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Long-Term Survival of Skin Allografts in Mice Treated with Fractionated Total Lymphoid Irradiation

Abstract. *Treatment of recipient Balb/c mice with fractionated, high-dose total lymphoid irradiation, a procedure commonly used in the therapy of human malignant lymphomas, resulted in fivefold prolongation of the survival of C57BL/Ka skin allografts despite major histocompatibility differences between the strains (H-2^d and H-2^b, respectively). Infusion of 10^7 (C57BL/Ka × Balb/c)F_1 bone marrow cells after total lymphoid irradiation further prolonged C57BL/Ka skin graft survival to more than 120 days. Total lymphoid irradiation may eventually prove useful in clinical organ transplantation.*

The immunosuppressive effects of ionizing radiation have been well known since the beginning of the century (*1*). Treatment of prospective allograft recipients with whole body radiation (WBR) became customary in organ transplantation studies of both laboratory animals and humans. However, the high dose of WBR required to prevent allograft rejection in animals with major histocompatibility differences produced severe injury to the bone marrow and gut (*2, 3*). By 1965, almost all clinical centers had ceased using WBR to prepare patients for kidney transplantation (*2–5*). Nevertheless, extensive research was devoted to overcoming the adverse consequences of radiation by limiting its effects specifically to immunocompetent lymphoid cells. These experimental procedures included local irradiation of the graft site (*4*), local irradiation of the allotransplant (*2–4*), intralymphatic administration of radioactive isotopes (*3–6*), intravascular implantation of a high-energy beta-emitting source (*4, 7*), and extracorporeal irradiation of blood (*2–5, 8–10*) and lymph (*2–5, 8, 11*). Technical difficulties, primarily in estimating the distribution of the radiation dose in the tissues, and the limited success in prolonging allograft survival have restricted the clinical use of these techniques (*3–5*).

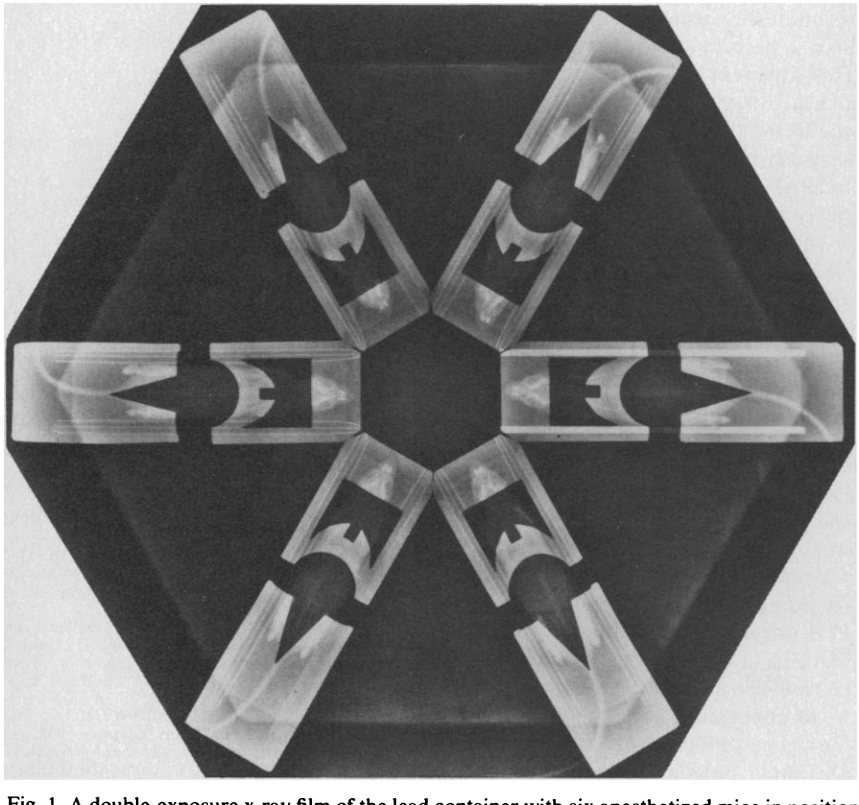

Fig. 1. A double-exposure x-ray film of the lead container with six anesthetized mice in position for irradiation.

