ANTIBODY PRODUCTION BY SINGLE CELLS DURING IMMUNIZATION OF ANIMALS WITH SOLUBLE PROTEIN

Ya. S. Shvartsman

UDC 612.017.12:612.014.1]:612.398

In many recent publications the production of antibodies by single cells isolated from animals immunized with bacterial antigens [5, 10-12], bacteriophages [6], and erythrocytes [2, 9] has been described.

A method has been developed in the author's laboratory [4] which can be used to study the production of antibodies by single cells against serum proteins, which are widely used in immunologic investigations.

It was therefore decided to examine whether antibody synthesis by single cells during immunization of animals with soluble protein differs from the same process evoked by bacterial antigens and erythrocytes.

EXPERIMENTAL METHOD

The experimental animals were chinchilla rabbits weighing 3-3.5 kg, and altogether 48 animals were used. The antigen was horse serum albumin (HSA). This preparation was injected once or twice subcutaneously into the plantar surfaces of both hind limbs. The dose of antigen per injection was 10 mg and the interval between the first and second injections was 2 months. In addition, a group of animals received a single injection of 20 mg protein each. The animals (4 rabbits in each group) were sacrificed before the first and second injections of antigen and on the 1st, 3rd, 5th, and 7th days after the last injection. The popliteal lymph glands were removed and cell suspensions prepared from their tissue [3]. To determine the number of producing cells in this suspension, the indirect hemadsorption test was carried out with sheep erythrocytes conjugated with HSA by means of bis-diazotized benzidine [4]. A mean number of 2500 cells was investigated from each rabbit. The antibody concentration in their blood serum was determined by the indirect hemagglutination test with erythrocytes treated with tannin and sensitized with HSA.

EXPERIMENTAL RESULTS

There are data in the literature to show that the immune erythrocyte agglutination test [1] may be "induced" passively [8] as the result of the presence of cytophilic [7] antibodies in the blood and lymph. It thus became necessary to verify that the indirect hemadsorption test is suitable for detecting cells producing antibodies to soluble proteins. For this purpose a suspension of cells from the lymph glands of unimmunized animals was divided into three parts: the first part was combined with 2 ml serum from the doubly immunized animals, the second part with the same volume of a saline extract of the lymph glands of the same rabbits, and the third part with normal rabbit serum. All three portions were incubated for 2 h at 37°, washed off three times and investigated in the indirect hemadsorption test (see table).

Specificity of Indirect Hemadsorption Test

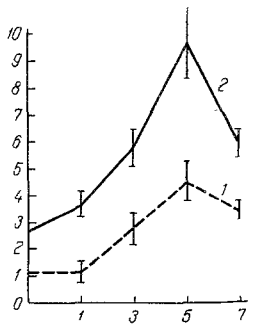
Preparation used to "induce" adhesion of erythrocytes	No. of cells investigated	No. of cells with adhesion of erythrocytes	
		absolute	%
Blood serum	11 974	11	0,10
Extract of lymph glands	11 348	16	0,13
—	10 639	14	0,12

The method of treatment used did not cause an increase in the number of cells with adhesion of erythrocytes. Evidently, cytophilic antibodies against HSA either were absent from the serum and extracts or were not adsorbed on the lymphoid cells [7, 13]. It was thus possible to use the indirect hemadsorption reaction in the main experiments (see figure, 1).

In the rabbits immunized with a single injection antibody-producing cells were found only from the 3rd day after

Leningrad Research Institute of Vaccines and Sera (Presented by Active Member of the Academy of Medical Sciences of the USSR L. A. Zil'ber*). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 10, pp. 65-67, October, 1967. Original article submitted September 3, 1966.

*Deceased.



