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HLA REGULATES POSTRENAL TRANSPLANT CML NONREACTIVITY[1]

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Previous studies have shown that lymphocytes from renal allografted patients with a good functioning graft display donor-specific cell-mediated lympholysis nonreactivity (CML-NR) in vitro. To define whether the HLA system influences the occurrence of the CML-NR, immunogenetic studies were carried out. Posttransplant lymphocytes derived from CML-NR patients were stimulated in vitro with lymphocytes *from unrelated healthy blood donors*, who were selected for the presence or absence of kidney donor-specific HLA antigens. The presentation of kidney donor-specific HLA-B (and -C) antigens on the lymphocytes of unrelated blood donors resulted in cytolytic nonresponsiveness, whereas presentation of the kidney donor-specific HLA-A locus antigens on lymphocytes of the unrelated blood donors revealed no cytolytic nonresponsiveness. The results, as displayed by posttransplant lymphocytes of renal allografted patients, demonstrate that the kidney donor HLA-B (and -C) antigens are responsible for the in vitro-observed, donor-specific CML-NR. Consequently, presentation of cells from panel members matched to the kidney donor at the HLA-B locus suppresses the response towards HLA-A locus antigens. The in vitro-observed cytolytic nonresponsiveness appeared not to be due to an absence of specific cytotoxic T lymphocytes, because the nonresponsiveness can be abrogated by addition of exogenous IL 2.

The cell-mediated lympholysis (CML)[2] technique is one of the cellular test systems that may be used as an in vitro reflection of the in vivo allograft reaction. Immunologic tolerance, as manifested by allograft acceptance, may be correlated in vitro with the *absence* of host cytotoxic T lymphocytes (CTL) specifically directed against the graft histocompatibility antigens. The development of posttransplant cell-mediated lympholysis nonreactivity (CML-NR) in recipients of HLA-non-identical related and unrelated donor kidneys has been documented in several reports (1–11). In a previous study of 82 related donor/recipient combinations, we reported that the failure of recipients' lymphocytes to elicit in vitro cytotoxic response against the kidney donor splenocytes (in 70% of the nonrejecting renal allografted recipients) correlated significantly with good kidney allograft function (6).

The development of specific anti-donor CML-NR after transplantation is presumably a complex of cellular and humoral events. Several mechanisms such as involvement of suppressor cells (11–13) or anti-idiotypic antibodies that inhibit specifically the proliferative responses in mixed lymphocyte cultures (MLC) against the kidney donor alloantigens (14, 15) have been suggested to account for this phenomenon.

To increase our insights into the mechanism(s) involved in the posttransplant development of donor-specific CML-NR, we investigated the cytolytic repertoire of recipients' lymphocytes. Immunogenetic studies were carried out to define more precisely the influence of the HLA system on the occurrence of CML-NR.

Consequently, the specific cytotoxic response of the recipients' lymphocytes towards a selected panel of unrelated blood donors as specific stimulator target cells was measured. A drastically diminished cytotoxic activity was observed against the kidney donor-specific HLA-B (and -C) antigens when presented on lymphocytes from *unrelated blood donors*. Furthermore, normal levels of cytolytic activity could be restored through the addition of exogenous interleukin 2 (IL 2) or by the use of HLA-B (and -C) mismatched stimulator cells.

MATERIALS AND METHODS

From a group of 82 unrelated donor/recipient combinations, 51 patients became CML-NR (after successful kidney transplantation) against the splenocytes of their specific kidney donor (6). Immunogenetic studies were performed by using the lymphocytes of 16 of these 51 CML-NR patients at different time intervals posttransplantation and of three patients with *CML reactivity* against the specific kidney donor splenocytes.

Table I lists the match grades of these patients with their respective kidney donors. The three *CML reactive patients* included patients 17, 18, and 19. Patient 17 rejected the graft; the lymphocytes obtained after graft nephrectomy showed CML reactivity against the specific kidney donor splenocytes. Lymphocytes from patient 18 showed donor-specific CML-reactivity 3 yr posttransplant, this patient had a functioning graft. Patient 19 developed specific kidney donor CML-NR only *after* 200 days posttransplantation, immunogenetic studies have been carried out with lymphocytes obtained during both CML-reactive and CML-nonreactive (day 500) periods. All patients had received blood transfusions before kidney transplantation, they received a first cadaveric graft under the auspices of Eurotransplant.

