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RENAL TRANSPLANT PATIENTS WITH STEROID WITHDRAWAL EVALUATED LONGITUDINALLY FOR THEIR DONOR-SPECIFIC CYTOTOXIC T CELL REACTIVITY[1]

Lymphocytes from renal allograft patients can display specific cell-mediated lympholysis nonreactivity (CML-NR) in vitro toward the splenocytes of the specific kidney donor. Recently we evaluated whether the CML status had a predictive value regarding graft prognosis. By assessing the correlation of the outcome of donor-specific CML reactivity (CML-R) and graft survival for different intervals, it appeared that only the posttransplant period between 2 weeks and 6 months showed a borderline significant correlation (P=0.05) between CML-NR and graft survival on one hand and CML-R and graft loss on the other hand (*1*).

It is recognized that long-term steroid immune suppression can produce serious side effects (*2–4*). It is therefore desirable to attempt to withdraw the steroid treatment in renal allograft patients with well-functioning grafts. More than two decades ago, the first studies on successful withdrawal of steroids were reported (*5–8*). Since then a large number of clinical trials, including our own (*9*), have reported on gradual reduction and complete withdrawal of steroids in related and unrelated renal transplant recipients.

Our clinical results are described elsewhere (*9*) and can be summarized as follows. In 36 of 102 patients a complete withdrawal of steroids was achieved. Twelve of them remained without steroids for a mean period of 59.6 months, ranging from 4 to 97 months (end of the study) and were, except for one, still off steroids 29 months later. In 24 patients steroids had to be reinstituted after 1–48 months. The mean number of incompatible donor HLA antigens was lowest in those 12 recipients with successful withdrawal of steroids, intermediate in those 24 with transient withdrawal, and highest in the remaining 66 patients in whom a complete withdrawal never could be obtained.

The objective of this study was to investigate whether the patient's CML status during steroid dosage tapering could guide the therapeutic strategy. The in vitro tests were carried out retrospectively without prior information concerning clinical status of the patient and steroid dosage. The donor-specific CML reactivity was tested prior to and during steroid dosage reduction in 43 recipients of cadaveric renal transplants. The CML follow-up time ranged from 49 to 1611 days, with a mean

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of 253 days. Serial samples of recipients' PBLs were collected at several intervals. All blood samples of a given patient were tested on the same day in the same experiment.

Donor lymphocytes were obtained from the spleen. All patients' blood samples, the donor spleen cells, and the control cells were frozen and stored in liquid nitrogen until used. The PBLs (i.e., 10^6 responder cells) were sensitized in vitro for 6 days against 10^6 irradiated splenocytes from the specific kidney donor (i.e., specific antidonor reactivity) as well as against 10^6 control cells from healthy unrelated individuals (i.e., control responder capacity of patients' lymphocytes). Irrelevant PBLs were used as responder cells to control the stimulator capacity of the donor splenocytes. Depending on the quantity of lymphocytes available, which was limited in most of the cases, either tissue-culture flasks or 2-ml cluster wells were used; the ratio of responder to stimulator cells, however, was identical under both culture conditions. After the culture period, the effector cells were harvested and tested in the standard CML assay against their specific stimulator cells (i.e., splenocytes of the specific kidney donor and control cells of healthy unrelated individuals) as target cells.

The terms CML-NR and CML-R are used to describe the CML-nonresponsiveness and CML-responsiveness, respectively, exhibited by the recipients' PBLs against the specific kidney donor splenocytes. Patients either remained CML-NR→NR or developed CML-NR (i.e., CML-R→NR), or were not NR at all—i.e., remained donor-reactive (CML-R→CML-R) during the whole CML follow-up period. Changes in CML reactivity over time are not observed in healthy individuals (*10*). Almost all the recipients showed a normal cytolytic response to HLA-incompatible control cells. The few cases in which the response to the control cells remained low in repeated experiments were excluded from the analyses. The CML assay has been described in detail (*1*). The percentages of lysis were determined using phytohemagglutinin-stimulated blast cells in a 4-hr ^{51}Cr assay. Cytotoxicity (i.e., the amount of isotope released from ^{51}Cr-labeled target cells) was determined and calculated according to a method described elsewhere (*1*). Standard errors of the mean of triplicate determinations were less than 5%. Positive and negative assignments were made on the basis of a 10% specific ^{51}Cr release value and on a positive slope—i.e., the various effector-to-target cell ratios are plotted and must give an S-shaped curve (or, in the case of transforming the percentage of lysis to a log scale, a straight line). All experiments were repeated at least twice at different effector:target cell ratios.

Patients transplanted before 1983 (n=18) received 100–200 mg/day azathioprine in addition to 15–25 mg prednisolone; whereas 25 patients transplanted since 1983 were maintained on 3–6 mg/kg/day cyclosporine. Steroid dosage reduction was performed gradually as described previously (*9*); reduction not exceeding the previous value of serum creatinine by more than 0.2 mg%, was considered as successful. The last patient entered this study in July 1987. The last CML determinations were performed in February 1988, and the period of observation ended September 1989.

