Fractionated Total Lymphoid Irradiation as Preparative Immunosuppression in High Risk Renal Transplantation

Clinical and Immunological Studies

JOHN S. NAJARIAN, M.D., RONALD M. FERGUSON, M.D., PH.D., DAVID E. R. SUTHERLAND, M.D., PH.D., SHIMON SLAVIN, M.D., TAE KIM, M.D., JOHN KERSEY, M.D., RICHARD L. SIMMONS, M.D.

Twenty-two patients at high risk to reject renal allografts have been treated with fractionated total lymphoid irradiation (FTLI) prior to transplantation of primary (2), secondary (16) or tertiary (4) renal allografts. All patients undergoing retransplantation had rapidly rejected previous grafts. At 24 months following transplantation, 72% of grafts were functioning in the TLI group compared with a 38% graft function in an historical control group of recipients receiving secondary or tertiary grafts and treated with conventional immunosuppression. Important variables in determining success of transplantation following fractionated TLI include the dose of TLI, the interval from radiation to transplantation, and maintenance post-transplant immunosuppressive therapy. Optimal results were achieved with 2500 rads delivered in 100 rad fractions followed by transplantation within two weeks, and a tapering prednisone schedule and maintenance azathioprine post-transplantation. Seventeen patients had significant complications of the radiation treatment and there was one death, prior to transplantation, associated with pneumonitis. *In vitro* assessment of immune function demonstrated marked peripheral T cell depletion and loss of *in vitro* responsiveness to mitogen and allogeneic stimulation following FTLI. The administration of donor bone marrow at the time of transplantation did not produce chimerism. The results suggest that when properly utilized FTLI can produce effective adjunctive immunosuppression for clinical transplantation.

From the Departments of Surgery, Therapeutic Radiology and Pediatrics, University of Minnesota, Minneapolis, Minnesota

THE APPLICATION of the immunosuppressive effects of ionizing irradiation to transplantation were first explored nearly 25 years ago when treatment of prospective renal allograft recipients with whole body irradiation was attempted. It was found that the irradiation dose required to produce allograft prolongation also produced such severe bone marrow and G-I toxicity that this approach was all but abandoned by the mid 1960s.[1]

Other less toxic applications of x-irradiation were directed at immunocompetent lymphoid cells. Experimental and clinical use of local graft irradiation,[2] intralymphatic administration of radioisotopes,[3] and extracorporeal irradiation of blood[4] and lymph[5] were all tested experimentally and clinically. The variability of radiation dose delivered and allograft prolongation achieved by these techniques limited their applicability to clinical transplantation. It was the Stanford group who first noted that patients treated with fractionated total nodal irradiation for Hodgkin's disease sustained a long-lasting impairment of cell-mediated immune functions.[6] This led to a series of experimental studies in rodents,[7,8] dogs,[9,10] and primates[11] that demonstrated greatly delayed rejection of skin and vascularized solid organ allografts following a preparative protocol of fractionated total lymphoid irradiation. Additionally observed was the indefinite survival of skin allografts in FTLI-treated rodents given donor bone marrow simultaneously with the graft.[12] The animals were permanently chimeric, and graft-*vs.*-host disease did not occur. The lack of toxicity to the bone marrow and G-I tract using this experimental protocol while maintaining a strong immunosuppressive effect gave the impetus for further experimental studies and a clinical trial of preparative FTLI in a group of renal transplant patients at a high risk of rejection of their grafts.

Patients in renal failure who have rapidly rejected previous kidney grafts are at high risk of graft loss following retransplantation. In our experience, only 46% of patients who reject first transplants within one year will have functioning second grafts one year after re-

Presented at the Annual Meeting of the American Surgical Association, Boston, Massachusetts, April 21–23, 1982.

Reprint requests: John S. Najarian, M.D., Box 195 Mayo Building, 420 Delaware St. SE, University of Minnesota, Minneapolis, Minnesota 55455.

Supported in part by Public Health Service grant no. AM-13083.

0003-4932/82/1000/0442 $01.35

transplantation. By four years this figure drops to 28% despite the use of antilymphocyte globulin and conventional prednisone and azathioprine therapy.[13] To improve results in this difficult group of patients, a more effective form of immunosuppression is required.

Since 1979 the authors have treated 22 patients at high risk for rejection of a renal allograft with total lymphoid irradiation prior to transplantation. FTLI was used primarily for its generalized immunosuppressive effect, but donor bone marrow cells were administered at the time of transplantation when it was logistically feasible. Azathioprine and prednisone were also administered post-transplant because there was an anticipated dissipation of the TLI effect with time.

Materials and Methods

Patient population

Between February of 1979 and July of 1981, 22 patients (ages 5 to 55) were transplanted after receiving a course of total lymphoid irradiation. Twenty patients received either second, third, or fourth transplants. The mean graft survival of all previous grafts was 4.5 months. An additional two first-transplant recipients of grafts from two-haplotype mismatched living donors received FTLI prior to transplantation. There was a spectrum of primary diseases including nine with diabetic nephropathy, six chronic glomerulonephritis, one obstructive uropathy, one Henoch–Schonlein purpura, one renal tubular acidosis, one hemolytic uremic syndrome, two focal glomerulosclerosis and one drug-induced nephritis. All but one patient underwent splenectomy prior to any irradiation. This patient received 1200 rads of FTLI and became profoundly leukopenic and thrombocytopenic. A splenectomy was performed with a subsequent rise in WBC and platelet count and completion of the FTLI course prior to transplantation. All patients irradiated were in chronic renal failure and required maintenance hemodialysis at the time FTLI was delivered. There were 18 cadaveric and four living donor transplants performed. All had negative warm T-cell cross matches with their potential donors and all were blood group compatible. The mean HLA A and B antigen match was 1.3 antigen per pair.

