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

K. A. Lebedev, S. A. Samsygin, T. A. Nechaeva, and N. A. Golovanova

UDC 616.155.321.02-008.939.624-092.4

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

Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Y. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii in Meditsiny, Vol. 82, No. 9, pp. 1092-1094, September, 1976. Original article submitted October 15, 1975.

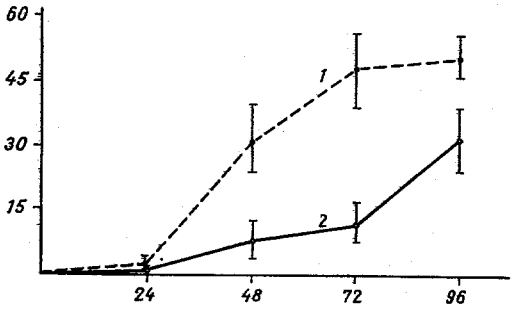

Fig. 1. Percentage of blast cells in cultures stimulated by PHA (1) and by SF (2). Here and in Fig. 2: ordinate: percentage of cells; abscissa, time of investigation (in h).

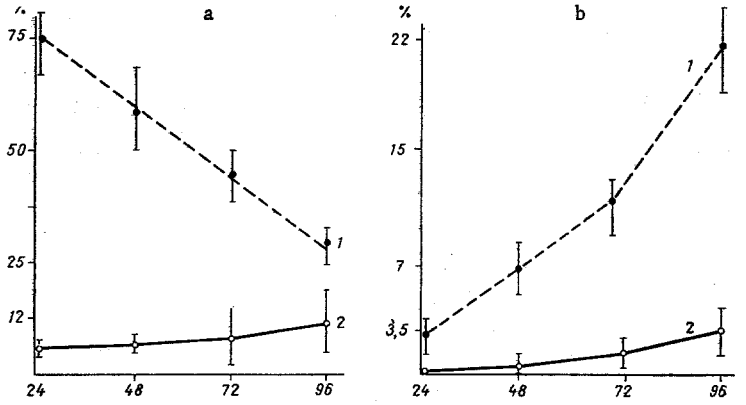

Fig. 2. Percentage of all (1) and only globulin-producing (2) lymphocytes (a) and blast cells (b) in culture stimulated by SF.

suspension of the cells in the cultures with PHA. Better results were obtained by adding a 5% solution of EDTA to the culture. The cell suspension was applied to a slide. After incubation for 10 min the slide with the deposited cells was fixed in 96% ethanol for 15 min at 37°C. Some of the preparations were stained with methyl green–pyronine, whereas others were treated by Coons' direct method to detect IG. Antiglobulin rabbit serum labeled with fluorescein isothiocyanate (batch 726, N. F. Gamaleya Institute of Epidemiology and Microbiology) was used. Before the staining process the serum was purified by absorption with rabbit liver and human embryonic powder. To confirm the specificity of staining controls were used in which fluorescence of lymphocytes was completely absent: adsorption and neutralization tests, and treatment of rat or guinea pig lymphoid tissue with human antiserum against IG.

EXPERIMENTAL RESULTS

In the unstimulated control cultures intermediate transformed types of cells (up to 7%) appeared in the course of growth; hardly any blast cells were seen. On stimulation with PHA blast cells appeared earlier and in larger numbers than on stimulation with SF (Fig. 1). The number of plasma-like cells increased during growth in both the control and the experimental series, although it was higher in the latter (up to 9%). These cells had an extensive but weakly pyroninophilic cytoplasm and an eccentrically placed dense nucleus 5-9 μ in diameter.

In cultures stimulated with both SF and PHA the following types of IG-containing cells were discovered: intermediate stimulated cells, blast cells, small and medium lymphocytes, and plasma-like cells. Fluorescent cells in the peripheral blood and control cell cultures were lymphocytes and plasma-like cells. In all the cells fluorescence was present in the cytoplasm. It was weak in most cells.

The numbers of IG-containing cells differed greatly in lymphocyte cultures from different donors. Not more than 1-3% of IG-containing cells could be detected in the leukocyte suspension at the time of transplan-

tation. These were small and medium lymphocytes and mature plasma cells. During cultivation without the mitogen the number of fluorescent cells increased after 24 h to 0.3% and remained at that level until 4 days.

On stimulation with SF the number of fluorescent cells in the cultures increased considerably, even after growth for 24 h; later their percentage rose slowly until the 3rd day, and then rose more sharply until the 4th day (up to 20% of fluorescent cells). During the 1st day most of the fluorescent cells were lymphocytes, but on the 4th day about half of the fluorescent cells were transformed types. After an increase on the 1st day the number of IG-containing lymphocytes thereafter remained at the same level, with only a small increase on the 4th day, accompanied by a steady decrease in the total percentage of lymphocytes. The dynamics of appearance of fluorescent plasma-like cells was similar to that described for lymphocytes, but without a decrease in the total number of the cells of that type. The number of fluorescent blast cells increased parallel with the increase in the total population of blast cells in the culture (Fig. 2).

In cultures with PHA the cells formed dense aggregates which gave nonspecific fluorescence. Since it was impossible to disaggregate the cells completely without at the same time destroying the cytoplasm, it was impossible to determine the exact total number or formula of the IG-containing cells. It could only be noted that after 24 h fluorescent cells were found in considerable numbers, whereas on the 2nd-3rd day they had almost completely disappeared, to reappear on the 4th day. They were substantially more numerous than in the control cultures. On the 1st-2nd day lymphocytes and plasma-like cells appeared, whereas on the 4th day intermediate forms and blast cells appeared among the fluorescent cells.

