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CORRELATION BETWEEN MIXED LYMPHOCYTE CULTURE PERFORMED BEFORE RENAL TRANSPLANTATION AND KIDNEY FUNCTION

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SUMMARY

Mixed lymphocyte cultures were performed before renal transplantation in 36 patients, using the cells from the recipient and the prospective living donor. A highly significant correlation was found between the results of mixed leucocyte cultures and (a) the renal function at one year or two years; (b) the occurrence of early or late rejection crises. A good correlation was also found between the results of mixed cultures and leucocyte typing based on HL-A haplotypes (but not with conventional typing based on the number of antigenic differences). As applied to the selection of the donors for kidney transplantation, the mixed culture has the advantage of recognizing all the 'good' cases, including the favourable cases of the HL-A semi-identical group; it will also select however a small number of poor cases. In contrast, the determination of HL-A haplotypes will select almost only 'good' cases but will discard a significant number of cases which will eventually prove to be favourable.

INTRODUCTION

The mixed lymphocyte culture (MLC) test has been proposed as a method for the selection of a graft donor (Bain, Lowenstein & MacLean, 1965; Fritz Bach & Amos, 1967). In thirty-six patients the MLC test was performed before a renal transplantation, and in most of them, leucocyte typing as well. The correlation between MLC and graft survival has been examined and compared with that observed for leucocyte typing.

METHODS

1. *Mixed lymphocyte culture*

Purified lymphocytes were obtained from heparinized blood after sedimentation with Dextran and passage through a nylon filter (Debray-Sachs *et al.*, 1968). The cells of uraemic

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patients were washed three times in culture medium, the cells of others twice. The technique used for lymphocyte culture was that described by Hirschhorn (1965); the plasma added to the medium was obtained from normal donors and never from uraemic patients. The degree of cell activation was measured on the 7th day of culture by counting the number of 'blast' cells and mitoses after fixation and staining with orcein and, in the more recent cases, by measuring the incorporation of ^{14}C thymidine into the cells. A good correlation between the two methods was found ($r = 0{\cdot}73$; $P<0{\cdot}001$).

The results of mixed cultures were classified as 'negative' if the transformation rate was lower than 5%, and 'positive' if the transformation rate was higher than 5%. These two categories were chosen on the following basis:

a. The 5% transformation rate is the upper value found in the cultures containing a single population of lymphocytes;

b. In mixed cultures repeated after 15 days or more, a change of category was observed only in two cases with a shift in the transformation rate from 2% to 6·2% and 3·8% to 7·9% respectively, whereas the reproductibility was very imperfect within each category.

2. *Leucocypte typing*

The methods used for leucocyte typing and determination of HL-A genotype (haplotyping) have been described (Kreis *et al.*, 1970, this issue).

3. *Clinical data*

Thirty-six MLC tests have been performed before kidney transplantation between the lymphocytes of uraemic patients and their donor. The thirty-six patients were transplanted between 1966 and 1968. Donor selection was based on the results of leucocyte typing and MLC tests. Immunosuppression was achieved in all cases with azathioprine and corticosteroids. None of the patients received antilymphocyte serum. The distribution of the donors was the following: 1 identical twin, 19 siblings, 13 parents, 3 unrelated living donors.

The assessment of the clinical results was based primarily on the *creatinine clearance* obtained 1 year and 2 years after transplantation. The choice of 1 year as a minimal period for the follow-up study is justified since a large fraction of the possible failures occurs within this period. Thus, all cases with a functioning transplant and a follow-up study shorter than 1 year were excluded. On the other hand, all cases with death or complete rejection (i.e. patients on a dialysis programme) during the 1st year, were considered in this study and their creatinine clearance was scored as zero. In cases with functioning grafts and the required follow-up period, transitory changes in the clearances might have resulted from rejection crises or non-renal complications; to minimize the influence of these factors, the values of creatinine clearance considered for the calculations were the average of the values obtained during the 2 months preceding and following the 12th and 24th month.

Rejection crises were also considered as a useful criterion in the clinical evaluation. They were defined as episodes of reversible renal failure unrelated to urological or infectious complications. Rejection crises were classified as 'early' and 'late' depending on their beginning before or after the 15th post-operative day. This date has been chosen after a careful evaluation of the occurrence rates of rejection crises and their related clinical features (Jean-François Bach & Leski, 1970). This clinical evaluation refers to the whole series of thirty-six patients. A 'selected group', supposed to be more homogeneous in several respects, was subsequently extracted from the larger one and used for correlation studies. This

selected group of twenty-two patients was obtained after exclusion of the following cases: (a) patients transplanted with unrelated donors (3 cases); (b) patients who died, but apparently without any significant rejection process, namely from hepatic (2 cases), pulmonary (4 cases), or urological (4 cases) complications with good renal function; (c) one case with early bone-marrow aplasia resulting in the interruption of azathioprine treatment during the three weeks after transplantation.