Protocol immunogenetic studies. Posttransplant lymphocytes of 16 CML-NR patients and of three CML-reactive patients were stim-

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[2] Abbreviations used in this paper: CML, cell mediated lympholysis, CML-NR, cell mediated lympholysis nonreactivity.

TABLE I

Match of patients and kidney donors

Recipients	Kidney Donors HLA Match				Immunogenetic Study[a]
	A	B	C	DR	Total number of selected stimulator cells tested
1 B	=[b]	=	=	#[c]	9
2 Be	=	=	=	#	10
3 He	=	=	#	#	9
4 Di	=	=	#	#	6
5 Ha	=	=	#	=	6
6 de S	#	=	=	#	5
7 La	#	=	=	#	14
8 Ko	#	=	=	#	17
9 de W	#	=	=	#	10
10 La	=	#	#	#	7
11 Kn	=	#	#	#	5
12 Di	=	#	#	#	15
13 Bo	=	#	#	=	18
14 Ku	#	#	=	#	11
15 Ha	#	#	#	#	10
16 Si	#	#	#	#	9
17 Ey	=	#	#	#	7
18 Le	=	#	#	=	5
19 v d R	=	=	#	#	11

[a] Lymphocytes from each patient have been stimulated in vitro with lymphocytes derived from different unrelated healthy blood donors
[b] =, compatibility
[c] # incompatibility

TABLE II

Immunogenetic protocol example of stimulator cell selection

	HLA Typing			
	A	B	C	DR
Patient (P)	1, w33	37, 58	w3	1
Kidney donor	1, 3	37, 35	w6	1, 5
Selected stimulator cells[a]				
1 A=, B=, C=, DR=	1, 3	37, 35	w6	1, 5
2 A=, B=, C=, DR#	1, 3	35	w6	2, 3
3 A=, B=, C#	1, 3	37, 35	w4	1, 7
4 A# B=, C=	1, 2	37, 35	w3	4
5 A#, B=, C#	3, 32	35	w4	1, 8
6 A=, B#, C=	1, 3	37, 57	w6	1, 7
7 A= B#, C#	1, 3	40, 35	w2, w4	4, 6
8 A#, B# C=	2 28	7, 13	w6	1, 2
9 A# B#, C#	23, 25	18, 45	w5	2, 7

[a] =, matched #, mismatched with the kidney donor for HLA-A, -B, -C, or -DR antigens The availability of 10,000 HLA-A,-B,-C typed individuals in the Department of Immunohematology and Bloodbank, Leiden, facilitated the stimulator cell selection

ulated in vitro against specific kidney donor splenocytes and selected stimulator cells from healthy unrelated blood donors matched or mismatched with the kidney donor for the HLA-A, -B, or -C antigens and combinations thereof (see Table I) Stimulator cells were considered as matched for a specificity when splits and/or main specificities were identical, i e , B7-B7, B44-B44; B14-B14, Bw57-Bw57 Mismatches were considered stimulator cells with different main specificities or other splits of the same specificity, i e , B7-B8, B44-B45, B14-B8, Bw57-Bw58 An example of the immunogenetic protocol is shown in Table II It demonstrates the selection of stimulator cells from healthy unrelated blood donors matched or mismatched with the kidney donor for HLA-A, -B, or -C antigens and combinations thereof

To control the *responder capacity* of the lymphocytes of CML-NR patients and *stimulator capacity* of the selected stimulator cells, lymphocytes from unrelated healthy individuals (HLA-A, -B, -C, and DR identical to the patient) were stimulated with the same array of stimulator cells (see above) on the same day in the same experiment of a given patient Kidney donor lymphocytes were obtained from the spleen and used without density centrifugation All blood samples, i e , the patient's lymphocytes, the kidney donor splenocytes, and the lymphocytes of the HLA-A, -B, -C, and -DR compatible and incompatible unrelated healthy blood donors, were frozen and stored in liquid nitrogen until tested

The responder/stimulator cell combinations, as mentioned above, were cultured for 6 days Depending on the amount of lymphocytes available either tissue culture flasks or 2 ml cluster wells were used After the culture period, the effector cells were tested in the standard CML assay against their specific stimulator cells as target cells

The CML assay has been described in detail (16) The percentages of lysis were determined in relation to 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 the described method (16) 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. All experiments were repeated at least twice at different effector to target ratios Prolonged growth of some effector cells populations was performed with the use of commercially available IL 2 (T cell growth factor containing a residual amount of ±150 ng PHA/ml, Biotest Cat. no. 812800), an appropriate final concentration is 20% in the culture medium. A preliminary report on the immunogenetic studies of five other CML-NR patients has appeared elsewhere (17) Typing for HLA-A, -B, and -C antigens was performed with the standard lymphocytotoxicity technique (18), typing for the HLA-DR antigens was performed with the two-color fluorescence test (19).

