EFFECT OF PRELIMINARY INCUBATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES ON INTENSITY OF TRANSFORMATION INDUCED BY PHYTOHEMAGGLUTININ

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Preliminary incubation of lymphocytes in a leukocyte suspension or isolated in a Verografin-Ficoll gradient at 37°C leads to more intensive blast transformation in response to stimulation by phytohemagglutinin (PHA). Preincubation in the presence of soy trypsin inhibitor (500 μg/ml) abolishes this effect. The results suggest that neutral proteinases of neutrophils or monocytes participate in modification of the response to PHA.

KEY WORDS: lymphocytes; transformation; phytohemagglutinin.

An increase in the intensity of activation of human peripheral blood lymphocytes, stimulated by mitogens or allogeneic cells after preliminary culture [5], was described in 1978.

Since the results of those investigations could make a significant contribution to the understanding of the mechanisms regulating lymphocyte activity, in the present investigation the effect of preliminary incubation of human peripheral blood lymphocytes on the intensity of their transformation by phytohemagglutinin (PHA) was studied.

EXPERIMENTAL METHOD

A leukocyte suspension was obtained from donors' blood. Erythrocytes were sedimented by the addition of gelatin solution (final concentration 1%). Lymphocytes were isolated from the leukocyte suspension by Boyum's method [2]. The lymphocytes were cultured and radioactive specimens prepared as described in [1]. The cell concentration for lymphocyte culture in a leukocyte suspension was 1×10^6/ml, and for culture of lymphocytes isolated in a Verografin-Ficoll gradient it was 0.25×10^6 cells/ml. Lymphocytes, monocytes, and other mononuclear cells accounted for 40-50% of the cells in the leukocyte suspension and not less than 95% after isolation in a Verografin-Ficoll gradient (d = 1.078). The term "leukocytes" will be used for lymphocytes in the leukocyte suspension, and the term "lymphocytes" to describe cells isolated from the leukocyte suspension by centrifugation in a Verografin-Ficoll gradient. A pool of sera (from three group AB donors) was used in the experiments and fetal calf serum was obtained from Sigma. The sera were inactivated by heating at 56°C for 40 min.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that preliminary incubation of the lymphocytes, whether isolated in a Verografin-Ficoll gradient or contained in a leukocyte suspension, stimulated the response to PHA in all cases. This effect was most marked in variants of the experiment in which suboptimal PHA concentrations were used to stimulate the lymphocytes.

The kinetics of activation of lymphocytes preincubated for 20 h and of the control cultures was similar, and maximal incorporation of thymidine-^{3}H into lymphocyte DNA was demonstrated 64-68 h after stimulation.

Replacement of the medium after preincubation was the essential condition for successful transformation. In experiments without a change of medium the level of transformation was practically indistinguishable in the control and experimental cultures (Fig. 1).

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TABLE 1. Dependence of Intensity of Lymphocyte Transformation on Conditions of Preliminary Incubation and PHA Concentration (in cpm per culture)

Experimental conditions	Leukocytes			Lymphocytes		
	PHA concentration, μg/ml					
	1,0	2,5	5,0	1,0	2,5	5,0
Control (cells stimulated by PHA immediately after isolation from blood):						
experiment No. 1	3 370	41 830	57 200	15 940	43 700	36 420
experiment No. 2	1 080	9 920	17 980	1 930	27 780	33 580
experiment No. 3	7 960	43 040	69 060	—	—	—
Cells preincubated for 20 h at 37°C						
experiment No. 1	12 500	99 000	87 660	32 500	52 900	45 250
experiment No. 2	2 850	40 140	37 880	4 610	50 080	51 400
experiment No. 3	11 480	87 360	111 018			
Cells preincubated for 20 h at 4°C						
experiment No. 1	2 850	39 480	60 300	—	—	—
experiment No. 3	8 310	51 710	65 000	—	—	—
Cells preincubated for 20 h at 37°C in the presence of soy trypsin inhibitor (500 μg/ml):						
experiment No. 2	—	10 220	15 340	—	—	—
experiment No. 3	—	44 900	66 500	—	—	—

Legend. Mean values of five determinations shown. Coefficient of variation $[(\sigma/\mu) \cdot 100]\%$ for each mean does not exceed 12%.

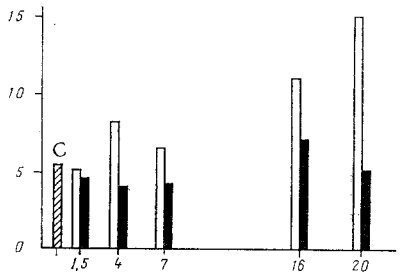

Fig. 1. Dependence of intensity of lymphocyte transformation stimulated by PHA (5 μg/ml) in a leukocyte suspension on duration of preincubation in experiments with and without change of medium. Abscissa, duration of preincubation (in h); ordinate, intensity of lymphocyte transformation (in cpm per culture $\times 10^{-4}$). C) Control; unshaded columns – with change of medium; black columns – without change of medium. Mean of five determinations. Coefficient of variation for each mean did not exceed 10%.

Dependence of the intensity of transformation of lymphocytes in a leukocyte suspension on the duration of preincubation is shown in Fig. 1. In this particular experiment incubation for 4 h was long enough to reveal intensification of transformation in response to subsequent stimulation. This time was not strictly fixed and could vary somewhat (3-6 h) in different experiments.

The results of investigation of the effect of the preincubation temperature on the intensity of lymphocyte transformation are shown in Table 1. Only when the lymphocytes were preincubated at 37°C was transformation intensified; preincubation at 4-6°C for 20 h had no effect on the intensity of the response.

Stimulation was always more marked in experiments with preincubation of lymphocytes in a leukocyte suspension than of lymphocytes isolated in a Verogafin-Ficoll gradient. This fact, and also dependence of the effect on the temperature conditions of preincubation suggested that proteinases of neutrophils and monocytes participate in modification of the response to PHA.

Preincubation of lymphocytes in the presence of soy trypsin inhibitor, which can interact with neutral proteinases of neutrophils [3, 7, 9], abolished the effect of intensification of transformation.

The results described in this paper were obtained in experiments in which lymphocytes were preincubated in medium No. 199 containing 5% inactivated human serum. They do not agree with the observations of McCombs et al. [5], who did not observe any increase in the intensity of transformation after preincubation in medium containing human serum, but did observe an effect after incubation in medium with 5% inactivated fetal calf serum. The reason may perhaps be the different duration of preincubation of the lymphocytes, which was 7 days in the experiments of McCombs et al. Under our experimental conditions, intensification of the response to PHA of comparable magnitude was obtained after preincubation both in medium containing inactivated human serum and in medium containing fetal calf serum.

So far as mechanisms are concerned, the action of proteinases of the accessory cells may be directed toward modification of lymphocyte membrane components and (or) may lead to the formation of suppressor peptides [4, 6, 8, 10].

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