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Weaning of Immunosuppression in Long-Term Recipients of Living Related Renal Transplants: A Preliminary Study

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ALTHOUGH renal transplantation continues to be associated with increasingly good short- and long-term graft survival, there remains a need for continued chronic immunosuppression and this can lead to significant complications.[1-3] This has prompted the evaluation of withdrawal or modification of immunosuppression by several investigators with varying results.[4,5] Emerging data proposing dendritic cell migration and repopulation with chimerism following solid organ transplantation as the basis for graft acceptance have further encouraged evaluation of titrated withdrawal of immunosuppression in select groups of patients.[6-8] The finding that donor-specific nonreactivity and chimerism occur simultaneously in long-term living related kidney recipients[9] led us to evaluate withdrawal of immunosuppression in these patients.

MATERIALS AND METHODS

Eight long-term recipients of continuously functioning living related renal allografts were weaned from immunosuppression. Three patients were noncompliant and had self-weaned their medications many years previously. Patient demographic data are listed in Table 1. An additional five patients were deliberately weaned from their immunosuppression according to the following criteria: the patients were recipients of living related kidneys, more than 5 years posttransplant, with at least 2 years of stable graft function. Baseline renal biopsy without evidence of rejection was required, as was a compliant medical history and complications related to immunosuppression.

Immunologic reactivity was assessed when available by in vitro mixed lymphocyte reaction (MLR) and cell-mediated lymphotoxicity (CML). Unidirectional human MLR cultures used 5×10^4 responder and 5×10^4 irradiated stimulator (donor and third party) cells in tissue culture media supplemented with human serum. The degree of labeled thymidine incorporation during the final 20 hours of incubation determined proliferation.

CML was assessed between recipient lymphocytes and donor and third-party targets by using effector lymphocytes incubated with Cr-labeled target cells at various effector:target ratios.

Weaning proceeded in the following manner. After a baseline renal biopsy was obtained, prednisone was weaned to less than 5 mg/d. The ACTH stimulation test was used to assess for intact adrenocortical function, as these patients had been on steroids for many years.[10,11] Patients with an appropriate incremental increase in cortisol levels following stimulation with ACTH were weaned off prednisone completely.[12] The remaining immunosuppressant (azathioprine in all cases except case no. 8) was sequentially reduced by 50% at monthly intervals. Patient weight, urine output, blood urea nitrogen, and serum creatinine were followed on a monthly basis.

RESULTS

Baseline immunosuppression, donor relationship, serum creatinine before and after withdrawal of immunosuppression, episodes of rejection, and time off immunosuppression are listed in Table 2.

Renal biopsies obtained on patients 4 to 8 had minimal background infiltrate with no evidence of rejection. One patient developed mild acute cellular rejection diagnosed on biopsy at 5 months postweaning and was treated with pulse steroids and a return to baseline therapy. His creatinine immediately returned to normal. One patient is still in the process of being weaned; to date, her immunosuppression has been decreased by 75% without any untoward effect. Current time off immunosuppression for the remaining six patients ranges from 3 months to 29 years (mean = 12.4 years). One patient is on maintenance steroids (5 mg/d prednisone) because of adrenal insufficiency.

Donor-specific reactivity by MLR in patients 3, 4, and 5 was unresponsive when initially evaluated for weaning of

Table 1. Patient Demographic Data

Patient No.	Age	Sex	Dx	Date of LRRT	Complications Related to Immunosuppression
1. KP	52	M	Chronic glomerulonephritis (GN)	2/9/63	—
2. SM	45	F	Chronic GN	2/17/64	—
3. JN	62	M	Chronic GN	1/15/64	—
4. JW	49	M	Chronic GN	7/10/64	SCCa/DJD hip
5. DS	45	F	Pyelonephritis	7/19/62	Verrucous warts
6. LN	52	M	Chronic GN	7/29/66	Obesity, hypertension, verrucous warts
7. SS	37	F	Pyelonephritis	5/10/78	Hypertension, verrucous warts
8. JK	50	M	Renal agenesis	2/25/87	Basal cell carcinoma, verrucous warts

*SCCa/DJD hip, squamous cell cancer/degenerative joint disease of hip.

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Table 2. Effect of Weaning Immunosuppression on Renal Function

Patient No.	Baseline Immunosuppression (mg/d)	Creat. Pre/Post	Rejection	Time Off Drug (y)
1	Azathioprine (AZA)	0.7/0.9	No	29
2	Pred 15 AZA 25	1.0/1.1	No	14[a]
3	AZA	0.8/0.9	No	28
4	AZA 125/pred 10	1.6/1.	No	2
5	AZA 75	0.8/0.7	No	1
6	AZA 100/pred 10	0.9/0.9	No	Still weaning; doses decreased by 75%
7	AZA 75/pred 10	1.4/1.0	No	3 months
8	AZA 5 7.5 pred <CyA 50	1.0/3.4—1.2	Yes	5 months (then restarted)

[a]Maintenance steroids 5/d prednisone (pred) for adrenal cortical insufficiency.

immunosuppression. Repeat MLR in patients 4 and 5 now show a significant response (Fig 1). These patients have remained off immunosuppression for 1 and 2 years, respectively, and have normal serum creatinines. The MLR of patient 3 would be expected to remain unresponsive, as he is a double haplotype match. CML activity remains absent in patients 3, 4, and 5 (Table 3).

