

Kidney Allograft Survival in Dogs Treated with Total Lymphoid Irradiation

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Total lymphoid irradiation (TLI) is immunosuppressive and, in rodents, can induce a state where transplantation of allogeneic bone marrow results in chimerism and permanent acceptance of organ allografts from the donor strain. We attempted to apply this treatment to a large animal model. Twelve splenectomized dogs were treated with TLI (150 rads per fraction, total dose 1950–3000 rads) before bilateral nephrectomy and renal allotransplantation. Eight dogs received bone marrow from the kidney donor. In 13 untreated control dogs renal allografts functioned (serum creatinine level < 2.0 mg/dl) for a mean \pm (SE) of 4.7 ± 0.3 days. In the four TLI treated dogs who did not receive bone marrow the renal allografts functioned for 15–76 days (two dogs died with functioning grafts). In the eight TLI treated dogs who received donor bone marrow, two died immediately after transplantation, two rejected at 3 and 13 days, one died at 13 days with a functioning graft, and two have had the grafts function for longer than 500 days. Chimerism was not detected in the one dog tested. The response of peripheral blood lymphocytes to stimulation with phytohemagglutinin and in mixed lymphocyte culture was suppressed for at least one month after TLI. The results confirm the immunosuppressive effect of TLI. The absence of kidney rejection in two recipients of donor bone marrow show the potential of this approach to induce long-term immunologic unresponsiveness as to an organ allograft, but the outcome is unpredictable and further experiments are needed to define the optimal conditions for administration of TLI and bone marrow to the recipients.

THE SURVIVAL OF SKIN and heart allografts is prolonged in inbred mice and rats treated with fractionated, high dose (3400 rads) total lymphoid irradiation (TLI).^{7,8} The combination of low dose TLI and administration of antithymocyte globulin, in a dose that, by itself, is not effective, can also greatly delay the rejection of cardiac allografts in Rhesus monkeys.²

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Permanent heart and skin allograft survival can be achieved in rodents if bone marrow is transplanted simultaneously.^{7,9} The bone marrow recipients are stable chimeras, and despite major histocompatibility differences, graft versus host disease does not occur. The mechanisms of tolerance induction by the combination of TLI and bone marrow administration is not understood, but Slavin et al.^{5,10} have demonstrated the presence of suppressor cells specifically directed against the donor in chimeric animals. This group has also reported that mongrel dogs that are administered TLI and bone marrow allografts become chimeras.^{6,11}

The experiments by this group show the great potential for the application of total lymphoid irradiation in organ transplantation, either for its generalized immunosuppressive effect or for its ability to facilitate the induction of specific immunologic tolerance by donor bone marrow administration. We report here the results of kidney allograft survival in dogs treated with total lymphoid irradiation before transplantation, with or without administration of donor bone marrow two or three days before transplantation.

Methods

Outbred beagles (10–12 kg) were used in this study. The dogs were tissue-typed for histocompatibility antigens, either prior to or after they were purchased, using a one-stage microcytotoxicity test¹³ with DLA typing sera. The typing sera were originally derived from the same source.³ Donor and recipient pairs were unrelated (nonlitter-mates, nonsiblings) and were mismatched for at least two DLA antigens.

Before irradiation, the dogs were splenectomized.

This was done so that the lateral abdomen could be shielded and the amount of intestine exposed to irradiation reduced. The radiation field extended from the base of the skull to the tail. The entire neck, both axilla, mediastinum, midabdomen and pelvis, and both groins were included in the irradiation field, but the lungs and lateral aspects of the abdomen were shielded (Fig. 1). The radiation was administered using a 4 MeV linear accelerator at 130 cm target-skin distance. The dogs were administered light anesthesia of sodium thiamyol before each irradiation dose. The dogs received 150 rads per day, five days per week, using alternating ventral and dorsal ports until the cumulative dose desired had been delivered.

