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GRAFT-VERSUS-HOST REACTIVITY AND RENAL ALLOGRAFT SURVIVAL IN RATS GIVEN ALLOGENEIC SPLEEN CELLS OR SPLEEN ALLOGRAFTS[1]

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SUMMARY

Selective recruitment of antigen-sensitive cells (ASC) into the spleen as a method of inducing specific suppression was attempted by intravenous injection of either DA or Lewis spleen cells 24 hr before a (DA × Lewis)F_1 renal allograft into a Lewis or DA recipient, either with or without a splenectomy. This led to suppression of rejection in the DA recipient and delayed rejection in the Lewis recipient. Splenectomy produced a minimal augmentation effect. Assay of graft-versus-host (GVH) reactions in (DA × Lewis)F_1 rats by a popliteal node assay showed that injection of allogeneic DA or Lewis spleen cells 48 hr before the assay significantly reduced the reaction produced by node lymphocytes but not spleen lymphocytes, suggesting a loss of ASC from the lymph nodes. Lewis spleen allografts did not produce such a significant reduction in the GVH reactivity of DA node lymphocytes as intravenous Lewis cells, whereas DA spleen allografts led to an increased GVH reactivity of Lewis node lymphocytes. From these studies, it is not possible to attribute the suppression produced by the intravenous injection of allogeneic cells to selective recruitment of antigen-sensitive cells to the spleen.

Within 1–2 days after antigen administration, there is a selective recruitment of specific antigen-sensitive lymphocytes from the circulating pool into lymphoid areas where the antigen has localised (*7, 12–14*). Presumably, the recruited cells are stimulated to undergo differentiation and proliferation to various effector cells. If it were possible to recruit all of the specific cells recognizing a particular antigen into a single lymphoid organ, removal of that organ at the appropriate time after antigen administration should provide a very effective and simple method of specific immunosuppression. This approach to immunosuppression has been attempted with some success using the popliteal lymph node in the sheep (*9*).

The purpose of the present series of experiments was to determine whether the spleen could be used as a potential site for selective recruitment of antigen-sensitive cells (ASC).[4] If so, splenectomy at some time after antigen administration might induce specific suppression. Extending this concept further, a spleen allograft might not only provide the source of antigen, but also the trapping mechanism for ASC, and subsequent removal of the spleen allograft might allow long-term survival of a subsequent renal allograft. Before adopting such a plan, it was decided to test the effect of allogeneic spleen cell injection on the graft-versus-host (GVH) reactivity of the host's lymph-

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[4] Abbreviations used in this paper: ASC, antigen-sensitive cells; GVH, graft-versus-host; IVI, intravenous injection.

oid cells and on its capacity to reject renal allografts, both with and without splenectomy. The effect of transplanting allogeneic spleen as a vascularized organ graft for 48 hr on the GVH reactivity of the recipient's lymphocytes was also examined.

MATERIALS AND METHODS

Rats. Inbred rats of the DA/SsWehi (AG-B^4 or H-1^a), Lewis/SsWehi (Ag-B^1 or H-1^l) and the (DA × Lewis)F_1 hybrid were used in these experiments.

Rats used as donors of kidney or spleen grafts were male and averaged 300 g, and rats used as recipients were female and averaged 190 g.

Cells suspensions. Spleen cell suspensions were prepared by slicing spleens into portions and grinding them through an 80-mesh stainless steel sieve. The heavier fragments were allowed to settle, and the cell suspension washed once before injection. In some experiments, lymphocytes were obtained from the spleen cell suspensions by using the Isopaque-Ficoll density gradient separation technique (*1*). Approximately 1.5×10^8 mononuclear leukocytes were present in suspensions obtained from a single spleen. Lymph node cell suspensions were prepared by teasing cervical, axillary, and mesenteric lymph nodes through the sieve and washing once before injection.

Kidney transplantation. Kidney transplants were performed according to the technique of Fabre et al. (*3*), except that a ureteric anastomosis was used instead of a vesico-vesical anastomosis. All transplant recipients had blood ureas determined at 1, 2, 3, 4, 6, 8, 12, and 20 weeks by the method of Kaplan (*11*). The survival of each rat was recorded in days, and survival at 100 days was considered a long-term survival.

