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Effects of donor pretreatment with antilymphocyte serum and cyclosporin on rejection and graft-versus-host disease after small bowel transplantation in immunosuppressed and nonimmunosuppressed rats

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Abstract. After fully allogeneic small bowel transplantation, both graft-versus-host disease (GVHD) and rejection may occur. Donor pretreatment may prevent GVHD, but this sometimes leads to accelerated graft rejection. To study a possible balance between GVHD and rejection, fully allogeneic total orthotopic small bowel transplantation was performed in rats using the WAG-to-BN donor-host combination. Untreated control grafts were rejected in 16.6 ± 2.7 days (mean ± SEM), and 35 % of the animals had mild, transient GVHD. Pretreatment of the donor with antilymphocyte serum on days -2 and -1 before grafting, either intravenously or intraperitoneally, completely eliminated the occurrence of clinical GVHD but led to significantly shortened survival times (12.3 ± 0.8 and 10.3 ± 0.9 days, respectively). Donor pretreatment with 50 mg/kg cyclosporin (CyA) on days -2 and -1 prolonged graft survival significantly to 22.1 days but had no significant effect on the incidence of GVHD. Administration of 25 mg/kg CyA on days 0, 1, 2, 4, and 6 after grafting prolonged survival to 38.3 days with no evidence of GVHD. Pretreatment of the donor with antilymphocyte serum (ALS), combined with the same postoperative, short-term CyA regimen, increased survival to more than 50 days, again with no evidence of GVHD. When CyA was used as both donor pretreatment and postoperative therapy, there was no survival advantage compared to the use of postoperative CyA alone. These results show that an in vivo balance between GVHD and rejection exists and that abrogation of GVHD leads to accelerated rejection. Immunosuppression of the recipient may overrule this accelerated rejection while preserving the beneficial effect of donor pretreatment: elimination of clinical GVHD.

Key words: Small bowel transplantation, rat – Donor pretreatment, small bowel transplantation – ALS, small bowel transplantation, rat – Cyclosporin, small bowel transplantation, rat

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As was first shown in the parent-to-F1 hybrid rat model, transplantation of the small bowel may produce a lethal graft-versus-host-disease (GVHD) [14]. This GVHD shows histological similarities to that induced by bone marrow transplantation [15], and is caused by T lymphocytes originating from the transplanted gut and its mesenteric lymph nodes [11, 22].

In fully allogeneic models of small bowel transplantation, in which both GVHD and host-versus-graft (rejection) reactions may occur, rejection predominates. However, 30 %–50 % of the animals transplanted with a total small bowel without immunosuppression show clinically overt GVHD [17]. This GVHD is characterized by a redness of ears, snout, and paws and, in more severe cases, also includes dermatitis, alopecia, and a hunched posture of the animals. It is distinguished from GVHD in the one-way model by its nonlethal, short-lived, transient nature. Immunomodulation of the recipient and/or the donor can dramatically alter this picture. From studies in one-way models it is known that the immunosuppressive agent cyclosporin A (CyA) has a greater impact on rejection than on GVHD [11]. When CyA is given at a critical dose following fully allogeneic transplantation, GVHD can be more severe than in untreated animals, while rejection is delayed [1].

In both the one-way and the fully allogeneic models of small bowel transplantation, GVHD can be eliminated by reducing the mass of lymphoid tissue in the graft [4, 12]. Surprisingly, eliminating GVHD sometimes leads to accelerated graft rejection in fully allogeneic models [18]. This finding suggests that there is an immunological balance between rejection and GVHD [2] and that GVHD in some way prevents the development of rejection by attacking the host immune system.

Previous data obtained in our laboratory substantiate this hypothesis. We have shown that irradiation of the donor with 5 or 10 Gy prevents the occurrence of clinical GVHD and leads to accelerated graft rejection. This study also gave indirect evidence that subclinical GVHD may benefit graft survival [18]. However, most studies on GVHD following small bowel transplantation have used

one-way models, and further study of the fully allogeneic model is required to substantiate this hypothesis [23].

