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CELL-MEDIATED LYMPHOLYSIS STUDIES IN RENAL ALLOGRAFT RECIPIENTS[1]

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SUMMARY

Cell-mediated lympholysis (CML) reactivity against the splenocytes of the kidney donor might be a good in vitro correlate of the homograft reaction. The present study was performed in an attempt to determine whether CML nonreactivity between unrelated donor-recipient combinations occurs and, if so, under what conditions.

We were able to show that CML nonreactivity occurs between unrelated donor-recipient combinations in 70% of the nonrejecting patients, whereas all of the rejecting patients were CML reactive. Patients with CML nonreactivity did clinically well more frequently than those that were CML reactive.

The question as to whether or not such variables as HLA-A, B, and DR match and sex, the number of pretransplant blood transfusions, and the degree of presensitization, etc. predispose to the development of donor-specific CML nonreactivity was studied as well. Sex and compatibility for HLA-B antigens between donor and recipient might be such factors.

HLA matching is able to improve graft survival in related donor-recipient combinations, but it is less effective for transplants in which donor and recipient are unrelated. Furthermore, both in related and unrelated donor-recipient combinations 30% or more of the renal transplants have good graft survival after 5 years, although they are mismatched for HLA-A and B. It is unclear why these major histocompatibility complex (MHC) nonidentical grafts are not rejected. Findings by others and our group suggest that survival of the graft even if MHC mismatches exist often coincides with donor-specific CML nonreactivity (*1–6*). Logically, one can postulate that the occurrence of CML donor-specific nonreactivity and good survival of MHC-mismatched grafts could be causally related. The present study attempts to document this and to delineate those factors which lead to donor-specific CML nonreactivity.

We will report on studies in which 20 unrelated donor-recipient combinations were investigated longitudinally (at various times before and after blood transfusion and renal allografting) and 45 who were studied only after transplantation.

Our results indicate that the development of CML donor-specific nonreactivity in the two groups of patients indeed correlates well with the good function of the graft. The occurrence of CML nonreactivity seems to be influenced by matching for HLA-B and to occur most frequently in male to male transplants. The results of mixed lympocyte cultures (MLCs) will be presented as well.

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MATERIALS AND METHODS

Sixty-five donor-recipient combinations were investigated for their cytolytic potential (with the CML assay) and proliferative response (on the basis of the mixed lymphocyte reactions). Twenty patients that were studied prospectively received only one planned random blood transfusion before kidney transplantation; 19 patients were transfused with 1 unit of washed (i.e., leucocyte-poor) erythrocytes and 1 patient received 1 unit of buffy coat-free blood. The remaining 45 patients received more than one blood transfusion and were studied retrospectively. Nine of the 65 patients rejected their grafts, one patient died during transplantation. In three cases graft failure was attributable to nonimmunological or technical reasons. Three patients were studied after they had been given a second graft. All patients received immunosuppressive therapy consisting of azathioprine and prednisone. No antilymphocyte serum or antithymocyte globulin was used.

Lymphocyte preparation. Blood was collected from the recipients in preservative-free heparin. For the longitudinal studies, serial samples of peripheral blood lymphocytes were collected from the recipients at several intervals, as follows: (1) immediately prior to blood transfusion, (2) at different times after blood transfusion, (3) on the day of transplantation, (4) at different intervals after transplantation, and (5) in the event of rejection, after transplantectomy. The lymphocytes were separated by Ficoll-Isopaque gradient centrifugation. Donor lymphocytes were obtained from the spleen without density centrifugation. All blood samples and the spleen cells of the specific kidney donors were frozen and stored in liquid nitrogen until tested.

MLC and CML techniques. Standard MLC and CML techniques were used in which the peripheral blood lymphocytes of the recipients were sensitized in vitro against the irradiated splenocytes of the specific kidney donor and against HLA-A, B-, C-, and DR-incompatible control cells of unrelated healthy subjects. MLC tests were performed with a microtechnique (*7*). The CML assay has been described previously in detail (*8*). Briefly, the effector cells (i.e., patient antidonor, patient anticontrol, control antidonor) were cultured for 6 days in tissue culture flasks.

