REACTIVITY OF HUMAN FETAL LYMPHOCYTES CULTIVATED IN VITRO

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The intensity of the blast-transformation reaction in a mixed culture of adult human lymphocytes is directly dependent on the degree of antigenic differences between the cultivated cells. Spleen cells from human embryos at the 16-28-week stage in mixed culture and in the presence of stimulators underwent the same differentiation as adult human lymphocytes. Fetal thymocytes were unable to undergo blast transformation.

The question of the immunologic competence of embryonic lymphocytes has not been adequately studied, and the answer remains in doubt. It is assumed that, because of immunologic immaturity of the fetal lymphocytes, injection of an antigen may bring about subsequent immunologic tolerance. On the other hand, investigations have shown that human fetal lymphocytes at the 20-24-week stage are capable of antibody formation and blast transformation in vitro [8, 9].

The object of this investigation was to compare the ability of lymphocytes from human fetuses aged 16-28 weeks and from adults to undergo morphological transformation. The blast transformation reaction (BTR) was used to investigate human fetal spleen cells and thymocytes and adult human circulating lymphocytes and thymocytes.

EXPERIMENTAL METHOD

The technique of the BTR was that used previously [2]. The results in monoculture were read on the 3rd day, and in mixed culture on the 7th day. In monoculture Difco phytohemagglutinin M (PHA) and antilymphocytic serum (ALS) obtained by immunization of rabbits with human thymocytes as described in [1] were used to stimulate blast formation.

EXPERIMENTAL RESULTS

There is still no direct evidence of any direct relationship between the immunologic competence of cells and their ability to undergo transformation into blasts in vitro. With this in mind, a preliminary study was made of the ability of lymphocytes from persons closely related genetically to undergo blast transformation.

The intensity of the BTR shows a reasonably clear dependence on genotypic and antigenic differences between individuals (Table 1). These results agree with those observed by other workers [4, 5, 7]. The immunologic nature of the BTR is also confirmed by the ability of blast cells to produce globulin [3, 6]. These facts suggest that the BTR in a mixed culture of lymphocytes reflects cell differentiation characteristic of the immunologic reaction. Consequently, the BTR can be used as an index of the immunologic reactivity of the cells.

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TABLE 1. BTR in Mixed Culture of Human Circulating Blood Lymphocytes

Group of subjects	Number of observations	Mean value of BTR and limits of variations in different investigations (in %)
Uniovular twins	6	0
Binovular twins	4	4.2 ± 0.84 (3.5-5.0)
Unrelated persons	100	17.3 ± 4.2 (4.0-42.0)

TABLE 2. Results of BTR in Mixed Culture of Fetal Lymphocytes

Cells	BTR (in %)	
	first and second donors	third and fourth donors
Spleen cells + spleen cells	12.5	8.0
Spleen cells + thymocytes	4.1	4.0
Thymocytes + spleen cells	5.7	Not tested
Thymocytes + thymocytes	0	0

TABLE 3. BTR in Monoculture of Human Adult and Fetal Lymphocytes

Source of cells	Number of investigations	BTR (in%)	
		with PHA	with ALS
Fetus			
spleen	8	32 ± 10.2	41 ± 12.2
thymus	8	0	0
Adults			
lymphocytes	22	42 ± 5.4	30 ± 5.1
thymus	2	27 ± 6.2	Not tested

In the experiments of series II the ability of embryonic lymphocytes obtained from two pairs of fetuses to undergo transformation was studied. The ages of the fetuses were as follows: first donor 28 weeks, second 20, third 16, and fourth 28 weeks.

The results given in Table 2 demonstrate that, unlike spleen cells, thymocytes of fetuses of this age cannot undergo blast transformation. When cells of both types were cultivated together, the intensity of the BTR was very low, and the reaction probably took place on account of differentiation of the spleen cells.

Similar results were obtained also in monoculture studies of the reactivity of lymphocytes. In the presence of PHA and ALS, fetal spleen cells and adult human lymphocytes and thymocytes underwent the characteristic transformation which was not observed in a monoculture of fetal thymocytes.

The results indicate that the ability of fetal lymphocytes to react by specific morphological transformation in the presence of a foreign antigen depends on the origin of these cells. Thymocytes from a 16-28-week human fetus were unable to react both in monoculture and in mixed culture. By contrast, fetal spleen cells of the same age were transformed in the BTR in the same way as adult human lymphocytes. In turn, the BTR of lymphocytes in mixed culture was directly proportional to the degree of isoantigenic difference between the individuals.

The results obtained (Table 3) cannot be reconciled with the view that the entire fetal lymphoid system is immunologically completely nonreactive. If it is assumed that the BTR reflects the primary immunologic reaction of competent cells, it then follows that tolerance must be the result of an active immunologic state, and not of the action of the antigen on a functionally immature lymphoid tissue.

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