In stimulated cultures on medium with the addition of autologous serum or embryonic calf serum an increase in the number of IG-containing cells compared with their number in the control cultures also was observed.

The results showed that the number of fluorescent cells increased during cultivation not only on human, but also on heterologous bovine serum. The increase in the number of IG-containing cells discovered in cultures stimulated by PHA and SF was therefore connected with the actual production of IG and not with its pinocytosis from the culture medium. To activate IG synthesis, serum antigens in the culture were not of decisive importance, for the activation also took place in cultures grown on autologous serum. Induction of IG synthesis by mitogens evidently takes place on activation of antigen stored in the cells [13]. Mitogens have been shown to stimulate antibody formation in an immunized cell culture [10]. IG-producing cells are the progeny of B lymphocytes and, for that reason, the appearance of IG-producing blast cells in cultures with PHA shows that PHA stimulates the transformation not only of T, but also of B cells, in agreement with the results of other investigations [9]. Failure of some workers to detect IG- producing cells in BTR during stimulation by PHA [2, 4] was evidently connected with the technical difficulties of disaggregating the cells of the culture before staining.

Mitogens stimulate IG synthesis only in a very small part of the population of B-cells. These could perhaps be immunological memory cells for different antigens, for no primary immune response can be obtained when these conditions of cultivation are used [6].

The dynamics of appearance of the IG-synthesizing cells points to the presence of two subpopulations of precursor cells. One is connected with the early transformation of small lymphocytes into IG-producing lymphocytes and plasma cells without division, as shown by the early increase in the number of fluorescent lymphocytes, before any dividing blast cells have yet appeared. This is in agreement with other findings [7, 10]. The other subpopulation of IG-producing cells arises from precursors transformed into actively dividing blast cells, as shown by the later appearance of fluorescent blast cells and the maintenance of or increase in the number of IG-producing lymphocytes in the later stages (Fig. 2).

The number of fluorescent cells revealed in the present investigations was greater than that found by Kreth and Herzenberg [5], although, admittedly, they used other mitogens for stimulation. The reason is evidently that these workers counted only brightly fluorescent cells.

LITERATURE CITED

1. K. A. Lebedev, and L. V. Van'ko, Byull. Éksp. Biol. Med., No. 8, 91 (1969).
2. N. R. Ling, Stimulation of Lymphocytes [Russian translation], Moscow (1971).
3. M. F. Greaves and G. Janossy, in: Cell Interactions, (ed. by L. G. Silvestri), Amsterdam (1972), p. 143.
4. G. Harris and R. J. Littleton, J. Exp. Med., 124, 621 (1966).
5. H. W. Kreth et al., Cell. Immunol., 12, 396 (1974).

6. R. I. Mishell and E. W. Dutton, J. Exp. Med., 126, 423 (1967).
7. B. S. Nilsson, B. M. Sultzer, and W. Bullock, J. Exp. Med., 137, 127 (1973).
8. F. Parenti, P. Frangeschini, and G. Farti, Biochim. Biophys. Acta, 123, 181 (1966).
9. B. Phillips and E. Weisrose, Clin. Exp. Immunol., 16, 383 (1974).
10. J. Quintaus and I Lefkovitz, J. Immunol., 113, 1373 (1974).
11. C. S. Ripps and K. Hirschhorn, Clin. Exp. Immunol., 2, 377 (1967).
12. T. W. Tao, Science, 146, 247 (1964).
13. E. R. Unanue and B. A. Askonas, J. Exp. Med., 127, 915 (1968).

EFFECT OF VARIOUS ANTISERA ON INTENSITY OF THE IMMUNE RESPONSE DURING INDUCTION OF ANTIBODY FORMATION BY ALLOGENEIC MACROPHAGES

T. V. Anfalova and V. G. Galaktionov

UDC 612.017.1.014.46:615.365.018.51/.53

Data on the effect of various antisera on the induction of antibody formation by immune allogeneic macrophages are described. A considerable decrease in the intensity of the immune response was observed after injection both of allogeneic antiserum and of antimacrophagal serum during the first 2 days after transplantation of the allogeneic macrophages. Injection of these sera on the following days had no significant effect on the intensity of the immune response. Antierythrocytic serum prevented the accumulation of antibody-forming cells if injected at various times after transplantation of the allogeneic macrophages.

KEY WORDS: macrophages; induction of immune response; antimacrophagal serum; allogeneic antiserum.

In previous investigations [1, 2] the writers showed that incompatibility between the donor of antigen-treated macrophages (MPH) and recipient with respect to the H-2 locus leads to marked depression of the immune response compared with its induction under conditions of complete syngeneity of the donor of immune MPH and the recipient.

To study whether immune rejection by an incompatible recipient is the decisive factor in the observed decrease in the accumulation of antibody-forming cells (AFC), the effect of various antisera was studied on the induction of antibody formation by immune allogeneic MPH in the early and later stages of induction. Antimacrophagal and allogeneic antisera were used as the model factor destroying the donor's MPH.

EXPERIMENTAL METHOD

Mice of strains CBA and DBA/2 were used, for this particular allogeneic pair gives the most marked depression of the immune response in the nonsyngeneic transfer system. Immune MPH were obtained as described earlier [2].

Mouse antierythrocytic serum was prepared from the blood of CBA mice immunized twice with sheep's red cells. The hemagglutinin titer was 1:64,000.

Rabbit antimacrophagal serum was prepared as described by Shortman and Palmer [5]. Anti-DBA/2

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1094-1096, September, 1976. Original article submitted October 31, 1975.