We have studied the effect of fractionated high-dose total lymphoid irradiation (TLI) on the survival of skin allografts between mice with major histocompatibility differences. In humans, TLI has been used extensively for treating malignant lymphomas (*12*). High, cumulative doses of irradiation to the lymphoid tissues can be used without causing the severe side effects of WBR, by selectively localizing the radiation to lymphoid tissues and fractionating the total radiation dose. In humans, TLI is immunosuppressive (*13*). If the radiation is selectively restricted to lymphoid tissues and the total dose is fractionated, high cumulative doses of irradiation do not have the severe side effects of WBR.

Adult (8-month-old) Balb/c (H-2^d) male and female mice were anesthetized with pentobarbital and positioned in an apparatus designed to expose the major lymphoid organs to x-rays but to shield the skull, chest (lungs, heart, ribs, and dorsal spine), hind legs, and tail (Fig. 1). Radiation was administered with a Phillips x-ray unit (250 kv at 15 ma, 40 r/min) 60 cm from source to skin; filter, 0.25-mm Cu plus 1.0-mm Al. The dosimetry was verified with a calibrating ionization chamber and by lithium fluoride thermoluminescence dosimeters. All mice received 200 r/day five times a week to a total dose of 3400 r. One day after completion of irradiation, full-thickness skin from C57BL/Ka (H-2^b) mice was grafted onto the flank of the Balb/c recipients (*14*). Grafts were checked daily and considered rejected when they had completely sloughed off or an eschar had developed. The mean survival time of C57BL/Ka skin grafts on 12 multiply anesthetized but not irradiated Balb/c control mice was 10.7 days (range, 10 to 13 days). The mean survival time of skin allografts on 16 mice receiving TLI was 49.1 days (range, 35 to 67 days) (Table 1). Full hair growth was noted on all experimental grafts at about 20 to 25 days. The combination of TLI and a single intravenous injection of 1×10^7 (Balb/c × C57BL/Ka)F_1 bone marrow cells on the day of skin grafting resulted in long-term acceptance (>120 days) of skin by seven of eight recipients. Although

deaths occurred during or after the transplantation procedure, there were no deaths among the irradiated mice that had not been given skin grafts. At least some deaths could be attributed to restriction of respiration by the plaster bandages. No deaths occurred as a result of infection at the site of the transplant.

Peripheral blood lymphocytes were counted and their function was studied in 12 mice given TLI but no skin graft. The TLI resulted in a severe leukopenia with marked depletion of peripheral blood lymphocytes (Fig. 2A). The absolute number of circulating polymorphonuclear cells rose quickly after irradiation, and the count was normal by the end of the third week. However, the absolute number of peripheral blood lymphocytes remained 2 standard deviations below the mean for normal mice for 90 days. Lymphocyte subpopulations followed different patterns of recovery (Fig. 2A). Thymus-derived (T) cells detected by a modified in vitro cytotoxicity assay with antiserum to θ antigen (*15*) were almost totally eliminated for 27 days. The absolute number of T cells rose steeply during the next 2 weeks after which the recovery rate increased more slowly. However, the number of T cells remained 2 standard deviations below the mean for normal mice at 90 days and 1 standard deviation below at 120 days. Repopulation of immunoglobulin (Ig)–bearing lymphocytes (B cells) detected by immunofluorescent staining—by a two-stage procedure with a rabbit antiserum to mouse Ig (*15*)—started as soon as TLI was terminated. The number of Ig-bearing cells became normal by day 60. However, after 120 days an absolute B-cell lymphocytosis with values 2 standard deviations above the mean normal level was noted. Summation of the percentage of T and B cells revealed a high percentage of null lymphocytes (bearing neither surface Ig nor θ antigen) within the first 28 days after TLI (up to 50 percent of lymphocytes).

The TLI totally suppressed the mixed-lymphocyte reaction (MLR) of responder Balb/c peripheral blood lymphocytes to C57BL/Ka lymph node cells as determined by tritiated thymidine uptake (*16*) for at least 30 days (Fig. 2B). The first positive response was noted by day 41, and the MLR was completely reinstated by day 70. The first rejections of skin allografts coincided with the return of MLR.

The efficacy of TLI in prolonging allograft survival can be compared with that of other forms of lymphocyte depletion. Sublethal WBR prolongs skin allografts by only 4 to 7 days in mice when there are major H-2 differences (*17*). Thoracic duct drainage or continuous beta irradiation of the spleen and cervical lymph nodes in rats before grafting does not prolong allograft survival in animals that differ at the major histocompatibility locus despite marked lymphocytopenia (*18*). Lymphocyte depletion by extracorporeal irradiation of the blood of calves (*9*) and humans (*10*) is of little if any value in prolonging allograft survival even when combined with Imuran and thymectomy (*9*). However, treatment with antilymphocyte serum and thymectomy prolongs skin graft survival to the range (41 to 80 days) observed with TLI alone (*19–21*).