The percentages of donor-specific lysis and control cell lysis were determined in relation to phytohemagglutinin-stimulated blast cells, in a 4-hr ^{51}Cr assay. The percentage of lysis was calculated with the following formula:

$$\frac{\text{experimental mean cpm} - \text{spontaneous release mean cpm}}{\text{maximum release mean cpm} - \text{spontaneous release mean cpm}} \times 100$$

The results are expressed on a scale in which the spontaneous release value was set to 0% and the maximum release value to

100% (9) Standard errors of the means of triplicates were always less than 5% Percentages equal to or below 10% were considered to be negative when only one effector to target ratio (50 1) was used Since cell numbers in several experiments were very limited, positive and negative assignments were made on the basis of a 10% specific Cr release value This criterion is among others also the assignment of the European CML study group who aimed to standardize the CML technique (A comprehensive guide to the European CML workshops, report from the European CML study group, edited by Tom Kristensen, to be published) In order to limit within experimental variation, all combinations with a given patient were tested on the same day in the same experiment

Besides the specific antidonor cytotoxicity, the cytolytic capacity of the recipient cells after priming in vitro with fresh or frozen unrelated HLA incompatible control cells were tested against these cells Furthermore, the lymphocytes of donors selected at random were used to test the stimulatory capacity of the splenocytes of the kidney donor in order to determine whether or not the CML nonreactivity could have been because of a defect in the stimulatory capacity of the kidney donor splenocytes

The culture medium for both the MLC and CML assays was RPMI 1640 supplemented with 20 mM L-glutamine, 100 IU of penicillin per ml 100 μg of streptomycin per ml, and 20% heat inactivated pooled human AB serum from male donors

Detection of suppressor cells The protocol used to study a suppressive effect of the recipient lymphocytes was as follows Recipient lymphocytes (which showed donor CML nonreactivity after grafting) were mixed in a 1 1 ratio with lymphocytes obtained at an earlier date from the same recipient (a pretransplant sample) Thereafter, they were cultured with irradiated splenocytes of the specific kidney donor and tested on the specific donor target cells according to the above described protocol

Detection of HLA A, B C, and DR antigens and screening for HLA antibodies Typing for HLA-A, B, and C was performed with the standard lymphocytotoxicity technique (10) Typing for the HLA-DR and the MB1, MB2, and MB3 antigens were performed with the two-color fluorescence test (11) Screening for HLA-A, B, and C antibodies was performed using the standard lymphocytotoxicity technique

Significance testing The χ^2 test with Yates' correction was used throughout the study

RESULTS

Table 1 lists the variables sex of recipient and donor, ABO blood group, rhesus blood group, and HLA-A, B, C, and DR types of the 20 patients studied who had received one single planned blood transfusion prior to transplantation If only one HLA DR antigen was recognized, it was assumed that the patient and/or donor were homozygous for that antigen This was considered permissible because the sum of the gene frequencies of HLA-DR1 to w8 is close to 1 0 in The Netherlands (van Rood et al submitted for publication) Only patient 17 had formed leukocyte antibodies The antidonor MLC reactivity (expressed in cpm $\times 10^{-1}$) and the percentage of lysis in CML against the cells of the kidney donor of each individual patient at different intervals before and after blood transfusion and after transplantation are listed Ten of the 15 patients with functioning grafts with a follow up time of at least 60 days after transplantation became negative in the CML test against the splenocytes of the kidney donor, although they were positive in this test with the lymphocytes from random donors In eight patients with a negative CML test against specific donor splenocytes 60 days or more post transplantation, earlier samples were positive with the same donor cells, thus suggesting the induction of donor-specific CML nonreactivity Three of the patients with functioning grafts still showed a positive CML test in their most recent samples

A pretransfusion blood sample was available for 19 of the 20 patients and the CML test results obtained with those samples were compared with the results obtained with one or two blood samples collected between blood transfusion and transplantation In some instances, the CML reactivity showed a tendency to increase 14 days after blood transfusion, followed by a decrease in CML reactivity 3 weeks after the transfusion A difference of 5% or less, which was the level of standard error of triplicates, was considered to be not significant This transient rise in CML is in agreement with earlier more extensive kinetic studies reported by Charmot et al (12)

The antidonor MLC activity measured as a proliferative response by ^{3}H-thymidine incorporation is also shown in Table 1 Some patients displayed a decrease, others an increase, or only a slight change in MLC activity before and after the blood transfusion In about one-third of the donor recipient combinations, a decrease of MLC reactivity was observed 14 days after blood transfusion Arbitrarily, only a decrease of 30% or more was considered to be significant