Irradiation

Irradiation was delivered to patients using either 10 MEV or 4 MEV linear accelerator in the Department of Therapeutic Radiology at the University of Minnesota. Both mantle and inverted Y fields were irradiated simultaneously. Individual lung blocks were made for anterior and posterior portals. Radiation regimen for earlier patients was to give 3200 rads total dose with 125 rads daily fraction. The total dose delivered, however, ranged from 1050 to 4050 rads because of the delay between the completion of radiation and poor tolerance of radiation by some patients. Daily fraction was reduced to 100 rads after the first nine patients. Three patients received maintenance radiation ranging from 625 to 800 rads, 125 rads per fraction, twice weekly while they are waiting for a compatible cadaver kidney after the completion of planned radiation.

Radiation dosimetry was verified by lithium fluoride measurement. Irradiation was withheld if patients became leukopenic (WBC $< 2000/mm^3$) or if the patient complained of gastrointestinal symptoms or had a febrile illness. After symptoms resolved, the radiation schedule was reinstituted.

Post-transplant Immunosuppression

All patients received maintenance azathioprine in a dose of 1 to 1.5 mg/kg/day adjusted for WBC. There were three prednisone protocols used. Fifteen patients received a standard prednisone taper beginning at 2 mg/kg/day tapered to 0.5 mg/kg/day by three weeks. Four patients received an accelerated taper of prednisone beginning at 2 mg/kg/day tapered to 0.3 mg/kg/day by two weeks. There were three patients who were begun on low-dose (0.4 mg/kg/day) prednisone therapy immediately following transplantation without initial high doses.

Bone Marrow Administration

Five of the patients received low-dose bone marrow (0.5×10^8 nucleated cells/kg) from their donor at the time of transplantation. A low dose was used to reduce the risk of graft-*vs.*-host disease. These five later received the standard taper post-transplant prednisone regimen. Four patients were transplanted with kidneys from mismatched living donors, therefore, there were no logistical problems associated with bone marrow procurement or administration. One patient received bone marrow and a kidney from a local cadaver donor. In each case, the harvested donor bone marrow was treated *in vitro* with heterologous human anti-T cell globulin in a further attempt to minimize the occurance of graft-*vs.*-host disease.

The 16 patients who did not receive donor bone marrow were recipients of cadaver kidneys procured from other transplant centers so fresh donor bone marrow was unavailable.

T-cell Enumeration

Peripheral blood mononuclear cells (PBMs) were isolated from patients following ficoll hypaque centrifu-

gation.[14] Interface mononuclear cells were washed and were greater than 95% viable. The cells were mixed with sheep red blood cells and the number of peripheral mononuclear cells forming SRBC rosettes were enumerated according to the methods of Jondal et al.[15]

PHA and Con A Responses

Mitogenic activation by PHA or Con A of 10^5 PBMs from each patient were performed[16]; 10^5 cells were placed in round-bottom microtiter plates (Linbro, Hamden, CT) in the presence of 20% pooled human serum and optimally mitogen concentrations of PHA or Con A. Samples were performed in triplicate. Eighteen to 24 hours before harvest, 1 uC of H^3 thymidine was added to each well. The lymphocytes were collected, dried, placed in scintillation fluid, and counted. Data were reported as the mean counts per minute ± the standard error of the mean.

Mixed Lymphocyte Culture Responses

Mixed lymphocyte cultures were performed using the microtechnique of Hartzman et al.[17] in which 10^5 PBM from patients were used as responder cells and placed in microtiter wells in the presence of 10^5 x-irradiated (2500 rad) stimulator cells that were pooled from fresh mononuclear cells harvested from three to five unrelated normal individuals. Cultures were incubated for six days at 37 C in humidified air and 5% CO_2. The triplicate cultures were pulsed with H^3 thymidine 18 hours prior to harvest. Counting was performed in liquid scintillation media in a scintillation counter.

Donor Bone Marrow Preparation

Donor bone marrow was harvested under general anesthesia using a sterile aspiration technique from bilateral iliac crests. The cell suspension was passed through sterile stainless steel filters to remove debris and placed in Hank's Balanced Salt Solution. The marrow was concentrated and then incubated for 30 minutes at 4 C with horse antihuman thymocyte sera[18] at a concentration of 0.1 mg per 10^8 cells. The ATG incubated donor bone marrow was then infused intravenously the day of transplant in a dose of 0.5×10^8 nucleated cells 1 kg over a six-hour period.

Results

Descriptive data on the patients included in this series is given in Table 1. The FTLI number denotes the sequence of the series, number one being the first patient to receive preparative FTLI and number 22 the last patient entered. All patients in the series were irradiated between January 1979 and November 1980 and underwent transplantation between February 1979 and July 1981.

Complications of TLI

Only seven of 24 patients irradiated experienced uncomplicated and uninterrupted courses of preparative radiation therapy. Seventeen had complications that required an interruption of their radiation therapy of from three to 38 days. Six patients experienced leukopenia with WBC <2500; seven patients had severe gastrointestinal symptoms of anorexia, nausea, and vomiting, three of whom required hospitalization and nutritional support with intravenous hyperalimentation for periods of five to 14 days. Three additional patients had unexplained fever that ran a self-limited course (Table 2).

Another patient developed bilateral pneumonitis after receiving 3000 rads. Bronchoscopy was performed for diagnostic purposes. The patient had a drug reaction and a cardiorespiratory arrest. She died before transplantation.

All patients experienced varying degrees of weight loss and anemia throughout their radiation course. In general, radiation was tolerated less well in diabetic patients who seemed to experience more severe weight loss and gastrointestinal symptoms then their nondiabetic counterparts.

Immune Consequences of TLI

Serial determinations of immune function beginning prior to irradiation treatments and at weekly intervals thereafter were performed on peripheral blood of all patients (Fig. 1). Total T-cell number as well as functional responses *in vitro* were assayed. Prior to any irradiation, T-cell numbers were normal with only a slight depression in functional responses (60 to 90% of control) (Fig. 1).