Spleen transplantation. The donor spleen was prepared as follows (see Fig. 1). The coeliac artery was cleared. The left gastric and common hepatic arteries were ligated and divided. The portal vein and splenic vein were cleared. The pancreas was completely separated from the splenic vessels all the way to the spleen using a cotton applicator swab. The superior mesenteric vein was ligated and cut. The portal vein and coeliac artery were anastomosed to the left renal vein and artery of a recipient after left nephrectomy (Fig. 1). A dissecting microscope

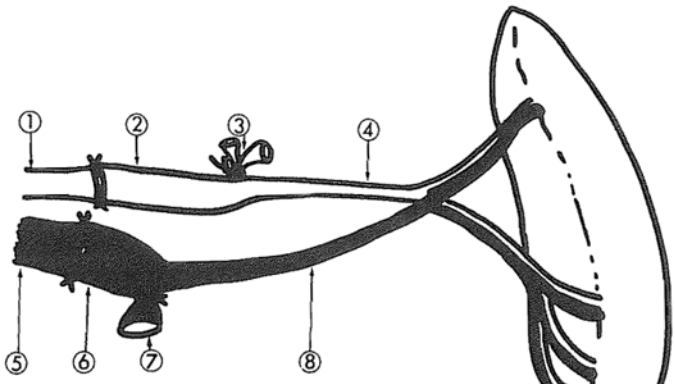

FIGURE 1. Technique used for spleen transplantation. The portal vein and the coeliac artery are anastomosed to the renal vein and renal artery, respectively.

was used (10×-24×) and anastomoses were accomplished with 10-0 interrupted nylon sutures. Ischemia times were between 20 and 30 min.

Graft-versus-host assay. The popliteal lymph node assay of Ford et al. (8) was used. The popliteal nodes were weighed 7 days after injection of 10^7, 2×10^7, or 4×10^7 lymphocytes into the footpads of (DA × Lewis)F_1 rats.

Statistical methods. All values are expressed as the mean with 1 standard error of the mean. Student's t test was used for evaluating significance. $P < 0.5$ was considered to be a significant difference.

RESULTS

Renal allografting 24 hr after allogeneic spleen cell injection. Groups of DA and Lewis rats were bilaterally nephrectomized and transplanted with (DA × Lewis)F_1 kidneys 24 hr after an intravenous injection (IVI) of 1.5×10^8 allogeneic spleen cells. Splenectomy was performed in some rats before renal transplantation. Control animals were given an IVI of syngeneic spleen cells followed by splenectomy before transplantation. The results are shown in Table 1. IVI of Lewis spleen cells in DA recipients (groups 1 and 2) virtually abolished rejection. Although all controls had signs of acute rejection at 7 days (group 3), three out of five recovered completely; one dying of acute rejection at 12 days, the other of chronic rejection at 84 days. Although all five DA rats splenectomised 24 hr after Lewis spleen cell injection were long-term survivors with no rejection episodes (group 1), this is not significantly different from group 2 animals who did not have a splenectomy, for four of these five animals also had complete suppression of graft rejection (group 2). One died at 13 days but the cause of death was not determined.

All control Lewis rats, injected with syngeneic spleen cells and splenectomised at the time of transplantation, had severe rejection episodes at 1 week (group 6), and four died by day 16. One animal survived more than 100 days. Rejection was delayed in Lewis rats injected 24 hr earlier with DA spleen cells either with (group

TABLE 1. The effect of allogeneic spleen cell injection 24 hr before splenectomy and transplantation, or transplantation alone, of an F_1 (DA × Lewis) kidney. DA rats receiving an F_1 (DA × Lewis) kidney have a mean blood urea at week 1 of 458 ± 93 mg/100 ml and a median survival time of 19 ± 12.6 days. Lewis rats given an F_1 (DA × Lewis) kidney have a mean blood urea at week 1 of 435 ± 51 mg/100 ml and a median survival time of 10.6 ± 1.8 days (6).