Furthermore, in the longitudinal studies, we observed that if after transplantation the antidonor MLC reactivity diminished, it diminished also against donors selected at random This is in contrast to the antidonor CML reactivity after transplantation which seemed to be kidney donor specific An example of this phenomenon is shown in Figure 1, where it can be seen that, while the anticontrol cell CML reactivity remained positive, the antidonor CML reactivity after transplantation is low This is in contrast to the MLC test which became low against both

Table 2 summarizes the relevant CML data and variables for 45 patients given two or more blood transfusions before transplantation Forty of 45 patients had functioning grafts for more than 1 year, 5 patients rejected their grafts CML results obtained with blood samples taken before transplantation were available for only a few patients and are not shown In several instances two blood samples were tested at different times after transplantation Donor specific CML nonreactivity was observed in 28 of the 45 patients, in 9 of them 2 to 12 months post-transplantation and in the remaining 19 more than 1 year after transplantation In the 12 nonrejecting patients who remained positive in the antidonor CML test, the percentage of kill ranged from 14 to 78

The question as to whether the fraction of patients which become CML nonreactive increases after transplantation (2, 13) cannot yet be answered In the group given only one blood transfusion, 11 of 15 functioning grafts became nonresponsive within 12 months post-transplantation and in the multitransfused group 19 of 28 were nonresponsive within the same period The duration of the period in which a patient develops cellular nonreactivity post transplantation can differ between individuals

Table 3 shows that the occurrence of CML nonreactivity did not significantly correlate with the difference between one and more than one pretransplant blood transfusions

CML reactivity, when it occurs, with some exceptions, rarely amounts to more than 35% after the first few weeks post-transplantation We observed nine cases in which it was substantially higher In five cases the high values coincided with a

TABLE 1 Longitudinal MLC and CML study of 20 singly transfused recipients

Patient		F/NF	Sex recipient/ donor	ABO (Rh)	HLA A	HLA B	HLA C	HLA w4 w6	HLA DR	SD≠	DR≠
1	B	F	M/M	A+	9 28	12 —	Cw3	w4	8	18 w32	2 LB58
2	K	F	M/M	A+	1 2	5 8		w4 w6	2 + 3	37	4
3	A	F	M/F	A+	2 11	15 40	Cw3	w6	1 + 2	7	4 5
4	M	F	M/F	A+	2 11	7 17			3	15	4 5
5	S	F	M/F	A+	1 10	8 41		w6	LB58 + 6	9	4 5
6	J	F	M/F	O+	1 11	7 21			2	2 3	6 7
7	E	F	M/M	A+	1 w32	8 40	Cw2	w6	3	—	—
8	H	F	F/F	A−	1 w19	17 37	Cw3	w4	1	3 w30	5
9	P	F	M/M	AB−	2 10	7 12	Cw4	w4 w6	2	w23	—
10	P	F	M/M	O+	2 3	12 15	Cw3	w6	4	9 28	3 6
11	B	F	M/M	O+	2 9	17 35		w4 w6	6 + 7	11	
12	S	F	F/F	A+	2	12 w41		w4 w6	3 + 5	37	4 8
13	M	F	M/F	O+	1 9	5 27	Cw2	w4 w6	2 +	18 w30	3
14	S	F	M/F	A+	11 —	7 40	Cw2		LB58 + 2	3 22	5
15	N	F	M/F	AB+	2 3	12 w41			1 + 7	18 w30	3 4
16	S	†	M/M	A+	3 9	35 —	Cw4	w6	?	1 17 40	
17	v D	N F	1 /M	A+	11 w30	13 w22	Cw3 Cw6	w4 w6	1 + 7	—	6
18	v P	N F	M/M	AB+	2 11	17 w22	Cw1	w4 w6	2 + 7	28 18	6
19	J	N F	M/F	A+	11 28	35 40	Cw3 Cw4	w4 w6	1 + 6	3	—
20	v A	N F	M/M	A−	2 11	40 w51			5	3	4 6