The consequence of increasing irradiation dose on circulating T-cell number was a dramatic decrease in the total circulating T-cell number with increasing dose of radiation to 10–15% of pretreatment values after 2500 rads. Despite T-cell depletion in the peripheral blood, the per cent of lymphocytes that formed sheep red blood cell rosettes remained constant throughout the course of irradiation. T-cell function, however, as assayed by response to mitogens or by responsiveness to allogeneic stimulation in a mixed lymphocyte culture, progressively decreased to a point prior to transplantation where 10–15% of control responsiveness was the rule. This decrease in responsiveness was not simply due to T-cell depletion. When the functional assays were performed, a constant number of lymphocytes were placed in culture. Because the percent of T cells did not change throughout the course of radiation therapy, there was

TABLE 1. *Descriptive Data and Outcome of 22 Patients Receiving Fractionated Total Lymphoid Irradiation Prior to Transplantation*

FTLI Pt. No.	Primary Disease	Age	Sex	Trans-plant No.	Survival of Previous Transplant* No.	Survival of Previous Transplant* Mos.	FTLI Dose (rads)	Interval FTLI to Transpl. (days)	Post-transplant Prednisone Std. Taper†	Post-transplant Prednisone Acc. Taper‡	Post-transplant Prednisone Low Dose§	Donor Source	Bone Marrow‖	Rejection Episode Time	F/U Mos	Curr. Status	Creat-inine
4	CGN	27	F	2	no. 1	6 mo	3875	40	+			Cad	–	–	33	Well	1.3
5	Diabetes	30	M	1	–		3500	6	+			Sib	+	–	31	Well	1.4
8	Diabetes	23	M	2	no. 1	6 mo.	2300	1	+			Cad	–	–	27	Well	1.9
11	Renal tubular acidosis	14	F	2	no. 1	72 mo.‖‖	3200	3	+			Step Father	+	–	24	Well	1.3
22	Chronic Pyelo-nephritis	41	M	4	no. 1 no. 2	1 mo. 2 mo.	2200	180	+			Cad	–	–	5	Well	1.1
18	Diabetes	38	M	1	–		1050	22	+			Sib	+	no. 1 5 wks.	18	Well	1.0
21	Focal sclerosis	14	M	2	no. 1	8 mo.	1600	330	+			Cad	–	no. 1 4 wks.	11	Well	3.5
2	Hemolytic uremic	5	M	3	no. 1 no. 2	1 mo. 10 mo.	2625	6	+			Uncle	+	no. 1 50 wks.	36	Well	2.4
10	CGN	26	F	2	no. 1	1 mo.	4000	180	+			Cad	–	no. 1 8 wks. no. 1 12 wks.	27	Well	4.1
7	Diabetes	44	M	2	no. 1	3 mo.	3125	35	+			Cad	–	Technical Failure§§			–
13	Diabetes	51	M	2	no. 1	5 mo.	2800	2	+			Cad	–	+ 2 wks	1	Died#	–
3	Obstructive uropathy	5	M	3	no. 1 no. 2	2 mo. 1 mo.	2500	2	+			Cad	+	–	12	Died¶	–
6	Focal sclerosis	14	M	2	no. 1	2 mo.	3250	6	+			Cad	–	–	5	Died**	–
9	H. S. purpura	14	M	2	no. 1	18 mo.‖‖	4150	118	+			Cad	–	+no. 1 8 wks. no. 2 12 wks. no. 3 24 wks.	7	Died**	–
12	Diabetes	45	M	2	no. 1	6 mo.	1300	55	+ (+1400 FTLI)††			Cad	+	no. 1 12 wks no. 2 16 wks.		Rej‡‡	–
14	CGN	29	M	2	no. 1	1 mo.	3200	21		+		Cad	–	–	21	Well	1.9
15	CGN	32	M	3	no. 1 no. 2	1 mo. 9 mo.	2500	24		+		Cad	–	–	21	Well	1.6
19	CGN	14	M	3	no. 1 no. 2	1 mo. 9 mo.	2000	1		+		Cad	–	–	18	Well	1.2
20	CGN	14	M	3	no. 1 no. 2	17 mo. 2 mo.	2000	36		+		Cad	–	no. 1 57 wks.	17	Well	1.6
1	Diabetes	35	F	2	no. 1	3 mo.	2750	1			+	Cad	–	no. 1 4 wks.	38	Well	1.1
16	CGN	38	F	2	no. 1	4 mo.	2000	30			+	Cad	–	no. 1 2 wks.	19	Well	1.2
17	Diabetes	29	M	2	no. 1	3 mo.	2000	5			+	Cad	–	no. 1 2 wks. no. 2 5 wks.	18	Well	1.3

* All previous transplants indicated were lost to rejection.

† Standard prednisone taper, 2 mg/kg/tapered to 0.5 mg/kg by three weeks.

‡ Accelerated taper, 2 mg/kg tapered to 0.3 mg/kg by two weeks.

§ No taper, 0.4 mg/kg begun the day of transplant.

‖ 0.5×10^8 cells/kg of ATG treated donor bone marrow given i.v. as a single dose the day of transplant.

Myocardial infarction at one-month post-transplant.

¶ Pneumococcal sepsis at 12 months post-transplant.

** Lymphomas at five and seven months post-transplant—see text.

†† 1400 rads of FTLI given post-transplant, days one to 25.

‡‡ Graft loss was due to acute and chronic rejection six months post-transplant. The patient died on dialysis seven months following transplant nephrectomy.

§§ Graft never functioned—see text. The patient removed himself from dialysis three months post-transplant and died.

‖‖ Both patients experienced early severe acute rejections of previous grafts and never returned to normal function.

TABLE 2. *Complications Occurring During the Course* of Fractionated TLI*

Uninterrupted	7
Interrupted	17
leukopenia	6
anorexia, nausea and vomiting	4
anorexia, nausea and weight loss requiring parenteral alimentation	3
fever	3
death†	1

* Any clinical event that caused interruption in the scheduled irradiation treatment course.