Twelve dogs were irradiated. The amount of the irradiation was varied in the first few dogs, while we tried to find a suitable dose. The first two dogs received 3000 rads each. These dogs lost approximately 20% of body weight, were very weak and both died the day after transplantation. The dose of irradiation was thought to be excessive. The third dog received 1950 rads before transplantation; this dog did not lose weight, but ultimately rejected the kidney graft. The fourth dog received 2850 rads; this dog survived the transplant operation, but, again, was in a weakened condition and died 13 days after the operation from intussusception. The last eight dogs received 2400 rads; these dogs all appeared to be healthy at the completion of the radiation and tolerated the transplantation operation with no problems.

Eight of the dogs received intravenous injection of bone marrow from their kidney donor one to two days after completion of TLI, and one to three days before the kidney operation. The bone marrow was aspirated from the femurs and humeri of the donor, placed in Hanks' balanced salt solution containing 25 U/ml-virgule heparin, and filtered through sterile gauze. Nucleated cells were counted. The two dogs that received 3000 rads received 4×10^6 and 2.5×10^8 cells/kg, respectively. Since these two dogs died the day after transplantation, they are excluded from further analysis. Of the six remaining irradiated dogs, one (FEY6) received 5×10^9 cells/kg, four 0.5×10^8 cells/kg, while one dog (BKY6, the one treated with 1950 rads) received only 2.2×10^6 cells/kg.

Kidney transplantation was performed according to the standard technique in our laboratory.¹² The dogs were administered an anesthetic combination of sodium thiamyol and a fentanyl-droperidol combination; succinyl choline was administered at the time of anesthesia induction for muscle relaxation. The recipient's own kidneys were removed at the

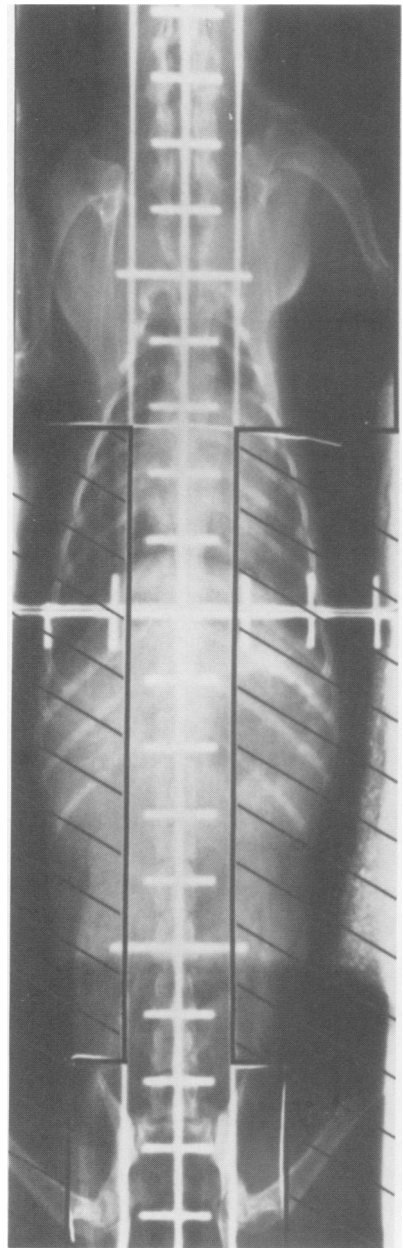


FIG. 1. X-ray of dog prepared for total lymphoid irradiation. Shielded areas are indicated by hatching.

time of transplantation. The donor kidney was transplanted to the left iliac fossa of the recipient with vascular anastomoses of the renal vessels of the donor to the iliac vessels of the recipient, and a ureteral neocystostomy was performed.

Serum creatinine levels were determined daily for the first few weeks after transplantation. In the long-term surviving dogs, serum creatinine levels then were determined weekly. The graft was designated a failure if the serum creatinine level rose to >2.0 mg/dl. Thirteen control dogs were not irradiated, and received renal allografts (without bone marrow) from DLA mismatched donors at the time of bilateral nephrectomy. No dogs received immunosuppressive drugs.

