BLAST TRANSFORMATION OF LYMPHOCYTES INDUCED BY A MONOLAYER OF MACROPHAGES TREATED WITH VARIOUS MITOGENS

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A macrophage monolayer treated with phytohemagglutinin P or streptolysin O induced blast transformation of autologous lymphocytes during combined cultivation. The intensity of the effect depended on the mitogen used and the age of the monolayer.

Macrophages have been shown to play an important role in the first stages of the immune response [2, 3]. The role of macrophages in blast transformation of peripheral lymphocytes as a response of immunocompetent cells in vitro has been confirmed by several investigations. Ability to undergo blast transformation during incubation with specific antigens is reduced in pure peripheral blood lymphocytes obtained after removal of phagocytic cells, while the normal blast transformation response to phytohemagglutinin (PHA) is preserved. On addition of macrophages, the normal response of the lymphocytes to specific antigens is restored [9, 10]. The mechanisms of interaction between macrophages and lymphocytes are not clear.

In the investigation described below the possibility of induction of blast transformation of lymphocytes by means of a monolayer of autologous macrophages, treated with various mitogens, was studied.

EXPERIMENTAL METHOD

All tests were carried out with human peripheral blood leukocytes obtained from healthy donors whose lymphocytes were shown to be capable of transformation under the influence of PHA and streptolysin O (SLO).

A monolayer of macrophages was grown on strips of coverslips in Leighton's tubes by the method of Hersh and Harris [8]. The proportion of macrophages in the monolayer was 98-99%. Monolayers of macrophages grown for 6-7 and 9-10 days were tested. Monolayers of macrophages grown for 9-10 days were indistinguishable in density from those grown for 6-7 days and from monolayers of embryonic fibroblasts.

The lymphocyte suspension was obtained by the method of Coulson and Chalmers [6]. The proportion of lymphocytes in the suspension was 76.7 ± 5.7%. Lymphocytes were cultivated in order to obtain blast transformation by a modified method of Bach and Hirschhorn [5]. The following mitogens were tested: PHA-P and SLO (Difco, USA).

Tests of the inducing ability of the macrophages were carried out as follows. Mitogenic doses of PHA (0.002 ml) or SLO (0.1 ml) were added for 3 h to the culture of macrophages. The macrophage layer was then washed with three batches of medium no. 199, each 1.5-2 ml in volume, to remove the mitogen, and 2 ml of a suspension of unstimulated autologous lymphocytes, containing 1 million cells per ml, was added to each tube with the macrophages.

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TABLE 1. Induction of Blast Transformation of Lymphocytes by Monolayer of Macrophages Treated with SLO or PHA

Test	Transformation of unstimulated lymphocytes (in %)	Transformation of lymphocytes on unstimulated monolayer (in %)*	SLO transformation of lymphocytes (in percent)*			PHA transformation of lymphocytes (in percent)*		
			Direct stimulation	Induction by means of treated monolayer	Index of induction†	Direct stimulation	Induction by means of treated monolayer	Index of induction†
Use of 6-7-day monolayer								
1	4,6	3,8	38,4	47,5	+23,6	—	—	—
2	1,4	2,1	34,6	40,6	+17,3	—	—	—
3	1,8	—	19,3	26,7	+38,3	71,1	34,1	—52,1
4	2,5	—	16,6	23,6	+42,1	68,1	33,8	—50,4
5	3,7	3,4	16,4	22,4	+36,5	60,4	21,6	—64,3
6	2,3	1,4	15,8	19,2	+21,5	58,1	40,2	—30,9
7	2,8	—	15,7	16,7	+6,4	—	—	—
				$M \pm m = +26,5 \pm 4,9$			$M \pm m = -49,4 \pm 6,9$	
				$P < 0,01$			$P < 0,01$	
Use of 9-10-day monolayer								
3	1,8	—	19,3	19,2	—0,5	71,1	45,4	—36,2
4	2,5	—	16,6	14,3	—13,8	68,1	52,6	—22,8
8	2,8	—	34,2	14,5	—57,0	66,5	48,5	—27,1
9	1,2	—	21,2	18,4	—13,2	63,2	39,1	—38,1
10	3,6	—	14,5	13,8	—4,8	—	—	—
				$M \pm m = -17,9 \pm 10,1$			$M \pm m = -31,1 \pm 3,6$	
				$P > 0,01$			$P < 0,01$	

*Mean value of transformation in two cultures of lymphocytes.
† Difference between degrees of blast transformation induced by macrophages and obtained by direct stimulation expressed as a percentage of the latter.