RESULTS

Posttransplant lymphocytes from 16 CML-NR patients have been analyzed for their specific cytolytic capacity against selected stimulator cells of unrelated, healthy blood donors. As already mentioned (*Materials and Methods*), the selection of the stimulator cells was based on the sharing of HLA-A, -B, -C, or -DR antigens, or combinations thereof, with the specific kidney donor.

Table III shows the CML results of the 19 patients studied in the immunogenetic protocol. Posttransplant patient's lymphocytes were stimulated in vitro with the specific kidney donor splenocytes and with a series of selected stimulator cells, and thereafter were tested against the specific target cells. The results of only one responder/stimulator cell combination per patient are shown in the table. As expected, none of the patients 1 to 16 showed CML activity against the specific kidney donor splenocytes. Absence of cytolytic activity of patient's lymphocytes was also observed after specific stimulation with stimulator cells 1, 2, 3, and 4, and in several cases with stimulator cells 5. The common denominator

TABLE III

Immunogenetic analysis of the CML nonreactivity in 19 patients tested posttransplantation

		Stimulator/target cells[a]								
		A= B= C= DR=	A= B= C= DR#	A= B= C#	A# B= C=	A# B= C#	A= B# C=	A= B# C#	A# B# C=	A# B# C#
Responder cells	Kd[b]	1	2	3	4	5	6	7	8	9
	0[c]	2	7	10	16	58	35	72	81	61
P1	1	NT[f]	NT	0	0	20	65	47	27	40
P2	1	4	0	8	10	3	47	22	40	34
P3	1	0	2	5	3	8	12	41	65	29
P4	5	0	NT	6	4	6	NT	18	47	39
P5	7	NT	NT	NT	0	NT	24	29	49	83
P6	9	2	7	0	0	1	48	56	54	38
P7	4	NT	NT	1	7	20	56	59	37	66
P8	3	7	0	4	5	27	21	55	20	39
P9	6	0	3	6	NT	10	0	42	31	73
P10	3	NT	NT	NT	3	NT	NT	48	NT	27
P11	0	NT	8	NT	10	9	27	55	37	37
P12	0	8	8	4	6	10	30	28	68	55
P13	0	4	2	0	0	2	NT	37	NT	24
P14	10	6	NT	1	8	37	64	28	19	39
P15	10	NT	10	NT	1	13	48	61	48	25
P16	99	NT	92	96	88	56	NT	NT	NT	92
P17	38	NT	22	27	43	51	48	56	71	64
P18	48	NT	62	79	37	39	59	62	NT	70
P19[d]	7	2	6	10	6	16	61	46	NT	54
P19[e]										

[a] Results of only *one* responder/stimulator cell combination are shown
[b] Kd = kidney donor
[c] Percentage of specific lysis at an effector target ratio of 50 1
[d] P19 first bleeding 200 days posttransplantation
[e] P19 second bleeding 500 days posttransplantation
[f] Not tested

among stimulator cells 1 through 5 and the kidney donor was that they all carried the same HLA-B (or -B *and* -C) antigens as present on the specific kidney donor.

On the contrary, positive CML reactions were obtained when patients' lymphocytes were stimulated with stimulator cells 6 to 9. None of the latter stimulator cells carried the same HLA-B (or -B and -C) antigens as present on the specific kidney donor.

Lymphocytes from *CML-reactive* patients 17 to 19 were used as positive controls. The posttransplant lymphocytes from these patients showed cytotoxic activity against both the specific kidney donor splenocytes and all (i.e., 1 to 9) stimulator cells tested. Patient 19 had been bled twice. Patient's lymphocytes from the first bleeding (i.e., 200 days posttransplantation) showed cytolytic activity against the specific kidney donor splenocytes, as well as against all stimulator cells. The second bleeding (i.e., 500 days posttransplantation) showed donor-specific CML-NR, and consequently also the absence of cytotoxic activity after stimulation with stimulator cells 1 to 4. The immunogenetic studies with lymphocytes from four different patients (i.e., P7, P8, P12, and P13, Table III) have been enlarged by "control combinations" (see *Materials and Methods*).