Group	Recipient rats		Pretreatment of recipient rats	Blood urea		Survival
	Strain	No.		1 week	2 weeks	
				mg/100 ml	*mg/100 ml*	*days*
1	DA	5	IVI of 1.5×10^8 Lewis spleen cells 24 hr before splenectomy and F_1 kidney transplant	64.6 ± 9.9[a]*	76.8 ± 19.2	>100, >100, >100, >100, >100
2	DA	5	IVI of 1.5×10^8 Lewis spleen cells 24 hr before F_1 kidney transplant	80.4 ± 15.2[b]	55.0 ± 3.6	13, >100, >100, >100, >100
3	DA	5	IVI of 1.5×10^8 DA spleen cells 24 hr before splenectomy and F_1 kidney transplant	296.0 ± 60.4[a, b]	58.3-5 ± 5.1	12, 84, >100, >100, >100
4	Lewis	5	IVI of 1.5×10^8 DA spleen cells 24 hr before splenectomy and F_1 kidney transplant	114 ± 21.6[c, d]	344.0 ± 74.9	15, 18, 21, >100, >100
5	Lewis	5	IVI of 1.5×10^8 DA spleen cells 24 hr before F_1 kidney transplant	195.0 ± 49.5[d]	481.0 ± 58.9	14, 16, 18, 19, 90
6	Lewis	5	IVI of 1.5×10^8 Lewis spleen cells 24 hr before splenectomy and F_1 kidney transplant	301.5 ± 35.5[c]		11, 11, 13, 16, >100

* The superscripts indicate which values have been compared to give the following probabilities of significant differences: [a] = $P < 0.001$; [b] = $P < 0.01$; [c] = $P < 0.002$; [d] = $P < 0.05$.

4) or without a splenectomy (group 5). Unlike the DA recipients, four of five Lewis rats that had not undergone splenectomy had a severe acute rejection episode at 2 weeks and died (group 5), whereas three of five splenectomized Lewis rats had an acute rejection episode at 2 weeks and died (group 4). Although the splenectomized Lewis rats appeared to fare somewhat better than the nonsplenectomized rats, the difference observed between groups 4 and 5 is not significant.

Graft-versus-host reactivity 48 hr after allogeneic spleen cell injection. Lewis and DA rats were given allogeneic spleen cells intravenously 48 hr before their lymph node or spleen lymphocytes were assayed for GVH reactivity. The results are summarized in Table 2. When Lewis control lymph node cells (group 7) are compared with DA (group 9), it is clear that the reactivity of the DA cells is stronger than that of Lewis cells in this assay, despite the fact that DA rats reject (DA × Lewis)F_1 kidneys less firecely than Lewis rats. But the weak reactivity of Lewis lymph node cells was more difficult to suppress by pretreating the donors with spleen cells (groups 7 and 8) than the stronger reactivity of DA lymph node cells (groups 9 and 10). Thus, DA rats injected with Lewis spleen cells 48 hr earlier had the GVH reactivity of their lymph node cells dramatically suppressed (group 10). By contrast, when DA spleen lymphocytes were assayed for GVH reactivity 48 hr after the injection of Lewis spleen cells, there was only minimal suppression (groups 11 and 12). These results suggest that in rats injected intravenously 48 hr before with allogeneic spleen cells, ASC were reduced in lymph nodes although not in spleen.

Lymph node cells from long-term survivors of renal allografts that had been pretreated by intravenous allogeneic spleen cells were tested for their reactivity in the GVH assay (Table 3). DA rats with long surviving F_1 kidneys had normal GVH reactivity (group 13) not significantly different from that of controls (group 9). This is in contrast to the markedly suppressed reactivity of lymph node cells from DA rats injected 48 hr before with Lewis spleen cells (group 10, Table 2). But Lewis rats surviving with an F_1 renal allograft had increased GVH reactivity as compared to that of controls (group 14). This, again, is in contrast to the moderate but significant reduction of GVH reactivity of lymph node cells from Lewis rats injected 48 hr before with DA spleen cells (group 8, Table 2).