Changes in number of antibody-producing cells during immunization with HSA. Ordinate) number of producing cells (in %); abscissa) time after injection of antigen (in days); 1) single injection; 2) 2 injections of antigen.

injection of antigen. Their number reached a maximum by the 5th day, and thereafter it fell rapidly. In contrast to this, when the reaction to repeated immunization was studied, an increase in the number of cells with adhesion of erythrocytes was observed even before injection of the antigen. A definite increase in their number could be observed during the first 24 h after immunization, and at all times of observation the number of active cells was much greater than in the animals immunized with a single injection (before injection of antigen, $P = 0.02$, at all other times $P = 0.01$). At the same time a simple doubling of the dose of antigen, which was given as a single injection, produced no visible increase in the number of producing cells (0.46% on the 5th day with a dose of 10 mg and 0.48% with a dose of 20 mg per injection).

The mean number of erythrocytes adherent to the active cell was also determined. In cells isolated from animals immunized with a single injection this figure did not exceed 1.2. By the 5th day after the second injection of antigen it had increased to 2, indicating an increase in the intensity of antibody formation in each cell.

In their morphological characteristics most of the producing cells were plasma cells. This also showed that cells with adherent erythrocytes were in fact the antibody producers.

Some increase in the antibody titers was observed from the 5th day after the first injection of antigen. Doubling the dose of antigen, giving the whole at a single injection, produced no great increase in antibody titers. Conversely, in the rabbits immunized with 2 injections a sharp increase in concentration of antibodies was observed from the 3rd day after the second injection, and their titers were 10 times or so higher than those of animals immunized once only.

As experiments to immunize rabbits with HSA showed, accumulation of antibody-producing cells in the animals immunized by soluble proteins is almost indistinguishable from that observed when other antigens are injected, although some special features do occur. All the antigens so far studied share in common an increase in the number of active cells during the response reaction, participation of only a small proportion of the population in immunogenesis [5, 9], and also an increase in the number of active cells following repeated immunization and an increase in the intensity of antibody synthesis in each cell in these conditions [11, 12]. At the same time, in contrast to the reaction to bacterial antigens [5], during immunization with soluble protein no increase was observed in the intensity of antibody production by single cells at the height of the reaction to the first injection of antigen. A slower accumulation of producing cells likewise was observed after the second immunization than was found by other investigators [10] who injected endotoxins of Gram-negative bacteria into animals. The probable cause of these differences is absence of adjuvant properties from serum proteins.

LITERATURE CITED

1. P. V. Osipova and O. A. Karasik, Byull. Éksp. Biol., No. 10, 100 (1964).
2. L. A. Pevnitskii, V. V. Solov'ev, and L. N. Fontalin, Byull. Éksp. Biol., No. 8, 85 (1965).
3. Ya. S. Shvartsman, Byull. Éksp. Biol., No. 12, 88 (1964).
4. Ya. S. Shvartsman and A. V. Ispolatova, Byull. Éksp. Biol., No. 5, 121 (1966).
5. Ya. S. Shvartsman, M. K. Karpov, and A. V. Ispolatova, In the book: Stimulation and Mechanisms of Antibody Biosynthesis [in Russian], Leningrad (1966), p. 77.
6. G. Attardi, M. Cohn, and K. Horibata et al., J. Immunol., Vol. 92 (1964), p. 335.
7. S. V. Boyden, Immunology, Vol. 7 (1964), p. 474.
8. A. Bussard, C. R. Acad. Sci. (Paris), Vol. 259 (1964), p. 3411.
9. R. Kearney and W. J. Halliday, J. Immunol., Vol. 95 (1964), p. 109.
10. M. Landy, R. P. Sanderson, and A. L. Jackson, J. Exp. Med., Vol. 122 (1965), p. 483.
11. G. J. V. Nossal and O. Mäkelä, Ann. Rev. Microbiol., Vol. 16 (1962), p. 57.
12. G. J. V. Nossal, Advanc. Immunol., Vol. 2 (1962), p. 163.
13. W. E. Parish, Nature, Vol. 208 (1965), p. 594.