Total orthotopic allogeneic small bowel transplantation in rats: effect of allograft irradiation combined with cyclosporine-A therapy

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Abstract

Rejection and graft *versus* host disease are prominent features in small bowel allotransplantation in rats. Cyclosporine treatment of the recipient and irradiation of the donor were used to circumvent these phenomena in the WAG to brown Norway rat model. Irradiation of the donor with five or 10 Gy did prevent graft *versus* host disease but resulted in a more vigorous rejection of small bowel allografts in untreated recipients (mean (SEM) survival times of 11.5 (0.4) (n=8) and 7.5 (0.9) (n=11) days respectively, *versus* 16.6 (2.6) days (n=17), $p<0.01$). Cyclosporine treatment of the recipient (25 mg/kg on days 0, 1, 2, 4, and 6 after transplantation) led to a mean (SEM) survival time of 38.3 (8.5) days (n=10); 20% of the animals developed graft *versus* host disease. Combined with 5 Gy donor pretreatment, a similar survival was obtained without occurrence of graft *versus* host disease. However, cyclosporine treatment combined with 10 Gy led to a significant shortening of graft survival (23.1 (6.8) days, n=9). These results suggest that although irradiation is very effective in preventing graft *versus* host disease, high dosages may accelerate rejection either by making the graft more vulnerable to rejection or by completely removing the immunosuppressive effect of graft *versus* host disease.

Various experimental studies on the rejection of small bowel allografts have shown that under certain immunological conditions, immunocompetent cells in these grafts can cause graft *versus* host disease (GVHD).¹⁻⁴ While the occurrence of this reaction in well defined inbred systems has been conclusively documented, its importance in outbred systems is uncertain. In a previous study we successfully ameliorated GVHD in the WAG to BN rat model using whole body irradiation of the donor with 10 Gy.⁵ The effectiveness of donor irradiation had been shown previously in a parent to F1 hybrid rat model in which the donors were irradiated with 7, 9.5, and 10 Gy before small bowel transplantation.^{2,6,7} In this study we investigated the effects of combined cyclosporine treatment and donor irradiation because we had evidence that irradiation of the donor leads to accelerated rejection of the graft.⁵ We wondered whether cyclosporine could reverse this effect. Earlier we found that cyclosporine, used alone, was not effective in consistently producing long term survivors in the WAG to BN donor-host combination.⁸ In this study we show that the effect of cyclosporine on GVHD is less pronounced than its effect on

rejection, as shown earlier by Kirkman.⁹ In large animal models cyclosporine proved to be less successful than in certain rat strain combinations.¹⁰⁻¹⁷ Two recent studies in pigs, using *ex vivo* irradiation of the transplant with 0.5 Gy, a very low dose, combined with cyclosporine therapy of the recipient showed no effect on host survival time compared with cyclosporine monotherapy.^{18,19} Because the immunological problems of large animal models resemble the problems in our WAG to BN rat model, this model is highly suitable for investigating the effect of irradiation combined with cyclosporine treatment.

Methods

ANIMALS

A fully allogeneic donor-host model was employed by using inbred WAG (RT1^u) rats as donors and inbred BN (RT1^a) rats as recipients. The rats weighed between 200-350 g.

OPERATIVE PROCEDURES

Donors were fasted for 24 to 48 hours with water *ad libitum*. All procedures were performed under ether anaesthesia. The method of orthotopic small bowel transplantation has been published recently.⁵ Briefly, the small bowel was double tied and cut 1 cm distally to the ligament of Treitz and 1-2 cm proximally to the caecum. The graft was harvested along with the attached vascular pedicles, consisting of the portal vein and the superior mesenteric artery, including an aortic cuff. The graft was flushed via the superior mesenteric artery with 1-2 ml cold Hank's balanced salt solution (HBSS), and stored in cold HBSS while the recipient was prepared. In the recipient, the infrarenal aorta and inferior caval vein were isolated and end to side anastomoses were performed between the graft superior mesenteric artery and the aorta, and the graft portal vein and inferior caval vein, respectively. The host's small bowel was resected and the donor small bowel was anastomosed end to end, proximally with the duodenum, distally with the remnant of the ileum at 1-2 cm from the caecum. At the end of the operation all rats received a single dose of 20 000 IU procaine penicillin G and 20 mg dihydrostreptomycin subcutaneously (Depomycine, Gist-Brocades, The Netherlands).