Table 1. Days of survival of C57BL/Ka skin allografts on Balb/c mice given total lymphoid irradiation (TLI) with and without intravenous administration of 10^7 (C57BL/Ka × Balb/c)F_1 bone marrow cells (BMC).

Survival (days)	Number of animals		
	Control	TLI	TLI+BMC
< 20	12		
21 to 30			
31 to 40		5	
41 to 50		4	
51 to 60		5	
61 to 70		2	
80 to 100			1
> 120			7
Mean survival (days)			
	10.7 (10 to 13)*	49.1 (35 to 67)	> 120

*Ranges are shown in parentheses.

Survival of the allografts in mice can be further prolonged by combining injection of lymphoid cell extracts or infusion of F_1 hybrid marrow with adult thymectomy and treatment with high doses of antilymphocyte serum (*19, 21*). Even more potent immunosuppression can be achieved in some adult mice by lethal WBR and bone marrow reconstitution with or without thymectomy (*22*). These procedures, however, are impractical for human organ transplantation owing to the drastic nature of recipient preparation.

Conversely, TLI, a routine regimen used for treating patients with Hodgkin's disease, prolonged the survival of skin allografts in mice at least fivefold when used alone. When TLI was combined with infusion of semiallogeneic bone marrow, skin grafts were accepted for a long term (>120 days); this result could be achieved previously only with much more drastic forms of therapy. Preliminary experiments with a small group of dogs failed to demonstrate prolonged survival of heart allografts after 3000-r

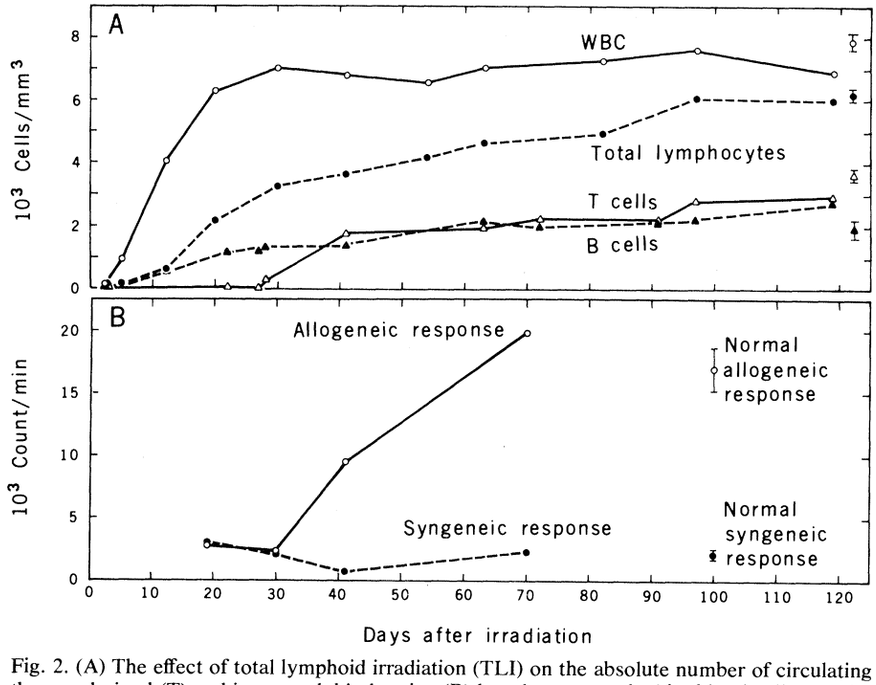

Fig. 2. (A) The effect of total lymphoid irradiation (TLI) on the absolute number of circulating thymus-derived (T) and immunoglobin-bearing (B) lymphocytes and white blood cells (WBC). Mean normal levels (± standard error) of 12 mice are shown on the right. Pooled heparinized blood samples were collected from 12 Balb/c mice that received TLI without skin transplantation. Lymphocytes were obtained after sedimentation of erythrocytes and leukocytes with dextran. (B) Mixed-lymphocyte reaction after 4 days of culture; responder cells from peripheral blood of Balb/c mice and irradiated stimulator lymph node lymphocytes from C57BL/Ka mice at various time periods after TLI compared with normal syngeneic and allogeneic responses (mean ± standard error). Blood samples were pooled (i) from 12 mice that received TLI without skin transplantation and (ii) from 12 control mice.

fractionated irradiation of the major lymphoid tissues (*23*). It is possible that differences in the lymphoid tissues, techniques of irradiation, choice of organ allograft, or transplantation techniques could explain the different results to date in dogs and mice. Further animal experimentation will be required to determine whether TLI may be of use in clinical organ transplantation.

