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## **I. New means of physical immunosuppression**



## MARROW AND ORGAN TRANSPLANTATION AFTER TOTAL LYMPHOID IRRADIATION (TLI)

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High-dose fractionated total lymphoid irradiation (TLI) is a safe, routine regimen used to treat patients with lymphoid malignancies (1). Fractions of 200-250 rads each are administered to the lymph nodes, spleen and thymus until at total dose of 4,400 rads is achieved. The skull, lungs, marrow and kidneys are shielded with lead(1). Although few side effects are associated with the regimen, a profound, suppression of cell-mediated immunity is observed for several years after therapy as judged by both *in vivo* and *in vitro* assays. The mixed leukocyte reaction (MLR) is eliminated for 2-3 years, and slowly returns to normal levels at about 5 years after treatment (2). Intradermal skin reactivity to dinitro-chlorobenzene is eliminated for almost 2 years, and T lymphocytopenia persists for at least 10 years (2). A profound immunosuppression has also been observed in mice and rats given TLI (3-5). Treated animals accept fully allogeneic bone marrow grafts without subsequent graft versus host disease (GVHD), and are rendered tolerant to the transplantation antigens of the donor strain (3-5). Recently, we have achieved similar results using TLI in non-matched bone marrow transplantation in outbred dogs (6,7). The experimental work in animals and implications for clinical transplantation are reviewed here.

## MATERIALS AND METHODS

### TLI in Mice

Almost all experiments were performed on BALB/c mice (H-2<sup>d</sup>) bred in specific pathogen free (SPF) conditions in the colony of Dr. R. Kallman, Department of Radiology, Stanford University School of Medicine. Mice were at least 4-5 months old before use, and both males and females were treated depending upon availability. All animals were maintained in conventional housing, but with mice also bred in the same SPF facility.

The radiotherapy regimen consisted of 17 fractions of 200 rads (total dose, 3,400 rads) administered to the cervical, axillary, abdominal and inguinal lymph nodes, as well as to the thymus and spleen (3,5). The skull,

lungs, tail, hindlimbs and forelimbs were shielded with lead. Animals were placed in the lead apparatus after anesthesia with pentobarbital, and received 5 treatments (fractions) per week until the total dose of 3,400 rads was achieved.

Animals lost up to one third of their body weight during irradiation, and regained their pretreatment weight within one month after radiotherapy if no illness intervened. The incidence of severe illness associated with diarrhea, and the mortality rate was quite variable. For periods of several months more than 90% of the animals survived the procedure well without diarrhea, and appeared normal after one month. During other periods, lasting weeks to months, animals developed diarrhea within one week of institution of therapy, and only 10-15% of animals were alive one month after treatment. The causes of this variability were not clear, but were assumed to be associated with changes in endemic viral and bacterial organisms in the conventional mouse rooms.

#### TLI in Rats

TLI in Lewis rats (AgB1/1) was performed using a procedure similar to that in mice. Adult rats weighing 250 to 350 g were given 17 fractions of 200 rads each during a period of about 3 weeks (4). The most satisfactory ports included all of the neck, mediastinum, axillae, humeri, the whole abdomen including the pelvis, and the proximal half of both femora (4). Rats lost up to one third of their body weight during irradiation, but usually regained their pretreatment weight within 4 to 6 weeks after radiotherapy.

#### TLI in Dogs

Male and female mongrel dogs weighing 15 to 20 kg were irradiated using a 6 Mev linear accelerator (6,7). Irradiation fields included the whole neck, axillae, mediastinum, humeral heads, the whole abdomen including the pelvis, and the femoral heads. The lungs and rectum were shielded with lead, and the distal parts of the limbs, skull and tail were outside of the perimeter of the field. A total dose of 1,800 rads was given in 100 rad fractions to the chest and abdominal fields in continuity during a period of three to four weeks (7). None of the dogs required antibiotic therapy or intravenous fluids during irradiation.

