

THE HLA SYSTEM

HLA-A, B, C (CLASS I) ANTIGENS

Immunogenetic Studies With In Vitro Generated Cytotoxic Lymphocytes of Cell-Mediated Lympholysis (CML) Nonreactive Kidney Graft Recipients

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ONE of the cellular test systems which, at the moment, can be used as an in vitro reflection of the in vivo homograft reaction is the cell-mediated lympholysis (CML) technique. The development of donor-specific CMI nonreactivity (CML-NR) in recipients of HLA-nonidentical related and unrelated donor kidneys has been documented in several reports.¹⁻³ Our studies, containing 82 unrelated donor/recipient combinations, showed that the development of donor-specific CML-NR correlated significantly with good kidney graft function. Furthermore, donor-specific CML-NR occurred more frequently in those donor/recipient combinations that were matched for (A) the HLA-B locus antigens and (B) male sex.⁴ Additionally, we observed that donor-specific CML-NR could not be abolished by pool stimulation of the recipients' lymphocytes,⁵ i.e., after stimulation of the CML-NR recipients' lymphocytes towards a pool of randomly selected stimulator cells, no cytolytic activity against the specific kidney donor splenocytes could be observed. However, despite a normal CML reactivity against control and pooled cells as target cells, absence of cytolytic activity after pool stimulation was observed not only against the spe-

cific kidney donor splenocytes as target cells, but also against some individual target cells from the pool. The latter CML-NR seemed to be influenced by the HLA match of the individual target cells from the pool.⁵

In an attempt to clarify this pattern of cytolytic nonreactivity, we subsequently studied the cytolytic capacity of the CML-NR patients' lymphocytes against a selected panel of unrelated blood donors as specific stimulator cells, e.g., target cells. Absence of CML-NR was not only observed against the specific kidney donor splenocytes, but also against

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several unrelated blood donors. These donors shared the HLA-B or HLA-B and C antigens with the specific kidney donors. Our results indicate so far that the occurrence of kidney-donor-specific CML-NR against unrelated blood donors as stimulator cells depends on the kidney donor HLA-A and C antigens.

MATERIALS AND METHODS

Donor-specific CML-NR occurred in 70% of 82 patients with a functioning graft. Immunogenetic studies were performed with the lymphocytes of 16 CML non-reactive patients.

Protocol immunogenetic studies Lymphocytes of CML nonreactive patients (posttransplantation) were stimulated *in vitro* against (1) specific kidney donor splenocytes, (2) HLA-A, B, C, and DR incompatible control cells, and (3) selected stimulator cells from unrelated blood donors that differed from the kidney donor for HLA-A antigens, HLA-B antigens, HLA-C antigens, and (4) combinations thereof.

CML technique The standard CML assay has been described before in detail.⁴

RESULTS

The results of immunogenetic studies with lymphocytes of an CML-NR patient are shown in Table 1. CML reactivity was observed against HLA-incompatible control cells and against specific stimulator/target cells 1, 3, 4, and 5. Absence of CML reactivity was observed, as expected, against the specific

kidney donor splenocytes and in addition against stimulator/target cells 2. It is remarkable that stimulator/target cells 2 carried the same HLA-B and C antigens as the original specific kidney donor.

To control the percentages of specific lysis obtained by patients' lymphocytes, responder cells that were HLA identical to the patient were stimulated following the same protocol, and subsequently tested against the same specific target cells. Positive lysis could be shown, without any exception, against all target cells.

Table 2 shows the CML pattern of 5 different patients stimulated and tested against specific kidney donor splenocytes; HLA-incompatible control cells and 7 selected stimulator/target cells differing either for HLA-A antigens, HLA-B antigens, HLA-C antigens, or combinations thereof. The responder cells of the 5 patients all showed positive lysis against HLA-incompatible control cells, target cells 5, 6, and 7, and in 3 of 5 cases, against target cells 4. CML-NR was observed not only against the specific kidney donor splenocytes, but also against target cells 1, 2, and 3, and in 2 of 5 cases against target cells 4. The most striking similarity between stimulator/target cells 1, 2, 3, and 4 is that

Table 1 Immunogenetics of CML Nonreactivity

Responder Cells	Donor†	Control	Stimulator/Target Cells				
			A ≠ B = C ≠ DR ≠ 1	A ≠ B C ≠ DR ≠ 2	A ≠ B ≠ C = DR ≠ 3	A B ≠ C - DR - 4	A - B ≠ C DR ≠ 5
R†	+ 10*	+ 39	+ 37	+ 8	+ 19	+ 28	+ 64
X	+ 22	+ 69	+ 65	+ 47	+ 66	+ 26	+ 58