The HLA DR typing of the lymphocytes of patients 11 and 16 was difficult to interpret Patient 10 died on day 86 (pneumonia) patient 15 died on day 18 (myocardial infarct) both with good kidney function Patient 16 died during transplantation and was therefore excluded from the statistical analysis Patients 17 and 18 rejected their grafts because of uncontrollable allograft rejection The grafts of patients 19 and 20 were lost because of nonimmunological causes Patient 19 received one buffy coat free blood transfusion Patient 8 was treated for a reversible rejection crisis on day 11 Patient 13 was treated for reversible rejection crises on days 12 and 37 Patient 14 was treated for reversible rejection crises on day 3 11 19 29 and 42 Antibodies were negative in all patients pretransplant samples except those from patient 17 Only patients 8 and 17 had been pregnant (three pregnancies each)

† functioning graft NF nonfunctioning graft

SD≠ DR≠ indicates the SD and DR antigens which were mismatched between donor and recipient

reversible rejection crisis An example is shown in Figure 1 (Table 2 patient 20)

Among the patients who rejected their grafts (a total of nine for the two groups) five showed extremely high levels of lysis in the CML assay (≈80%) after transplantectomy against the spleen cells of their donor These high levels of cytotoxic effector cells persisted for more than 450 days after transplantectomy

Since 70% of our nonrejecting patients demonstrated CML nonreactivity the frequency of CML nonreactivity in a non transplanted control group of 31 responder and target cells with similar HLA mismatches was examined In this group only 10% had a negative CML test (data not shown) which is significantly lower than in our patient material but is about the same as the occurrence of CML nonreactivity obtained with pretransfusion blood samples (Table 1 10%) In the combinations in which the recipients antidonor lysis were negative we never observed positive lysis of donor cells after in vitro sensitization of the recipient lymphocytes with the lymphocytes of third party cells Furthermore stimulation of recipient lymphocytes toward a pool of stimulator cells obtained from five unrelated donors (pool stimulation) with subsequent testing on the specific kidney donor splenocytes as target cells induced no cytolytic activity of the recipient lymphocytes against the specific donor cells CML results of the pool stimulation of four patients are shown in Table 4

It has been suggested that donor specific CML nonreactivity is attributable to the presence of suppressor cells (*1 4*) However we could demonstrate suppressor cell activity in only one (Table 5 patient 1) of seven patients studied A decrease of specific antidonor lysis from 54 to 21% in the mixture with the pretransplant sample was observed

In order to determine what factors could be predictive of donor specific CML nonreactivity the CML results from the patients were compared with all of the variables shown in Tables 1 and 2 Table 6 gives the significant and/or informative

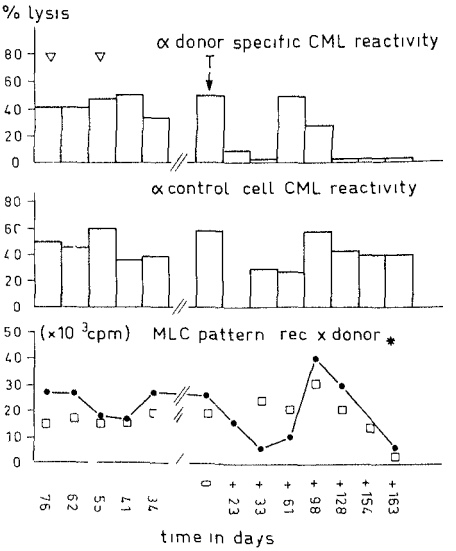

FIGURE 1 Longitudinal CML and MLC study patterns of cytotoxic and proliferative responses against donor and control cells The patient received two HLA A and B compatible blood transfusions prior to transplantation He was treated for two reversible rejection crises (on days 7 and 58 post transplantation) and was released from the hospital with good graft function on day 83 post transplantation ● proliferative response of patient s lymphocytes versus irradiated kidney donor splenocytes □ same against irradiated lymphocytes from a donor selected at random

