INVESTIGATION OF ACTIVITY OF ANTILYMPHOID SERA IN CULTURES OF HUMAN CIRCULATING BLOOD LYMPHOCYTES

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Individual heterologous antilymphoid sera act differently on the blast-transformation reaction in mixed cultures of homologous human circulating blood lymphocytes. The effect of the antilymphoid sera depended on their activity in a lymphocyte monoculture.

Heterologous antisera against lymphocytes (ALS) or thymocytes (ATS) of a recipient give rise to a marked immunodepressive effect during allografting [1, 2, 7-10, 12]. A convenient working model for studying the mechanism of action of antilymphoid sera on lymphoid cells is a culture of circulating blood lymphocytes. In the presence of ALS, mitotic activity of lymphocytes in culture is stimulated [3, 5, 6, 11]. However, there is no information in the literature concerning the effect of ALS on a mixed culture of lymphocytes.

The object of this investigation was to study the action of various heterologous antisera against human lymphoid cells on monocultures and mixed cultures of circulating blood lymphocytes.

EXPERIMENTAL

Antisera were obtained by immunizing rabbits and horses with a suspension of human fetal lymphocytes or thymocytes. The scheme of immunization and method of obtaining antisera were described by the writers previously. Altogether 8 antisera were obtained for testing: 4 rabbit ATS, 2 rabbit ALS, and 2 horse ALS. The antibody titer in the antisera was tested by the lymphocyte-agglutination reaction (LAR) and the lymphocytotoxic test (LCT).

The blast-transformation reaction in monocultures and mixed cultures of lymphocytes (BTR) was carried out by the writers' modification of the method described in [4]. Monocultures and mixed cultures of homologous lymphocytes obtained from 23 apparently healthy persons were studied.

The activity of the antilymphoid sera was estimated by their ability to cause blast transforming, cytotoxic and leukocyte-agglutinating effects in vitro. The cytotoxic effect was assessed quantitatively from the relative number of lysed and degenerated cells (under 10% +, 10-20% ++, 30-40% +++, 50-60% ++++, and 60% +++++). The effect of the antisera on the BTR in a mixed culture was assessed from the change in intensity of reaction of cells preliminarily tested with ALS or ATS.

In all series of experiments the following standard scheme of treatment of lymphocytes was used. Whole antiserum, in a dose of 0.1 ml per million lymphocytes, was added to the monoculture. Treatment of the lymphocyte partners during cultivation was carried out in the presence of a dose of 0.2 ml per million cells. After incubation for 30 min at 37°, the cells were washed with medium No. 199 and mixed in the proportion of 1:1 for subsequent cultivation by the usual scheme.

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TABLE 1. Activity of ALS and ATS in Vitro

Antiserum No.	Antiserum	No. of tests	Activity in monoculture: mean values of BTR (in %)	Activity in monoculture: cytotoxic effect	Titer of antibodies: cytotoxic	Titer of antibodies: leukocyte-agglutinating
1	Rabbit ALS	16	69± 6,1	±	1:64	Not determined
2	" ATS	4	50±12,2	+	1:256	" "
3	" ALS	2	35±0	+	1:256	" "
4	" ATS	3	20±2,2	++	1:32	" "
5	" "	4	19±5,4	++	1:32	" "
6	» »	2	15±4,3	+++	1:32	" "
7	» »	3	4±9,5	+++	1:128	" "
8	Horse ALS	13	3±3,1	++	1:256	1:320
9	" "	14	0	+++	1:320	1:320

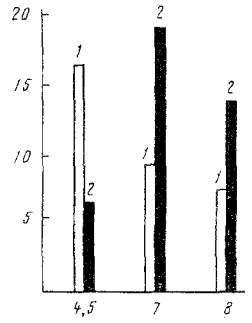

Fig. 1. Changes in BTR in mixed culture of homologous lymphocytes treated with ALS or ATS. Abscissa: No. of individual antisera with which lymphocytes were treated; ordinate: BTR (in %); 1) BTR in mixed culture of native lymphocytes; 2) BTR in mixed culture of the same lymphocytes but preliminarily treated with antiserum.

The reaction in a mixed culture of lymphocytes, both treated and untreated with antiserum, was read on the 6th day. Activity of the antisera in the monoculture was estimated on the 3rd day of cultivation. All test sera were inactivated by heating them at 56° for 30 min.