### RESULTS

#### Transplantation Tolerance in Mice After TLI

Skin grafts from C57BL/Ka(H-2<sup>b</sup>) donors showed a mean

survival time of about 50 days when transplanted 1 day after irradiation of the BALB/c recipients (3). The mean survival time of skin grafts on untreated mice was about 10 days. The intravenous infusion of  $30 \times 10^6$  C57BL/Ka bone marrow (BM) cells 1 day after the irradiation regimen rendered at least 90% of the BALB/c mice tolerant to C57BL/Ka tissues (3,5). The recipients were found to be stable chimeras with 50 to 90% of donor-type lymphocytes in the peripheral blood. Chimerism of the red blood cells was also documented (3). Although BALB/c mice given a single dose of lethal whole body irradiation (1,000 rads) and  $30 \times 10^6$  C57BL/Ka BM cells develop a vigorous GVHD, none of the chimeric animals given TLI and a similar dose of allogeneic marrow cells developed GVHD (5). C57BL/Ka skin grafts given simultaneously with BM cells after TLI survived for more than 250 days in the chimeric recipients (3). However, third party, C3H(H-2<sup>k</sup>), skin grafts were rejected within 2 to 3 weeks by the chimeras. Thus, the BALB/c recipients were found to be specifically tolerant of the tissues of the BM donor strain.

#### Effect of Presensitization of BALB/c Mice

BALB/c mice sensitized to C57BL/Ka transplantation antigens by two intravenous injections of whole blood failed to accept C57BL/Ka BM grafts after TLI (5). Sensitization to minor histo-compatibility antigens also prevented BM engraftment. Table 1 shows that BALB/c mice given intravenous injection of whole blood from B10.D2(H-2<sup>d</sup>) donors which share only the minor antigens with C57BL/Ka mice, failed to accept C57BL/Ka BM cells after TLI. Injection of B10.D2 blood during rather than before the irradiation procedure, still prevented marrow engraftment in the majority of animals (Table 1). Thus, the potential clinical use of TLI in organ or marrow transplantation might be limited by the prior exposure of prospective recipients to major or minor transplantation antigens of the graft donor.

#### Transplantation Tolerance in Rats After TLI

In order to achieve uniform chimerism after TLI (3,400 rads) a large dose of BM cells ( $300 \times 10^6$ ) from ACI (AgB<sup>4/4</sup>) donors was required, and the entire pelvis had to be included in the irradiation fields (4). The stable ACI→Lewis chimeras were found to have about 50% donor-type lymphocytes in the peripheral blood, and showed no evidence of GVHD (4). ACI skin grafts given simultaneously with the marrow cells survived for at least 6 months with full hair growth. Chimeric recipients with long-term ACI skin grafts rejected third party, BN(AgB<sup>3/3</sup>) skin grafts within 3 weeks. Thus, evidence for specific

transplantation tolerance was achieved in both mice and rats after TLI.

In order to evaluate the effect of TLI on the survival of whole organ allografts, Lewis rats were given TLI, and ACI or BN hearts were directly anastomosed to the abdominal aorta and inferior vena cava (4). Graft function was determined by direct palpation and by electrocardiography. The heart transplants survived between 50 to 300 days in recipients given TLI, and no more than 10 days in untreated recipients. Four out of 5 animals given TLI and both heart and BM transplants maintained functioning heart grafts for at least 300 days.

TABLE 1. Effect of Presensitization of BALB/c Mice to Non-H-2 Transplantation Antigens of the BM Donor on the Survival of C57BL/Ka BM Allografts After TLI

Recipient treatment	No. of definite chimeras/ No. of mice tested+
TLI	42/50
TLI, B10.D2 blood transfusions during TLI*	0/10
TLI, B10.D2 blood transfusions during TLI**	2/13

+Fraction of animals with > 16% donor-type (H-2b) PBL at least 40 days after transplantation.

\*0.1 ml whole blood injected intravenously 18 and 11 days before TLI

\*\*0.1 ml whole blood injected after irradiation fraction numbers 1 and 3.

#### Allogeneic Bone Marrow Transplantation in Outbred Dogs

One day after the completion of TLI, dogs received an intravenous infusion of a mean of  $0.70 \times 10^9$  BM cells/Kg body weight from mongrel donors. All donors were opposite in sex from the recipients, so that chimerism could be determined by the presence of sex-chromosome markers in karyotype analysis of spontaneous mitoses in bone marrow aspirates of the recipients (6,7). In addition, some donors were selected for the presence of blood group (DEA) antigens which were not present in the recipients. The appearance of the latter antigens on red blood cells of the BM transplant recipients could also be used to determine chimerism.