† Respiratory arrest during diagnostic bronchoscopy of pulmonary infiltrates after 3200 rads of FTLI and prior to transplant.

always a constant number of T cells present in each assay system. Therefore, not only was there T-cell depletion but there was a qualitative change in the functional ability of the T cells that remained. Preliminary studies on two patients have demonstrated that this decrease in responsiveness correlates with a reversal of the ratio of functional T-cell subpopulations as detected by the OKT4 and OKT8 monoclonal reagents[18] and therefore represents a relative helper cell depleted state of the peripheral blood.

Figure 1 shows the proportional decrease in T-cell number and function early in the course of TLI. The most significant drop is noted in the first 1000 rads with less of a decline between 1000 and 2000 and very little from 2000 to 3000 rads. Based on these data, a dose of 2500 rads of fractionated TLI appears to produce the maximum immunosuppression activity as assayed *in vitro*, with additional radiation producing little demonstrable immunosuppressive effect.

Test for Chimerism

The authors could find no evidence of chimerism in the five recipients of donor bone marrow. Red cell genotyping at six months did not detect any donor red cells in the three patients tested. In addition, HLA typing of donor and recipient cells, and sex chromosome analysis, could not detect any donor derived marrow cells in any recipient.

In addition, there was nothing in the clinical course of these patients that would distinguish them from those who received TLI without donor bone marrow. Of the five patients receiving donor bone marrow, two were recipients of first transplants from two haplotype mismatched related donors. Two were recipients of third transplants and one of a second transplant. Four never experienced a rejection episode, and one patient currently has chronic rejection 33 months following transplantation. One patient died at 12 months of pneumonococcal sepsis. Three patients who received donor marrow had no complications and have normal renal function for from 15 to 28 months post-transplant.

Patient and Graft Survival

Twenty-two patients have been transplanted following a course of total lymphoid irradiation. There have been two primary grafts from two haplotype mismatched living donors and 20 patients who were retransplanted after the rapid rejection of one or more previous grafts. There was one technical failure in the 22 grafted kidneys. Because of either an embolic or thrombotic event, the graft never functioned. A biopsy at five weeks post-transplant revealed multiple infarcts with no cellular infiltrate suggestive of rejection or antibody or compliment detected by immunofluorescence. This technical failure is excluded from further analysis.

Of the 21 patients presented in Figure 2, 16 are alive and well with functioning grafts from five to 36 months following transplant. One graft was lost to acute and chronic rejection at seven months following transplantation. There were four deaths that accounted for the other four graft losses. The overall one-year actuarial survival is 79% graft function with 86% patient survival. At two years there is a 74% graft function and a 78% patient survival.

When the two primary grafts are excluded, and only retransplants are analyzed and compared with the authors' past experience using conventional immunosuppressive therapy (Fig. 3), at 24 months there is a 72% function in the TLI secondary and tertiary transplant group, with only a 38% graft function in the historical conventionally treated group. This increase in graft function is not associated with increased patient loss. In fact,

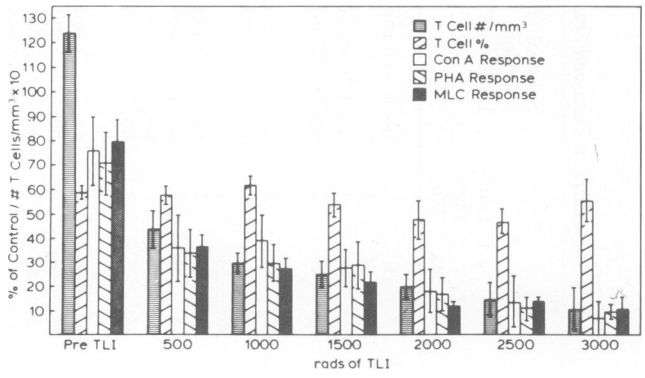

FIG. 1. Sequential immune monitoring of 22 patients receiving FTLI in 100–125 rad fractions delivered to mantle and inverted Y fields simultaneously. T-cell enumeration was performed by E rosette formation. Mitogen and mixed lymphocyte responses are expressed as per cent of normal control cells in cohort culture performed in parallel with the patient cells. Percent responses are given as ± the standard error of the mean.

patient survival is slightly better in the TLI-treated group than in the conventional secondary and tertiary transplant group.

Cause of Graft and Patient Loss

Four of the 22 patients have died. The causes of death have included pneumococcal sepsis in a six-year-old recipient of his third transplant at 12 months following transplantation. The patient's serum creatinine was 0.9, and he had not been treated for rejection. He had had a previous splenectomy and had been receiving prophylactic gantrisin or cortimosazole. Two patients died of disseminated lymphomas. The first patient received a cadaveric kidney after 3200 rads of total lymphoid irradiation. He had normal renal function after transplantation and never experienced acute rejection. An episode of cytomegalovirus infection with pneumonitis was present at two months and clinically resolved. At five months he developed a parotid mass and rapidly progressive neurologic symptoms. The diagnosis of disseminated lymphoma was made, but prior to institution of any therapy the patient suddenly died. At autopsy, lymphoma was found in the brain, parotid and thyroid glands, lung, colon, and heart. In addition, cytomegalovirus inclusion bodies were present in the lung, colon, and liver. This patient had positive cultures for cytomegalovirus as well as Epstein–Barr (EB) virus, and had EB nuclear antigen positive lymphoma cells from both brain and parotid. The second patient was transplanted following 4050 rads and a long interval between radiation and transplantation. He experienced a rejection episode at one month that was treated with increased prednisone and antilymphocyte globulin. One month later he developed clinically significant cytomegalovirus infection that resolved over a three-week period. At two months he was treated for a second rejection episode.

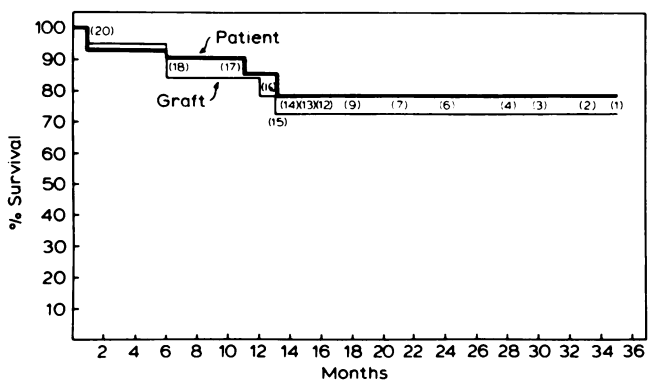

FIG. 2. Actuarial graft and patient survival in two primary and 19 retransplanted patients. Median patient follow up is 17 months. Mean follow up is 18.3 months. Number in parenthesis are the number of patients at risk at the time indicated.

