LETTER TO THE EDITOR

Total lymphoid irradiation for pretransplant immunosuppression and recurrence of focal segmental glomerulosclerosis

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Recurrence of nephrotic syndrome after renal transplantation in patients with focal segmental glomerulosclerosis (FSGS) is a major issue in renal transplantation. The mechanisms of recurrence are not clear. Early recurrence of proteinuria and subsequent development of FSGS lesion in almost 100% of retransplanted patients suggest that humoral factors work in the pathogenesis of the disease [1–3]. Involvement of the immune system has also been suggested [4,5], and both T and B cells may participate in the pathogenesis of nephrotic syndrome in FSGS [6–8].

Different treatments have been used in the management of FSGS recurrence with variable success, such as cyclosporine (CsA) [9], cyclophosphamide [10], plasma exchange, immunoabsorption and mycofenolate mofetil (MMF) [7,11,12]. Pre-emptive plasmapheresis associated or not with CsA has also been used to treat FSGS recurrence, but 20–30% of those patients also failed to respond to treatment [12].

Total lymphoid irradiation (TLI) is an immunosuppressant treatment, which promotes a more pronounced impairment of T-dependent immunological functions compared with conventional immunosuppression [13]. Because of these characteristics and before of the improvement of immunosuppressant drugs, TLI was used in the management of renal transplant (TX), especially in high risk patients, like those with retransplants and diabetics, as well as in the treatment of refractory recurrent cardiac rejection and autoimmune diseases [14–16].

As immune system (B and T cells) and/or a circulating factor produced by these cells have/has been involved in the recurrence of FSGS, and to date no treatment showed to be efficient in preventing the disease in the renal graft, we proposed a different and higher immunosuppressive treatment. In this report, we show the results of a study protocol using TLI plus conventional immunosuppressant drugs MMF, CsA and prednisone (PRED) (ClinicalTrials.gov/Study ID Numbers: TLIFSGSusp/ NLM Identifier: NCT00353535).

We studied 12 patients with biopsy-proven primary FSGS transplanted from 2000 to 2001 (Table 1).

Table 1. Clinical data of the patients.

Patient	Sex	Age (years)	Time of FSGS (years)	Time to CR failure from first diagnosis (years)	NS	Allograft no.	Donor*
1	M	37	9	7	Yes	1	LNRD
2	M	21	7	6	Yes	1	LRD brother
3	F	38	4	0.4	Yes	1	LRD brother
4	M	41	6	5.5	Yes	1	LRD brother
5	M	31	3	2	Yes	1	LRD sister
6	M	28	2	1	Yes	1	LRD sister
7	M	54	5	4.4	Yes	1	LNRD
8	F	29	10	0.6	Yes	3†	LRD sister
9	M	21	3	2.9	Yes	1	LRD mother
10	M	26	22	19	Yes	1	LRD brother
11	M	21	19	12	Yes	2‡	LRD brother
12	F	20	6	5	Yes	1	LNRD

FSGS, focal segmental glomerulosclerosis; CR, chronic renal; NS, nephrotic syndrome; LNRD/LRD, living no related and related donor.
*All cross-matched negative.
†First graft lost by rejection and second by recurrence.
‡First graft lost by recurrence.

Total lymphoid irradiation

All the supradiaphragmatic lymph nodes were irradiated in a daily midplane dose of 1.8 Gy (5 days per week), until a total dose of 18 Gy, in a linear accelerator (6MV). The field of irradiation was then changed to the infradiaphragmatic nodes with no pause in the treatment and with the same daily and total dose (18 Gy).

Drugs

Cyclosporine was started after TLI and 3 days before TX. It was given in two divided oral doses of 3–4 mg/kg/day, wt/day to maintain a low therapeutic through blood levels. MMF was initiated after TLI and 5 days before TX, in an oral dose of 1 g/day before and 2 g/day after TX. Metilprednisone (PRED) was used intravenously (i.v.), in a dose of 1 mg/kg/day on the TX's day. The interval

between TLI plus drugs and transplant was 5 days. After TX, all patients continued to receive CsA, MMF and PRED in doses adjusted for white blood cells rate and toxicity of these drugs.

Nine patients received the initial full course of TLI. One patient who abandoned TLI after two doses and two patients who refused to participate in the protocol were transplanted using the same drugs (CsA, MMF and PRED) and dosages, which were initiated 5 days before TX. Some irradiated patients ($n = 5$) did not use MMF pre-TX and some patients ($n = 3$) had the MMF suspended after TX because of leucopoenia.

Hemogram and immunophenotyped lymphocyte (T and B cells) subsets using flow cytometric were done before, during TLI, and 30, 60 and 90 days after TX. Functional analysis of lymphocytes was assessed through phytohemagglutinin (PHA). Pokeweed mitogen blastogenesis (PWM) was performed before and after TLI.

A biopsy of the allograft was performed, if recurrence/rejection was suspected or 3 months after transplant.