The aim of the present study was to determine whether donor pretreatment with antilymphocyte serum (ALS) or CyA could also suppress GVHD, and what effect this pretreatment might have on graft survival following total orthotopic, fully allogeneic, small bowel transplantation.

Materials and methods

Animals

Rats of the inbred WAG ($Rt1^u$) and BN ($Rt1^n$) strains were used as donors and recipients, respectively. The animals were bred under specific pathogen-free conditions and weighed between 200 and 300 g.

Operative procedure

Small bowel transplantation was performed as described earlier [17]. In brief, the total small bowel was harvested from Treitz's ligament to the terminal ileum, along with a vascular pedicle consisting of the superior mesenteric artery and portal vein. In the recipient the infrarenal aorta and caval vein were clamped and end-to-side anastomoses were performed between the recipient aorta and caval vein and the donor superior mesenteric artery and portal vein, respectively. The recipient small bowel was resected and the graft was placed in an orthotopic position by end-to-end anastomoses proximally with the host's duodenum and distally with the remaining 1–2 cm of terminal ileum. After transplantation rats received a single subcutaneous dose of 20,000 IU of penicillin and 20 mg of streptomycin (Depomycine, Mycofarm, De Bilt, The Netherlands).

Postoperative monitoring

After surgery, animals received standard laboratory rat chow (Hope farm diet for rat and mouse # 1410) and water ad libitum. The animals were weighed three times a week and were inspected daily for signs of clinical GVHD. Three grades of GVHD were distinguished: grade 1: light redness of ears, snout, and paws; grade 2: moderate redness of ears, snout, and paws, light hair loss, and diarrhea; and grade 3: severe redness of ears, snout, and paws, alopecia, generalized dermatitis, and diarrhea. Rats that died within 4 days of transplantation were considered technical failures. After death, autopsy was performed to confirm or exclude rejection.

Cyclosporin A. Commercially available CyA (Sandimmun, Sandoz, Basel, Switzerland) was obtained and dissolved in olive oil to a concentration of 50 mg/ml before intramuscular administration.

Antilymphocyte serum. Rabbit anti-rat antilymphocyte serum was produced by subcutaneous injection of 10^8 rat thymocytes in complete Freund's adjuvant. Subcutaneous booster injections with 10^8 thymocytes were given after 14 and 28 days. Blood was collected 1 week after the last immunization and the serum was prepared and decomplemented at 56 °C for 1 h. The serum was shown to be effective in a rat heart allotransplantation model in which it prolonged graft survival from 8.5 ± 0.5 days to 27 ± 1.1 days (MST ± SD) when it was given to the recipient subcutaneously on days 0, 1, and 2 after transplantation in a volume of 4 ml/kg.

Experimental groups

The following groups were studied using the WAG-to-BN fully allogeneic orthotopic total small bowel transplantation model:
Group 1. Control group, no immunosuppressive therapy ($n = 17$)
Group 2. Donor pretreatment on days -2 and -1 prior to transplantation with 4 ml/kg ALS intravenously ($n = 7$)
Group 3. Donor pretreatment on days -2 and -1 with 4 ml/kg ALS intraperitoneally ($n = 10$)
Group 4. Donor pretreatment on days -2 and -1 with 50 mg/kg CyA, given intramuscularly ($n = 8$)
Group 5. Recipient treatment with 25 mg/kg CyA intramuscularly on days 0, 1, 2, 4, and 6 after transplantation ($n = 10$)
Group 6. Donor pretreatment on days -2 and -1 with 4 ml/kg ALS intravenously; recipient treatment with 25 mg/kg CyA on days 0, 1, 2, 4, and 6 after transplantation ($n = 7$)
Group 7. Donor pretreatment on days -2 and -1 with 4 ml/kg ALS intraperitoneally; recipient treatment with 25 mg/kg CyA on days 0, 1, 2, 4, and 6 after transplantation ($n = 7$)
Group 8. Donor pretreatment on days -2 and -1 with 50 mg/kg CyA intramuscularly; recipient treatment with 25 mg/kg CyA on days 0, 1, 2, 4, and 6 after transplantation ($n = 9$).

Statistics

The Wilcoxon rank-sum test and the chi-square test were used for statistical analysis of the data.