Table 2 summarizes the experimental results obtained with 12 dogs given TLI and non-matched BM transplants. The first 3 dogs were treated as outlined above. The subsequent 9 dogs were given 1 or 2 infusions of whole blood from random donors during the irradiation procedure to determine whether exposure to blood elements might sensitize the recipient and prevent marrow engraftment. Stable chimerism was documented in the first 3 dogs for at least 40 weeks after BM transplantation (last observation point) by blood group and/or karyotype analysis (Table 2). None of these dogs showed evidence of (GVHD) such as weight loss, diarrhea or liver dysfunction.

TABLE 2. Summary of Unmatched Allogeneic Bone Marrow Transplantation in Dogs After TLI

Recipient Treatment	Weeks After BM Transplantation	Karyotype Analysis (%donor-type)	Blood Group Analysis (donor-Antigen*)
1,800 rads	40	40%(♂)	1.1,5*,6
1,800 rads	40	32%(♀)	1.1*,3,4,6,
1,800 rads	40	40%(♂)	4,6
1,800 rads 1 transfusion+	10	24%(♀)	1.1,4*,6
1,800 rads 2 transfusions	8	20%(♀)	--
1,800 rads 2 transfusions	8	33%(♂)	--
1,800 rads 2 transfusions	8	40%(♂)	--
1,800 rads 2 transfusions	6	37%(♀)	--
1,800 rads	6	29%(♀)	--
1,800 rads 2 transfusions	6	40%(♀)	--
1,800 rads 1 transfusion	6	25%(♀)	1.1*,4,5*,6,8
1,800 rads	6	20%(♀)	1.1,6,8*

+Transfusions (150-250ml whole blood from random donors) were given at various times during irradiation.

-- indicates blood group typing was not done. 15 to 20 karyotypes were analyzed per BM sample.

## DISCUSSION

The mechanism of transplantation tolerance and lack of GVHD in TLI - chimeras is unclear. We recently documented the presence of cells which specifically suppress the MLR of host responder cells to donor stimulator cells (8). These suppressors may maintain recipient tolerance to donor tissues. We have not assayed for the presence of putative donor suppressor cells to stimulators obtained from normal recipients. However, it is possible that T cell maturation of both donor and recipient cells is altered due to changes in the lymphoid micro-environment after TLI. The altered maturation of both donor and host cells might result in the preferential activation of suppressor cells upon exposure to alloantigens. Thus, mutual tolerance may be explained by the presence of suppressor cells of both donor and host origin which have as their targets immunocompetent T cells of the donor and host respectively. Although the suppressor cell of the MLR has not yet been characterized, studies of the induction of tolerance to bovine serum albumin (BSA) after TLI showed that the antigen specific suppressors are T cells, since they are eliminated by treatment with anti-Thy-1 antiserum and complement (9).

The application of TLI to bone marrow and whole organ transplantation in human merits further study, since TLI offers several advantages over presently used therapeutic modalities. Current regimens used to prepare patients for marrow transplantation are lethal in the absence of allogeneic marrow engraftment, and marrow donors are restricted to HL-A matched siblings due to the danger of GVHD (10,11). On the other hand, TLI is a non-lethal procedure which has been used successfully in animals to transplant allogeneic marrow from unmatched donors without the development of GVHD. Thus, TLI might allow for marrow transplantation in all patients with a single sibling, whereas conventional procedures are feasible in only one out of four such cases (probability of finding a single HL-A matched sibling). In addition, the induction of transplantation tolerance with TLI would obviate the requirement for the use of maintenance immunosuppressive drugs after whole organ transplantation. Systemic infections associated with the use of these drugs account for the majority of deaths in heart transplant patients at present (12). Serious infectious complications associated with TLI are rare, such that this therapeutic regimen may offer considerable improvement in the long-term survival of organ graft recipients as compared to that presently obtained with immunosuppressive drugs.



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