Our results showed high morbidity and did not prevent or decrease the recurrence of FSGS. One patient had acute humoral rejection and was excluded from the clinical analysis. There was a 37.5% (three of eight) clinical and biopsy-proven recurrence in renal allograft in irradiated patients and two recurrences in partially or nonirradiated patients ($n = 3$) (Table 2).

There was a significant decrease in T and in B lymphocyte counts and functional response to PHA (Friedman's ANOVA; $P \leq 0.05$). The three recurrent irradiated patients did not have significant response to treatment compared to the nonrecurrent ones. Partially or nonirradiated patients did not show significant reduction in all parameters except for the mutagen-response to PWM ($P < 0.05$) (Fig. 1).

Irradiation induces ablation of memory cells. Replacement is done for short-lived hypofunctional 'naive' T cells, which characterize the state of immune amnesia [17]. Despite the profound immunosuppressant effect and consequence observed, many rejection episodes (60%) were unexpectedly detected in our study, although most transplants were the first patient's transplant (eight of nine) and from live related donors (seven of nine). To our knowledge, this is the first time that TLI was used with FSGS patients. We could speculate that the high rates of rejection episodes are related to the FSGS. In fact, in a former publication, more frequent rejection episodes and higher incidence of acute tubular necrosis (ATN) were reported when compared with the non-FSGS transplanted patients. [18,19]. Another aggravating problem in FSGS patients is that the differential diagnosis between rejection and recurrence is difficult, and some authors consider that the high incidence of ATN would actually be recurrence episodes [5,19,20]. In our study, despite adequate time of ischaemia/reperfusion, which is an ATN inductor factor, we observed that three of nine patients with ATN (33%) and two out of three patients with FSGS recurrence also had ATN and rejection.

Modern immunosuppressant regimens reduced the acute rejection (AR) rate sharply. Because of the interac-

Table 2. Outcomes of 12 patients with focal segmental glomerulosclerosis.

Patient	Rejection time (days)	ATN	Time to recurrence	Intercorrences	TLI	Creatinin	Graft survival	Follow-up (months)
1	–	–	–	2 gastric CMV 1 HZ	18 Gy	1.5	–	58
2	ACR	–	Yes/5 days	Pneumonia	18 Gy	Lost Dialysis	30 months	56
3	CCR/Banff I/240 days/ACR	Yes	–	Pneumonia	18 Gy	Cr = 3.0 Reno-vascular hypertension	–	55
4	AHR/10 days	–	–	Pneumonia	18 Gy	Lost Dialysis	10 days	54
5	–	–	–	2 Lung CMV staphylococcus	18 Gy	1.6	–	52
6	ACR/8 days	–	–	Gastric CMV	18 Gy	1.5	–	51
7	ACR/7 days	–	–	Gastric CMV, Hodgkin's lymphoma	18 Gy	1.7	–	41
8	ACR/150 days	Yes	Yes/150 days	Enterococus infection, 2 pneumonia, HZ, sepsis and death	18 Gy	Dyalisis, death	5 months	8
9	–	Yes	Yes/10 days	Fungal pneomonitis, ARDS, septic shock, trombosis with feet amputation	18 Gy	1.2	–	37
10	–	–	Yes/3 days	1 gastric CMV	3.6 Gy	1.5	–	42
11	–	–	Yes/5 days	No	Refused	1.4	–	42
12	–	–	–	No	Refused	1.2	–	41

ACR, acute cellular rejection; CCR, chronic cellular rejection; ATN, acute tubular necrosis; AHR, acute humoral rejection; CMV, cytomegalovirus; HZ, herpes zoster; ARDS, angst respiratory distress syndrome.