The reaction patterns of the lymphocytes from each patient were compared with the cytotoxic activity obtained with lymphocytes from healthy individuals who were HLA-A, -B, -C, and -DR identical to the patient (see *Materials and Methods*). Normal levels of cytotoxic activity to all stimulator/target cells were observed (Table IV).

To further establish the immunogenetic requirements that are associated with CML-NR, cells from unrelated individuals with different HLA-A, -B, and -C antigens have been selected in each group of stimulator cells (i.e., 1 to 9), and used to stimulate the posttransplant patient's lymphocytes. Table V summarizes the total number of stimulator cells that have been tested in each selected group against the lymphocytes of 16 CML-NR recipients. Table VI compares the total number of CML-positive and -negative reactions obtained after stimulation of patients' lymphocytes with lymphocytes from healthy blood donors that were matched vs the donor-specific splenocytes for HLA-B, (and -B and -C) or mismatched for HLA-B, (and -B and -C) locus antigens. It is clear that a strong influence on CML-NR ($p = 0.0001$) is found in the groups of stimulator cells that were selected for the presence of the HLA-B, or -B and -C antigens, as expressed on the specific kidney donor cells.

One of the possible mechanisms that might explain the HLA-dependent, donor-specific CML-NR is clonal deletion. To answer the question as to whether donor-specific CTL were indeed absent, we expanded the 6 day effector cell cultures of lymphocytes from 10 CML-NR patients against the specific kidney donor splenocytes by the addition of IL 2. In nine out of 10 of the latter effector cell combinations, kidney donor-specific CTL were observed. In addition, the lymphocytes of two CML-NR patients were stimulated according the immunogenetic protocol and were subsequently expanded. The results of these experiments are shown in Table VII. Two short culture cycles with the addition of exogenous IL 2 resulted in detectable levels of cytotoxic activity, as well as responses against the specific kidney donor splenocytes similar to

TABLE IV

Comparison of patterns of cytotoxic activity between the lymphocytes of four patients (P) and the lymphocytes of unrelated individuals (X) who are HLA identical to these patients upon stimulation with kidney donor cells or cells selected for the presence or absence of kidney donor HLA antigens

	Stimulator/Target Cells[a]								
	A= B= C= DR=	A= B= C= DR#	A= B= C#	A# B= C=	A# B= C#	A= B# C=	A= B# C#	A# B# C=	A# B# C#
Responder cells	Kd[c] 9[a]	1 2	2 7	3 0	4 0	5 1	6 48	7 56	3 54	9 38
P7[b]	50	23	17	27	23	27	44	49	54	39
X										
P8	4	NT[e]	NT	1	7	20	56	59	37	66
X	44	NT	NT	25	25	33	67	79	47	54
P12	0	NT	8	NT	10	9	27	55	37	37
X	23	NT	19	NT	25	27	35	63	42	33
P13	0	8	8	4	6	10	30	28	68	55
X	55	38	29	28	37	38	25	24	50	60

[a] The specific stimulator cells/specific target cells were selected for the presence (=) or absence (#) of the kidney *donor-specific antigens*. In most cases more than one stimulator cell in each group (i.e., 1 to 9) has been studied. The results of only one responder/stimulator cell combination are shown in this table.

[b] Responder cells P7 = patient 7, responder cells X = unrelated healthy individual, HLA-A -B, -C, and -DR identical to each patient.

[c] Kd = kidney donor.

[d] Percentage specific lysis.

[e] Not tested.

TABLE V

Analysis of CML activity of posttransplant patients' lymphocytes against stimulator cells selected for the presence (or absence) of the kidney donor HLA antigens

	Selected Stimulator Cells/ Target Cells	Total Number of Stimulator Cells Tested	CML responses of post transplant patient s lymphocytes[a]	
			+	−[b]
1	A=B=C=DR=	10	0	10
2	A=B=C=DR#	11	0	11
3	A=B=C#	15	1	14
4	A#B=C=	25	3	22
5	A#B=C#	22	13	9
6	A=B#C=	18	14	4
7	A=B#C#	36	34	2
8	A#B#C=	16	16	0
9	A#B#C#	25	25	0

[a] Posttransplant lymphocytes from 16 CML-NR patients were stimulated with a number of stimulator cells selected on the HLA typing of the original kidney donor (see *Materials and Methods*).

[b] Positive and negative assignments were made on the basis of a 10% ^{51}Cr-release value.