POSTOPERATIVE CARE

After operation, the rats were permitted their

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Accepted for publication
3 August 1990

normal diet (Hope Farm diet for rat and mouse No 1410) and water ad libitum. They received no antibiotics in the postoperative period. To get an overall clinical impression of graft function and occurrence of GVHD, the rats were weighed five times a week. Increase in weight was considered to be the most important indicator of acceptable small bowel function. All deaths within four days were considered to be technical failures. After death, necropsy was performed to confirm or exclude rejection.

IMMUNOSUPPRESSION

Cyclosporine (Sandoz, Basel, Switzerland) was dissolved in olive oil to a concentration of 25 mg/ml and was administered intramuscularly in doses of 25 mg/kg on days 0, 1, 2, 4, and 6 after transplantation. The first dose was given immediately after operation.

IRRADIATION

Whole body irradiation with doses of 5 or 10 Gy was performed with a Gammacell 40 caesium 137 irradiation unit (Atomic Energy of Canada, Ltd), one to four hours before transplantation.

GVHD

The severity of GVHD was estimated by clinical grading as follows:

Grade 1, light redness of ears, snout, and paws;
Grade 2, moderate redness of ears, snout, and paws; light hair loss and diarrhoea;

Grade 3, severe redness of ears, snout, and paws; alopecia, generalised dermatitis; and profuse diarrhoea.

EXPERIMENTAL GROUPS

Six groups were distinguished (see Table) as follows:

Group 1, controls, WAG to BN, no immunosuppressive therapy, $n=17$;

Group 2, irradiation of the donor with 5 Gy, $n=8$;

Group 3, irradiation of the donor with 10 Gy, $n=11$. The results for this group have been published before.⁵

Group 4, administration of 25 mg/kg of cyclosporine on days 0, 1, 2, 4, and 6 after transplantation, $n=10$.

Group 5, irradiation of the donor with 5 Gy in combination with 25 mg/kg of cyclosporine given on days 0, 1, 2, 4, and 6 after transplantation to the recipient, $n=9$.

TABLE I Schedule of graft pretreatment and immunosuppression

Group	No	Pretreatment*	Immunosuppression†
1	17	—	—
2	8	5 Gy, in vivo	—
3	11	10 Gy, in vivo	—
4	10	—	CsA
5	9	5 Gy, in vivo	CsA
6	9	10 Gy, in vivo	CsA

* Whole body irradiation was performed 1–4 hours before transplantation using a caesium 137 gamma source.

† Cyclosporine A (CsA) was given intramuscularly in doses of 25 mg/kg on days 0, 1, 2, 4, and 6 after transplantation.

TABLE II The effect of donor pretreatment and immunosuppression on the survival of orthotopic small bowel transplantation in rats

Group	Survival (days)	Mean (SEM)
1	6, 6, 6, 7, 8, 8, 13*, 14, 14, 15, 15, 19*, 21*, 24, 27*, 31*, 47*	16.6 (2.6)
2	10, 11, 11, 11, 11, 12, 12, 14	11.5 (0.4)
3	5, 5, 6, 6, 6, 6, 6, 7, 10, 12, 13	7.5 (0.9)
4	8, 11, 13, 13, 21, 55, 59†, 65, 66*, 72	38.3 (8.5)
5	11, 17, 31, 34, 49, 55, 57, 58, 72	42.7 (6.8)
6	5, 6, 7, 7, 7, 35, 39, 48, 54	23.1 (6.8)

* Grade 1–2 severity of graft versus host disease; † grade 3 severity of graft versus host disease.

Groups 2 and 3 significantly worse compared with group 1, $p<0.01$; group 2 significantly better compared with group 3, $p<0.02$; group 5 not significantly different from group 4, $p<0.01$; group 6 significantly worse compared with group 4, $p<0.01$.

Group 6, irradiation of the donor with 10 Gy in combination with 25 mg/kg of cyclosporine given on days 0, 1, 2, 4, and 6 to the recipient, $n=9$.

