Treatment with total lymphoid irradiation, cyclosporin A and a monoclonal anti-T-cell antibody in a hamster-to-rat heart transplantation model: Graft survival and morphological analysis


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Abstract. Treatment with preoperative total lymphoid irradiation and post-transplant cyclosporin A has been shown to have a synergistic effect on graft survival in allo- and xenotransplantation. Specific monoclonal antibodies against T cells and T cell subpopulations could offer new ways of preventing graft rejection in xenotransplantation. Graft survival and histology were examined after total lymphoid irradiation plus cyclosporin A treatment versus cyclosporin A plus a monoclonal antibody in a concordant, heterotopic, hamster-to-rat heart transplantation model. Preoperative total lymphoid irradiation was given at a dose of 1.25 Gy, 12 times over a period of 3 weeks. Cyclosporin A at a dose of 12.5 mg/kg per day was administered perorally and OX-19, a pan T cell monoclonal antibody, was given as intraperitoneal injections at doses of 100 μg or 500 μg/kg per day from day 0 until graft rejection. While total lymphoid irradiation alone prolonged graft survival to 9.4 days, total lymphoid irradiation plus cyclosporin A extended graft survival to a mean of 22 days. Cyclosporin alone or combined with the monoclonal antibody could not increase graft survival significantly when compared to untreated animals, which rejected their grafts within 3.7 days. Vascular rejection was the characteristic morphological finding, even after some weeks of excellent graft function. In conclusion, total lymphoid irradiation and cyclosporin A had a synergistic effect on graft survival in this concordant xenotransplantation model, although recent impressive results from other groups could not be reproduced. Total lymphoid irradiation combined with cyclosporin A appears to delay a primary humoral graft rejection, while the mechanism of rejection, judged by histology, stays the same.

Key words: Xenotransplantation – Total lymphoid irradiation, in xenotransplantation – Immunosuppression, in xenotransplantation

In allogeneic and concordant xenogeneic heart transplantations, a synergistic immunosuppressive effect of total lymphoid irradiation (TLI) and cyclosporin A (CyA) has been reported [3, 10, 18, 19]. The mechanisms of rejection in concordant xenotransplantation are complex, involving preformed natural antibodies, incompatibility of enzyme systems, and most likely the sensitization and induction of specific antibodies [7].

During recent years, monoclonal antibodies (MAB), acting as specific T cell or T-subpopulation markers and inhibitors, have been used clinically in the treatment of graft rejection [15] and in different experimental allograft transplantation models with the aim of improving conventional immunosuppressive treatment strategies [9, 11, 23]. These specific MABs could possibly influence the sensitization and induction phases in concordant xenotransplantation.

We report here on the results of studies of graft survival and histology in a hamster-to-rat model for evaluation of CyA and TLI versus CyA and MAB treatment.

Materials and methods

Animals

Outbred Sprague-Dawley rats (SPF, Møllegård Breeding Center, Copenhagen, Denmark) served as recipients and inbred Syrian hamsters (albino variant, Department of Odontological Research, Göteborg University, Göteborg, Sweden) as donors. All animals were male, 3–5 months of age, and were maintained under standard laboratory conditions, receiving humane care.
Surgical procedure

Fifty-three concordant xeno heart transplantations were performed using a modified microvascular technique described by Ono and Lindsey [14]. Briefly, the donor hamsters were anesthetized with fentanyl/fluanisone (1 mg/0.02 mg per 100 g body weight) and diazepam (0.5 mg per 100 g body weight).

Heparin (500 IU) was injected into the inferior vena cava, after which the animals were bled and the anterior chest wall was opened. The inferior and superior venae cavae were clamped and the donor heart was flushed with 5 ml aqueous heparin solution (50 IU/ml) via the inferior vena cava. After ligation of the venae cavae and the pulmonary veins, the aorta and the pulmonary artery were divided.

Recipient rats were anesthetized with pentobarbital (3.5 mg/100 g body weight). A segment of the infrarenal aorta and inferior vena cava was freed and clamped. End-to-side anastomosis was performed with 9-0 running sutures. In contrast to the original description by Ono and Lindsey, the heart was turned 180 degrees with the heart apex pointing to the right, resulting in our experience in an improved venous outward flow.

Irradiation technique

Preoperatively, TLI was performed using a 4 MV linear accelerator (Philips SL 75-5) with pentobarbital-sedated rats in a custom-built perspex box. The head, most of the lungs and heart, half of the liver, and the tail were shielded by lead alloys (Fig. 1). Treatment was given four times a week for 3 weeks; 1.25 Gy per session to a total central dose of 15 Gy. The dose rate was 0.41 Gy/min at a source-animal distance of 211 cm. The dose was calculated using a perspex phantom and continually checked by Baldvin-Farmer air ionization chambers during the irradiation sessions.

Monoclonal antibodies

Hybridoma cells secreting MRC OX-19, a MAB directed against all rat T cells [2], was a gift from Dr. A.F. Williams, MRC Cellular Immunology Unit, Oxford, UK.

Immunosuppressive treatment

CyA (Sandoz), dissolved in oleum tenue vegetabile to a concentration of 7.5 mg/ml, was given perorally from day-8 to graft rejection.

CyA levels were analyzed by radioimmunoassay (RIA; Sandoz method) on day 0 and at 14-day intervals after transplantation or at the time of graft rejection.

MRC OX-19 was administered intraperitoneally from day 0 to graft rejection.

Total white blood cells and differential counts were determined before TLI treatment, on day 0, and with 14-day intervals after transplantation or at the time of rejection.

Experimental groups

The following eight groups were studied:

Group 1: Control group, no immunosuppressive treatment  
(n = 11)

Group 2: 12.5 mg CyA/kg per day  
(n = 5)

Group 3: 25 mg CyA/kg per day  
(n = 4)

Group 4: 50 mg CyA/kg per day  
(n = 7)

Group 5: 5 Gy TLI  
(n = 7)

Group 6: 5 Gy TLI plus 12.5 mg CyA/kg per day  
(n = 9)