also the incidence of spontaneous leukemia among them is evidence that failure of one component of immunity does not always lead to an increased risk of tumor development in vivo. The results of these experiments are in harmony with the view that relations between immunity and carcinogenesis are complex and not always consistent in character [1, 3, 4, 6, 7].

LITERATURE CITED

1. V. M. Bergol'ts, The Problem of Leukemia [in Russian], Moscow (1973).
2. V. I. Levin, in: Proceedings of the 2nd Republican Congress of Hematologists and Transfusion Specialists of White Russia [in Russian], Minsk (1973), pp. 49-52.
3. G. Ya. Svet-Moldavskii, in: The Immunology of Tumors [in Russian], Kiev (1975), pp. 222-224.
4. V. S. Ter-Grigorov, in: The Immunology of Tumors [in Russian], Kiev (1975), pp. 246-249.
5. J. Cunningham, Immunology, 14, 599 (1968).
6. N. Haran-Ghera, M. Ben-Yackov, A. Peled, et al., J. Nat. Cancer Inst., 50, 1227 (1973).
7. R. T. Prehn, Science, 176, 170 (1972).

PARTICIPATION OF ANTIBODIES SYNTHESIZED IN VITRO IN ROSETTE FORMATION

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The possibility of rosette formation by cells binding antibodies synthesized in vitro was investigated. Within the period of the rosette-formation test at 37°C and with prolongation of the incubation time, antibodies capable of inducing the formation of pseudorosettes appeared in the culture fluid. If the rosette formation test was performed in the cold the formation of pseudorosettes in the residue after centrifugation was minimal.

KEY WORDS: rosette-forming cells; cytophilic antibodies.

One method of detecting the population of immunocompetent cells formed in response to injection of an antigen in vivo is the rosette-formation test. Existing methods of performing the test differ from one another in the time of incubation of the mixture of lymphocytes and red cells and also the incubation temperature. It has been suggested that at 4°C mainly cells carrying immunoglobulin receptors on their surface (antigen-recognizing cells) are revealed, whereas at 37°C antibody-forming cells (AFCs) can also be detected, for this temperature does not prevent antibody synthesis in vitro [1, 4, 6]. According to Wilson et al. [9], AFCs can also be detected if incubation is prolonged in the cold. However, the causes of formation of more rosettes at 37°C or on prolongation of the incubation time have not been finally elucidated. It may be that some of the antibodies synthesized during the reaction are cytophilic, i.e., they have the property of attachment to certain cells, as a result of which the cells become capable of specifically adsorbing antigen [3]. The pseudorosettes formed as the result of this process can be a serious obstacle to the use of the rosette-formation test.

The object of this investigation was to study whether cells binding antibodies synthesized in vitro can take part in rosette formation and to establish the optimal conditions for the reaction.

EXPERIMENTAL METHOD

Male CBA mice weighing 18-21 g, unimmunized or immunized 6 days before the experiments with sheep's red cells ($0.5 \cdot 10^9$ cells, intraperitoneally) were used. There were three series of experiments. In series I

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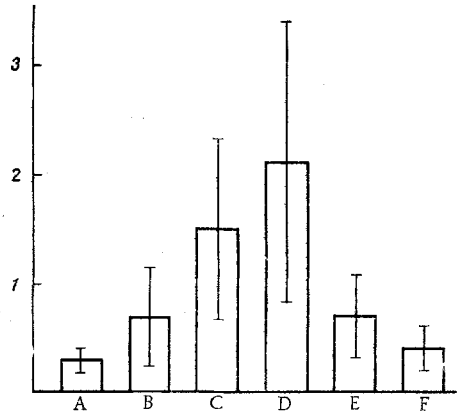

Fig. 1. Number of RFC in suspension of spleen cells of unimmunized mice after addition of CFS. Ordinate, number of RFCs/10^3 nucleated cells (arithmetic means and standard deviations); abscissa: A) control (addition of fresh medium No. 199, n = 7); B, C, D, E, F) addition of CFS obtained after incubation of cells of immunized mice: B) at 37°, 1 h (n = 3), C) at 37°C, 2 h (n = 4), D) at 37°C, 2 h, and at 4°C, 20 h (n = 10), E) at 4°C, 20 h (n = 3), F) with red cells at 37°C, 2 h and at 4°C, 20 h (n = 3); n is number of determinations.

