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Mixed Leukocyte Culture in Uraemia

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Summary. The activities of mixed leucocyte cultures (MLC) prepared from pairs of healthy subjects were estimated from the uptake of tritiated thymidine by the cultures. It was confirmed that MLC is a practical method for detecting identical twins amongst siblings. MLC has not often been employed for donor selection for renal transplantation, mainly because of the impaired MLC reactivity of leukocytes from uraemic patients. An attempt was made to separate lymphocytes from uraemic leukocyte-rich plasma, which always has a high proportion of neutrophils. A lymphocyte-rich fraction of uraemic leukocytes has MLC reactivity comparable to that of non-uraemic leukocytes.

Key words: Mixed leukocyte culture, histocompatibility test, uraemic subjects.

When leukocytes from two unrelated subjects are cultured together in vitro, some lymphocytes are stimulated to undergo mitosis, offer a phase of DNA synthesis (1). The correlation between mixed leukocyte culture (MLC) reactivity and graft survival between two subjects has been investigated both in experimental animals (2, 3), and man (4). It has been suggested that MLC could be used as reliable in vitro histocompatibility test. The technique of MLC testing has been improved by measuring the incorporation of radioactive thymidine as an indicator of the reaction, and adopting the one way reaction using irradiated (5) or mitomycin C-treated (6) leukocytes as "stimulating" cells.

Lymphocytes from monozygotic twins (1) or siblings (7) who are identical at the major histocompatibility locus HL-A are mutually non stimulatory in MLC. The survival rate of kidney allografts from HL-A identical siblings has been reported to be considerably higher than that from HL-A non-identical siblings (8). In clinical practice the prospective recipients of kidney allografts are always uraemic. Kasakura and Lowenstein reported diminished reactivity of uraemic leukocytes in MLC and questioned its applicability in uraemic subjects (9).

The MLC reactivity of leukocytes from uraemic subjects has been studied to determine whether they could be used either as the responding cells or stimulating cells in MLC testing of histocompatibility for renal transplantation.

Materials and Methods

Mixed leukocyte culture. Heparinized blood collected by venepuncture was centrifuged for 5 min at 450 g. The plasma and buffy coat were collected together, allowed to stand for 30-60 min at 37° C until the erythrocytes had sedimented and the leukocyte-rich plasma separated. Cells to be used as "responding cells" were suspended in TC 199 containing 100 units/ml penicillin, 100 µg/ml streptomycin and 20% autologous serum, at a concentration of 5×10^5 lymphocytes. Cells to be used as "stimulating" cells were suspended in 2-5 ml of TC 199 containing 20% autologous serum, incubated for 20 min at 37° C with mitomycin C (25 µg/ml), and washed twice with TC 199. The sedimented cells were then mixed with a small amount fo serum, counted, and resuspended in the same fashion as for responding cells. For the two-way reaction, 0.65 ml each of the responding cell suspension from two subjects were mixed, and for the one way reaction, 0.65 ml of the responding cell suspension from one and 0.65 ml of the stimulating cell suspension from the other subject were mixed. The culture were incubated at 37° C. After 168 h of incubation tritiated thymidine (specific activity 5.0 Ci/mmole, Daiichi Pure Chemicals Corp. Tokyo) was added to each culture to give a concentration of 1 uCi/ml. Two hours later the cultured cells were harvested by the addition of cold saline followed

by immediate centrifugation for 5 min at 30-55 g. Five ml of cold 5% TCA were added to each tube and the contents are stirred with a Vortex mixer. The precipitates were collected by centrifugation and dissolved in 1 ml of 0.1 N NaOH; the precipitation procedure was repeated twice. The final precipitate was washed once with methanol, dissolved in 0.2 ml 2 N NaOH and mixed in a counting vial with 0.7 ml BIO-SOLV BBS-2 (Beckman), one drop of 4% $SnCl_2$ in 0.1 N HCl and 5 ml toluene scintillator. The radioactivity was measured by a liquid scintillation counter (Beckman LS 200). The results are expressed as counts per minute for each sample; the data given are the means of duplicate cultures.

Serum exchange. Usually culture media contained 20% human serum - 10% from each leukocyte donor (autologous serum). For exchanging autologous and homologous sera, the culture tube was centrifuged for 5 minutes at 100 g, the supernatant discarded and the same volume of culture medium containing 20% homologous serum added.

Purification of lymphocytes using a tetron fiber column. A column was prepared by tightly packing 0.7 g of tetron fiber (No. 421, Tore Corp. Tokyo) in a 10 ml disposable syringe. Leukocyte-rich plasma (2 ml) was applied to the sterilized column, which was then washed through with 6 ml of fresh serum. Cells were concentrated by centrifuging the washings at 100 g for 5 min. The rate of recovery of lymphocytes after purification varied from 80-90%.