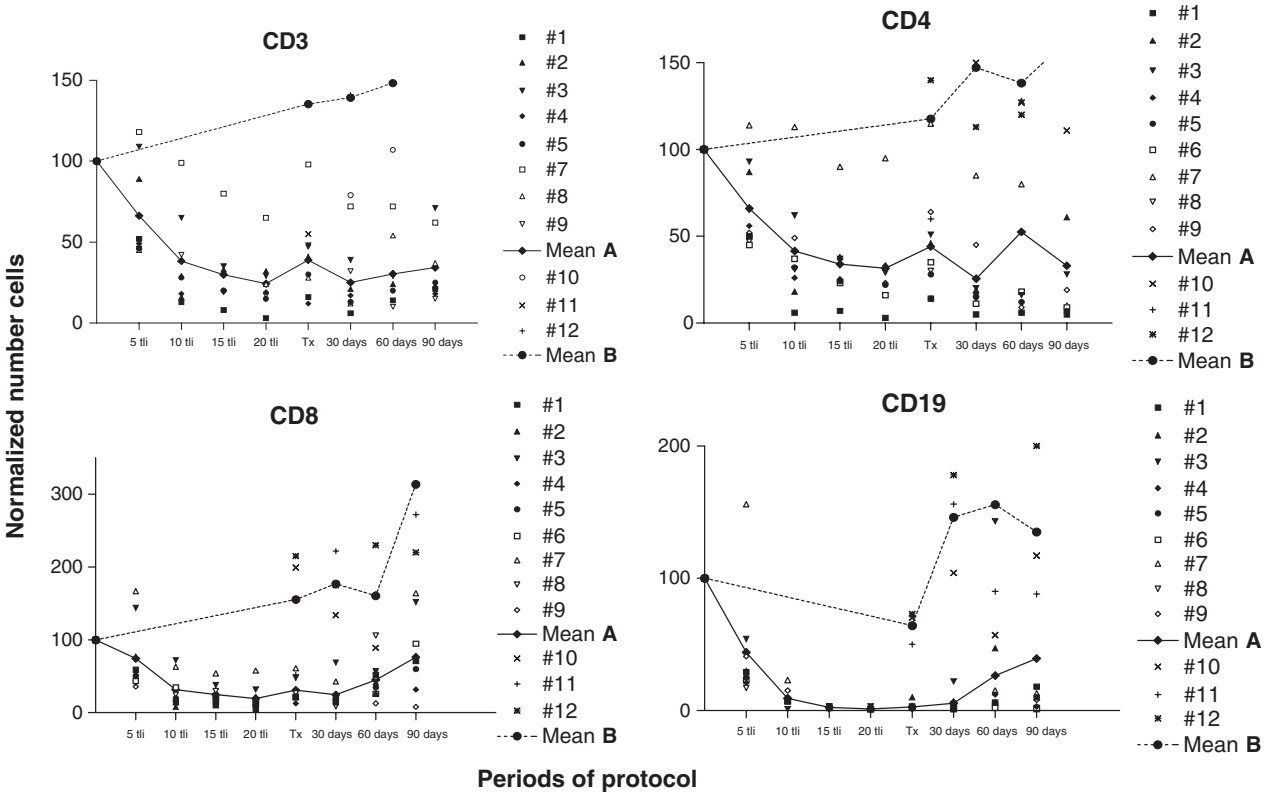

Figure 1 Individual and mean (dose–response curve) changes in the different lymphocytes subsets during total lymphoid irradiation and immunosuppressant drugs before and after (30, 60 and 90 days) renal transplant (TX) in nine patients (mean A). Three patients received the same drug treatment but were partially or nonirradiated (mean B).

tion between MMF and calcineurin inhibitors concerning the pharmacokinetic behaviour of drugs, the association of CsA and MMF requires higher MMF doses [21]. However, CsA withdrawal has been more associated with AR than MMF withdrawal [22]. Ideal doses of immunosuppressants associated with TLI were unknown. As at the time of the study we used triple drug regimen (CsA, MMF and PRED) with 30–40% FSGS recurrence, we decided to keep the regimen and associated TLI. We wanted to compare results with the experience of our group. The association resulted in high and significant immunosuppression during TLI. Toxicity was high and tolerance to regimen was low, so the doses of drugs were reduced or even suspended, especially MMF. Despite evident immunosuppression, the regimen was not sufficient to prevent recurrence. Some other aspects might have influenced our results; the high rate of cytomegalovirus infections (and consequent rejections) could have presented antigens to the memory T cells and reversed the amnesia state induced by our protocol. Maintenance of native and sick kidneys in our patients could also be responsible for activating memory T cells. Fujisawa *et al.* [23] observed more memory T cells in patients with recurrence of FSGS than in stable patients.

Our protocol induced a significant decrease in B cells and mutagen-response to PWM, but it was not sustained for a long time after TX. Pescovitz *et al.* [8] described that using an anti-C20 monoclonal antibody induced long-term remission in a patient with nephrotic syndrome, suggesting that activated B cells may play a pivotal role in FSGS. This fact could make our protocol more susceptible to recurrence, for recovery of B cells occurred few months after TLI. However, in most patients, recurrence occurred a few days after transplant with an evidently low rate of B cells and using immunosuppression after TLI, which suggests that immunological factors may not be the main 'trigger' of recurrence.

Concluding, treatment of idiopathic FSGS remains elusive. More contributions to the knowledge of FSGS physiopathology and recurrence are necessary to provide a rationale for new therapeutic approaches.

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Rosangela C. Villar,[1] Pedro R. Chocair,[2] Wladimir Nadalin,[1] Luiz E. Ianhez,[2] Carmen Tzanno-Martins,[3] Alberto J. S. Duarte[3] and Dewton M. Vasconcelos[3]

1 Division of Radiotherapy, Institute of Radiology, 2 Division of Urology, Renal Transplant Unit, 3 Laboratory of Medical Investigation on Immunology, Hospital das Clinicas, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil

E-mail: villardias@uol.com.br and rcvillar@hcnet.usp.br

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