CML Nonreactivity After Kidney Transplantation

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THE occurrence of donor-specific cell-mediated lympholysis (CML) nonreactivity in unrelated and related donor–recipient combinations has been documented in several reports.[1-3] Our results showed that the development of CML nonreactivity correlated significantly with good graft function. Furthermore, we found that compatibility for the HLA-B locus antigens between donor and recipient and sex match in male patients predisposed to the occurrence of CML nonreactivity.[3,4]

In this study we investigated whether the absence of CML reactivity of recipient lymphocytes towards the specific kidney donor splenocytes could be due to lack of helper cells. Our results indicate that even after stimulation of the recipient lymphocytes towards a pool of stimulator cells, no cytolytic activity against the donor cells could be observed. However, when such pool-stimulated effector cells were tested on each cell of the pool individually as a target, absence of cytolytic activity was observed in effector–target cell combinations that were HLA-B identical.

MATERIALS AND METHODS

From a group of 65 unrelated donor–recipient combinations, 39 patients became CML nonreactive after kidney transplantation against the splenocytes of their specific kidney donor [4]

Pool Stimulation

Seven of the 39 CML nonreactive patients were further studied Peripheral blood lymphocytes of the recipients were sensitized in vitro against the irradiated splenocytes of the specific kidney donor, against HLA-A, B, C, and DR incompatible control cells of unrelated healthy individuals selected at random, and against a pool of stimulator cells (minimum of 5) carrying different HLA antigens

CML Technique

The standard CML assay has been described before in detail [5]

RESULTS

Table 1 lists the percent lysis in CML in 7 patients obtained after in vitro pool stimulation. The results show that no cytolytic activity of recipient lymphocytes against the specific kidney donor splenocytes could be induced even after pool stimulation. They all show a normal cytolytic capacity towards the pool as a target cell.

Subsequently, 4 of the 7 patients were tested against each of the target cells of the pool individually.

Table 2 shows the CML pattern of the recipient lymphocytes tested against the following target cells: (A) specific kidney donor splenocytes; (B) HLA-incompatible control cells; (C) a pool of cells selected at random; and against (D) the target cells from the pool individually (5 informative target cells are shown).

The lymphocytes of patient 1 showed CML nonreactivity against the specific kidney donor splenocytes and also against target cell 1 and target cell 3. Positive lysis was observed against an HLA-incompatible control cell (B), against the pool as target cell (C), and also against targets 4 and 5.

The lymphocytes of patient 2 showed no

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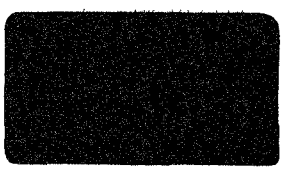


Table 1. Percent Lysis Obtained After Pool Stimulation

		Targets	
		Splenocytes of Specific Kidney Donor*	
Patient	Pool	Before Pool Stimulation	After Pool Stimulation
1	32	1	5
2	28	8	4
3	26	3	2
4	35	10	7
5	42	3	8
6	45	7	7
7	33	1	−1

CML was carried out with PBLs from renal allografted patients. The lymphocytes of these patients became CML nonreactive to their specific kidney donor after kidney transplantation. They were stimulated with a pool of lymphocytes obtained from 5 unrelated donors and thereafter tested on the pool and on the splenocytes of the specific cell donor.* Note that although lysis to the pool occurred in all instances, the reactivity towards the specific donor remained the same.

lysis against the donor cells and against target cell 2, but reacted strongly with the HLA-incompatible control target cell (B), with the pool as a target cell (C), and with target cell 5.

The lymphocytes of patient 3 showed no lysis against the donor target cells but reacted positively against HLA-B incompatible control cells, the pool, and target cell 4 from the pool.

The lymphocytes of patient 4 showed CML nonreactivity towards the donor target cells, but failed also to lyse target 1, although good cytolytic capacity was observed on the pool as a target.

DISCUSSION

Several groups have already investigated the occurrence of indirect CML reactivity in renal allograft donor–recipient combinations. With special regard to our own studies,[3,4] the change from CML reactivity towards CML nonreactivity could be demonstrated in 70% of nonrejecting patients. The occurrence of CML nonreactivity appears to be under the influence of the HLA system, because compatibility between donor and recipient for the HLA-B locus antigens increases the chance for the development of CML nonreactivity.

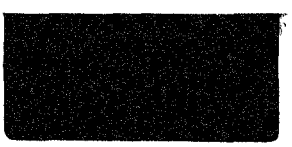
From the results from the pool stimulation experiments (Table 1) we can conclude that CML nonreactivity is not due to a lack of helper cells. Even after pool stimulation, the patients' lymphocytes showed no cytolytic capacity towards the specific kidney donor splenocytes. (The abolished capability of patients' lymphocytes to generate cytotoxic effector cells against the specific donor even after pool stimulation has been described earlier.[6]) The anti-control cell and the anti-pool cell lysis always showed normal CML reactions.

However, when the cytolytic capacity of recipient lymphocytes after pool stimulation was tested separately against the target cells

Table 2. Pattern of Lysis in CML of CML Nonreactive Patients

		Targets								
Patient	Sex	(A) Donor	Sex	(B) Control Cell	(C) Pool	1*	2	3	4	5
1.	♀	−1	♂	+43	+28	+3		+6	+60	+32
2.	♂	0	♀	+80	+42		+8			+36
3.	♀	+7	♂	+49	+45				+35	
4.	♂	−1	♂	+25	+33	+1				

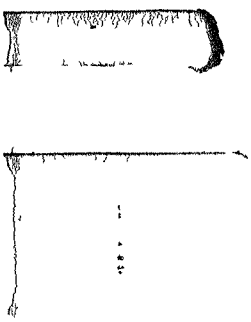
*Individual targets from the pool:
target 1 HLA-A =, −B =, −DR ≠, sex: male
target 2. HLA-A ≠, −B =, −DR ≠, sex female
target 3. HLA-A ≠, −B =, −DR =, sex male
target 4: HLA-A =, −B ≠, −DR =, sex female
target 5. HLA-A ≠, −B ≠, −DR =, sex. female
HLA-B identical, CML nonresponsive, versus HLA-B nonidentical, CML responsive. $p_{exact} = 0.01$



from the pool, an HLA-B dependent pattern of cytolytic nonresponsiveness-was observed (Table 2).

Investigation of the HLA types of the individual target cells from the pool showed that in the case of a lack of lysis, the relevant target cell showed HLA-B compatibility with the original kidney donor cells

Although only 4 patients have been studied, the data seemed to be consistent and significant. These findings are in agreement with our previous results, which indicated that the development of CML nonreactivity is influenced by matching for the HLA-B locus antigens between kidney donor and recipient. The study reported here is in agreement with our earlier observations and reinforces the hypothesis that CML nonreactivity is most likely to occur in HLA-B identical combinations. The reason for this and for the exceptions that also occur awaits clarification.



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