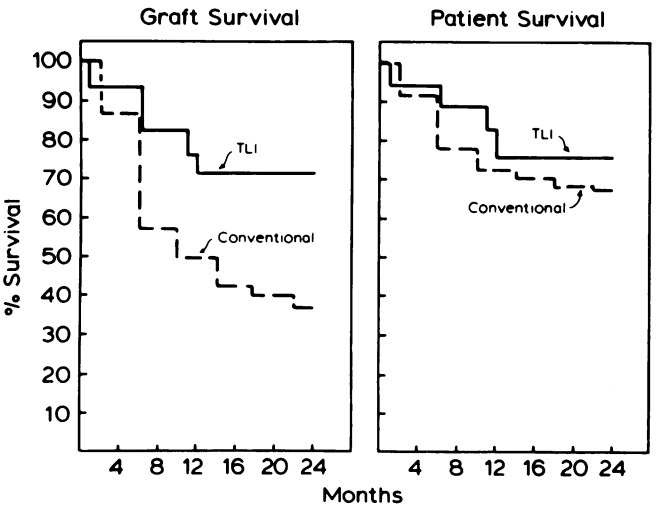

FIG. 3. Comparison of graft and patient survival for retransplantation in patients treated with conventional immunosuppression (ALG, prednisone, azathioprine) with pretransplant FTLI and post-transplant prednisone and azathioprine. The conventional treatment group represents an historical control group of 45 patients transplanted between 1974 and 1979.

At six months the patient presented with a neck mass that was biopsied and diagnosed as a post-transplant lymphoma. Cultures for cytomegalovirus, Herpes simplex virus, and Epstein–Barr virus were all positive. Bone marrow aspiration at this time was also positive for tumor. The patient's immunosuppression was decreased, and he was treated with the antiviral drug acyclovir. The neck mass disappeared, the bone marrow became negative for tumor, and cultures for EB virus became negative. He maintained positive cultures for CMV. Six weeks later he was treated for a third rejection episode. This was followed by recrudescence of his lymphoma to which he ultimately succumbed. At autopsy, tumor was present in the brain, liver, lung, transplanted kidney, retroperitoneum, and gastrointestinal tract.

These two cases of lymphoma are the subject of a much more detailed report.[20,21] Briefly, however, these both can be viewed as Epstein–Barr associated lymphoproliferative disorders. Whether these are true malignancies or the result of an abnormal host immune response associated with an EB virus infection is not clear.

A fourth patient died at one month following retransplantation. After 2800 rads of FTLI the patient received a cadaveric graft. At one week a subtotal gastrectomy was necessary because of multiple bleeding gastric ulcers. Azathioprine and prednisone were discontinued at three weeks. Renal function deteriorated. A transplant nephrectomy was performed that demonstrated a predominant humoral rejection with strongly positive IgM straining of vessels by immunofluorescence. The patient died of a massive postoperative myocardial infarction.

TABLE 3. *Relationship Between Dose of FTLI, Interval from FTLI to Transplant and Post-transplant Immunosuppression*

Post-transplant prednisone therapy	FTLI Dose (rads)	Time FTLI to Transplant Days	T cell Number/mm³		MLC Responsiveness at Transplant
			Pre FTLI	At Transplant	Per cent of Control†
Standard taper					
Rejection episodes (5)*	2240 ± 800	102 ± 32	1093 ± 150	888 ± 170	27 ± 7
		$p < .01$	NS	$p < .002$	$p < .05$
No rejection episodes (9)	2740 ± 401	3 ± 1	1017 ± 131	91 ± 14	14 ± 3
Accelerated taper					
No rejection episodes (4)	2425 ± 286	20 ± 7	1047 ± 91	178 ± 9	13 ± 2
Low dose					
Rejection episodes (3)	2250 ± 255	15 ± 6	1114 ± 44	170 ± 44	11 ± 2

* Number of patients in each group.

† The responsiveness of patient PBLs compared with normal cells in cohort cultures performed in parallel. $\frac{\text{cpm patient cells}}{\text{cpm normal cells}} \times 100$.

Analysis of Rejection Episodes

Of the 21 patients analyzed, eight of them have experienced a total of 16 acute rejection episodes. The most significant contributing factors were the postoperative immunosuppressive protocol utilized and the interval between completion of FTLI and transplantation (Table 3). Three protocols for post-transplant prednisone therapy were utilized. Fourteen patients received a standard tapering dose of prednisone beginning at 2 mg/kg/day tapered to 0.5 mg/kg/day by three-weeks post-transplant. Four patients received an accelerated taper of prednisone beginning at 2 mg/kg/day but tapered to 0.3 mg/kg/day by two weeks. Three patients did not receive a tapering schedule but were begun immediately post-transplant on a low dose of 0.4 mg/kg/day. All patients received azathioprine titrated to their WBC and platelet counts. Usually this was between 1 and 1.5 mg/kg/day.

Five of the 14 patients who received the standard prednisone taper (Table 3) experienced acute rejection episodes within the first two months post-transplant. These five were the only patients in this group who had a significantly longer ($p < 0.01$) length of time between completion of the radiation course and transplantation—a problem that was necessitated by the prior unavailability of a crossmatch negative cadaveric kidney. During this time, immunologic recovery, as assayed by *in vitro* testing, was observed. In the group undergoing early rejection episodes, T-cell number had returned to near normal levels with partial recovery of MLC reactivity prior to transplantation when compared with the group that never experienced an acute rejection episode. It should be emphasized that the increase in T-cell number and MLC reactivity is a function of time and represents partial immunologic recovery from the immunosuppressive effects of radiation. All patients including those experiencing early rejection episodes, had been profoundly T-cell depleted with markedly depressed MLC responsiveness immediately following the course of FTLI (Fig. 1).