Antidonor MLC reactivity (cpm × 10) and % antidonor lysis in CML									
Between transfusion and transplantation			0	Days after transplantation					Anticontrol cell lysis
				+1 → 15	+16 → 30	+31 → 60	+61 → 120	+121 → 360	≥360	
27/39 (86)	31/54 (72)	21/24 (65)		28/42	23/14			10/1		(+46)
24/16 (36)	33/8 (22)	NT/NT	34/2					14/0		(+2)
NT/20 (21)	7/30 (7)	NT/NT	3/20					1/−2		(+46)
9/14 (21)	NT/NT	NT/NT			7/31				9/3	(+42)
16/26 (128)	9/27 (114)	11/22 (107)	6/17	10/15	7/12				10/7	(+23)
45/11 (464)	18/74 (450)	24/39 (443)	24/12				9/2			(+42)
1/13 (71)	2/21 (63)	2/13 (56)			1/5				2/1	(+66)
15/18 (84)	17/24 (70)	17/17 (63)		4/42	7/10			26/30	30/10	(+64)
19/8 (40)	14/14 (26)	NT/NT	13/4			7/13	6/16	8/5		(+58)
8/12 (46)	13/44 (32)	NT/NT	3/NT		−2		?			(+36)
48/31 (116)	39/69 (102)	NT/NT	39/41		28/33				24/4	(+54)
NT/2* (73)	NT/21 (59)	NT/18 (51)	NT/26					NT/26		(+50)
41/26 (558)	23/22 (544)	26/36 (537)	29/35			14/81	6/30			(+63)
8/26 (274)	7/28 (260)	8/28 (253)	11/34	1/64			23/36	20/25		(+56)
26/40 (38)	18/35 (24)	26/13 (17)	18/11	7/40						(+25)
10/40 (15)	3/14 (21)	3/28 (14)								(+16)
18/1 (135)	34/14 (121)	40/25 (114)		Tx* 2/96	3/80		NT/96	12/88	NT/98	(+22)
32/26 (139)	20/48 (125)	27/58 (118)	37/42	Tx	21/73		12/95	3/84		(+38)
9/23 (100)	7/26 (86)	9/28 (79)		Tx			NT/16	3/36		(+30)
NT/NT	NT/13 (128)	NT/21 (121)	NT/12	NT/4	NT/2		NT/6	Tx NT/13		(+65)

Antidonor MLC reactivity (cpm × 10) and percentage of specific antidonor lysis measured on the day of transplantation
Antidonor MLC reactivity (cpm × 10) and percentage of specific antidonor lysis measured prior to the planned blood transfusion Numbers in parentheses days before transplantation
NT not tested
* Tx transplantectomy

results A statistical analysis of all variables which could influence the occurrence of CML nonreactivity was performed for 55 nonrejecting patients Nine cases of rejection and one patient who died during transplantation were excluded from the analysis The results indicate clearly that CML nonreactivity is indeed influenced by several factors The best correlation with CML nonreactivity is good kidney function as judged by laboratory tests (creatinine clearance, etc) and clinical judgment Furthermore kidneys from male donors into male recipients do better than all other possible sex combinations Compatibility for HLA B is also significantly associated with CML nonreactivity The presence of HLA DR4 in the recipient shows a trend which is not yet significant for the occurrence of the CML nonreactivity

With regard to the presence of particular HLA antigens in the donor, we found that CML reactivity seems to occur more often in the presence of HLA-DR2 or DR3 antigen on donor lymphocytes

Matching for MB was not significantly correlated with CML nonreactivity (see Table 7)

DISCUSSION

The CML results obtained in our patients can be divided into three groups group 1 with extremely high percentages of lysis in patients who rejected their graft, group 2 with weakly positive CML values (>10 to 30%) often correlated with a poorer function of the grafted kidney, and group 3 comprising the CML nonreactive recipients with the highest number of good functioning grafts (see Table 6)

Specific antidonor CML nonreactivity has been described also by other authors (*1–4, 6*), but most of the reports concern post transplant studies in related donor recipient combinations The phenomenon of CML nonreactivity occurs, as is shown in this study, in unrelated donor recipient pairs as well

In 39 of our 65 transplant patients, we found donor specific CML nonreactivity at various times after transplantation, which in this study was defined as ≤ 10% lysis against the splenocytes of the specific kidney donor Donor specific nonreactivity can occur quite soon after transplantation, in some instances even within 14 days Almost all of the recipients showed a normal cytolytic capacity toward HLA incompatible control cells In only one case the anticontrol cell lysis remained low in repeated experiments (Table 1, patient 2) This might have been because of the influence of immunosuppressive drugs on the MLC and CML activity as postulated by Keown et al (*14*) That is certainly not the cause of the donor specific CML nonreactivity in the other 38 patients However, the possibility cannot be excluded that immunosuppressive drugs influence the proliferative capacity of the patients lymphocytes Furthermore, diminished proliferation cannot explain the inability to develop cytotoxic effector cells We have observed previously (*15*) that, even with a low stimulation index in MLC, strong lysis can occur In the present study, the specific antidonor ^{3}H thymidine uptake in the patients who rejected their grafts showed low proliferation and extremely high cytotoxic activity in some cases Although a drop in MLC reactivity toward the kidney donor splenocytes after blood transfusion was observed in about one-fourth of the patients (Table 1), this decrease does not seem to correlate with the occurrence of donor specific CML nonreactivity