TABLE 1. Outcome After Transplantation of DLA Mismatched Renal Allografts in Bilaterally Nephrectomized Dogs Treated with Total Lymphoid Irradiation and with and without Donor Bone Marrow

Dog	TLI Dose (Rads)	BM	Graft, Survival*† (Days)	Dog Survival‡ (Days)	Comments
BKY6	1950	+	13	36	Delayed and slowly progressive rejection. Cr = 5.9 at death
FEY6	2850	+	13‡	13	Died of intussusception. Cr = 0.6 at death
LG16	2400	+	>500	>500	F to M transplantation. Chimerism not detected. Current Cr = 0.9
EY47	2400	+	>500	>500	F to F transplantation. Chimerism not tested. Current Cr = 0.8
CV16	2400	+	?	51	Partial ureteral obst. No rejection. Cr never <3.5 and 15.7 at death. No rejection
ABK48	2400	+	3	11	Hemorrhagic kidney at autopsy, PMN infiltration consistent with humoral rejection
CB14	2400	-	20	26	Delayed rejection. Cr = 11.0 at death
FCX7	2400	-	76‡	76	Died of intussusception. Cr = 1.4 at death
QF65	2400	-	15	25	Delayed rejection. Cr = 8.5 at death
TOY8	2400	-	15‡	15	Died of peritonitis. Cr = 1.2 at death

* Graft survival defined as days serum creatinine (Cr) < 2.0 mg/dl.
 † Thirteen control dogs rejected their renal allografts at a mean of

4.7 ± 0.3 (S.E.) days and died at a mean of 11.2 ± 1.0 (S.E.) days.
 ‡ Died with functioning graft.

Immune function studies of peripheral blood lymphocytes were performed after or before and after TLI and transplantation in the dogs that received 2400 rads. These studies included mixed lymphocyte culture (MLC) reactions to either the donor or indifferent dogs, and the response to phytohemagglutinin (PHA). The methods used for these studies were previously published.¹²

Results

The outcome after kidney transplantation is summarized in Table 1. The 13 control dogs that were not treated with TLI rejected the kidney grafts between three and six days after transplantation (median graft survival: five days) and died between five and seventeen days (median dog survival: 11 days).

All of the dogs that were treated with TLI alone had renal allograft function longer than the control dogs. One dog rejected the graft at 20 days and one dog at 15 days after transplantation. The other two dogs died 15 and 76 days, respectively, after transplantation. At the time of death, they had functioning grafts.

Of the six dogs that received donor bone marrow after TLI, in only one was graft survival not prolonged. This dog (ABK48) received the donor bone marrow three days before the transplant. The serum creatinine level increased to >2.0 mg/dl on the third day. Histologic examination of the kidney at death, 11 days after transplantation, showed no mononuclear cellular infiltrate in the kidney, but

interstitial hemorrhage and neutrophils were present, consistent with humoral rejection. Immunofluorescent staining was not done.

The dog that received 1950 rads and donor bone marrow (BKY6) slowly rejected the graft beginning at 13 days. The other four dogs that were treated with TLI plus donor bone marrow did not reject the graft. Two of the dogs died with functioning grafts at approximately two and seven weeks after transplantation, one died of intussusception (FEY6) and one died of a technical complication (CV16). In dog CV16, the serum creatinine level remained above 3.5 ml/dg after transplantation. At 42 days after transplantation, re-exploration demonstrated hydro-nephrosis and a ureteral stenosis at the ureterocystostomy site. A renal biopsy specimen demonstrated dilated tubules, but no evidence of rejection. The ureter was reimplanted in the bladder, but the dog died 51 days after transplantation because of peritonitis and uremia. Again, no evidence of rejection was seen on histologic examination at autopsy.

The remaining two dogs treated with TLI and donor bone marrow (LG16 and EY47) have survived with normal graft function for more than one year. Intravenous pyelograms were obtained six months after transplantation in both dogs, and showed normal function of a solitary kidney (allograft) in the left iliac fossa. One of the dogs (LG16) was a male that received a graft from a female. Chromosome analysis of peripheral white blood cells was performed one year after transplantation according to standard techniques.⁴ Examination of 30 cells from PHA

and pokeweed mitogen stimulated peripheral blood lymphocytes showed no female karyotypes, and examination of 500 polymorphonuclear leukocytes showed no drumsticks. Spontaneous blasts of a bone marrow aspirate performed at 15 months were also examined for the female karyotype, and none were seen in 50 cells. Thus, we have no evidence of chimerism in the one bone marrow recipient that was tested.