*Percent specific lysis

†HLA phenotypes (responder stimulator/target cells) kidney donor—A1 A3, Bw35 B37 Cw6 DR1 5, recipient—A1 Aw19, B17 B37, Cw3 DR1

X, HLA identical to the patient

Control cells A1, A2 B8 Bw44 Cw5 DR3, 4

Stim /target cells 1 A3, Aw32 Bw35 Cw4 DR1 8

Stim /target cells 2 A3, A9 Bw35 B37 Cw6 DR4

Stim /target cells 3 A3, A26, B7 B13 Cw6 DR1 2

Stim /target cells 4 A1, A3, Bw35 B40 Cw2, 4, Dr1

Stim /target cells 5 A1 A3 B8, B17 Cw6 DR3

Posttransplant patients lymphocytes and lymphocytes from X were sensitized *in vitro* against different stimulator cells (see Materials and Methods) and thereafter tested in CML against the specific target cells

Table 2 Immunogenetics of CML Nonreactivity

Responder Cells*	Stimulator/Target Cells								
	Donor	Control	A-	A-	A≠	A≠	A-	A	A≠
			B	B-	B-	B	B≠	B≠	B≠
			C-	C≠	C	C≠	C	C≠	C-
			1	2	3	4	5	6	7
1	+4†	+51	+1	-1	+1	+20	+56	+72	+37
2	+1	+36	1	-1	-1	+38	+65	+47	+42
3	+3	+27			+2	+1	+21	+48	
4	+3	+83	+7		+4	+6	+48	+56	+54
5	1	+43	+8	+4	2	+22	+43	+31	+68

*Responder cells 1-5 represent lymphocyte populations of 5 different patients posttransplantation

†Percent specific lysis

Patients' lymphocytes were sensitized in vitro against different stimulator cells (see Materials and Methods) and thereafter tested in CML against the specific target cells

they carried the same HLA-B (or B and C) antigens as the original kidney donor

DISCUSSION

HLA matching does improve graft survival in unrelated donor/recipient combinations, and moreover, influences the development of posttransplant CML-NR. We observed that the occurrence of CML-NR, besides its significant correlation with good graft function, occurred more frequently in donor/recipient combinations that were matched for HLA-B locus antigens⁴

It was the aim of this immunogenetic study to define the more precise role of the HLA system on the occurrence of CML-NR. The results presented in Tables 1 and 2 (which are representative for 16 patients studied) demonstrate that donor-specific CML-NR does occur not only against the specific kidney donor splenocytes as target cells, but also against other target cells. Immunogenetic studies show that donor-specific CML-NR also occurs against the kidney donor-specific HLA-B or HLA-B and C antigens presented on lymphocytes of selected unrelated blood donors as stimulator cells. Thus, sharing of HLA-B or HLA-B and C antigens with the specific kidney donor causes significantly less lysis

The possible mechanisms of the occurrence of CML-NR against donor-specific HLA-B

or HLA-B and C antigens are

(1) HLA-B or HLA-B and C antigens are the major cytotoxic determinants. This would implicate that HLA-A antigens are poor targets in CML. This seems unlikely, since alloimmune HLA-A2 CTLs (generated between unrelated responder/stimulator cell combinations differing only for the HLA-A2 antigen) showed good cytotoxicity⁶

(2) Elimination or clonal deletion of specific anti-donor cytotoxic clones of effector cells by antidiotypic antibodies. Evidence in this direction has been reported by Miyajima et al,⁷ who showed specific inhibition of certain MLR by antidiotypic antibodies in a patient with a functioning renal graft. In addition, Singal et al⁸ showed that antibodies directed against recognition sites on T lymphocytes can also be induced by blood transfusions. In both latter reports, specific antibodies capable of inhibiting proliferative responses in MLC were directed against HLA-B antigens of the kidney donor.^{7,8} This observation is very striking, especially because in our immunogenetic studies, occurrence of kidney donor-specific CML-NR against unrelated blood donors as stimulator cells depends on the kidney donor HLA-B (or HLA-B and C) antigens

(3) Compatibility for HLA-B (or HLA-B and C) between donor and recipient can induce suppression. Liburd,⁹ Thomas,¹⁰ and

several other authors described the presence of suppressor cells responsible for the occurrence of CML-NR. With special reference to the latter studies and our observations, it seems important to search for the influence of the HLA system on the induction of suppres-

sion of the specific anti-donor CML reactivity posttransplantation.

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