Bone Marrow Transplantation in DLA-Haploidentical Canine Littermates
Fractionated Total Body Irradiation (FTBI) and in Vitro Treatment of the Marrow Graft with Anti-T-Cell Globulin (ATCG)


A. Introduction

Allogeneic bone marrow transplantation requires tolerance of the host versus the graft as well as tolerance of the graft versus the host. This mutual tolerance can be induced in most instances of marrow transplantation between DLA-identical siblings by conditioning the host with total body irradiation (TBI) or Cyclophosphamide and post grafting treatment with Methotrexate [1].

These methods are not adequate for induction of lasting tolerance across major histocompatibility differences [2]. Marrow grafts from histoincompatible family members were significantly less successful than those from DLA-matched littermates, but somewhat better than those of histoincompatible unrelated donors [3].

A treatment regimen which allows marrow grafts between one haplotype different family members would provide possibilities of marrow transplantation for almost every patient lacking an HLA-identical sibling. In the present study bone marrow was grafted between canine littermates which differed in one DLA-haplotype. We investigated, whether fractionated total body irradiation with high total doses can suppress the hosts immune reaction against the DLA-incompatible graft and whether in vitro treatment of the graft with an anti-T-cell globulin from which crossreacting antibodies against hemopoietic stem cells have been absorbed can suppress the grafts reaction against a DLA-incompatible host.

B. Materials and Methods

I. Dogs

The dogs used for transplantation studies were beagles bred in the kennels of the Gesellschaft für Strahlen- und Umweltforschung. Their ancestors were acquired from five different commercial breeding colonies in Europe. The dogs weighing 8 to 15 kg were vaccinated against distemper, canine hepatitis and leptospirosis, regularly dewormed, and at least 6 months old. Absorption material was obtained from mongrel dogs.

II. DLA-Testing

Antigens of the DLA-A and -B locus were determined with the microlymphocytotoxicity test [4, 5], those of the DLA-D locus were defined in mixed leukocyte culture (MLC) with DLA-D homozygous typing cells as previously described [6]. DLA-haplotypes were defined by segregation analysis. DLA-homozygous donors and DLA-heterozygous littermate recipients sharing the DLA-haplotype

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of the donor were selected for graft-versus-host disease (GVHD) experiments. In these combinations mixed leucocyte cultures (MLC) regularly showed the response of the donor against the host and no response of the host against the donor.

DLA-heterozygous littermates sharing one DLA-haplotype and differing in the second haplotype showed mutual MLC responses and were selected for experiments involving host versus graft and graft versus host reactions.

### III. Total Body Irradiation

Total body irradiation was applied from two opposing 60Co-sources to the dog placed in a wooden cage midway between the sources. The cage had an interior width of 55 × 97 cm and a height of 44 cm in which the dog could move around. The dose rate was 5.5 R/min delivered at a source target distance of 400 cm. The single dose of TBI consisted of 1200 R given on day −1, fractionated total body irradiation (FTBI) was given as 1200 R on day −1 and additional fractions of 600 R on days −7 and/or −5 and −3. 600 R were chosen as a large dose fraction without clinical symptoms of gastrointestinal toxicity. Dogs were kept off food and water on the days of irradiation and for five days after the last irradiation. They were given parenteral fluids twice daily and antibiotics as clinically indicated. Marrow cells were given 24 hours after the last irradiation.

### IV. Bone Marrow Transplantation

Bone marrow was obtained from the anaesthetized donor by aspiration from humeri, femora and pelvic crest. It was mixed with tissue culture medium TC 199 containing heparin and processed as described by Thomas and Storb [7]. In cases of “in vitro” treatment 200 ml bone marrow was mixed with one or two ml absorbed ATG diluted in 10 ml TC 199 and incubated at 4°C for 30 min with constant shaking. Thereafter it was infused into the recipients within 10 to 20 min. Hemopoietic recovery was evident by a rise of blood counts following the postirradiation nadir (granulocytes of more than 350/mm³, platelets more than 20,000/mm³). GVHD was diagnosed clinically by the typical skin rash, jaundice and diarrhea. It was confirmed with complete post mortem examination including histology.

Proof of chimerism was obtained by cytogenetic analyses and DLA-typing. Karyotypes were analysed in direct 3-hour cultures of bone marrow and in 3-day cultures of blood lymphocytes stimulated with phytohemagglutinin.

### V. Production, Absorption and Testing of Antithymocyte Globulin

Antithymocyte serum was produced by i.v. injection of 10⁸ thymocytes of new born dogs into rabbits followed by three daily booster injections three weeks later and exsanguination four weeks later.

Rabbit anti-dog thymocyte globulin was absorbed with 2 × liver/kidney homogenate, 3 × new-born spleen, 2 × erythrocytes at 4°C for 30 min at a weight ration of sediment: antiserum of 1:4. The antiserum was purified as globulin fraction by ammonium sulfate precipitation, DEAE ion exchange chromatography and ultracentrifugation [8]. ATG was reconcentrated to 10 mg/ml.

Two antisera were produced and tested in complement fixation tests before and after absorption. The titer against marrow cells was selectively lowered in both (Table 1). The remaining titer of 5 and 7.5 may in part be caused by admixture of blood in the aspirated marrow sample.

4 × 10⁶ mononuclear cells were incubated with the antibody preparation in a final volume of 0.5 ml of TC 199 medium for 30 min at 4°C. Fresh dog serum was added as the source of complement (25% final dilution) and the suspension further incubated for 45 min at 37°C. The cells were then washed once and 10⁶ mononuclear marrow cells were plated in semisolid agar (0.3%). As a source of colony stimulating activity 20% serum from dogs irradiated and bled 10 days later was added. Colonies (defined as groups of 50 and more cells) were counted after a culture period of 8 days.

The unabsorbed antisera inhibited colony growth up to titers higher than 1:32 and 1:128, while absorbed antisera did not inhibit colony growth at titers of 1:8 and 1:128 (Table 2).

### VI. Cryopreservation of Bone Marrow

The cryopreservation of bone marrow was performed as described by Buckner et al. [10]. Aspirated marrow was given in 50 ml portions into 600 ml Travenol-Fenwal bags mixed with 50 ml tissue culture