whether such changes can be attributed to hemopoietic engraftment or to the GVH-induced immune response.

## Material and Methods

### *Bone marrow transplantation and GVH*

Twelve-week-old C57BL/6 (H-2b,IA<sup>b</sup>) mice and (C57BL/6xCBA)F1 hybrids (H-2b,IA<sup>b</sup>xH-2k,IA<sup>k</sup>)F1 were raised in our institute. The parental strains were originally obtained from the Jackson laboratory (Bar Harbor, ME). Spleen cells, rich in T cells, were added to bone marrow in order to simulate acute mortality of GVH which occurs in dogs and primates following transfer of incompatible bone marrow contaminated with blood T lymphocytes.  $5 \times 10^7$  spleen and  $2 \times 10^4$  bone marrow cells of donor strain were injected into the tail vein of cells of preirradiated (C57BL/6xCBA)F1 mice. The latter had been exposed 24 h earlier to 9 Gy using <sup>137</sup>Cs (Gammacell, HWM-D-2000) at 131 rad/min, target distance 35 cm. Syngeneic donors were (C57BL/6xCBA)F1 mice. Acute GVH lethal within 2 weeks occurred when cells from C57BL/6 donors were administered.

### *GVH prophylaxis with monoclonal antibodies (moab)*

RmT1, a rat anti-Thy-1 moab of IgG2b isotype was produced as described [5]. It was applied as ascitic fluid equivalent to 1 mg antibody. A single subcutaneous injection 4 h after irradiation of (C57BL/6xCBA)F1 mice prevented GVH resulting from subsequent transfusion of C57BL/6 donor marrow [5].

### *Determination of biopterin*

The spleens (half of each) were homogenized in 5 ml 0.2 N HCl in a teflon-fitted homogenizer (Braun, Melsungen) with 500 strokes/min. Iodine oxidation of tetrahydro- and dihydrobiopterin, protein precipitation by trichloroacetic acid and prepurification by Dowex H<sup>+</sup> ion exchange chromatography was performed according to Fukushima and Nixon [9]. Slight modifications of this method, the instrumentation assembly for HPLC separation and the fluorometric determination of biopterin as described in [10]. Each data point was obtained from 4–6 determinations.

## Results

The radiation-induced aplasia caused a decrease of spleen weight during the first 3 days in all three types of BMT (Fig. 1a and 1b). During the subsequent period (days 5–11) hemopoietic reconstitution took place in spleen and bone marrow of syngeneic transplant recipients; donor cell proliferation by allogeneic bone marrow caused splenomegaly. Both types of BMT caused increase in spleen weight to about 3-fold normal levels. Application of Thy-1 moab had no effect. Animals which had received allogeneic bone marrow without *anti*-Thy-1 moab, developed acute GVH and died within 13 days after transplantation.

During the period of aplasia (0–4 days after transplantation), spleen cell biopterin declined slightly in all types of BMT (Fig. 2a). Subsequently (days 5–9), spleen reconstitution by syngeneic transplants was manifested by a transient increase of biopterin up to 3-fold levels. Normal levels were resumed during the following 6 days. In the recipients of allogeneic transplants, proliferation of the histoincompatible bone marrow progeny cells occurred. These proliferating donor cells were not competent for biopterin synthesis. The levels of this pteridine fluctuated within the range which was observed during the period of aplasia. Immunotherapy with Thy-1 moab restores fully the commitment of the donor cells for biopterin synthesis, together with hematopoietic and lymphopoietic reconstitution (Fig. 2b). The time course of the transient pteridine accumulation was identical with that obtained during syngeneic transplantation.

## Discussion

The results demonstrate that biopterin synthesis in the spleen during BMT is dependent on normal hematopoietic and lymphopoietic reconstitution which only takes place in case of histocompatibility between marrow graft and the recipient. The *de novo* biopterin synthesis pathway is completely absent in proliferating donor cells in case of histoincompatibility. The model system emphasizes the value of monitoring either blood cell biopterin or urinary neopterin during BMT in patients. Their take peak indeed, indicates a successful engraftment and the onset of unimpaired hemopoietic reconstitution (for review see ref. [8]).