The following controls were set up: 1) a monoculture of lymphocytes in the presence of autologous serum; 2) a monoculture of lymphocytes in the presence of a nonimmune heterologous serum (rabbit or horse); 3) a monoculture of lymphocytes preliminarily treated with antiserum and washed with medium No. 199; 4) a monoculture of rabbit or mouse lymphocytes in the presence of the test antisera; 5) a mixed culture of human homologous lymphocytes, not treated with antiserum.

EXPERIMENTAL RESULTS

The addition of ALS and ATS to a monoculture of lymphocytes caused agglutinating, blast-transforming, and cytotoxic effects (Table 1).

With a decrease in transforming activity of the antisera, their cytotoxic action increased (Table 1). No strict correlation could be found between the cytotoxic activity of antisera in monoculture and the titers of leukocyte antibodies. Heating the serum appreciably reduced the cytotoxic effect in a culture of lymphocytes. Of all the antisera tested, horse serum (No. 9) had the greatest cytotoxicity, and caused virtually total death of the cells in the monoculture.

Heterologous nonimmune sera, inactivated by heating to 56° for 30 min, caused weak cytolysis and gave no transforming effect. The antisera possessed species-specificity, so that their addition to a monoculture of mouse or rabbit lymphocytes caused no changes other than a weak cytolytic action.

It follows from these results that the properties of individual heterologous antilymphoid sera differ when tested in monoculture. The preliminary results obtained by testing Soviet and imported specimens of immune globulins isolated from ALS indicate that they also may possess different cytotoxic and blast-transforming activities in a lymphocyte culture in vitro. These differences evidently depend on many factors: the scheme of immunization, the characteristics of the antigen used, the species of animal from which the antiserum was obtained, and so on. Similar observations have been made previously by the writers during experiments in vivo after injection of various antisera into rabbits with skin allografts [2].

To investigate the relationship between the cytotoxic and transforming activities of the immune antisera, a series of tests was carried out using different dilutions. With increasing dilution of antiserum No. 9 (Table 1) to 1:20, a gradual decrease in its cytotoxic activity and an increase in its ability to stimulate the BTR were observed, after which both effects diminished. In experiments in which antisera Nos. 4 and 5 were diluted (Table 1), the gradual disappearance of both their transforming and their cytotoxic activity was observed. On the basis of these results it can be postulated that the change in the quantitative relationship between the studied parameters during dilution of ALS and ATS is characteristic only of the most active sera.

In whole sera Nos. 4 and 5 the content of transforming and cytotoxic factors was evidently at the threshold level, so that a 1:10 dilution led to the complete disappearance of these properties.

In the experiments of series II the BTR was studied in a mixed culture of human lymphocytes preliminarily treated with ALS or ATS. The control monoculture of lymphocytes treated with antiserum was virtually indistinguishable from a monoculture of untreated lymphocytes. The exception was horse ALS No. 9, which, because of its marked toxicity, caused considerable destruction of cells even after treatment for only 30 min. The lymphocytes thus underwent the characteristic morphological transformation only in the presence of antiserum in the culture. However, in a mixed culture of homologous cells, preliminary contact between lymphocytes and antiserum, even for only a short time, affected their ability to undergo morphological transformation (Fig. 1). Antisera with marked blastogenic and cytotoxic action in monoculture (No. 1, 2, 9; Table 1) caused death of lymphocytes in a mixed culture. Rabbit ATS (Nos. 4, 5), giving a mean transformation of 20% and a moderate cytotoxic effect in a monoculture of lymphocytes (++), lowered the BTR in a mixed culture by more than half. Horse ALS No. 8 and rabbit ATS No. 7, which in monoculture caused weak transforming and marked cytotoxic effects, almost doubled the BTR in a mixed culture. These results suggest that the effect of immune heterologous antisera depends on the ratio between the content of transforming and cytotoxic factors. It should be noted that the character of the change in BTR was independent of the test system, for when the same antiserum was used during cultivation of different populations of lymphocytes, similar results were obtained.

Hence, in a mixed culture antilymphoid sera can produce both stimulation and inhibition of the BTR. The best situation for manifestation of the immuno-depressive effect on the BTR in mixed culture is evidently that in which both the transforming and cytotoxic activities of the antisera are sufficiently strong. However, the final answer to the question of optimal parameters of biological activity of antilymphoid sera can only be obtained after a parallel investigation of different antisera in vivo and in vitro.

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