TABLE VI

CML responses of posttransplant lymphocytes of renal allograft patients

Selected Stimulator Cell used	CML Response[a] +	CML Response[a] −	Selected Stimulator Cell used	CML Response[a] +	CML Response[a] −
HLA-A= and HLA-A and -C=	49	41	HLA-B= and HLA-B and -C=	17	66
HLA-A# and HLA-A and C#	32	31	HLA-B# and HLA-B and -C#	64	6
	$\chi^2 = 0.20$ $p = 0.65$			$\chi^2 = 76.72$ $p = 0.00001$	

[a] Total number of positive and negative CML reactions obtained with posttransplant lymphocytes from 16 CML-NR patients that were stimulated with lymphocytes selected for the presence or absence of the kidney donor HLA antigens.

TABLE VII

Reappearance of donor specific CTL by addition of IL 2

	Stimulator/Target Cells								
	A= B= C= DR=	A= B= C= DR#	A= B= C#	A# B= C=	A# B= C#	A= B# C=	A= B# C#	A# B# C=	A# B# C#
Responder cells	Kd[a]	1	2	3	4	5	6	7	8	9
P8[a]	6[c]	6	NT[d]	7	2	15	60	49	51	39
P8 + IL 2[b]	58	54	NT	48	83	76	65	61	60	69
P13	3	6	NT	8	10	9	28	36	48	66
P13 + IL 2	70	30	NT	29	72	51	55	77	65	82

[a] Kd = kidney donor

[b] Lymphocytes from patient 8 (P8) have been stimulated with a series of different stimulator cells (i e kidney donor splenocytes and stimulator cells 1 to 9) see also *Materials and Methods*

[c] P8 + IL 2 prolonged growth of the 6 day effector cell population was carried out

[d] Percentage specific lysis at an effector target ratio of 50 1

[e] Not tested

the response against selected stimulator cells 1, 3, 4, and 5 These results indicate that clonal deletion is *not* the cause of the observed donor specific CML-NR

DISCUSSION

Lymphocytes from patients with a well functioning graft may display an absence of cytotoxic potential in vitro towards the splenocytes of their specific kidney donor (1–11) We have previously analyzed the cytolytic activity of recipients' lymphocytes towards a pool of randomly selected stimulator cells, and found that the absence of CML reactivity of recipients' lymphocytes was observed not only against specific kidney donor cells but also against some individual target cells from the pool (20) Investigation of these target cells revealed a systematic absence of cytolytic activity against target cells that partially shared HLA antigens with the specific kidney donor (20)

In this study, the influence of the HLA system on the spectrum of the in vitro cell-mediated cytotoxic responses of posttransplant recipients' lymphocytes are described Immunogenetic analyses of the CML results obtained with the lymphocytes from 16 recipients who had received a renal allograft from an unrelated donor show that selected stimulator cells may induce donor-specific CML-NR (Tables III and IV)

All patients were able to exhibit normal effector cell function after in vitro stimulation with mismatched lymphocytes vs the kidney donor for the HLA-B (or -B and -C) antigens (i e , stimulator cells 6 to 9) Some positive reactions have been observed with stimulator cells 5 Apparently mismatching for the HLA-C locus products seemed to lead to positive reactions in some cases, therefore additional matching for HLA-C antigens seemed to be necessary in some cases but not in others The additive effect of matching of HLA-C to matching for HLA-B antigens on the CML response is remarkable (Table V)

Sharing of HLA-A or HLA-A and -C antigens with the specific kidney donor resulted in almost all combinations in the generation of cytotoxic effector cells Consequently the presence of kidney donor-specific HLA-B (and C) antigens on stimulator cells from healthy unrelated individuals leads to the absence of the generation of CTL (Table VI) These results clearly demonstrate the influence of the HLA-B region products on the posttransplant donor-specific CML-NR Because stimulator cells that share the HLA-B or HLA-B and -C antigens with the kidney donor are associated with the induction of donor-specific CML-NR, whereas HLA-B region disparity between the stimulator cells and the kidney donor is associated with CML activity, it appears that the genetic region between HLA-B and -C is involved in the induction of CML-NR posttransplantation Consequently, the immunogenetic analyses of the CML-NR status of renal allografted patients demonstrate not only a lack of response against kidney donor-type HLA-B antigens but a more profound immunoregulation that prohibits the response against other loci