TABLE 1. Number of RFCs Detected by Different Variants of Rosette-Formation Test

Conditions of obtaining suspension and of incubation	n	Number of RFCs per 1000 cells ($\bar{x} \pm s_{\bar{x}}$)	
In protein-free medium without removal of cells adherent to glass			
a) 4°C, 30 min, with preliminary centrifugation	21	4,5±0,40	(3,7—5,3)
b) 37°, 2 h	12	8,2±0,94	(6,1±10,3)
c) 4°, 20 h	12	8,0±1,28	(5,2—10,8)
37°C, 2 h and 4°C, 20 h	9	13,3±1,52	(10,0—16,6)
In protein-free medium without removal of cells adherent to glass			
a) 4°C, 30 min, with preliminary centrifugation	5	3,8±0,87	(1,4—6,2)
b) 37°, 2 h	5	5,8±1,23	(2,4—9,2)
37°C, 2 h and 4°C, 20 h	5	10,4±2,14	(4,5—16,3)
In medium with 10% normal mouse serum after removal of cells adherent to glass			
a) 4°C, 30 min, with preliminary centrifugation	12	4,1±0,50	(3,0—7,1)
b) 37°, 2 h	12	3,8±0,45	(2,8—4,8)
c) 4°, 20 h	7	4,2±0,87	(2,1—6,3)
37°C, 2 h and 4°C, 20 h	6	3,3±0,42	(2,2—4,4)

Legend. n is number of determinations. Confidence intervals for P = 0.05 given in parentheses

suspensions of spleen cells of immunized and unimmunized mice were obtained by the usual method and washed twice in medium No. 199 without the addition of mouse serum. In some experiments, to detect antibodies in the culture medium, spleen cells of immunized animals were incubated in precipitation tubes as in one variant of the rosette-formation technique currently in use, and a suspension of red blood cells (20:1) was added to some of the tubes. By repeated centrifugation (3 min at 3000 rpm, TsLN-2 centrifuge) a cell-free supernatant (CFS) was obtained and was added to the residue of spleen cells of unimmunized mice, after which the cells were incubated for 15 min at 37°C and for 20 min at 4°C. In the control, spleen cells of unimmunized mice were incubated in fresh medium No. 199. After incubation the rosette-formation test was carried out in the residue in the cold [6].

In series II macrophages were removed from the suspension of spleen cells of the immunized mice by a modified Modabber's method [7] (repeated incubation in Petri dishes at 37°C for 30 min in an AVU-1 oscillator at a speed of 14 oscillations/min).

In series III macrophages were removed and the rosette-formation test carried out in medium No. 199 with the addition of 10% normal mouse serum (inactivated for 30 min at 56°C and absorbed with sheep's red cells at 37°C for 30 min).

The suspensions used in the experiments contained 90-95% of living cells (test with Trypan Blue). The rosette-formation test was carried out in four variants: in the cold in the residue [6], at 37°C for 2 h [10], by sedimentation at 4°C [2], and by immunocellular adhesion [8].

Several variants were used to investigate each suspension obtained. Counting was carried out in a phase-contrast microscope (magnification 600×) in a 102 of the large squares of a Goryaev's chamber. About 5000 nucleated cells were examined. A single nucleated cell with five or more red cells adherent to it was taken as a rosette-forming cell (RFC). Repeated counting of samples from the same tube showed that the error of the method is about 5%. The number of RFCs was calculated per 1000 nucleated cells. The results were subjected to statistical analysis in the usual way.

EXPERIMENTAL RESULTS

The number of spontaneous RFCs in suspensions of spleen cells from the unimmunized mice used as the control did not exceed $0.4/10^3$ and averaged $0.3/10^3$. After the addition of CFS the number of RFC in the suspension increased, indicating evidently that antibodies could appear in the culture medium during incubation of cells from immunized animals at 37°C and also, to a lesser degree, at 4°C. During incubation of spleen cells of immunized mice with sheep's red cells the CFS lost its ability to increase the number of RFC in the cell suspension from unimmunized mice (Fig. 1).