STATISTICS

The survival data were statistically analysed using the Wilcoxon test for group comparison.

Results

Table II shows that untreated control rats died after a mean (SEM) survival time of 16.6 (2.6) days. Six of 17 rats showed grade 1–2 symptoms of GVHD for three to four days. In groups 2 and 3, whole body irradiation of the donor with 5 and 10 Gy respectively resulted in a complete absence of GVHD. Surprisingly, the survival times in both groups were significantly shorter than those in the control group (11.5 (0.4) and 7.5 (0.9) days respectively *v* 16.6 (2.6) days, $p<0.01$). The accelerated graft rejection was more vigorous in group 3 than in group 2 ($p<0.02$). Rats in group 4 that received 25 mg/kg cyclosporine, survived for a mean (SEM) of 38.3 (8.5) days; two of 10 rats developed clinical symptoms of GVHD. Pretreatment with 5 Gy in combination with cyclosporine (group 5), produced no significant difference in survival time when compared with group 4 (47.2 (6.8) *v* 38.3 (8.5) days). However, none of the rats in group 5 showed signs of GVHD or peritonitis. One rat died on day 58 as a result of a late technical complication: stenosis of the proximal intestinal anastomosis. In group 6, irradiation with 10 Gy and cyclosporine therapy resulted in a mean survival time that was significantly shorter than that found in group 4 (23.1 (6.8) *v* 38.3 (8.5) days, $p<0.01$). At necropsy, four of the recipients that died in the first week showed symptoms of peritonitis as a result of a vulnerable, glassy transplant. No GVHD was observed in this group. Most rats in groups 4, 5, and 6 that survived more than three to five weeks increased in weight. In comparison with the weight gain observed earlier in a syngeneic (WAG–WAG) study, in which the donor was irradiated with 10 Gy, however, the weight gain was minimal.⁵

Discussion

This study clearly shows that irradiation of the donor very effectively prevents the occurrence of

clinical GVHD. In addition, when GVHD is ameliorated or absent in a fully allogeneic rat model, the acute rejection in untreated recipients is accelerated. This vigorous rejection can be explained by the assumption that irradiation with 10 Gy releases more immunocompetent cells in the peripheral circulation of the recipient than irradiation with 5 Gy. Although we cannot exclude this effect, it is our opinion that the vigorous rejection can only be controlled by cyclosporine therapy when GVHD is not completely abrogated. The mechanisms that are probably responsible for these results have been described in experiments on GVHD in bone marrow transplantation.^{20,21} These studies showed that a GVH reaction can induce a state of permanent suppression of cell mediated and of humoral immune responses.

This functional abnormality of T cells or B cells, or both, is probably related to a maturation defect.²⁰ Therefore the absence of GVHD, with its inherent immunosuppressive effect, is the most plausible explanation for the vigorous rejection that occurred in irradiated donors. From studies in a semiallogeneic parent to an F1-hybrid rat model it is known that irradiation with 5 Gy, in contrast to 10 Gy, failed to prevent the onset of GVHD.⁸ Extrapolating these data to our results, we presume that the immunosuppressive effect of subclinical GVHD was responsible for the beneficial effect on the survival time when donors were irradiated with 5 Gy compared with 10 Gy. Moreover, the absence of clinical GVHD may be of clinical importance.

Until now it has been uncertain to what degree, if any, GVHD will complicate human small bowel transplantation. Under the assumption that GVHD will develop after small bowel transplantation, strategies have to be developed to circumvent this complication. In theory, drug treatment or irradiation of the transplant before surgery would be the ideal method to inactivate donor lymphocytes. Practically, irradiation is the easiest procedure and can be performed *ex vivo* without losing its effect.⁷

Obviously, the major problem in small bowel transplantation remains the vigorous rejection process and its suppression. Cyclosporine will be the most important drug in any standard immunosuppressive regimen. This study gives indirect evidence that irradiation of the donor

in combination with cyclosporine is useful only when some immunocompetent cells in the graft, capable of producing a subclinical GVHD whose immunosuppressive effect is beneficial to the survival of the allografts are left. It is necessary to quantify further the donor irradiation dose that will result in the optimal interaction between the occurrence of GVHD and rejection of the graft.

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