Graft-versus-host reactivity 48 hr after allogeneic spleen transplantation. Forty-eight hours after a DA spleen was transplanted into a Lewis rat, the GVH reactivity of the lymph node cells of the recipient was markedly in-

TABLE 2. GVH reactivity in a popliteal node assay 48 hr after allogeneic spleen cell injection

Group	Donor rats			No. of (DA × Lewis)F_1 rats injected	Popliteal lymph node weights (mg) of (DA × Lewis)F_1 rats 7 days after intrafootpad injections of:		
	Strain	Pretreatment	Cells assayed		10^7 cells	2×10^7 cells	4×10^7 cells
7	Lewis	None	Lymph node		8.8 ± 1.3[a]	10.6 ± 2.0[b]	27.6 ± 5.8[c]
8	Lewis	IVI of 1.5×10^8 DA spleen cells 48 hr before assay	Lymph node	5	6.8 ± 0.8	8.6 ± 1.1	15.2 ± 3.8[c]
9	DA	None	Lymph node	6	31.3 ± 10.5[d]	67.4 ± 14.4[a, b]	
10	DA	IVI of 1.5×10^8 Lewis spleen cells 48 hr before assay	Lymph node	5	6.0 ± 0.9[e]	5.2 ± 3.7	11.0 ± 2.5
11	DA	None	Spleen	4	28.6 ± 4.6		
12	DA	IVI of 1.5×10^8 Lewis spleen cells 48 hr before assay	Spleen	5	20.0 ± 4.6[e]	36.8 ± 3.8	

[a] $0.10 < P < 0.5$.
[b] $P < 0.01$.
[c] $0.10 < P < 0.05$.
[d] $P < 0.001$.
[e] $P < 0.02$.

TABLE 3. GVH reactivity of lymph node cells of long-term survivors of renal allografts and of normal rats

Group	Donor rats			No. of (DA × Lewis)F_1 rats injected	Popliteal lymph node weights (mg) of (DA × Lewis)F_1 rats 7 days after intrafootpad injection of:	
	Strain		Group no. in Table 1		2×10^7 cells	4×10^7 cells
13	DA	long survival	1 and 2	4	56.0 ± 8.2	96.8 ± 13.8
14	Lewis	long survival	4 and 5	6	34.7 ± 8.0	59.2 ± 5.5[a]
9	DA	normal		6	67.4 ± 14.4	27.6 ± 5.8[a]
7	Lewis	normal		5	10.6 ± 2.0	

[a] $P < 0.002$.

creased (cf., group 15, Table 4, with group 7, Table 2). By contrast, 48 hr after a Lewis spleen was transplanted into a DA rat, the reactivity of the lymph node cells of the DA rat was moderately suppressed (cf. group 16, Table 4, with group 9, Table 2). These results are different from those seen after the injection of allogeneic spleen cells where complete suppression of reactivity occurred in DA rats and moderate suppression occurred in Lewis rats.

DISCUSSION

The (DA × Lewis)F_1→ DA combination represents a weak AgB histocompatibility barrier and specific immunosuppression is obtained easily. An IVI of 0.5 ml of donor blood 1 week before transplantation completely suppressed kidney rejection (*4*, *5*). If the blood was given only 1 day before transplantation, there was no suppression of rejection. In the present experiments, the spleen cell suspension represents a much larger dose of antigen, and injection 1 day before transplantation resulted in complete protection against rejection of the F_1 renal allograft with or without splenectomy in 9 of 10 animals. However, splenectomy did seem to produce some degree of suppression in comparison to controls receiving no pretreatment reported in other studies from the same laboratory (*4*, *5*).

The (DA × Lewis)F_1→ Lewis combination represents a very strong histocompatibility barrier and it is difficult to induce specific immunosuppression. Injection of 0.5 ml of donor strain blood 1 day prior to renal allografting resulted in an earlier, more severe rejection than in control animals (*4*, *5*). When donor blood was given 7 days before allografting, there was little difference from controls. Injection of DA spleen cells 1 day before allografting caused an impressive delay in rejection and better survival. Presumably because of a larger antigen dose, donor strain spleen cell suspension was much more effective than 0.5 ml of donor strain blood in causing specific immunosuppression. Splenectomy in this combination also may have produced some augmentation of the suppression.

No control group had splenectomy alone, but these results suggest that splenectomy itself does contribute some degree of suppression. The suppressive effect of splenectomy was particularly evident in the recent studies of Fabre and Batchelor (*6*) in an (AS × August)F_1→ August renal allograft model, where they showed that splenectomy at the time of grafting or even 7 weeks before grafting resulted in either partial or complete suppression of rejection. As they could not reconstitute this suppressive effect of splenectomy with normal spleen cells, they attributed the effect to loss of a major site of proliferation of ASC after exposure to the allograft.