The participation of antibodies in rosette formation was revealed by investigation of the number of RFCs in the suspension of spleen cells of the immunized animals by different variants of the test (Table 1). When the suspension of lymphocytes was obtained by the usual method and the test carried out in protein-free incubation medium at 37°C for 2 h and at 4°C for 20 h, more RFCs were detected than in the residue in the cold. Cytophilic antibodies are known to have the greatest affinity for macrophages [4]. If cells adherent to the glass (mainly macrophages) were removed, the differences in the number of RFCs detected by the various methods were reduced. However, despite the possibility that some AFCs could adhere to the glass [7], the tendency remained for the number of RFCs detected to be greater at 37°C and when the incubation time was lengthened. In one series of experiments, after removal of macrophages, the rosette-formation test was carried out in medium with 10% mouse serum, for it has been shown that the presence of a large excess of homologous serum protein prevents the antibodies from adhering to the lymphocytes in a quantity sufficient for rosette formation [7]. Under those conditions the number of RFCs revealed by the methods used did not differ significantly from their number revealed by the use of the rosette formation technique in the residue in the cold. The results suggest that performing the rosette-formation test at 37°C and lengthening the incubation time of the lymphocytes with the erythrocytes leads to the detection not only of AFCs, but also of cells binding antibodies synthesized in vitro. Evidence has now been obtained that antibody molecules are held on the surface membrane of AFCs during their passage from the liquid phase inside the cell into the surrounding medium [5]; this may be the basis for an hypothesis regarding the possibility of rosette formation by AFCs synthesizing hemaglutinins on close contact with red cells in the cold.

LITERATURE CITED

1. J. F. Bach et al., Transplant Proc., 4, 335 (1972).
2. G. Biozzi et al., Immunology, 16, 349 (1969).

3. C. V. Boyden, Immunology, 7, 474 (1964).
4. C. J. Elson et al., Immunology, 22, 291 (1972).
5. E. Helmreich et al., J. Biol. Chem., 236, 464 (1961).
6. I. McConnel et al., Int. Arch. Allergy Appl. Immunol., 35, 209 (1969).
7. F. Modabber et al., J. Immunol., 108, 1447 (1972).
8. N. R. Nota et al., C. R. Acad. Sci. (Paris), 259, 1277 (1964).
9. A. B. Wilson et al., Immunology, 24, 1059 (1973).
10. O. B. Zaalberg et al., J. Immunol., 100, 451 (1968).

GLOBULIN-PRODUCING CELLS IN CULTURES OF BLAST-TRANSFORMED LYMPHOCYTES IN VITRO

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Globulin-producing cells, consisting of lymphocytes, intermediate transformed cells, blast cells, and plasma-like cells were discovered by Coons' method in human peripheral blood lymphocyte cultures after stimulation by staphylococcal filtrate and phytohemagglutinin (PHA). Analysis of the dynamics of appearance of these cells suggests that they arise from a special subpopulation of B lymphocytes: immunological memory cells. Some globulin-producing cells arise from precursors without division, whereas for others differentiation is combined with proliferation.

KEY WORDS: blast transformation; B-lymphocytes; globulin-producing cells.

Under the influence of various mitogens the overwhelming majority of T or B lymphocytes cultivated in vitro starts to divide actively and are transformed into blast cells. Although all mitogens used for this purpose have weak antigenic activity, it has been shown clearly that the transformation of T and B cells under their influence is an immunologically nonspecific phenomenon [2]. It has not yet been finally established whether immunoglobulin (IG) synthesis is activated or not during the blast-transformation reaction (BTR). Data in the literature on this matter are contradictory, especially in the case of BTR taking place under the influence of phytohemagglutinin (PHA), a mitagen to which mainly T cells respond [2–4, 8, 11, 12].

The object of this investigation was to find globulin-producing cells in a culture of lymphocytes stimulated in vitro.

EXPERIMENTAL METHOD

Fifteen healthy persons aged 20-30 years served as donors. Leukocytes obtained after sedimentation of heparinized blood in gelatin were transferred in a dose of $2 \cdot 10^6$ cells in 4 ml medium No. 199 to suitable flasks. The medium was enriched by adding 20 μg asparagine, 0.2 mg glutamine, 100 units penicillin, 50 units streptomycin, and 15% human group IV serum to each 1 ml. The CO_2 concentration in the gaseous phase in the flasks was made up to 5%. At the time of inoculation of the cells, 0.01 ml PHA-P (Difco) or 0.2 ml staphylococcal filtrate (SF) per 1 ml medium was added to the experimental cultures.

The cultures were analyzed for a period of 96 h. The cells were washed twice with medium No. 199 and sedimented by centrifugation (8 min at 150g). To break up clumps of cells the suspension was repeatedly but carefully passed through the thin end of a drawn out capillary tube. The greatest difficulties arose during

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