A second important variable that may predispose to acute rejection episodes following preparative FTLI is the postoperative immunosuppressive regimen. All three patients given low-dose prednisone (0.4 mg/kg/day) from the day of transplant experienced acute rejection episodes within the first month. The dose of TLI and time interval in which it was delivered were not significantly different from the other groups (Table 1). These three patients were successfully treated, have had no further rejection episodes, and currently have normal renal function 18, 19 and 38 months following transplantation.

A third group of patients received an accelerated tapering prednisone schedule following FTLI that began at 2 mg/kg/day tapering to 0.3 mg/kg by two weeks. None of these four patients have had any acute rejection episodes 17 to 21 months later.

One Year Follow-up Data

Fourteen of the authors' 16 patients with functioning grafts, received their transplants more than one year ago. Of these 14 patients, three have biopsy-documented chronic rejection with serum creatinines of 4.1, 3.5, and 2.4 mg/dl, and 11 have normal renal function (mean serum creatinine, 1.3 ± .3). The results of immune function studies performed one year following transplantation are shown in Table 4. There is no significant difference in the per cent or number of T cells in the peripheral blood of those patients with chronic rejection and those with normal renal function. There is, however, a highly significant difference in the *in vitro* functional responsiveness of lymphocytes from patients within each group. Those patients with chronic rejection are highly mitogen and MLC reactive, whereas those with

TABLE 4. In Vitro *Immune Function One Year Following Transplantation*

	T Cell		PHA	Con A	MLC
	Per cent	Number	Per cent of Control†		
Chronic rejection (3)*	53 ± 8	697 ± 397	80 ± 16	98 ± 3	77 ± 9
		$p < .5$	$p < .001$	$p < .001$	$p < .001$
Normal function (11)	46 ± 7	346 ± 112	16 ± 4	21 ± 5	28 ± 3

* Number of patients in each group.
† The responsiveness of patient PBLs compared with normal cells in cohort cultures performed in parallel. $\frac{\text{cpm patient cells}}{\text{cpm normal cells}} \times 100$.

normal functioning grafts have maintained a low level of both mitogen and allogeneic reactivity, presumably the result of chronic azathioprine and prednisone therapy. Despite continued immunosuppression, recovery from the immunosuppressive effect of irradiation can occur and appears to be associated with the development of chronic allograft rejection.

Discussion

Previous analysis of the authors' results[18,22,23] as well as others[24] suggest that an important determinant of the success of secondary and tertiary transplantation is the duration of function of the first allograft. Those patients undergoing retransplantation who have lost a previous graft to acute rejection within the first several months following transplantation have a diminished chance of success when compared with either primary transplantation from the same donor source or retransplantation in patients who had maintained their first grafts for 12 months or more.[13] Other factors influencing success of retransplantation are donor source, recipients of secondary or tertiary cadaveric grafts having less successful outcome than living related grafts, patient age, older patients undergoing retransplantation doing less well then younger patients, and the presence of diabetes. Therefore, based on the authors' previous experience, the 22 patients transplanted in this series represent a population at high risk of graft and patient loss. Twenty of the 22 patients had lost previous grafts early following transplantation to acute rejection, and six of those 20 were diabetic. In addition, 20 of the 22 grafts were from cadaveric donors, and the two living related donor grafts were from totally HLA mismatched individuals. It would be predicted that this group of patients would have approximately 35% graft function at two years if conventional immunosuppression had been applied.

To offer retransplantation as a viable option in the therapy of renal failure in a population as presented in this series, therefore, requires an altered approach to immunosuppressive management in the post-transplant period. A pretransplant course of FTLI provides an acceptable immunosuppressive alternative and can be delivered safely to patients with chronic renal failure while maintained on hemodialysis. Adequate immunosuppression can be achieved when irradiation is delivered simultaneously to mantle and inverted Y ports in 100 to 125 rad fractions.

FTLI is immunosuppressive in this group of patients, and when combined with postoperative pharmacologic immunosuppression, can produce prolonged survival and a rejection-free post-transplant course. In addition, *in vitro* monitoring of immune function has clearly demonstrated a profound T-cell depleting effect in peripheral blood and an attendant qualitative reduction of *in vitro* immune responsiveness to approximately 10% of pretreatment levels.

There appears to be an important relationship between the total dose of TLI delivered and the pharmacologic immunosuppression given post-transplant. The authors' results suggest that 2500 rads of fractionated TLI followed by transplantation within two weeks of completion of the radiation course, and a postoperative immunosuppressive protocol of tapering prednisone and maintenance azathioprine therapy can achieve good patient and graft survival in an historically difficult group of patients.

The importance of the length of time between completion of radiation and transplantation is apparent (Table 1) and can potentially limit the patient population to which TLI could be used effectively. Ideal candidates would have a living donor available or would have a low percentage of cytotoxic antibodies to a panel, insuring the availability of a crossmatch negative cadaver kidney within a reasonable period of time after completion of a course of irradiation.

It has been reported that patients with Hodgkin's disease treated with fractionated TLI develop long-lasting immune defects.[6] The authors' results suggest that patients with chronic renal failure treated with FTLI recover from this immunologic insult more rapidly than patients with Hodgkin's disease. Trentheim et al. used fractionated TLI to treat refractory rheumatoid arthritis and had similar results.[25] Recrudescence of disease activity and recovery of *in vitro* immunologic function occurred one year after therapy. This however, was not

the case in a similar group of rheumatoid arthritis patients treated by Kotzin et al.[26] They found long-lasting and persistent immunologic defects in their patients seven to 18 months later. Fourteen of the authors' patients have been followed for over one year. Eleven of the 14 patients maintained markedly reduced *in vitro* immune reactivity. These patients all have normal renal function. Three patients recovered functional immune reactivity *in vitro* despite chronic immunosuppressive therapy. All three of these patients have documented chronic allograft rejection.