Results

Some of the results of one-way MLC tests between various kinds of pairs of healthy subjects are shown in Fig. 1. All seven monozygotic twin pairs tested were MLC-identical and their leukocytes did not react in this test. One out of five pairs of dizygotic twins was MLC-identical. Forty out of 158 sib-sib pairs were MLC-identical (25.3%). There were no MLC-identical pairs amongst the parent-child pairs or unrelated pairs. We considered that thymidine uptake less than 300 cpm represented lack of stimulation and counts exceeding 500 cpm represented positive stimulation in our cultures.

The results of MLC reactions between non-uraemic and uraemic subjects are shown in Fig. 2, using uraemic cells as responding cells (left column) and as stimulating cells (right column). Abnormally low uptake was found when uraemic patients were the source either of responding or stimulating cells (U-Nm and N-Um), whereas normal results were obtained when the same non-uraemic stimulating cells or responding cells were cultured with a control non-uraemic partner (N'-Nm, N-N'm).

The effect of uraemic serum on the MLC reaction between unrelated non-uraemic subjects was then studied by replacing autologous serum with

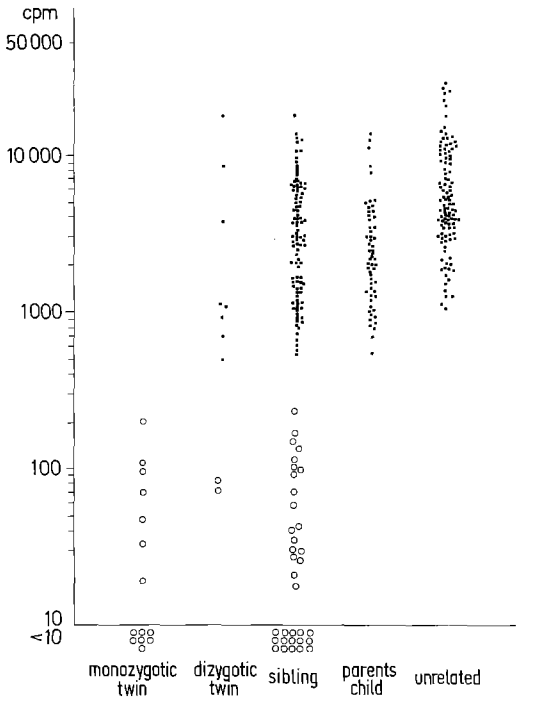

Fig. 1. Results of one way MLC in related and unrelated pairs

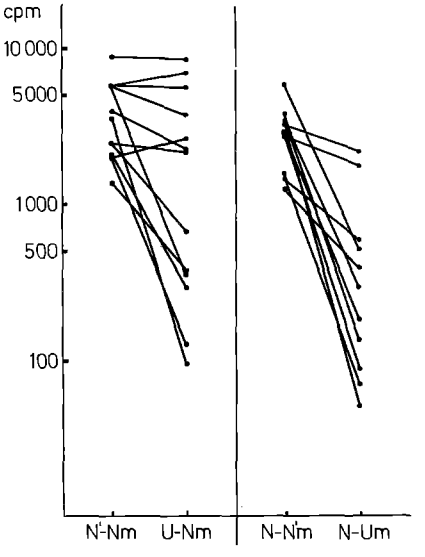

Fig. 2. MLC reaction of uraemic leukocytes with the control of non-uraemic leukocytes

uraemic serum. The results are presented in Fig. 3 (middle column); without exception, stimulation (represented by cpm) was less in MLCs containing uraemic serum. There was no depression of uptake, however, when homologous non-uraemic serum was used instead of autologous serum, as shown in Fig. 3 (left column).