Clonal deletion of these donor-directed CTL might be one of the mechanisms accounting for this in vitro-observed CML-NR, and might also be responsible for the graft tolerance Clonal deletion is not a likely cause of the observed absence of cytolytic activity We observed a significant increase of specific cytotoxic activity of CML-NR lymphocytes after explanding of the 6 day effector cells cultures by IL 2 (Table VII) Limiting dilution assays have to be carried out to determine the exact number of circulating donor directed CTL Apparently, in vivo graft tolerance and in vitro CML-NR exist in the presence of a strongly decreased number of donor-directed CTL, because the addition of exogenous IL 2 resulted in proliferation and differentiation of these donor directed CTL

It might be possible that the CML nonreactivity to the donor's alloantigens could be abrogated For example in the case of viral infections, the decreased number of circulating donor directed CTL could be activated, eventually leading to graft damage

Our observations differ from the results of Pfeffer et al (21), who reported an in vivo depletion of donor-specific cytotoxic cells in patients with well functioning kidney allografts from HLA disparate related donors, which could not be additionally activated in vitro However, the latter authors used a different protocol to expand the anti-donor-specific clones i e , exogenous T cell growth factor was added to the cultures *during* the induction phase of the effector cells, which did not result in augmentation of the donor-specific cytotoxic activity In view of the discussion on clonal deletion it has to be stressed that in this study, the immunogenetic analysis of the CML-NR status has been concentrated on the CTL responses towards HLA class I antigens Information concerning pre and postrenal transplant CTL activity against HLA class II antigens is fairly limited

Another possible explanation that might influence the occurrence of HLA-B (or -B and -C)-dependent CML non responsiveness could be that strongly diminished proliferation influences the development of cytotoxic effector cells Therefore, all responder/stimulator cell combinations have been checked for proliferative capacity by means of [^{3}H]thymidine uptake No correlation was found between the stimulation index in MLC and the presence or absence of cytotoxic T cells (Table VIII) The latter observation is in agreement with studies reported by others (9 13 22)

The exact mechanism(s) of the donor-specific HLA dependent CML nonresponsiveness is not clear The diminished number of donor-specific HLA-B-dependent cytotoxic T cell clones observed in renal allografted patients could be explained by the action of specific sup

TABLE VIII

Absence of correlation between proliferative capacity and cytotoxic activity of the same responder/stimulator cell combination

Responder Cells	Stimulator Cells	Proliferative Capacity[a]	CTL Activity[b]
P7[c]	Kidney donor	8 614	9
"	1	23 455	2
"	2	43 489	7
"	3	21 062	0
"	4	22 347	0
"	5	19 370	1
"	6	19 178	48
"	7	38 988	56
"	8	17 598	54
"	9	35 316	38

[a] The means of [^{3}H]thymidine uptake from triplicate cultures
[b] Percentage of specific lysis at an effector target ratio of 50 1
[c] P7 = patient no 7

pressor cells or by anti-idiotypic antibodies. First, suppressor cells responsible for the CML-NR, as manifested in the kidney allograft tolerant situation, have been documented in several reports (11–13). Second, evidence has been presented by Miyajima et al. (14) for the presence of anti-idiotypic antibodies; these authors demonstrated the inhibitory activity of patients' sera on the proliferative response in mixed lymphocyte reactions. Similarly, Singal and Joseph (15) described the induction IgG antibodies by blood transfusion directed against the recognition sites of the responder T lymphocytes. According to the latter authors (15), the antibodies capable of inhibiting responses in MLC could be induced by blood transfusion. The specific antibodies inhibiting responses in MLC against antigens present in the kidney donor were demonstrated in renal transplant patients with functional allografts, but not in patients who rejected the transplant. In these reports (14, 15), the antibodies were capable of inhibiting proliferative responses against the kidney donor HLA-B antigens and against stimulator cells which shared the kidney donor HLA-B antigens. These observations are striking, because in our immunogenetic studies, it was found that the occurrence of kidney donor-specific CML-NR apparently depends on the kidney donor HLA-B (or HLA-B and C) antigens. The mechanism underlying this in vitro-observed state of tolerance induced by the HLA-B (and -C) region described in this article encompasses most probably a combination of both the cellular and the humoral arms of the immune regulatory system. A large body of information concerning this topic has been obtained in animal studies (23). Recently, evidence has been presented for the presence and function of anti-idiotypic T cells in renal allografted rats with prolonged graft survival (24).

It would seem important to evaluate whether the same state of tolerance, as observed in CML after kidney transplantation, can also be obtained after blood transfusion. If that would be the case, selection of cadaveric kidney donors for pretransplant-transfused potential recipients to ensure good kidney graft survival could become much more likely.

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