Skin lesions were a prominent complication in these

EFFECT OF WEANING IMMUNOSUPPRESSION ON DONOR-SPECIFIC MLR

WITHDRAWAL OF IMMUNOSUPPRESSION

Fig 1. Effect of weaning immunosuppression on donor-specific MLR. T. P. = third party cells.

long-term recipients. One patient had metastatic squamous cell cancer. Four others had disfiguring verrucous warts. Patients 4 to 7 have had dramatic improvement of their verrucous lesions. Patient 4 had successful surgical therapy for his metastatic squamous cell lesion.

DISCUSSION

Long-term survival following renal transplantation has been previously reported.[13] Multiple drug immunosuppressive therapy based on steroids and azathioprine was found to prevent or treat renal allograft rejection in the early clinical experience.[14,15] The addition of cyclosporine (CyA) to the immunosuppressive regimen significantly improved graft survival.[16]

The risks of chronic immunosuppression have been well described,[17] and include opportunistic infection and neoplasia.[18,19] Less life-threatening but equally disabling sequelae of immunosuppression such as steroid-related weight gain, edema, growth retardation, and osteopenia have also had significant morbidity.[20] CyA-related nephrotoxicity and hypertension have also been described extensively.[17]

The benefits of withdrawal of immunosuppression[21,22] must be weighed against the dangers of graft rejection and loss. Our data suggest that weaning of immunosuppression can be carried out in a select group of patients. Only one patient has demonstrated rejection during weaning of immunosuppression. Absolute or relative donor-specific unresponsiveness with MLR prior to weaning was shown in three patients available for study who maintained normal renal function after weaning. Patients 4 and 5 have had return of antidonor reactivity on MLR but not CML; clinically, they have had no evidence of rejection. The recovery of MLR in the absence of cytotoxic effector cells is under investigation.

The study remains preliminary and the enrolled patient number is small. It is known that serum creatinine is not completely accurate as an assessment of renal function. Studies using creatinine clearance or glomerular filtration will be more conclusive. We continue to follow up these
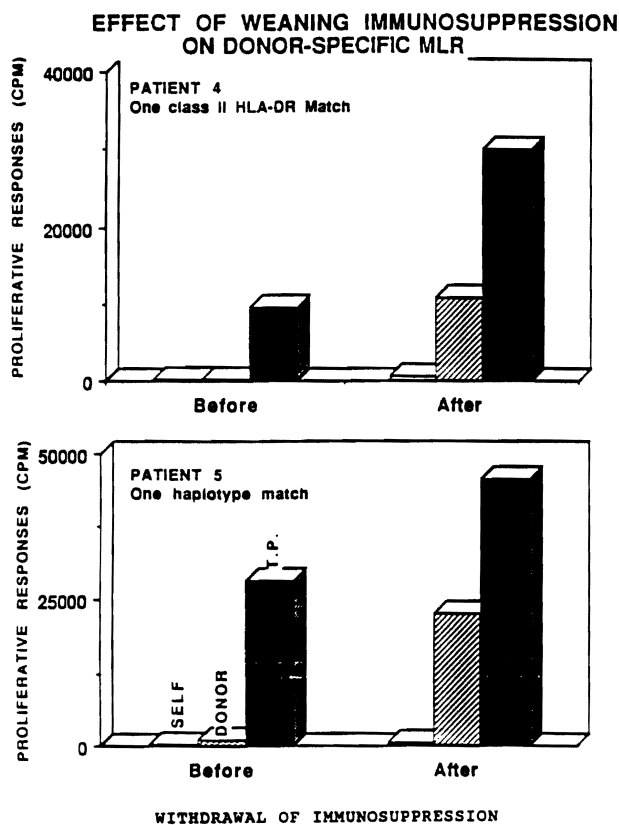
Table 3. MLR and CML Data

Patient No.	Donor	Haplotype Match	MLR	CML
1	Fraternal twin	Double	Not available (N/A)	N/A
2	Father	Single	N/A	N/A
3	Sister	Double	Unresponsive	Nonresponsive
4	Aunt	No haplotype; one class II match	Unresponsive → responsive	Nonresponsive
5	Mother	Single	Unresponsive → responsive	Nonresponsive
6	Brother	Single	N/A	N/A
7	Brother	Single	N/A	N/A
8	Sister	Single	N/A	N/A

patients as well as consider additional suitable patients for withdrawal of immunosuppression.

CONCLUSION

How to safely wean immunosuppression in kidney recipients is still unknown. The kidney is known to be susceptible to rapid rejection from preformed antibodies, whereas the liver, for example, has been said to be tolerogenic.[23] We have postulated that the critical difference may be the leukocyte component of the organ, thus explaining why the leukocyte-poor kidney is less likely to be tolerogenic. Thus, weaning is expected to be more dangerous for kidney than for liver recipients and should not be recommended except for disabling or life-threatening indications. Therapies to augment cell migration with infusion of donor bone marrow at the time of solid organ transplantation are a strategy to balance cell traffic in the direction of graft acceptance,[24] and these are expected to increase the eventual possibility of achieving a drug-free state. CyA may be safely withdrawn from recipients of HLA identical living related grafts after a year of stable graft function,[5] yet others report lack of predictive immunologic parameters in this same "good risk" category of patients.[25] Our data suggest that some recipients of living related HLA nonidentical grafts may be able to discontinue immunosuppression. The clinical "tolerance" exhibited here may be explained by the leukocyte microchimerism previously demonstrated. An attempt at complete weaning may be indicated in long-term survivors of living related kidney grafts, especially in those with significant complications, but close monitoring of renal function is required and long-term results are unknown.

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