The usefulness of donor bone marrow in the five recipients to whom it was administered is doubtful. No evidence of chimerism has been detected, and nothing in the clinical post-transplant course distinguishes them from the patients who received TLI without donor bone marrow. Whether the use of larger doses of bone marrow combined with pretransplant FTLI could induce a state of donor specific unresponsiveness is not clear. Before larger doses of bone marrow are used, assurance that graft-*vs.*-host disease will not occur would be necessary. If this cannot be achieved, then the potent generalized immunosuppressive effect of fractionated TLI combined with post-transplant pharmacologic immunosuppression will be needed for long-term prevention of graft rejection. Animal experiments have shown that nontoxic doses of TLI combined with low-dose antithymocyte globulin[11] or cyclosporin A[27] are synergistic in preventing graft rejection without steroid therapy. Whether such protocols will be more clinically efficacious than that described in this report are at present unknown.

From the data presented here, FTLI can produce superior graft survival in patients undergoing retransplantation after rapid rejection of previous grafts. To gain maximum benefit of the immunosuppressive effects of FTLI, the interval between completion of radiation therapy and transplantation should be minimized, for with time immunologic recovery can occur and can be clinically significant enough to cause early acute rejection despite post-transplant immunosuppressive therapy.

In its present form FTLI can be used only for its generalized immunosuppressive effect unlike the circumstances in experimental models. The administration of donor bone marrow did not produce any detectable chimerism, nor was there any clinical effect demonstrated. Post-transplant pharmacologic immunosuppression is necessary. Our results suggest that optimal immunosuppression is achieved with a tapering schedule of prednisone combined with maintenance azathioprine.

When these principles are applied to patients at high risk for graft loss, FTLI can provide an adjunctive immunosuppressive effect that can be safely applied and produce improved graft survival.

References

1. Hamburger J, Vaysse J, Crosnier J, et al. Renal homotransplantation in man after radiation of the recipient. Am J Med 1962; 32:854–71.
2. Hume DM, Wolf JS. Abrogation of the immune response: irradiation therapy and lymphocyte depletion. Transplantation 1967; 5:1174–91.
3. von Bekkum DW. Use of ionizing radiation in transplantation. Trans Proc 1974; 6(4):59–65.
4. Cronkite EP, Chanana AD, Schnappauf HP. Extracorporeal irradiation of blood and lymph in animals. N Engl J Med 1965; 272:456–61.
5. Joel DD, Chanana AD, Cronkite EP, Schiffer LM. Modification of skin allograft immunity by extracorporeal irradiation of lymph. Transplantation 1967; 5:1192–97.
6. Fuks Z, Strober S, Bobrove AM, et al. Long-term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease, J Clin Invest 1976; 58:803–14.
7. Slavin S, Strober S, Fuks Z, Kaplan HA. Long-term survival of skin allografts in mice treated with fractionated total lymphoid irradiation. Science 1976; 193:1252–54.
8. Slavin S, Reitz B, Bieber CP, et al. Transplantation tolerance in adult rats using total lymphoid irradiation: permanent survival of skin, heart, and bone marrow. J Exp Med 1978; 147:700–07.
9. Strober S, Slavin S, Gottlieb I, et al. Allograft tolerance after total lymphoid irradiation (TLI). Immunol Rev 1979; 46:87–112.
10. Howard RJ, Sutherland DER, Lum CT, et al. Kidney allograft survival in dogs treated with total lymphoid irradiation. Ann Surg 1981; 193:196–200.
11. Bieber CP, Jamieson S, Raney A, et al. Cardiac allograft survival in Rhesus primates treated with combined total lymphoid irradiation and rabbit antithymocyte globulin. Transplantation 1979; 28:347–50.
12. Slavin S, Strober S, Fuks Z, Kaplan HS. Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. J Exp Med 1977; 146:34–48.
13. Gifford RRM, Sutherland DER, Fryd DS, et al. Duration of first renal allograft survival as indicator of second allograft outcome. Surgery 1980; 88:611–18.
14. Boyus A. Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest 1968; 21(suppl 97):9–106.
15. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J Exp Med 1972; 136:207–15.
16. Schmidtke JR, Najarian JS. Synergistic effects on DNA synthesis of phytohemagglutinin or Concanavalin A and lipopolysaccharide in human peripheral blood lymphocytes. J Immunol 1975; 114:742–46.
17. Hartzman RJ, Bach ML, Bach FH, et al. Precipitation of radioactively labeled samples: a semi-automatic multiple-sample processor. Cell Immunol 1972; 4:182–86.
18. Miller W, Branda R, Flynn P, et al. Antithymocyte globulin treatment of severe aplastic anemia. Blood 1982 (in press).
19. Reinherz EL, Schlossman SF. Regulation of the immune response-induced and suppressor T-lymphocyte subsets in human beings. N Engl J Med 1980; 303:370–73.
20. Hanto DW, Fizzera G, Purtilo DT, et al. Clinical spectrum of lymphoproliferative disorders in renal transplant recipients and evidence for the role of Epstein–Barr virus. Cancer Res 1981; 41:4253–61.
21. Hanto D, Fizzera G, Gajl-Peczalska KJ, et al. Epstein–Barr virus-induced B-cell lymphoma after renal transplantation: poly-

clonal to monoclonal B-cell proliferation. N Engl J Med 1982; 306:913–18.
22. Casali R, Simmons RL, Ferguson RM, et al. Factors related to success or failure of second renal transplants. Ann Surg 1976; 184:145–154.
23. Ascher N, Ahrenholz D, Simmons RL, Najarian JS. 100 second renal allografts from a single transplantation institution. Transplantation 1979; 27:30–34.
24. Kountz SL, Belzer FO. The fate of patients after renal transplantation, graft rejection, and retransplantation. Ann Surg 1977; 176:509–520.
25. Trentheim DE, Belli JA, Anderson RJ, et al. Clinical and immunologic effects of fractionated total lymphoid irradiation in refractory rheumatoid arthritis. N Engl J Med 1981; 305:976–82.
26. Kotzin BL, Strober S, Engleman EG, et al. Treatment of intractable rheumatoid arthritis with total lymphoid irradiation. N Engl J Med 1981; 305:969–76.
27. Rynasiewicz JJ, Sutherland DER, Kawahara K, Najarian JS. Total lymphoid irradiation: critical timing and combination with cyclosporin A for immunosuppression in a rat-heart allograft model. J Surg Res 1981; 30:365–71.