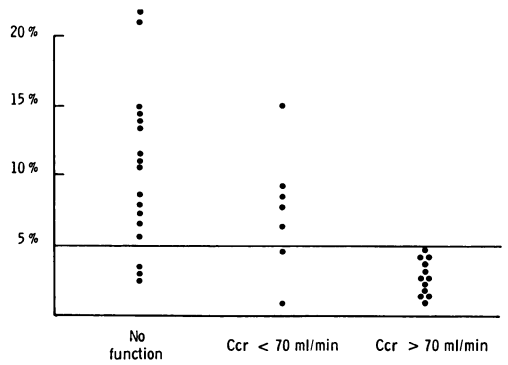

FIG. 1. Correlation between MLC and renal function at one year in all of the thirty-six cases ($P < 0{\cdot}001$).

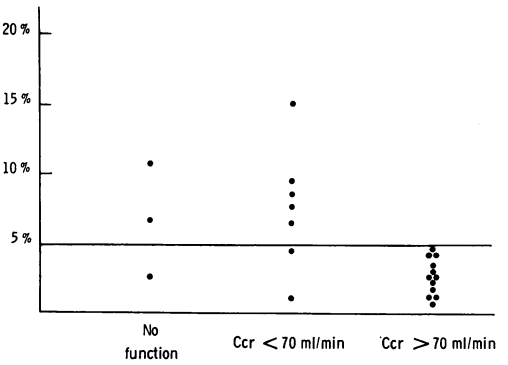

FIG. 2. Correlation between MLC and renal function at one year in the 'selected group' ($P < 0{\cdot}001$).

RESULTS

1. *Correlation of MLC and renal function*

At 1 year, (Fig. 1) the correlation between renal function and results of MLC tests is very significant ($P<0{\cdot}001$). It is also very significant ($P<0{\cdot}001$) when the 'selected group' of patients is considered (Fig. 2). It can be seen that none of the patients with a creatinine clearance over 70 ml/min had a transformation rate over 5%. The correlation at 2 years (Fig. 3) is also very significant ($P<0{\cdot}005$), although the number of cases is small. A good correlation is also demonstrated (Table 1) between the results of MLC test and the occurrence of rejection crises, especially of late crises, i.e. those beginning after the 15th day.

2. *Correlation of MLC and leucocyte typing*

As already shown (Debray-Sachs *et al.*, 1968) the correlation between the results of MLC and the number of antigenic differences is poor. Conversely, a good correlation is found when the HL-A haplotypes are considered ($P<0{\cdot}01$, Fig. 4). All of the HL-A identical pairs were negative in MLC tests, while only 30% of semi-identical pairs were found to be negative.

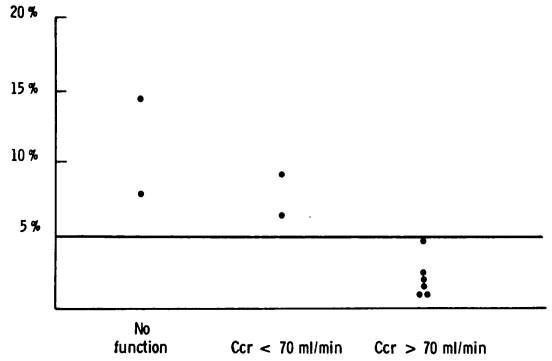

FIG. 3. Correlation between MLC and renal function at 2 years ($P < 0{\cdot}005$).

TABLE 1. Correlation of MLC test and occurrence of rejection crises

		MLC test		
		Negative (5%)	Positive (5%)	
Early crises	No crisis	14	5	$P < 0{\cdot}001$
	Crisis	2	10	
Late crises	No crisis	11	0	$P < 0{\cdot}001$
	Crisis	4	11	

DISCUSSION

The mixed lymphocyte culture test probably represents an *in vitro* immunological reaction between lymphocytes, each population responding to the HL-A alloantigens present in the other population (Fritz Bach & Amos, 1967).

Cultures obtained after killing one of the cell populations, for example by mitomycin C, allows the study of the response of a single population. These one-way cultures have been proposed for evaluation of the degree of histo-compatibility between two subjects (Fritz Bach & Amos, 1967; Amos & Fritz Bach, 1968). However, the rate of transformation in MLC also depends on the immunological reactivity of the lymphocytes which is variable within a group of uraemic patients (Wilson & Kirpatrick, 1964, Huber *et al.*, 1969).