It has been shown previously in this laboratory that following antigen-induced immunosuppression, long-term survivors with renal allografts had normal GVH reactivity against rats of the donor strain (*5*). This was found also in the present experiments (Table 3). However, with 48 hr of antigen injection, the GVH reactivity of the lymph node cells was dramatically suppressed in DA rats and marginally reduced in Lewis rats (Table 2). There was only minimal suppression of the GVH reactivity of the spleen lymphocytes at that time. One interpretation of these results would be that ASC in rats pretreated by an IVI of allogeneic spleen cells are

TABLE 4. GVH reactivity 48 hr after allogeneic spleen transplant[a]

Group	Donor rat			No. of (DA × Lewis)F_1 rats rejected	Popliteal lymph node weights (mg) of (DA × Lewis)F_1 rats 7 days after intrafootpad injection of:		
	Strain	Spleen transplant	Cells assayed		10^7 cells	2×10^7 cells	4×10^7 cells
15	Lewis	DA spleen 48 hr before assay	Lymph node	5	15.1 ± 4.2	47.7 ± 15.7	90.0 ± 9.1
16	DA	Lewis spleen 48 hr before assay	Lymph node	5	15.0 ± 4.4	30.6 ± 8.3	54.5 ± 8.4

[a] Comparing 4×10^7 cells groups 7 (Table 2) and 15, $P < 0.001$. Comparing 4×10^7 cells groups 8 (Table 2) and 15, $P < 0.0001$. Comparing 2×10^7 cells groups 9 (Table 2) and 16, $P < 0.05$. Comparing 2×10^7 cells groups 10 (Table 2) and 16, $P < 0.0001$.

recruited out of the lymph nodes into the spleen where they undergo differentiation and proliferation to effector cells which enter the circulation again within 3 days, as has been demonstrated in a mouse model (*13*). Splenectomy, at the time of renal allografting, may have produced some minimal suppression by removing a primary environment for further differentiation and proliferation of these ASC. However, it is apparent from these experiments that the spleen is not the only site of recruitment or proliferation of ASC.

It was hoped that by presenting the recipient with antigen in the form of a donor spleen allograft instead of intravenous spleen cells, all antigen-sensitive lymphocytes of the recipient would be selectively recruited to the spleen allograft. If this proved to be the case, there were important clinical implications. A donor spleen could be connected temporarily to the arteriovenous shunt of a prospective recipient, and then disconnected at 48 hr, leaving the recipient depleted of specific antigen-sensitive cells. But GVH assays of lymph node cells from allogeneic spleen transplant recipients at 48 hr were moderately depressed in the weak (DA × Lewis)F_1→ DA model, and greatly increased in the stronger (DA × Lewis)F_1→ Lewis model. This approach was thus less effective than the IVI of spleen cells in causing specific immunosuppression. Presumably, cells from the spleen allograft emerged and engaged specifically sensitive cells of the recipient in areas other than the spleen. It is conceivable that if the spleen graft were first perfused to remove its migratory cell population, a more localized selective recruitment of antigen-sensitive cells to the transplanted spleen might have been obtained. We do know from other studies that there is some selective recruitment of ASC to a spleen allograft, although not of sufficient intensity to have a significant effect on the host (*10*). Further studies are in progress in order to determine whether effective recruitment can be achieved following short-term transplantation of an allogeneic spleen after in vitro perfusion.

The injection of a large dose of allogeneic spleen cells 24 hr before renal transplantation will suppress or delay rejection of the renal allograft depending on the strength of the histocompatibility barrier. The mechanism by which this occurs is not clear from these or earlier studies (*5*), although it would seem that the injection does lead to loss of ASC certainly from nodes, although not from the spleen. This could be sufficient to delay the induction of the immune response such that rejection is delayed or even suppressed by the development of blocking factors or a suppressor cell population. These are similar to previous speculations by ourselves and others in comparable experimental models. However, it does not appear that it has been possible to recruit a population of antigen-sensitive cells to the spleen so that its removal produces a state of specific suppression of the immune response against a particular antigen.

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