The following controls were set up:

1) lymphocytes cultivated on a macrophage monolayer untreated with mitogen;

2) a macrophage monolayer treated with mitogen without the addition of lymphocyte suspension;

3) lymphocytes cultivated on a monolayer of human embryonic fibroblasts treated with the same mitogens and by the same method as the macrophage monolayers;

4) cultures of lymphocytes without a macrophage monolayer stimulated by SLO, by PHA, or unstimulated.

The blast transformation reaction was read in cultures stimulated with PHA on the 3rd day and in cultures stimulated with SLO on the 5th day. After the end of cultivation films were prepared from the cell suspension and stained, and the percentage of blast cells and of mitoses was determined under the microscope by the examination of 1000 cells.

Each result was calculated as the mean number of blast transformations in two duplicated cultures. If the reactions in the two cultures differed sharply from each other (for SLO $m > 4$, for PHA $m > 6$), the result was disregarded because of the possible intervention of irrelevant factors.

EXPERIMENTAL RESULTS

The results showed that a macrophage monolayer, if stimulated either by SLO or by PHA and then washed to remove the mitogen, induces blast transformation of autologous lymphocytes which have not been in contact with the mitogen when cultivated on the same monolayer (Table 1).

Induced transformation of lymphocytes by stimulated macrophages always exceeded the spontaneous blast transformation of lymphocytes cultivated in the absence of the mitogen by many times. During analysis of the results, the intensity of blast transformation of lymphocytes induced by a monolayer treated with the mitogen was therefore compared with the degree of transformation obtained by direct stimulation of the lymphocytes with the corresponding mitogen (without the monolayer).

As Table 1 shows, the level of transformation induced by the macrophage monolayer depended on the type of mitogen with which the monolayer was treated and on the time of cultivation of the monolayer.

A 7-day monolayer of macrophages stimulated by SLO induced transformation of lymphocytes to a degree greater than the blast transformation response of the lymphocytes to SLO with direct stimulation by the mitogen by 26.5 ±4.9% ($P < 0.01$). During stimulation of a similar monolayer by PHA, induction of blast transformation was observed in the lymphocytes but its intensity did not reach the level of blast transformation obtained by direct stimulation of the lymphocyte by PHA (the reaction was reduced by 49.4 ± 6.9%; $P < 0.01$).

With an increase in the age of the monolayer used to 9-10 days the induction phenomenon continued to be exhibited when the macrophages were treated by both mitogens, but in this case the reaction of the lymphocytes to treatment of the monolayer by SLO was not significantly different from the level of transformation by direct stimulation ($P > 0.1$). Transformation induced by PHA remained at the same level as when the 7-day monolayer was used (decrease of 31.1 ±3.6%; $P < 0.01$ compared with the direct reaction).

In lymphocytes cultivated on an unstimulated monolayer no difference was found in the level of blast transformation by comparison with the control culture without stimulator. When a macrophage monolayer stimulated by SLO or PHA was grown without the addition of lymphocytes, no blast transformation was observed.

During the examination of the phenomenon of transmission of the stimulus to lymphocytes by means of a macrophage monolayer, the possibility could not be ruled out that the lymphocytes may have been stimulated by residual amounts of mitogen resulting from its incomplete removal from the monolayer. To test this hypothesis control experiments were carried out in which the macrophage monolayer was replaced by a monolayer of embryonic fibroblasts. Cultivation of the lymphocytes on this monolayer, whether treated with SLO or with PHA, was accompanied by blast transformation to an intensity which did not differ significantly from the level of transformation of the unstimulated lymphocytes.

It can be concluded from these results that the phenomenon of induction of lymphocytes is connected with the biological function of the macrophages, although its mechanism is at present difficult to explain. In accordance with views regarding the role of macrophages deduced from experiments using other model systems [1, 4, 7] it can be postulated that in the system used in the present investigation induction took place through processing of the mitogen within the macrophages and the transmission of immunogenic material to the lymphocytes. The possibility of transmission of mitogen fixed on macrophages with special surface properties to the lymphocytes likewise cannot be ruled out.

The difference in the ability of the monolayer to induce transformation of lymphocytes when treated with PHA and SLO could be the result of differences in the response of these mitogens to processing by macrophages and also to differences in the course of the specific and nonspecific reactions of the lymphocytes during the appearance of additional contact resulting from the presence of large numbers of macrophages in the culture.

The decrease in the inducing power of the macrophages with increasing age of the monolayer was probably due to morphological transformation of the cells. Changes in macrophage morphology were observed with an increase in the age of the monolayer: the number of polynuclear cells increased, and giant cells measuring 100-200 μ and fibroblasts appeared. Evidently macrophages exhibit an immunogenic function only at certain stages of their development.

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