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Localization of Apolipoprotein B in Intestinal Epithelial Cells

Abstract. *Indirect immunofluorescence techniques were employed to determine the distribution within intestinal epithelial cells of apolipoprotein B, a protein essential for the normal transport of fat. Isolated intestinal cells were prepared from rats either during active lipid absorption or after biliary diversion. Specific immunofluorescence from an antiserum to apolipoprotein B was detected in the apical portion of epithelial cells from bile-diverted animals, demonstrating that a pool of apolipoprotein B is present in the nonabsorptive epithelial cell and may be a component of intestinal cell membranes. During lipid absorption in normal rats, an early and sustained increase in immunofluorescence was demonstrated, consistent with an increased synthesis of apolipoprotein B during lipid absorption. This study demonstrates the presence of apolipoprotein B within intestinal epithelium and provides evidence for the participation of this apoprotein in intestinal lipid transport.*

The intestine has recently been recognized as an active site of synthesis of lipoproteins that transport endogenous as well as exogenous lipid (*1*). Long chain fatty acids and monoglycerides, the products of luminal lipolysis of triglyceride, cross the microvillus membrane and are resynthesized to triglyceride in the endoplasmic reticulum of the epithelial cell. Endogenous fatty acids may be derived from biliary lipid and are similarly resynthesized to triglyceride. The newly resynthesized triglyceride is then subjected to a series of synthetic steps with the addition of phospholipid, specific apoproteins, and carbohydrate to form a completed lipoprotein particle (chylomicron or very low density lipoprotein). This completed particle is then ready for egress from the intestinal cell to enter the lymph and systemic circulation.

While there is information on the individual synthetic events in lipoprotein formation, little is known concerning the sequence of additions or the steps that are critical to the process. Although quantitatively small, the protein moiety of the $d < 1.006$ lipoproteins, especially apolipoprotein B (apo LDL), is thought to be of fundamental importance in the process of intestinal triglyceride transport. Indirect evidence for the importance of apolipoprotein B in intestinal lipid transport is provided by the disease abetalipoproteinemia where the presumed inability to synthesize this apoprotein is associated with a failure of intestinal lipoprotein formation (*2*). While apolipoprotein B is known to be synthesized by the intestinal epithelial cell during chylomicron formation (*3*, *4*), the precise subcellular distribution of this apoprotein and whether a pool of apolipoprotein B is present in the nonabsorptive state is unknown. Using fluorescent antibody techniques we have demonstrated the presence of apolipoprotein B in intestinal epithelial cells in the fasting and absorptive state.

Antiserum to apolipoprotein B was prepared in rabbits immunized with rat low density lipoprotein (LDL) isolated from fresh rat plasma between densities 1.025 and 1.050 (*5*). Rabbits were immunized with purified LDL in complete Freund's adjuvant in multiple intracutaneous sites along the back; booster injections were given 2 weeks later, and blood was withdrawn 1 to 2 weeks subsequently. Antiserums were characterized by double diffusion in agarose (Fig. 1) and by immunoelectrophoresis against whole rat serum and purified LDL, and they gave a single precipitin line which stained with oil red O. Additional antiserums to apolipoprotein B were prepared by injecting intact chylomicrons isolated from rat mesenteric lymph and purified on 2 percent agarose columns (*4*). Immunization and bleedings were carried out as described above. Early bleedings yielded an antiserum that was monospecific for apolipoprotein B (Fig. 1) when tested against rat plasma or delipidated chylomicrons.

Isolated rat intestinal epithelial cells were prepared from fasting animals, bile-diverted animals, or from animals during active lipid absorption. Isolated intestinal cells were prepared by the method of Weiser by incubating jejunal segments with phosphate-buffered saline (*p*H 7.4) containing EDTA and dithiothreitol (*6*). Isolated rat colonic cells were similarly prepared. To produce active lipid absorption, corn oil emulsion in 10 m*M* taurocholate was instilled into isolated jejunal segments in situ for varying periods of time as in-