Discussion

DR. ISRAEL PENN (Denver, Colorado): I would like to confine my comments to one aspect of the authors' findings, and that is the development of lymphomas in two of their 24 patients. A well recognized complication of various forms of conventional immunosuppression is an increased incidence of certain malignancies. (slide) Lymphomas, mostly of non-Hodgkin's type, have an incidence 45 to 100 times greater than that seen in aged-matched controls. Skin cancers are increased about sevenfold in areas of low sunshine exposure, and are increased about twenty-onefold in areas of high sunshine exposure. The other type of common malignancy in these patients is *in-situ* carcinomas of the cervix of the uterus, which are increased approximately fourteenfold over their incidence in the general population.

(slide) In the last 1229 patients that were reported to the Denver Transplant Tumor Registry, skin and lip cancers made up 501 malignancies, solid lymphomas 248, and carcinomas of the cervix, most of which are *in-situ* carcinomas, made up 97 of the neoplasms.

If we take these crude figures, the lymphomas make up approximately 18% of the malignancies. However, if we exclude nonmelanoma skin cancers and *in-situ* carcinomas of the cervix of the uterus, which are excluded from most cancer statistics, then the lymphomas become the most important single group, making up approximately 26% of all cancers. This contrasts with a 3 to 4% incidence in the general population.

There has been some experience with the development of malignancies following the use of TLI in patients with Hodgkin's disease, and this was reported by Kaplan and his colleagues at Stanford (Transplant Proc 1981; 13: 425–428.). They found that if TLI only was used in the treatment of more than 300 patients with Hodgkin's disease, there were no cases of leukemia or lymphoma. However, when TLI was used in conjunction with cancer chemotherapy in nearly 700 patients, there was a 3 to 5% incidence of leukemia or lymphoma.

A similar situation may be present in renal transplant recipients who receive not only TLI but other forms of immunosuppression, as you have heard, such as splenectomy, Imuran, and prednisone.

At first glance, the incidence of lymphoma in Dr. Najarian's series may appear to be rather high. However, the series is a small one, and with further experience it is quite possible that the incidence of lymphomas will be no higher than that seen with other forms of immunosuppressive therapy.

DR. PAUL S. RUSSELL (Boston, Massachusetts): As I see it, clinical transplantation is in an extraordinarily interesting phase right now, with at least five major possibilities for early advances in the control of rejection reactions. Those five are the following: the management of blood transfusions from the donor or from other individuals—it is not clear which is better; the possibility that the alteration of what are called "passenger leukocytes" in the donor tissue, and particularly from among that class of cells, the dentritic cell population, which have been found to be ubiquitous through many of the organs of the body, and are believed to be strongly immunogenic. Perhaps eliminating these cells in certain ways will make quite a difference to the antigenicity of transplants.

Third, Cyclosporin A you have heard quite a lot about, and for my lights, properly so; I think it is important, but I am not so sure it will be all important.

Fourth, TLI is a very interesting possibility that you have just heard raised for immunosuppression. Finally, our particular interest has been in the use of antibodies, especially monoclonal antibodies, directed toward lymphoid cells, and, in particular, subclasses of T-cells.

Now, the Minnesota group has reported in this paper the use of TLI for what are termed "immunoreactive" patients. I share their belief that there probably are such individuals. One could imagine that there are at least two reasons why patients might be particularly immunoreactive. One is a non-specific and poorly understood status of greater reactivity. Perhaps a lot of things may be involved in this, nutritional status, and other things must have a lot to do with one's inert immunoreactivity. Genetic factors are also known to play a role.

Also previous exposure to transplantation antigens will, of course, make an individual specifically more immunoreactive to those same antigens seen again, and we make every effort to avoid that kind of reactivity by appropriate cross-match tests before transplantation.

So this group of patients selected by John Najarian and his colleagues may be quite a mixed bag; if, in fact, previous immunity is part of their increased reactivity, cyclosporin A or TLI will be relatively ineffective as they are not very active against preexisting immune reactions.

Now, in our small studies with primates using TLI in cynamolgus monkeys, Dr. Gary Haas and Ben Cosimi have been looking carefully at what happens to T cells in the course of treatment with 2000 rads over three weeks. These cells do plunge right down in numbers, as John Najarian showed. It is interesting that in returning back up toward normal levels at the cessation of the radiation, the suppressor cells seem to come up quite a lot faster than do the helper/inducer subset cells. Whether that is important or not, I do not know, but I do know that if we put heart transplants into those animals during the period of their lymphopenia, they will do a great deal better than if you wait a few days until after the cells start to return again to the circulation. This raises the question I would like to ask Dr. Najarian.

Does he think that the timing of the last dose of radiation makes very much difference in regard to the time when the transplant is put in?

DR. ANTHONY P. MONACO (Boston, Massachusetts): I would like to follow up on the remarks of Dr. Wilson and Dr. Russell.

The fundamental issue up to this time for those patients who have rapidly rejected their transplants within 12 months, statistically, in North America, and within six months in European studies, is the fact that these individuals reject their transplants with the formation of broadly reactive cytotoxic antibodies to a large panel of HL-A antigens represented in the human population. Therefore, these individuals become untransplantable by virtue of the fact that, first, they reject their kidneys, but secondly, up until this time, we have not been able to find a kindey for them that theoretically we could transplant because of broadly reactive antibody to most donors.

Now, Dr. Najarian has clearly shown that when patients like this are subjected to TLI, they do not change their antibody titer during