The following factors could explain the occurrence of CML nonreactivity in more than one-half of the grafted recipients Preoperative blood transfusions could be one such factor CML nonreactivity occurs with the same frequency in the both single and multitransfused patients However, a nontransfused control group is lacking, although patient 19 (Table 1) and patients 42 and 45 (Table 2) received only buffy coat-free transfusions Such blood transfusions have been shown to be unable to reduce graft facilitation (*16*) and all three grafts were rejected In addition, suppressor cells could be responsible for the CML nonreactivity However, we have been able to demonstrate the presence of suppressor cells in only one of seven cases studied

TABLE 2 Post transplantation CML study of 45 multitransfused recipients

	Patient	F/NF[b]	Sex recipient/donor	Preg nancy	Abs	BTs[d]	ABO (Rh)	HLA	
								A	B
1	de L	F	F/F	3	+	4	A+	2	12 40
2	J	F	F/F	2	+	2	O+	2 28	5 12
3	K	F	F/M	1	+	≧30	O+	w19 28	12 14
4	de W	F	F/M	3	+	14	A−	10 29	8 —
5	de W	F	F/M	3	+	10	O+	2 w31	12 27
6	K	F	F/M	3	−	5	A+	2 3	7 15
7	B	F	F/F	3	−	5	A+	2 3	7 40
8	v R	F	F/F	2	−	8	B+	1 3	7 15
9	Y	F	F/M	2	−	≧11	A+	2 11	7 w16
10	T	F	F/M	0	−	5	O+	2 3	7 12
11	v T	F	F/M	0	−	4	A+	1 2	12 17
12	D	F	F/M	0	−	≧8	O+	2 9	12 15
13	L	F	M/F	−	+	≧1	B+	2 —	17 —
14	L	F	M/M	−	+	6	O+	2 11	12 w35
15	D	F	M/M	−	+	2	AB+	1 9	27 w35
16	K	F	M/M	−	+	≧1	A+	1 10	8 27
17	R	F	M/M	−	+	>1	O+	2 3	14 27
18	K	F	M/M	−	−	≧1	O+	1 2	8 12
19	v T	F	M/F	−	−	1ɔ	A+	2 w30	13 15
20	R	F	M/M	−	−	3	O−	2 w19	5 12
21	vd R	F	M/M	−	−	≧15	O+	3 11	14 40
22	W	Γ	M/M	−	−	5	A+	2	7 15
23	L	F	M/F	−	−	2	A−	2 28	12 22
24	M	F	M/M	−	−	11	A+	9 w32	12 16
2ɔ	H	F	M/M	−	−	8	A+	3	27 w35
26	H	F	M/M	−	−	10	O−	1 3	w35 37
27	G	Γ	M/F	−	−	≧1	A+	1 2	8 15
28	A	F	M/M	−	−	≧1	O+	2 w19	12 40
29	L	F	Γ/M	3	+	9	O+	1 w30	17 18
30	Z	F	F/F	1	+	7	B+	2 3	7 40
31	S	F	F/Γ	−	+	≧7	B+	3 10	7 18
32	C	F	Γ/M	−	+	≧6	A−	1 w19	8 12
33	v D	F[c]	F/M	0	+	≧1	A+	11	12 w35
34	V	F	Γ/F	0	−	≧1	AB+	2 9	w16 27
35	de P	F	M/F	−	+	4	O+	2 9	40 w41
36	M	F	M/F	−	−	4	O+	3 9	7 40
37	S	Γ	M/M	−	−	≧5	A+	2 3	7 40
38	C	Γ	M/Γ	−	−	3	A+	1 28	7 12
39	de V	F	M/Γ	−	−	≧1	A+	2 —	12 —
40	R	F	M/M	−	−	4	O−	2 9	15 22
41	B	N F	M/F	−	−	≧1	A+	1 28	8 15
42	B	N F	F/M	3	+	≧1	O+	2 11	7 12
43	S	N F	Γ/M	2	−	15	B+	3 9	7 12
44	D	N F	F/M	2	+	≧13	B+	2 28	12 27
45	v Z	N F	M/F	−	−	3	B+	3 w31	5 7

