ABILITY OF ANTIBODY-FORMING CELLS CULTIVATED IN VITRO TO REACT WITH SPECIFIC ANTIGEN

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The ability of immune lymphocytes, cultivated in vitro, to exhibit the phenomenon of rosette formation was studied. Despite the preservation of large numbers of direct and indirect plaque-forming cells in the culture, the lymphocytes completely lost their ability to form rosettes during incubation with sheep's erythrocytes. On the 2nd and 4th days after transfer of 10^7 cells cultivated in vitro into the spleen of normal syngeneic recipients, the number of direct plaque-forming cells was 40 and 14 times, respectively, higher than the number of "background" cells. However, the number of rosette-forming cells in the recipient's spleen was unchanged as a result of the transfer.

A previous investigation showed that under certain conditions of cultivation in vitro, many hemolysin-synthesizing cells can survive for a long time in suspensions of lymphocytes isolated from the spleen of immunized mice [1].

The object of the present investigation was to study the ability of immune lymphocytes, when cultivated in vitro, to exhibit the phenomenon of rosette formation.

EXPERIMENTAL METHOD

A suspension of lymphocytes was obtained on the 4th day after primary and secondary (at intervals of 40 days) intraperitoneal immunization of CBA mice with 5% sheep's erythrocytes in a dose of 0.5 ml. The cells were grown on Hottinger's tryptic digest or on Eagle's medium with the addition of hydrocortisone and insulin. The method of cultivation is described elsewhere [1, 2]. At different times the number of direct plaque-forming cells was determined in the suspension by the method of Jerne and Nordin [4]. Rosette-forming cells were identified by Zaalberg's method [5]. In cultures obtained at the height of the secondary immunological response, the number of indirect plaque-forming cells also was determined by the method of Dresser and Wortis [3]. Altogether, 24 independent cultures were investigated. Spleen cells obtained from once-immunized animals and cultivated for 7 days were injected intravenously into normal syngeneic recipients, and the number of direct plaque-forming and rosette-forming cells was determined in these animals at different times after the transfer. In each experiment 5-7 mice were studied.

EXPERIMENTAL RESULTS

During cultivation of a suspension of lymphocytes in Hottinger's digest after their removal at the height of the primary immunological response, the number of plaque-forming cells was reduced by almost half on the 1st day of incubation. Later their number became stabilized, and on the 5th-7th day an increase in antibody formation actually was observed. At these times the number of antibody-forming cells per 10^6 nucleated cells was 501 and 672, respectively. In cultures obtained at the height of the secondary response, the decrease in number of hemolysin-synthesizing cells and, in particular, in the number of indirect

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plaque-forming cells, was greater, but on the 5th-7th days their number gradually increased to 59 direct and 209 indirect per 10^6 nucleated cells.

Rosette-forming cells were absent throughout the period of cultivation both in cultures isolated from primarily immunized animals and from animals receiving a second injection of antigen. Similar results were obtained when the cells were cultivated in Eagle's medium.

On the 2nd day the number of hemolysin-synthesizing cells in the spleen of recipients receiving 5 million and 10 million cultivated cells was 2475 and 4186, respectively. On the 4th day their number was reduced by 3-4 times. On both the 2nd and the 4th days the number of rosette-forming cells in the recipient's spleen was no higher than the background level.

Consequently, when cultivated on Eagle's medium with the addition of hormones and on Hottinger's digest the antibody-forming cells thus completely lost their ability to react with the specific antigen, as reflected in the phenomenon of rosette formation. This may be connected with certain changes in the surface membranes of the antibody-forming cells when cultivated in vitro.

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