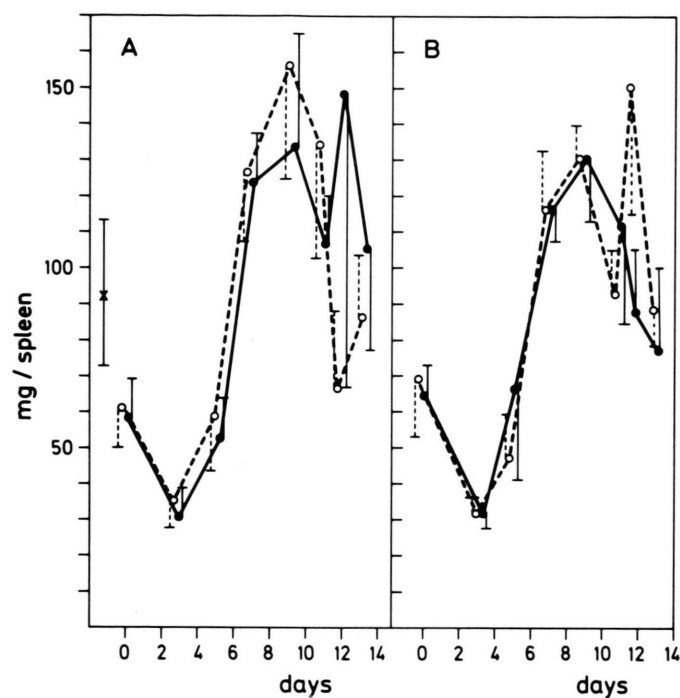


Fig. 1. Spleen weight during syngeneic and allogeneic bone marrow transplantation in mice. Aplasia had been introduced by irradiation prior to transplantation on day 0.

A: ●—● syngeneic transplantation;  
○---○ allogeneic transplantation;  
B: ●—● syngeneic transplantation together with application of Thy-1 moab;  
○---○ allogeneic transplantation together with application of Thy-1 moab.

TT S.D.

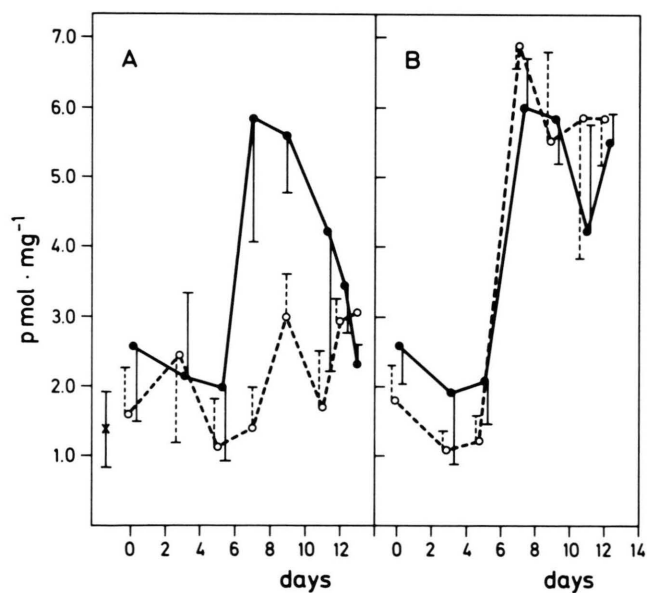


Fig. 2. Biopterin concentrations during syngeneic and allogeneic bone marrow transplantation in mice. Aplasia had been introduced by irradiation prior to transplantation on day 0.

A: ●—● syngeneic transplantation;  
○---○ allogeneic transplantation;  
B: ●—● syngeneic transplantation together with application of Thy-1 moab;  
○---○ allogeneic transplantation together with application of Thy-1 moab.

TT S.D.

Lectin stimulation of mouse spleen lymphocytes [10] or of human peripheral blood mononuclear cells [11] induces an episode of gradual pteridine accumulation which extends over a period of 3 days. During the following 24 h pteridine levels drop to initial values. A second episode of pteridine accumulation occurring in primed T cells upon binding of interleukin 2, reaches a maximum after 8–12 h and declines in the subsequent 6–10 h [12]. A short-term and transient pteridine synthesis is triggered by replacing the lymphokine with phorbol ester [13]. On the other hand, interleukin 2-mediated proliferation is modulated by tetrahydrobiopterin. This cooperation occurs at the receptor level [12].

The link of tetrahydrobiopterin synthesis with lymphokine-receptor interaction may offer an interpre-

tation of biopterin deficiency in the system of allogeneic bone marrow recipients. Interleukin 2 receptor dysfunction is reported for mice undergoing a GVH [14]. The early loss of interleukin 2 receptor expression [14] is accompanied by deficient biopterin synthesis; both events may cooperate in GVH associated immunodeficiency [15]. Immunotherapy by *anti*-Thy-1 moab repairs both immunodeficient syndrome and synthesis of the immunomodulator tetrahydrobiopterin.

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