[a] The percentage of antidonor lysis has only been measu ed post transplantation In some patients two samples have been tested

[b] Γ functioning graft NF nonfunctioning graft

[c] The sera of the recipients were tested for the presence of cytotoxic antibodies against the lymphocytes of 50 selected donors Leukocyte antibodies were presumed to be present if one clearly positive reaction (more than 50% dead cells) with at least one cell of the panel was found Patients 42 and 45 received only buffy coat free blood transfusions

[d] BTs blood transfusions

Consequently we suggest that in the majority of the patients suppressor cells are not likely to be the cause of the CML nonreactivity The results of the pool stimulation experiments make it unlikely that nonreactivity is attributable to a lack of helper cells

Finally another possible explanation for the occurrence of specific CML nonreactivity could be that the specific antidonor cytotoxic clones of the effector cells are eliminated by anti idiotypic antibody or have been absorbed by the graft These possibilities remain open

In an attempt to identify the variables which predispose for the induction of donor specific CML nonreactivity we investi gated as many variables as possible As shown in Table 6 several factors appeared to have a significant influence on the occurrence of CML nonreactivity The best correlation was found between CML nonreactivity and good kidney function

HLA			SD≠[a]	DR≠	Antidonor % lysis in CML after transplantation		Anticontrol cell lysis
C	w4 w6	DR			+61 → 360	≥360 days	
Cw3Cw5		5 + 6	10	7		+1	+73
	w4	2 + 4	—	5 7	+1		+30
	w4 w6	2 + 7	2	1 5		+8	+25
		7	1 16	4 6		+1	+32
		4 + 8	9	7		+3	+43
Cw3	w6	2 + 4	—	6 7		+2	+12
Cw3	w6	4 + 6	—	2 8	+2		+36
Cw3	w6	3 + 4	27	2 8		+2	+47
	w6	2 + 8	3	5 7	+19	+7	+35
	w4 w6	2 + 4	9	9		+3	+53
		6	19	1 7		+8	+11
Cw3Cw5	w4 w6	4 + 6	—	7 8	+1		+46
Cw6	w4	7 + 9	29 7	1	+6		+21
	w4 w6	5 + 9	24	4 6	+3	+2	+83
Cw2Cw5	w4 w6	4 + 5	—	1		+1	+15
Cw1	w4 w6	1 + 4	17	7	+16	+7	+73
Cw2	w4 w6	4 + 7	12	5	+12	+2	+79
	w4 w6	4 + 6	—	3	+2		+66
Cw3	w6	4 + 7	—	—	+1		+35
Cw4	w4	4 + 7	11	2 5	+49	+2	+40
Cw2	w6	5 + 6	7	3 4		+5	+44
Cw3		2 + 4	28	5 9		+1	+80
Cw1	w4 w6	1 + 4	37	8	+3	+6	+73
		5 + 8	2 41	4 7		+9	+68
Cw2Cw4	w4 w6	3	1	2 7		+3	+27
Cw4	w4 w6	1 + 5	—	4 7		+2	+34
	w6	4 + 5	—	7		+2	+22
Cw3Cw4	w6	1 + 3	—	5 6	+2		+33
	w6	2 + 3	2 7 35	—	+25	+21	+79
Cw2	w6	2 + 7	—	6		+78	+72
	w6	5 + 6	15	2 4		+51	+87
	w4 w6	LB58 + 3	—	7		+34	+48
	w4 w6	4 + 5	3	—	+22		+74
Cw2	w4	1 + 2	37	LB58	+68		+22
		3 + 6	22	9	+25	+18	+29
Cw3	w6	2	—	3	+30		+38
Cw3	w4 w6	4	28	2 3	+18		+29
Cw5	w4 w6	1 + 2	8	5 9	+52	+29	+31
Cw6	w6	3 + 7	9 18	5	+14		+21
Cw3	w6	2 + 4	7	1	+14		+57
Cw2	w6	3 + 4	18	LB58	+34	Tx[b]	+29
		4	5	2 5	Tx +93		+100
		2 + 7	2 25	—	Tx	+97	+92
	w4 w6	2 + 5	—	4 8	Tx	+31	+98
	w4 w6	2	11 24 35	1 9	Tx +41	+76	+35

[a] SD≠ DR≠ indicates the SD and DR antigens which were mismatched between donor and recipient

[b] Second graft. Patient 41 rejected his second graft. Patients 42, 44, and 45 rejected their first grafts because of uncontrollable allograft rejection. Transplantectomy was performed in patient 43 because of thrombopenia and cytomegaly infection.