The result of replacing uraemic by non-uraemic

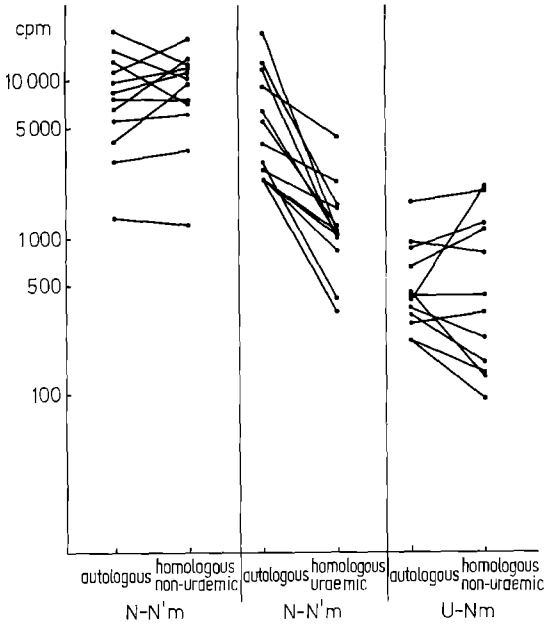

Fig. 3. Influence of serum exchange on MLC reaction

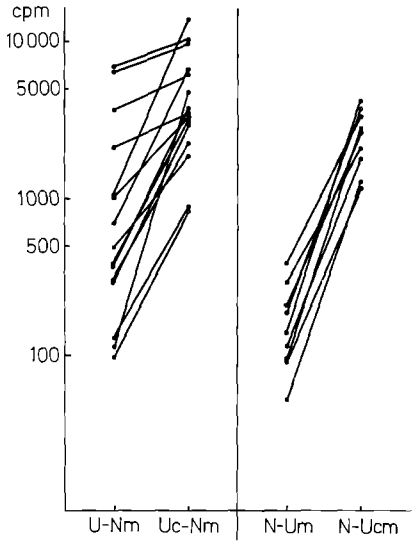

Fig. 4. Influence of lymphocyte purification and serum exchange on MLC reaction between uraemic and non-uraemic subjects

serum in MLC reactions between uraemic and non-uraemic patients is also shown in Fig. 3 (right column). It was exceptional to find an increase in cell stimulation to normal values.

Fig. 4 shows the reactions of purified uraemic responding cells (Uc-Nm) and stimulating cells (N-Ucm) with non-uraemic partner cells in the presence of non-uraemic serum. The thymidine uptake (cpm) found in this series were remarkably high compared to those obtained by the use of unpurified uraemic cells as responding cells (U-Nm) and stimulating cells (N-Um).

Discussion

Kasakura and Lowenstein reported abnormally low MLC reactivity of uraemic leukocytes and showed that uraemic plasma usually depressed MLC reactions between non-uraemic subjects. They also showed that replacing uraemic plasma in cultures by non-uraemic plasma did not restore the depressed thymidine uptake, i.e. leukocyte reactivity, to the normal range in most experiments. They used leukocyte-rich plasma as "responding" cells without any lymphocyte purification procedure, and adjusted the lymphocyte count to approximate equality in the final cell suspensions, as was done here for the MLC tests between uraemic and non-uraemic subjects using unpurified leukocytes. The present results using unpurified leukocytes (Fig. 2) were similar to those obtained by Kasakura and Lowenstein.

A further attempt was made to elucidate the factors responsible for the impaired MLC reactivity of uraemic leukocytes. The leukocyte preparations from uraemic subjects always showed a great preponderance of neutrophils, so lymphocytes from these donors were purified by passing leukocyte-rich plasma (initial lymphocyte content 2-50%) through a tetron fibre column (final lymphocyte content 80-97%). Using the purified lymphocytes and homologous non-uraemic serum, all the uraemic cells showed MLC reactivity comparable to that of non-uraemic leukocytes. It is still uncertain whether uraemic lymphocytes themselves have sightly impaired MLC reactivity, but after purification by the tetron fibre column, all the uraemic leukocyte preparations showed reactivities against unrelated leukocytes which could be accepted as indicating MLC-non-identity. Using a lymphocyte-rich fraction in MLC tests it should be possible, therefore, to select an MLC-identical partner for a uraemic subject from his siblings. The MLC test can be used as a histocompatibility test in uraemic subjects, at least for the selection of a living donor from the siblings of a prospective recipient.

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References

1. Bain, B., Vas, M.R., Lowenstein, L.: The development of large immature mononuclear cells in mixed leukocyte cultures. Blood 23, 108 (1964).
2. Dutton, R.W.: Spleen cell proliferation in response to homologous antigens studied in con-

genic resistant strains of mice. J. exp. Med. 123, 665 (1966).
3. Wilson, D. B.: Quantitative studies on the mixed lymphocyte interaction in rats. J. exp. Med. 126, 625 (1967).
4. Bach, F. H., Kisken, W. A.: Predictive value of results of mixed leukocyte cultures for skin allograft survival in man. Transpl. 5, 1046 (1967).
5. Kasakura, S., Lowenstein, L.: Irradiated and preserved leukocytes in mixed leukocyte cultures. Proc. Soc. exp. Biol. Med. 125, 355 (1967).
6. Bach, F. H., Voynow, N. Y.: One-way stimulation in mixed leukocyte cultures. Science 153, 545 (1966).
7. Bach, F. H., Amos, D. B.: Hu-1: major histocompatibility locus in man. Science 156, 1508 (1967).
8. Singal, D. P., Mickey, M. R., Terasaki, P. I.: Serotyping for homotransplantation. Transplantation 7, 246 (1969).
9. Kasakura, S., Lowenstein, L.: The effect of uremic blood on mixed leukocyte reactions and on cultures of leukocytes with phytohemagglutinin. Transplantation 5, 283 (1967).

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