THE USE OF A PROTEIN-CELLULOSE COMPLEX TO INDUCE INTENSIVE ANTIBODY FORMATION IN MICE

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KEY WORDS: protein-cellulose complexes; immunization; antibody formation; mice.

To intensify the immune response various stimulators (adjuvants) [4, 10] and immunization with complexes of antigens with polyelectrolytes [7] are widely used. However, immunization by methods so far suggested often leads to undesirable complications and is not always sufficiently effective [1].

Antisera with an extremely high content (up to 10-15 mg/ml) of antibodies were obtained previously [5, 6] in the writers' laboratory by injection of proteins, covalently bound with cellulose or Sephadex particles, into rabbits.

In the present investigation antibody formation was studied in C57BL/6 and BALB/c mice on immunization with a protein-cellulose complex.

EXPERIMENTAL METHOD

Female BALB/c and C57BL/6 mice weighing 18-20 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were vaccinated initially with smallpox vaccine and were used not less than 1 month thereafter.

Horse γ-globulin (HGG), from Miles Laboratories (England), was used as the antigen. For primary immunization the HGG was either bound covalently with a suspension of oxidized cellulose (SOC-HGG) or mixed with an equal volume of Freund's complete adjuvant (FCA + HGG) from Calbiochem (USA). The protein-cellulose complex (SOC-HGG) was prepared by adding 100 mg of HGG to 1 g of a suspension of reprecipitated cellulose, oxidized with sodium periodate, and reducing the resulting preparation with sodium borhydride [3]. During primary immunization the antigen was injected subcutaneously or intraperitoneally in a volume of 0.2 ml. On reimmunization 0.2 ml of a solution of HGG in 0.85% NaCl solution was injected intravenously.

Antibody-forming cells (AFC) were detected by Herne's method using sheep's red blood cells sensitized with HGG through $CrCl_3$ [9]. Since extremely few AFC were found in Jerne's "direct" test, the "indirect" version of this test was adopted, using serum from rabbits immunized with mouse γ-globulin. The serum was exhausted beforehand with SOC-HGG sorbent to remove antibodies cross-reacting with HGG. The specificity of passive hemolysis in Jerne's test was confirmed by the inhibition of plaque formation by 98% in the presence of 10 μg HGG in the top layer of agarose in the dish.

In most experiments antibodies in the mouse sera were determined by a semiquantitative double diffusion method in agar with a test system: a solution of HGG (100 μg/ml) and serum from a rabbit immunized with HGG (130 μg of antibodies in 1 ml). The absolute antibody concentration was determined by a quantitative method based on the increase in the quantity of protein on the immunosorbent [2].

The results were subjected to statistical analysis with calculation of the geometric mean and standard error ($M_g \pm m$).

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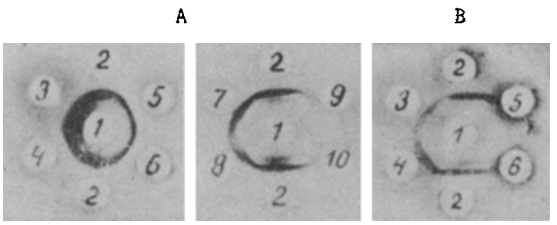

Fig. 1. Semiquantitative assay of antibodies in sera of BALB/c (A) and C57BL/6 (B) mice on immunization with SOC-HGG. Animals immunized by optimal scheme (see text), antisera obtained on 7th day after reimmunization. 1) Antigen of test system (HGG, 100 μg/ml); 2) antiserum of test system (serum of rabbits immunized with HGG; 130 μg of antibodies in 1 ml); 3-10) dilution of test antisera: 3) undiluted, 4) 1/2, 5) 1/4, 6) 1/8, 7) 1/16, 8) 1/32, 9) 1/64, 10) 1/128.

TABLE 1. Comparison of Intensity of Antibody Formation in BALB/c and C57BL/6 Mice

Strain of mice	Number of AFC/10^6 spleen cells	Antibody content in sera (μg/ml)
BALB/c	7 415 (6 490 ÷ 8 472)	2 600
C57BL/6	204 (164 ÷ 254)	230

Legend. Mice immunized subcutaneously in the flank with SOC-HGG in a dose of 1 mg (=100 μg protein); solution of HGG (10 μg) injected intravenously 50 days later; AFC counted on 4th day, antibodies in sera determined on 7th day after reimmunization.

TABLE 2. Comparison of Different Methods of Immunization of BALB/c Mice

Primary immunization (100 μg protein)		Number of AFC/10^6 spleen cells after reinjection of HGG (10 μg) in solution
form of antigen	mode of injection	
HGG in solution	Subcutaneously into flanks	16 (9 ÷ 29)
FCA + HGG		211 (142 ÷ 313)
SOC-HGG		5 282 (4 748 ÷ 5 875)
HGG in solution	Intraperitoneally	112 (52 ÷ 242)
SOC + HGG (mixture)		93 (63 ÷ 138)
FCA + HGG		2 333 (2 117 ÷ 2 571)
SOC-HGG (complex)		6 789 (5 104 ÷ 9 030)

Legend. SOC + HGG: mixture of reduced SOC (1 mg) with solution of HGG (100 μg).