Results

Untreated controls (group 1) died from rejection after a mean survival time (MST) ± standard error of the mean (SEM) of 16.6 ± 2.7 days. Of these animals, 35 % showed grade 1–2 symptoms of GVHD for 3–4 days between 9 and 12 days after transplantation (Table 1). Donor pretreatment with ALS, either intravenously of intraperitoneally (groups 2 and 3), successfully prevented the occurrence of GVHD; none of the animals displayed clinical signs of GVHD. Graft survival in these groups was significantly shortened as compared to untreated controls (12.3 ± 0.8 and 10.3 ± 0.9 days, respectively; $P < 0.05$).

Donor pretreatment with CyA (group 4) significantly prolonged graft survival (MST ± SEM 22.1 ± 3.4 days; $P < 0.01$), while 62 % of the animals developed clinical GVHD. Of these, 60 % had grades 1–2 and 40 % had grade 3 GVHD.

CyA given to the recipient at a dose of 25 mg/kg on days 0, 1, 2, 4, and 6 after grafting (group 5) led to a significant prolongation of graft survival time (38.3 ± 8.5 days; $P < 0.01$), with 20 % of the animals developing GVHD.

Pretreatment of the donor with ALS, either intravenously or intraperitoneally, combined with CyA treatment of the recipient (groups 6 and 7, respectively), led to survival times that were not significantly different from CyA treatment of the recipient alone (51.7 ± 17.6 and 54.8 ± 16.1 days, respectively). None of these animals showed signs of GVHD.

Donor pretreatment with CyA combined with recipient CyA treatment (group 8) gave the same MST as CyA treatment of the recipient alone (38.4 ± 10.2 days). All animals showing GVHD (33 %) had grade 1–2 severity.

Table 1. The effect of donor pretreatment and recipient immunosuppression on small bowel allograft survival in the fully allogeneic WAG-to-BN donor-host combination. Donors were given antilymphocyte serum (ALS) i.v. (groups 2 and 6) or intraperitoneally (i.p.) (groups 3 and 7) on days -2 and -1 prior to transplantation in a volume of 4 ml/kg. Cyclosporin (CyA) pretreatment was given i.m. on days -2 and -1 prior to transplantation at a dose of 50 mg/kg. Recipients were given 25 mg/kg CyA i.m. on days 0, 1, 2, 4, and 6 after transplantation. $P < 0.01$ for survival of group 1 vs groups 2, 4–8; $P < 0.05$ for survival of group 1 vs group 3 (Wilcoxon rank-sum test)

Group	Donor pretreatment	Recipient treatment	Survival in days	MST ± SEM (days)	% animals showing clinical signs of GVHD
1	None	None	6, 6, 6, 7, 8, 9, 13[a], 14, 14, 15, 15, 19[a], 21[a], 24, 27[a], 31[a], 47[a]	16.6 ± 2.7	35
2	ALS i.v.	None	10, 11, 11, 11, 13, 14, 16	12.3 ± 0.8	0
3	ALS i.p.	None	5, 8, 8, 9, 10, 12, 12, 13, 13, 13	10.3 ± 0.9	0
4	CyA i.m.	None	13, 13[a], 14[b], 20[a], 24, 25, 27[a], 41[b]	22.1 ± 3.4	62
5	None	CyA i.m.	8, 11, 13, 13, 21, 55, 59[b], 65, 66[a], 72	38.3 ± 8.5	20
6	ALS i.v.	CyA i.m.	7, 11, 13, 62, 63, 69, 137	51.7 ± 17.6	0
7	ALS i.p.	CyA i.m.	7, 12, 16, 62, 84, 99, 104	54.8 ± 16.1	0
8	CyA i.m.	CyA i.m.	9, 14, 17, 22, 24[b], 25[b], 70[b], 79, 86	38.4 ± 10.2	33

