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COMPARATIVE STUDY OF PROLIFERATIVE ACTIVITY OF HUMAN LYMPHOCYTES FROM DIFFERENT PAIRS OF DONORS IN MIXED CULTURE *IN VITRO*

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Mixed lymphocyte culture (MLC) is a widely used method of studying functional properties of human immunocompetent cells. The level of proliferative response in MLC is characterized by marked individual variability [9]: This phenomenon has received little study and is usually associated with the degree of difference between the partner cells with respect to HLA antigens. However, participation of spontaneous regulator cells in determination of the intensity of the proliferative response in MLC is highly probable [4].

In the investigation described below an attempt was made to analyze the causes of differences in the intensity of the proliferative response of cells in MLC. For this purpose the proliferative response of the lymphocytes in a two-way MLC with the partner cells in different ratios was first investigated. MLC in which the partner cells from two donors were alternately responders and stimulators also were studied in pairs. The effect of factors modulating the functional properties of human lymphocytes (preincubation of lymphocytes *in vitro*; addition of thymosin) also was analyzed in this investigation.

EXPERIMENTAL METHOD

Mononuclear cells (MC) were isolated by centrifugation of heparinized venous blood from healthy blood donors in a Ficoll—Verografin density gradient [1].

Macro- and micromodifications of the two-way MLC technique were used. In the first case separated MC from two different donors were cultured as described previously [4]. When the micromodification of the MLC method was used, MC were cultured in wells in plates used for immunologic tests (from the Leningrad Medical Polymers Factory) in culture medium consisting of 80% medium RPMI-1640 (from Serva, West Germany), 20% human group IV (AB) serum inactivated by heating, and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) and in atmosphere of air with 5% CO_2. The total number of cells in the two-way MLC was $0.2 \cdot 10^6$ MC in 0.15 ml of culture medium in each well. The number of responding and stimulating

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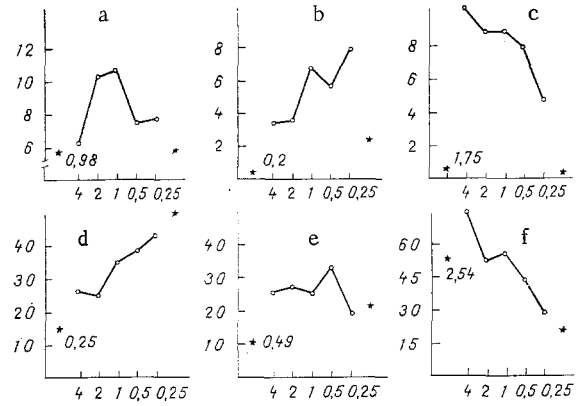

Fig. 1

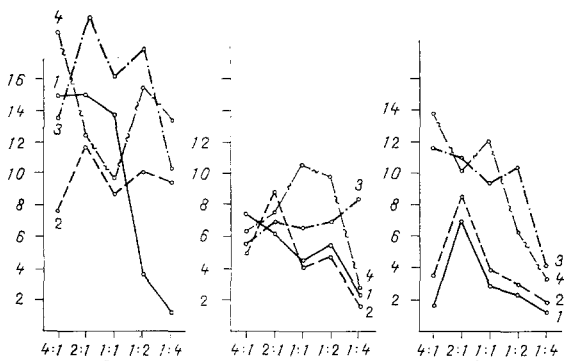

Fig. 2

Fig. 1. Proliferative response of lymphocytes in MLC. Results of six experiments (six pairs of donors) are shown. Abscissa, ratio of number of cells of one donor (A) to number of cells of second donor (B) in two-way MLC; ordinate, intensity of proliferative response in MLC (in cpm•10^{-3}). Values of proliferative response in one-way MLC marked by asterisks: on left — response of cells of donor A (responders) against mitomycin C-treated cells of donor B (stimulators); on right — response of cells of donor B against cells of donor A. Ratio of response in one-way MLC (A anti B) to response in one-way MLC (B anti A) indicated by numbers on left. a) A — donor 1, B — donor 2; b) donors 1 and 3; c) donors 2 and 3; d) donors 4 and 5; e) donors 4 and 6; f) donors 5 and 6, respectively.

Fig. 2. Effect of thymosin on proliferative response of lymphocytes in two-way MLC (macromodification). Results of three experiments shown. Abscissa, ratio between donor and partner cells in MLC; ordinate, intensity of proliferative response (in cpm•10^{-3}). 1-4) Thymosin concentration 0 (control), 0.1, 0.3, and 1.0 mg/ml respectively. Total number of cells $1 \cdot 10^6$/ml in each culture flask.

cells in the one-way MLC was $0.1 \cdot 10^6$ MC in each well. Stimulating cells were treated beforehand with mitomycin C (from Serva, West Germany) in a dose of 30 µg/ml for 40 min. The intensity of proliferation in MLC was estimated on the 5th day of culture radiometrically from incorporation of [^{3}H]thymidine into the nucleoprotein fractions of the cells. Preincubation of the cells was carried out as described previously [4]. Thymosin (IV or fraction of calf thymus extract), obtained by the method in [6], was added to MLC at the beginning of culture in doses of 0.1, 0.3, and 1.0 mg/ml.