Table 1 shows three representative experiments of patients who either developed or remained CML-NR or remained CML-R posttransplant. In two patients (one CML-NR, one CML-R), successful steroid withdrawal was obtained. In the third stable CML-NR patient only partial steroid reduction was achieved.

Table 2 gives the results of 57 and 74 CML assays carried out with lymphocytes from 18 patients on AZA and 25 patients on CsA, respectively, in whom steroid reduction was attempted.

For the longitudinal CML studies, either stable CML-NR or CML-R, or developing CML-NR, during the CML follow-up period are indicated. From Table 2 can be seen that successful complete steroid withdrawal was achieved in 11 (i.e., in 5 of the AZA- and in 6 of the CsA-treated patients) of 43 patients studied during the CML determination period. In the latter 11 patients CML-NR was observed in 3 of 5 AZA- and in 5 of 6 CsA-treated patients. All 8 CML-NR patients have a functioning allograft. In 9 AZA and in 12 CsA patients the CML-NR already established before steroid dosage tapering remained stable during steroid reduction. Moreover, tapering of steroids

TABLE 1. Longitudinal analysis of kidney donor-specific CTL activity during steroid withdrawal

Patients	Follow-up time (days)	Steroid dosage[a]	% Specific Lysis[d]		
			kd[b]	Control[c]	Splenocytes[e]
CML:R → NR (successful steroid withdrawal)	356	20	+27	+92	+81
	475	15	+37	+70	
	1125	5	+3	+66	
	2022	0	0	+70	
CML:R → R (successful steroid withdrawal)	50	20	+49	+22	+79
	168	15	+29	+50	
	196	10	+13	+89	
	398	15	+52	+93	
	591	7.5	+24	+77	
	1321	0			
CML:NR → NR (partial steroid withdrawal)	653	25	0	+56	+43
	883	15	0	+51	
	1037	10	0	+55	
	1154	7.5	0	+64	

[a] Prednisolone dosage (mg/day).
[b] % antidonor lysis.
[c] % anticontrol cell lysis.
[d] % lysis at an effector:target ratio of 50:1.
[e] % irrelevant responder cells stimulated with and tested against kidney donor splenocytes.

TABLE 2. CML status during steroid reduction

CML status during follow-up	Steroid withdrawal		
	Unsuccessful	Partial	Complete
Steroid reduction in AZA-treated renal allograft patients (n = 18):			
CML-NR→NR[a]	2 (2)[b]	6 (2)	3 (3)
CML-R→NR		4 (1)	
CML-R→R	1 (1)		2 (2)
Steroid reduction in CsA-treated renal allograft patients (n = 25):			
CML-NR→NR		8 (6)	4 (4)
CML-R→NR		3 (2)	1 (1)
CML-R→R		8 (6)	1 (1)

[a] Patients either remained or developed CML-NR or remained CML-R during the CML follow-up period.

[b] The number of patients with renal graft function at the end of the observation period is given in parentheses.

seemed not to be an obstacle to development of CML-NR in 4 AZA and 4 CsA patients. In one of the latter patients total steroid withdrawal was accomplished.

It should be noted, however, that the successful steroid withdrawal is not a phenomenon that is unique to CML-NR patients only. The CML-R status was maintained in 2 AZA patients and in 1 CsA patient with functioning allografts in whom complete withdrawal was achieved. Moreover, CML-NR is not a guarantee of successful steroid withdrawal—complete steroid withdrawal could be obtained only in a small fraction of patients with the CML-NR status. Previously, two studies on steroid withdrawal in CsA-treated recipients of living-related donor allografts reported on in vitro measurements of acquired graft tolerance by means of the mixed lymphocyte culture reactivity (*11*, *12*). In another report (*8*) three patients who underwent total-lymphoid irradiation prior to cadaveric renal allografting, were described as showing specific unresponsiveness to donor cells after complete withdrawal of immunosuppressive drugs. In all three studies, MLC hyporesponsiveness was used as the selection criterion for entrance into the steroid withdrawal protocol. Similar to what we describe here, Strober et al. (*8*) demonstrated long-term maintenance of not only donor-specific CML-NR but also MLC unresponsiveness. The patients involved in our study were not subjected to the steroid withdrawal trial on the basis of the donor-specific CML-NR but based on serum creatinine levels. The CML assays have been carried out retrospectively in a double-blind fashion.

In conclusion, we report here on the occurrence of CML-NR not only in patients treated with AZA as described earlier (*1*) but also in patients who received CsA as an immunosuppressive agent. More important, however, we observed that in 29 of 40 AZA- or CsA-treated patients in whom either partial or entire steroid withdrawal was achieved, donor-specific CML-NR was maintained or developed in the course of steroid tapering. Thus, donor-specific CML-NR can be demonstrated using lymphocytes of recipients of unrelated kidney allografts who received AZA or CsA as immunosuppressive therapy with low dosages or no steroids at all. Nonetheless, successful complete steroid reduction appears only to coincide with CML-NR, since partial (n=8) and complete (n=3) steroid withdrawal was achieved in CML-R patients as well. It must be stressed that CML responder status beyond 6 months posttransplantation is neither significantly associated with good renal allograft function (*1*) nor a contraindication for steroid withdrawal.

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