[c] Tx, transplantectomy.

(χ 10.186). This indicates that what has been studied is indeed clinically relevant. More studies are needed however to determine its value in renal transplantation. The following factors showed also a significant correlation: sex match—a male recipient who received a male graft had the greatest chance to become CML nonreactive and *HLB B* locus antigen match—compatibility between donor and recipient for the *HLB B* locus antigens increased the chance for the development of CML nonreactivity. These two factors reinforce each other (see Table 3). Furthermore, CML nonreactivity might also be influenced by the presence of HLA DR4 antigen on the recipient lymphocytes (Table 6). Conversely, the presence of HLA DR2 and DR3 on donor lymphocytes coincides with a positive CML.

Duquesnoy et al (*17*) recently have reported that compatibility between donor and recipient for the MB antigens (which are B cell antigens different from but closely linked to the HLA

DR antigens) seemed to be correlated with successful transplants and CML nonreactivity in intrafamilial donor and recipient combinations We were not able to confirm that finding in unrelated combinations (see Table 7)

Immunogenetic studies are under way to define more precisely the influence of the HLA system on the occurrence of CML nonreactivity and the importance of this phenomenon for kidney graft survival

TABLE 3 Relationship between the number of blood transfusions and the occurrence of CML nonreactivity against the splenocytes of the specific kidney donor[a]

		Blood transfusions	
		1	≥1
CML	+	4	12
	−	11	28

[a] $\chi^2_1 = 0.008$, $P \approx 0.88$ Rejections and patient 16 (Table 1) are excluded, second transplantations are included

TABLE 4 Percentage of lysis after pool stimulation

Patient[a]	Targets	
	Pool	Donor
1	+32	5
2	+36	0
3	+28	4
4	+37	7

[a] Patients 1 to 4 have been stimulated with a pool of stimulator cells (*minimum of five*) carrying different HLA antigens

TABLE 5 Changes in antidonor specific lysis (in percentage)[a]

Patient	Pretransplantation	Post transplantation	Mixed
1	54	2	21
2	27	7	18
3	21	5	22
4	39	2	38
5	17	10	47
6	38	18	42
7	49	2	38

[a] As responder cells, the pretransplantation and CML nonreactive post transplantation blood samples were mixed in a 1 1 ratio (only one ratio has been carried out)

TABLE 7 Influence of MB compatibility (=) on CML reactivity[a]

		MB=	MB≠
CML	+	9	7
	−	17	21

[a] No = 54 $\chi^2_1 = 0.23$, $P = >0.6$ Rejections and patients 11 and 16 (Table 1) are excluded, second transplantations are included

TABLE 8 HLA B compatibility (≈) and sex (male to male) matching reinforce each other[a]

	CML	
	−	+
M M and/or HLA-B =	32	6
Others	7	10

[a] $\chi^2_1 = 8.56$, $P = 0.003$

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TABLE 6 Statistical analysis

	+/+	+/−	−/+	−/−	No	χ^2	P
CML − / Fu good[a]	36	3	8	8	55	10 186	0 001
CML − / B ≈	26	13	5	11	55	4 436	0 035
CML − / M-M	18	21	2	14	55	4 194	0 041
(no further significant correlations could be detected)							
	CML versus HLA antigens present on patient s lymphocytes						
CML − / HLA DR4	17	22	3	13	55	2 047	0 153
	CML versus HLA antigens present on donor lymphocytes						
CML + / HLA-DR2	8	8	7	31	54	4 133	0 039
CML + / HLA DR3	5	10	3	36	54	3 795	0 05

[a] Fu good, kidney function good, B, zero mismatches for the *HLA B* locus antigens between donor and recipient M M, male recipient/male donor

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