# INCREASE IN NUMBER OF ANTIBODY PRODUCERS DURING COMBINED CULTURE OF LYMPH-GLAND CELLS OF IMMUNIZED ANIMALS AND INTACT BONE-MARROW CELLS*

R. N. Stepanenko and A. A. Mikhailova

UDC 612.428.017.1+612.419.017.1].014.46:615.37-085.23

The number of antibody producers in a mixed culture of lymph-gland cells obtained at the peak of the primary or secondary response in mice immunized with sheep's red cells and intact bone-marrow cells was determined by Jerne's method. During combined culture of these cells the number of antibody producers was 2-4 times greater than in a monoculture of immune lymph-gland cells; by 9-11 h of combined cultivation the number of antibody-synthesizing cells reached a maximum. Meanwhile the rate of survival of the cells was identical in monocultures and mixed cultures throughout the period of cultivation. It is postulated that during combined incubation of immune lymph-gland cells and intact bone-marrow cells, an extra contingent of cells from one of the two populations in culture participates in antibody synthesis.

KEY WORDS: immune lymph-gland cells; intact bone-marrow cells; combined cultivation; immune response; stimulation.

Recent investigations have shown the need for interaction between two or more types of cells for induction of the immune response to occur [6, 7, 10]. Besides cell cooperation, interaction leading to an increased immune response at the level of mature antibody producers is also possible in the initial stages of immunogenesis [11]. For instance, combined culture of lymph-gland cells taken at the peak of the immune response from animals immunized with horse $\gamma$-globulin with intact spleen or bone-marrow cells leads to a two- or threefold increase in the quantity of antibodies and of nonspecific immunoglobulins [2, 3]. This increase in the quantity of antibodies is not due to stimulation of protein synthesis in existing antibody producers, but to the appearance of new cells synthesizing antibodies of the same specificity [9]. The stimulation effect in a mixed culture of immune lymph-gland and intact bone-marrow cells was first obtained with the cells of animals immunized with horse $\gamma$-globulin.

To determine whether this phenomenon is observed when corpuscular antigens are used, in the investigation described below cells of animals immunized with sheep's erythrocytes were used.

## EXPERIMENTAL METHOD

Mice of lines CBA, A, C57BL, and $F_1$ (CBA × C57BL) weighing 18-22 g were immunized with 0.1 ml of a 10% suspension of sheep's red cells, injected into the plantar pad. The animals were killed by decapitation, and the axillary and inguinal lymph glands were removed from them with sterile precautions. Preparation of the cell suspensions and cultivation of the cells was carried out as described earlier [2]. After cultivation for 18-20 h the number of antibody-producing cells in the cultures was determined by Jerne's

*This research was directed by Doctor of Medical Sciences R. V. Petrov.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 6, pp. 76-79, June, 1975. Original article submitted August 20, 1974.

TABLE 1. Stimulation of Immune Response during Combined Cultivation of Cells of Different Histological Origin

| Character of immune response | Components of mixture and genotype of animals | | Number of plaque-forming cells per $10^7$ nucleated cells | | | | Coefficient of stimulation | P |
|---|---|---|---|---|---|---|---|---|
| | immune lymph glands | intact bone marrow | monoculture | | mixed culture | | | |
| | | | n[1] | M ± m | n[1] | M ± m | | |
| Primary | CBA | CBA | 5 | 15±1 | 5 | 23±1,8 | 1,5 | 0,05 |
| | $F_1$(CBA×C57BL) | $F_1$(CBA×C57BL) | 11 | 24±1,8 | 12 | 40±2,9 | 1,7 | 0,05 |
| | A | C57B | 6 | 25±1,7 | 6 | 40±2,1 | 1,6 | 0,01 |
| Secondary | A | A | 5 | 100±8,9 | 6 | 430±28,6 | 4,3 | |
| | CBA | CBA | 5 | 140±4,5 | 5 | 300±8,9 | 2,1 | 0,01 |
| | A | CBA | 11 | 510±42 | 10 | 1290±72,6 | 2,5 | |
| | CBA | A | 4 | 230±45 | 4 | 1000±55 | 4,3 | |

[1]n) Number of cultures investigated

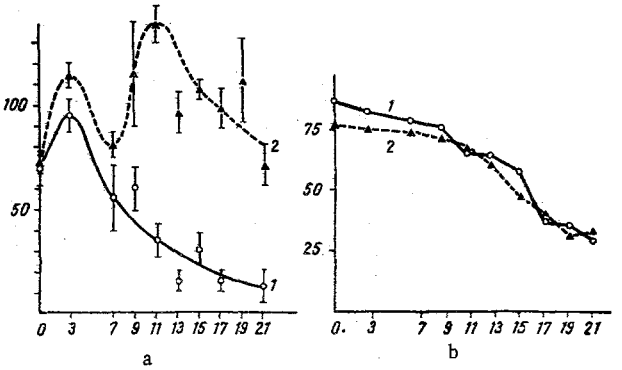


Fig. 1. Changes in number of antibody producers (a) and viable cells (b) in monocultures (1) and mixed cultures (2) during cultivation. Abscissa, time of cultivation (in h); ordinate: a) number of antibody producers (per $10^6$ nucleated cells); b) number of living cells, in %.

method [8]. The change in viability of the cells during culture was estimated from the staining properties with trypan blue.