No clinical material with a number of cases large enough for a statistical analysis of the correlation with MLC was available thus far. The only published data are those of Nelson,

Russell & McGeown (1967) dealing with six cases of renal transplantation: these data are compatible with a good correlation between MLC and kidney graft survival, but this correlation is not statistically demonstrated. We have found a very significant correlation between the results of mixed lymphocyte culture, performed before transplantation, and the function of the kidney one year after transplantation in thirty-six patients. This correlation was established by comparing positive (over 5%) versus negative results for MLC, and good (Ccr > 70 ml/min) versus poor renal function.

It could be argued that the criteria used to classify the results of MLC tests in 'positive' or 'negative' are to some extent arbitrary, and also that a prospective choice of the limit for negativity could have led to different results. The selected level of 5% transformation rate has been justified, however, and the correlations found are good enough to remain significant with other levels.

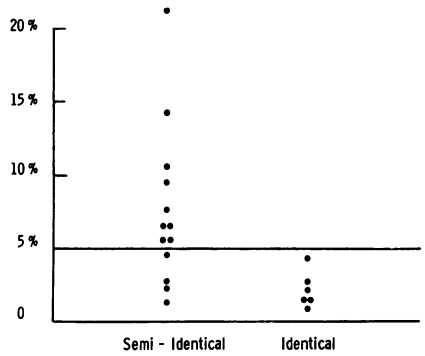

FIG. 4. Correlation between MLC and haplotyping in nineteen pairs ($P < 0{\cdot}01$).

All of the twelve patients with a good function had a negative MLC in contrast with most of those with mediocre or poor results (five negative MLC out of twenty-four cases). Moreover, all of the eleven patients with a positive MLC had at least one late rejection crisis, in contrast with eleven out of fifteen with a negative MLC. These results have been obtained with two-way cultures, with microscopical counting of blast cells and mitoses. This indicates that the one-way culture with thymidine incorporation is not a technical prerequisite for the selection of the donor of an organ to be transplanted, at least when pairs with negative MLC are considered. However, the one-way method has proved useful for quantitating different degrees of incompatibility.

The determination of haplotypes of patients and members of their family has recently become available, at least in some cases. Each pair is classified as HL-A identical, semi-identical or different for both haplotypes. When using this classification, a very good correlation was demonstrated between MLC and leucocyte typing (Fig. 4). But, in agreement with previous publications (Fritz Bach & Amos, 1967; Debray-Sachs *et al.*, 1968) no correlation was shown between the number of antigenic differences found by leucocyte typing and the result of MLC. This is in keeping with the critical value of the HL-A genotype instead of phenotype determination as reported in another paper (Kreis *et al.*, 1970).

In the case of transplantation with related donors, both haplotyping and MLC can be used for the selection of the most compatible donor. As shown in Table 2, a decision based on

the determination of HL-A haplotypes would select almost only 'good' cases with a few exceptions, but would discard a number of cases which could eventually prove to be favourable. Conversely, MLC will select all the 'good' cases (including the favourable cases of the semi-identical group), but in addition, a significant number of 'poor' cases.

The problem is more complicated when cadaver transplants are considered. Statistical correlations with graft survival and lymphocyte antigens matching (phenotype) were found by Morris *et al.* (1968), Terasaki, Vredovoe & Mickey (1967), van Rood *et al.* (1969) and Batchelor & Joysey (1969), but individual discrepancies are still not exceptional. Haplotyping is not possible in unrelated pairs. A selection based on MLC is not yet possible, due to the present conditions of organ storage and the 6 or 7 days duration of the culture. The study of early changes in stimulated cells (Marilyn Bach, personal communication, 1969) and the progress in organ storage might, however, make this study feasible in the future, in order to select good pairs among those preselected by leucocyte typing.

TABLE 2. Predictive values of HL-A determination and MLC test for the eventual fate of kidney transplants

	Ccr > 70 ml/min		Ccr < 70 ml/min	
	Selected	Discarded	Selected	Discarded
HL-A determination				
Criterion =	6	2	1	13
Genotype identity	(75%)	(25%)	(7%)	(93%)
MLC				
Criterion =	12	0	5	19
Transformation < 5%	(100%)	(0%)	(20%)	(80%)

The study of MLC in a two-way system demonstrates the critical importance of HL-A genetic identity for the eventual success of the transplantation. However, some HL-A non-identical cases with little or no transformation in MLC, do fare very well. Such situations might offer a basis for a more refined analysis of some of the alleles of the HL-A system and their role in graft rejection.

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