EXPERIMENTAL RESULTS

In the experiments of series 1 optimal conditions of immunization were selected on the basis of data on dependence of the intensity of antibody formation on the quantity of antigen injected and the time interval elapsing between injections. Antibodies in the sera were determined by the double diffusion method. Immunization of the animals initially with the protein-cellulose complex SOC-HGG (subcutaneously, into the flank), and again 1 month later with a soltion of HGG (intravenously) led to the appearance of large quantities of antibodies in the blood. For instance, if 1 mg of the SOG-HGG complex, containing 100 μg of HGG was injected first into C57BL/6 mice, and 10 μg of HGG in solution was injected 1 month later, the sera

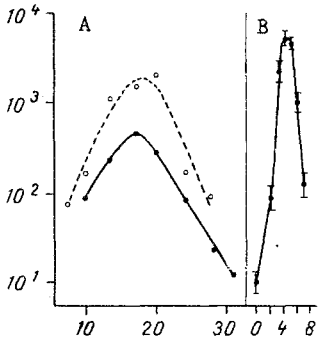

Fig. 2

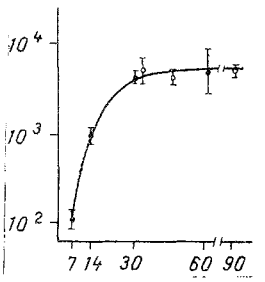

Fig. 3

Fig. 2. Time course of number of AFC in primary (A) and secondary (B) immune response. Abscissa, time (in days) after primary (A) and reimmunization (B); ordinate, number of AFC per 10^6 spleen cells (continuous line) and submandibular lymph node cells (broken line). A) AFC counted in cell pools from four animals at different times after subcutaneous injection of 1 mg of SOC-HGG (100 µg protein) into intermaxillary region; B) AFC determined individually after intraveneous reimmunization (1 month after injection of SOC-HGG) with 10 µg HGG in solution.

Fig. 3. Role of interval between primary immunization with SOC-HGG and reimmunization with HGG for development of secondary immune response. Abscissa, interval between two immunizations (in days); ordinate, number of AFC per 10^6 spleen cells. AFC counted on 4th day (filled circles) or on 5th day (empty circles) after reimmunization.

obtained on the 7th day contained about 0.26 mg of antibodies in 1 ml (Fig. 1A). On immunization of BALB/c mice by the same scheme the intensity of antibody formation was considerably higher: The antibody content in the sera was about 2 mg/ml (Fig. 1B). If, however, the animals received 100 µg of HGG in solution (and not in the form of the SOC-HGG complex) at the first immunization, no antibodies could be detected in the blood by the method indicated.

Quantitative assay of antibodies in the sera by the immunosorbent method confirms the results of the semiquantitative precipitation method, and also revealed a marked difference in the intensity of antibody formation between mice of the two strains. The antibody titer in sera from BALB/c mice was 10 times higher than in sera from C57BL/6 mice (2.6 and 0.23 mg/ml, respectively). An equally marked difference also was observed in the number of AFC in the spleen in mice of the two different strains (Table 1).

In the experiments of series 2 the number of AFC was compared in the spleen of BALB/c mice immunized primarily with SOC-HGG complex, HGG in Freund's complete adjuvant, and HGG in solution. On immunization with SOC-HGG, many more AFC were formed after repeated injection of the antigen than on immunization with HGG, even if mixed with Freund's complete adjuvant (Table 2).

The time course of the change in numbers of AFC was determined in BALB/c mice after primary immunization with SOC-HGG (subcutaneously, into the intermaxillary region) and after reimmunization with HGG in solution (Fig. 2). The results in Fig. 2A show that a primary immune response developed after injection of SOC-HGG both in the spleen and in the regional submandibular lymph nodes. The time course was similar in character. The number of AFC rose slowly to reach a maximum after 17-20 days, and then fell slowly until the 30th day. Although the absolute number of AFC in the spleen at the peak of the primary immune response was about twice the number found in the regional lymph nodes, the relative intensity of antibody formation (AFC/10^6 cells) in the regional lymph nodes was about 4 times higher than in the spleen. In other lymphoid organs (axillary, inguinal, popliteal, and mesenteric lymph nodes and bone marrow) comparatively few AFC were discovered. After reimmunization with soluble antigen there was a rapid increase in the number of AFC in the spleen (Fig. 2B). The number of AFC reached a maximum on the 4th-5th day, and then fell sharply. In the secondary immune response the contribution of the spleen (to the number of AFC) exceeded 90%.

With lengthening of the interval between injection of SOC-HGG and reimmunization the intensity of the response increased and reached a maximum after 1 month (Fig. 3). With a further increase in this interval (to 3 months) the intensity of the secondary response remained at the same level.

The SOC-protein complex which we used to immunize the mice is easily prepared [3]. It consists of unmetabolized particles with a very large total surface area, on which single protein molecules are covalently immobilized. Immunization with this complex induces much more intensive antibody formation in animals than immunization with protein in solution or even mixed with Freund's complete adjuvant. The formation of a large AFC population makes the suggested method of immunization extremely promising for hybridoma production. The great difference between the intensity of antibody formation in mice of strains BALB/c and C57BL/6 provides wide opportunities for the analysis of the phenomenon observed.

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EFFECT OF CHOLINOMIMETICS AND ADRENOMIMETICS ON PROLIFERATION OF MOUSE B LYMPHOCYTES DURING PRIMARY IMMUNE RESPONSE TO PROTEIN ANTIGEN

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The writers showed previously that the sensitivity of mouse B lymphocytes to adrenalin changes during the primary immune response [2]. It was postulated that this is associated with proliferation and differentiation of B lymphocytes, which promote the development of precursor cells into a clone of B lymphocytes producing specific antibodies. This change in the sensitivity of B lymphocytes to neurotransmitters during development of the immune response suggests that these transmitters may influence the processes of formation of the immune response through regulation of cell proliferation. There have been only isolated studies to show the effect of cholinomimetics and adrenomimetics on the level of proliferative activity of lymphocytes [7, 8].

The aim of this investigation was to study the effect of neurotransmitters on proliferation of B lymphocytes induced by specific antigen.

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