The presence of interaction between the cells was judged from the coefficient of stimulation (the ratio between the number of antibody producers in the mixed culture and the number in monoculture). The significance of the results was estimated by means of the Fisher–Student criterion, and the curve of survival of the lymphocytes as a function of cultivation time was smoothed by the sliding-mean method [5].

## EXPERIMENTAL RESULTS

After a single immunization of the mice with sheep's red cells the number of antibody-forming cells in the lymph glands reached a maximum on the 5th day, but after reimmunization this occurred on the 4th day. Since the effect of stimulation of γ-globulin synthesis in the mixed cultures was most marked when lymph-gland cells obtained from animals at the peak of the immune response were used [4], in the present experiments lymph glands were taken on the 5th day after immunization when studying the primary response and on the 4th day after reimmunization.

As the result of combined cultivation for 18–20 h of immune lymph-gland cells obtained from mice during both the primary and the secondary immune response, with syngeneic and allogeneic intact bone-marrow cells, an increase in the number of antibody producers was observed (Table 1). The intensity of the effect when cells from primarily immunized animals were used was rather weaker (coefficient of stimulation

1.6) than with cells from reimmunized animals (coefficient of stimulation 2-4). The effect of interaction between cells of different histological origin was the same whether syngeneic or allogeneic mixtures were cultivated together. This confirms yet again the earlier conclusion that the allogeneic barrier does not play an important role in interaction between cells in the stages of active antibody synthesis [11].

The dynamics of the change in the number of antibody producers in monoculture and in mixed culture of immune lymph-gland and intact bone-marrow cells is illustrated in Fig. 1a.

After an initial increase in the number of antibody-producing cells in both cultures, it fell toward the 7th hour. However, after 7 h of cultivation in mixed culture the number of antibody-synthesizing cells was increased and reached a maximum at 9-11 h. Whereas the coefficient of stimulation after cultivation for 20 h varied between 1.5 and 4.3, after cultivation for 11-17 h it was 5-7. The number of viable cells in the monocultures and mixed cultures decreased by an equal degree in both monocultures and mixed cultures in the course of cultivation (Fig. 1b). The identical mortality of the cells during cultivation indicates that the effect of an increase in the number of plaque-forming cells could not be explained by reduced mortality of the cells in the mixed cultures. The comparatively rapid manifestation of the effect of an increase in the number of antibody producers from the beginning of combined cell culture agrees with data on the kinetics of the stimulation of immunoglobulin synthesis during immunization of animals with horse $\gamma$-globulin [1].

The results described above indicate that stimulation of the immune response during combined culture of lymph-gland cells from immune donors and intact bone-marrow cells takes place if the animals are immunized not only with soluble antigens but also with corpuscular antigens, and it is observed whether the immune response in the mice is secondary or primary. However, during the primary response the stimulation effect is weaker than during the secondary. It is difficult at present to say anything about the causes of this difference. The presence of a maximum of the number of plaque-forming cells in the mixed culture 9-11 h after the beginning of cultivation and data showing the equal rate of death of the cells in the mono- and mixed cultures suggest that interaction at the level of mature antibody producers leads to the recruiting of new cells into antibody synthesis. Which of the cell populations – lymph-gland or bone-marrow – is the source of the new plaque-forming cells cannot yet be stated and further investigation of this problem is envisaged.

## LITERATURE CITED


1. L. A. Zakharova, Byull. Éksperim. Biol. i Med., No. 9, 73 (1973).
2. A. A. Mikhailova, R. V. Petrov, and L. A. Zakharova, Dokl. Akad. Nauk SSSR, 197, 209 (1971).
3. A. A. Mikhailova, L. A. Zakharova, and R. V. Petrov, Dokl. Akad. Nauk SSSR, 203, 948 (1972).
4. R. V. Petrov and A. A. Mikhailova, Dokl. Akad. Nauk SSSR, 187, 922 (1971).
5. P. F. Rokitskii, Biological Statistics [in Russian], Minsk (1964), p. 169.
6. H. N. Claman, E. A. Chaperon, and R. F. Triplett, Proc. Soc. Exp. Biol. (New York), 122, 1167 (1964).
7. D. L. Groves, W. F. Lever, and T. Makinnodan, Nature, 222, 821 (1969).
8. N. K. Jerne, Cold Spring Harbor Symp. Quant. Biol., 32, 591 (1968).
9. A. A. Mikhailova (A. A. Michajlova), J. Madar, and T. Hraba, Folia Biol. (Prague), 19, 315 (1973).
10. G. F. Mitchell and J. F. Miller, J. Exp. Med., 128, 821 (1969).
11. R. V. Petrov and A. A. Mikhailova (A. A. Michajlova), Cell. Immunol., 5, 392 (1972).