We were not able to detect any differences in the immune responses between the dogs that received and those that did not receive donor bone marrow. Stimulation indices of <5.0 for PHA and <3.0 for MLC reactions were considered to be a low response.¹² The mean (\pm S.E.) responses of all dogs to PHA and in MLC before TLI were 22.7 ± 4.4 and 9.5 ± 3.2 , respectively. All dogs lost the ability to respond to PHA or to respond in MLC, all during the course of TLI administration. PHA responsiveness returned by one month after TLI in the four dogs tested (the others died before 1 month had elapsed). None of the dogs had a significant response in MLC when tested between nine and 44 days after TLI and transplantation. Only the two long-term survivors (LG16 and EY47) were tested after this time, and they regained the ability to respond in MLC to indifferent dogs by 175 and 56 days, respectively (their donor was dead). Mean stimulation indices after these times (tested every two to four weeks, up to 500 days) for each dog were 4.7 ± 0.9 and 6.2 ± 1.5 , respectively.

The two recipients of bone marrow that have had their renal allografts function for greater than 500 days (LG16 and EY47) had low reactivity in MLC to their donors before TLI. However, this situation alone could not explain the failure to reject the graft. Dog CB14 also had low reactivity to MLC to the donor before TLI; this dog did not receive bone marrow, and rejected the graft 20 days after transplantation. In addition, of the nonimmunosuppressed control dogs, five had low reactivity in MLC to the donor, and rejected their grafts at a mean of 4.5 ± 0.3 days, nearly the same as the control dogs that did react in MLC to their donors (mean graft survival: 5.0 ± 0.5 days).

Discussion

The results of these preliminary studies show that total lymphoid irradiation can prolong renal allograft survival in dogs. TLI by itself, however, is not sufficient to prevent rejection indefinitely.

The administration of a low dose of donor bone marrow after TLI was associated with indefinite (>1.5 years) graft survival in two dogs (LG16 and EY47), in the absence of other immunosuppressive

treatments. A tolerant state may have been achieved in these two dogs. We were unable, however, to demonstrate chimerism in the one dog (LG16) tested.

In contrast, Slavin et al.^{5,7} have demonstrated chimerism in both mice and dogs, following TLI and bone marrow transplantation. Further experiments by Strober et al.⁸ have also shown that chimerism can be achieved in dogs treated with TLI and bone marrow transplantation, but the survival of organ allografts in their dogs has not been reported. In their experiments, a high dose of donor bone marrow ($>5 \times 10^8$ cells/kg) was used.

We used a low dose (0.5×10^8 cells/kg) of donor bone marrow in most of our dogs, including LG16, and perhaps chimerism was not induced for this reason. It may be that the administration of bone marrow after TLI can induce a tolerant state, without the establishment of hematopoietic chimerism. However, bone marrow administration did not have the desired effect in all of our dogs, since two of the dogs who received bone marrow after TLI rejected their renal allografts.

It is possible that the low degree of MLC response before treatment in the two dogs (LG16 and EY47) with long-term functioning grafts made it easier for a tolerant state to be induced by TLI and donor bone marrow administration, but the fact that another dog (CB14) with low reactivity in MLC rejected the graft after receiving TLI alone shows that this is not the sole factor. Other investigators have found that the degree of responsiveness to the donor in MLC before treatment correlates with the duration of renal allograft survival in immunosuppressed dogs,¹³ but in our own experiments the reactivity in MLC has not influenced the onset of rejection in nonimmunosuppressed dogs.¹²

The immune function studies in the dogs treated with TLI show a depression of lymphocyte responsiveness *in vitro*. However, recovery eventually occurred. This finding suggests that the clinical application of TLI without donor bone marrow will require adjunctive chemical immunosuppressants for long-term graft survival. Bone marrow administration may result in donor-specific unresponsiveness without the need for long-term pharmacological immunosuppression, but this outcome is unpredictable.

A variety of protocols will probably have to be tried before the optimal schedule for total lymphoid irradiation, with or without donor bone marrow administration, is found. The results of our trial of total lymphoid irradiation in dogs, however, shows the potential of TLI for application to renal transplantation.

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