[a] Animals showing grade 1–2 GVHD
[b] Animals showing grade 3 GVHD

Discussion

Monchik and Russel [14] first used parent and F1 hybrid models in small bowel transplantation. They showed that both unidirectional rejection and GVHD can be induced by small bowel grafts and that pretreatment of the donor with 7 Gy total body irradiation prior to transplantation completely eliminates GVHD. These findings have been confirmed by other studies [4, 12]. Observations in these unidirectional GVHD or rejection models are of uncertain relevance to the clinical situation, in which a two-way reaction between rejection and GVHD can occur [10]. In fully allogeneic small bowel transplantation, rejection rather than GVHD seems to predominate, but little documentation is available on the interaction between rejection and GVHD in these models. In some fully allogeneic rat models, the animals die from rejection while GVHD is not clinically present [6, 13, 14]. Cohen et al. [2] investigated the effect of graft irradiation with 0.5 and 1.5 Gy prior to transplantation in a canine small bowel allograft model. They found that pretreatment with 1.5 Gy leads to rejection of the small bowel allografts in 9.2 days; pretreatment with 0.5 Gy, however, prolongs graft survival to a mean of 28 days. This interesting finding has led to the hypothesis that there is a balance between rejection and GVHD, and that the existence of subclinical GVHD after 0.5 Gy irradiation results in prolonged graft survival.

Since the early 1960s it has been known that GVHD depresses the host's immunological reactivity [9]. This is best shown by clinical results obtained with T-cell-depleted bone marrow transplantation. On the one hand, T-cell depletion significantly reduces acute GVHD; on the other hand, it substantially increases graft rejection [21]. GVHD is also known to be immunosuppressive after experimental spleen cell and small bowel transplantation [7].

Histopathologically, GVHD is characterized by a loss of the normal architecture of the spleen, lymph nodes, and thymus [3, 7, 19]. This leads to a profound immunosuppression with impaired humoral and cell-mediated immune responses [7]. This immunosuppression probably accounts for the observed in vivo balance between rejection and GVHD.

Diflo et al. [5] observed the occurrence of a short, sublethal GVHD approximately 4–6 weeks after fully allogeneic transplantation in immunosuppressed animals. Donor pretreatment with ALS completely eliminated GVHD but had no effect on graft survival in these immunosuppressed hosts. Gundlach et al. [8] found that mesenteric lymphadenectomy, a method that has been shown to eliminate GVHD [16], does not influence the course of acute graft rejection in nonimmunosuppressed recipients. However, CyA was not effective in preventing chronic rejection following mesenteric lymphadenectomy, whereas the same dosage of CyA fully prevented rejection of normal small bowel grafts. They suggested that the absence of an immunosuppressive effect caused by a GVH reaction had led to chronic rejection in this model.

In the present study, we showed that pretreatment of the donor with ALS eliminated clinical GVHD and led to significantly accelerated rejection. Intraperitoneal and intravenous administration of ALS were equally effective in preventing GVHD. As Shaffer et al. [20] have shown that subcutaneous treatment is as effective as intraperitoneal treatment, the route of administration seems to be unimportant.

When our recipients of a graft pretreated with ALS received immunosuppressive treatment with CyA, no adverse effect on graft survival was seen anymore, whereas clinical GVHD remained suppressed. This important finding is in accordance with earlier findings from our laboratory that show that irradiation of the donor with 5 or 10 Gy eliminated clinical GVHD and led to significantly accelerated graft rejection [18]. When recipients of a graft that was irradiated with 5 or 10 Gy received immunosuppressive treatment with CyA, graft survival was prolonged in both groups, whereas clinical GVHD did not occur.

After CyA treatment of the donor, we found prolonged graft survival but no significant difference in the percentage of animals developing GVHD. However, two

out of five animals that developed GVHD had grade 3 severity. It is known that CyA is more effective against rejection than it is against GVHD [11]. Hence, the prolonged survival we observed could have been due to the immunosuppressive effect of GVHD and the CyA transferred with the graft. Treatment of both donor and recipient with CyA had no beneficial effect on GVHD or rejection compared to recipient treatment alone. Thus, ALS pretreatment of the donor, combined with CyA treatment of the recipient, results in significantly prolonged graft survival, while clinical GVHD is suppressed. Whether manipulation of this balance will be of use in future clinical small bowel transplantation remains to be established.

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