EXPERIMENTAL RESULTS

Analysis of the proliferative response of the cells in two-way MLC showed that the intensity of proliferation was highest as a rule in cultures containing unequal numbers of MC from donors and partners (Fig. 1b-f; Fig. 2). Different values of the proliferative response were obtained in one-way MLC containing lymphocytes from one pair of donors, but performing functions of responders and stimulators alternately (Fig. 1). Comparison of proliferative activity of cells from one pair of donors showed that a peak of proliferation in two-way MLC is observed only in cultures containing an excess of both lymphocytes which, when used in one-way MLC as responders, gave a stronger response (than in the case of responding cells consisting of their partner lymphocytes in MLC). Lymphocytes from different donors thus possess different proliferative activity in MLC; predominance of functional activity of cells from one of two donor-partners as a rule is found, moreover, in both one- and two-way MLC.

Thymus extract in culture *in vitro* is known to modify the functional properties of lymphocytes [8]. The effect of thymosin on the proliferative response was studied in two-way MLC with different ratios between partner cells. Thymus extract in concentrations of 0.3 and 1 mg/ml was found to stimulate cell proliferation, mainly in cultures with a low initial (control) level of response. As a result of this, the initially asymmetrical curve of intensity of proliferation as a function of ratio between partner cells in MLC became equalized. In other words, thymosin stimulates activity of cells with a low initial proliferative potential.

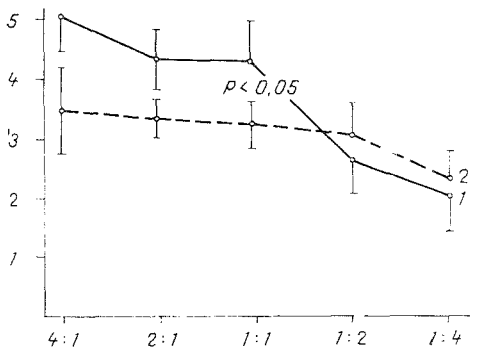

Fig. 3. Effect of preincubation of lymphocytes on intensity of proliferative response in two-way MLC (macromodification). Abscissa, ratio between cells from donors and partners in MLC. Total number of cells $1 \cdot 10^6$/ml in each culture flask. Ordinate, intensity of proliferative response (in cpm$\cdot 10^{-3}$). 1 and 2) Proliferative response of freshly isolated and preincubated cells respectively; P) significance of differences between responses of freshly isolated and preincubated cells (Wilcoxon's paired T test).

Previous investigations showed that preincubation of human lymphocytes for 18 h in culture *in vitro* is accompanied by changes in activity of proliferation-regulating cells. One of the most probable mechanisms of the effect of preincubation is differentiation of young post-thymic precursor T cells into mature lymphocytes with different functional properties [3, 7]. Preincubation of MC before testing in two-way MLC is accompanied (just as on addition of thymosin) by equalization of the curve of intensity of proliferation as a function of ratio between partner cells (Fig. 3). However, equalization was achieved (unlike with the action of thymosin) by a decrease in the intensity of initial proliferation in cultures with the most marked initial response.

These results indicate that the intensity of the proliferative response in MLC is determined not only by differences between the HLA antigens of the cells, but also by functional properties of lymphocytes of each donor-partner. Cells of one of them in this case are more active as responding lymphocytes. Incidentally differences described in the intensity of the response probably cannot be attributed purely to individual variations in the number of stimulating (B lymphocytes, monocytes) and responding (T lymphocytes) cells, for even after a considerable change in the ratio between partner cells in two-way MLC (from 1:1 to 19:1) the proliferative response, as was shown previously, is comparable in intensity [5]. There is no doubt that changes in quantitative content and relative numbers of responders and stimulators in this particular experimental system considerably exceed the maximal possible variability in the ratio between T and B cells in human peripheral blood. These investigations also showed that experimental procedures affecting activity of the regulator cells (preincubation, thymosin) modify the intensity of proliferation and equalize activity of the partner lymphocytes in MLC. It can consequently be postulated that spontaneous (i.e., not induced) helper and (or) suppressor cells participate in determination of the intensity of the proliferative response. It must be pointed out that thymosin and preincubation have opposite actions on the intensity of the proliferative response in two-way MLC. This fact confirms the antagonistic effect of these experimental procedures, discovered previously, on activity of nonspecific spontaneous suppressor cells [2, 10].

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