SECONDARY IMMUNE RESPONSE IN MICE TO IMMUNIZATION WITH A PROTEIN-CELLULOSE COMPLEX

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UDC 612.017.1.014.46:615.373.6:615.451.22:547.458.81

KEY WORDS: protein-cellulose complex; secondary immune response; antibodies; antibody-forming cells.

The writers showed previously that a protein component of a protein-cellulose complex possesses much greater immunogenicity than the same protein in solution [2, 4, 5].

A single injection of the protein-cellulose complex into animals induces a long process of antibody formation, and subsequent injection of this protein, in the dissolved form, induces an intensive, although brief secondary response [2].

The aim of this investigation was to study the specificity of the secondary immune response and dependence of its intensity on the quantity and form of injected antigen.

EXPERIMENTAL METHOD

Male BALB/c mice weighing 18-20 g were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. The mice were vaccinated on receipt with smallpox vaccine and used not earlier than 1 month after vaccination.

Equine gamma-globulin (EGG), from Miles Laboratories (England), was used as the antigen. In experiments to study the specificity of the immune response, bovine gamma-globulin (BGG), produced by the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, and hen's egg albumin (HEA), produced by the Olaine Chemical Reagents Factory, also were used.

The complex formed by a suspension of oxidized cellulose with EGG (SOC-EGG) was prepared by the method described previously [2, 3].

During primary immunization the SOC-EGG complex was injected subcutaneously into the flank of the mouse, once or three times (at intervals of 7 days). On reimmunization the protein antigen, dissolved in 0.85% NaCl, was injected intravenously or intraperitoneally, and the protein-cellulose SOC-EGG complex was injected intraperitoneally. Secondary immunization was carried out not less than 1 month after primary immunization.

Antibody-forming cells (AFC) were detected by the indirect passive local hemolysis in gel test with the aid of sheep's red blood cells (SRBC), sensitized with EGG (SRBC-EGG) or with BGG (SRBC-BGG) through $CrCl_3$ [6, 7].

The absolute content of antibodies in the sera was determined from the increase in the protein level on SOC-EGG immunosorbent [1].

The experimental results were subjected to statistical analysis, with calculation of the arithmetic mean (M_a) or the geometirc mean (M_g) and the standard deviation (m) for each group.

EXPERIMENTAL RESULTS

To discover the specificity of the secondary immune response, mice were immunized primarily with the SOC-EGG complex, and 1 month later they were given an intravenous injection of one of the following three antigens: homologous (EGG), cross-reacting (BGG), and not cross-reacting (HEA). SRBC-EGG or SRBC-BGG were used to test for AFC. Secondary immunization with

Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 12, pp. 736-738, December, 1986. Original article submitted January 9, 1986.

 0007-4888/86/0012-1738$12.50

TABLE 1. Specificity of Secondary Immune Response to Injection of Various Proteins into Mice Immunized Beforehand with SOC-EGG Complex

Protein injected on reimmunization	Number of AFC per 10^6 splenocytes on 4th day ($M_g \pm m$)	
	SRBC-EGG	SRBC-BGG
EGG	4587 (3729—5644)	719 (502—1013)
BGG	115 (71—188)	27 (16—45)
HEA	90 (65—125)	6 (4—8)

Legend. On primary immunization 100 μg of immobilized protein, in the composition of the SOC-EGG complex, was injected once. One month later, 10 μg of that protein in physiological saline was injected intravenously.

TABLE 2. Dependence of Intensity of Secondary Immune Response on Dose of Dissolved Antigen Injected during Reimmunization

Dose of EGG on reimmunization, μg		Number of AFC per 10^6 splenocytes ($M_g \pm m$)
intravenously	intraperitoneally	
0	0	10 (7—14)
0,1	0	1 062 (904—1249)
1,0	0	2 713 (2 201—3 345)
10,0	0	4 978 (4 517—5 486)
10,0	1600	35 317 (29 320—42 540)

Legend. On primary immunization 100 μg of immobilized protein, in the composition of SOC-EGG complex, was injected once. A second injection of EGG in solution was given 3 months later and the number of AFC was counted on the 5th day.

solutions of BGG and HEA were found not to cause the appearance of any substantial number of cells synthesizing antibodies against EGG and BGG. If a solution of EGG was injected, however, besides very many cells synthesizing antibodies to that protein, many cells forming antibodies to BGG also were found in the animals (Table 1). This result is evidently connected with the presence of common antigenic determinants on the EGG and BGG molecules.

In the next series of experiments dependence of the secondary response on the dose of antigen injected into the mice in the composition of the protein-cellulose complex during primary immunization and the possibility of reducing this dose by fractional injection of the complex, were studied. It will be clear from Fig. 1 that mice which received three injections, each of 0.5 μg of EGG in the form of the SOC-EGG complex, making a total of 1.5 μg of immobilized protein, developed a secondary immune response of the same intensity as mice which received 100 μg of protein in the composition of this complex as a single injection during primary immunization. A single injection of SOC-EGG in a dose of 1.5 μg of immobilized protein was ineffective (Fig. 1). Replacement of a single injection of the SOC-EGG complex by triple injections at intervals of 7 days thus enables the effective immunizing dose to be sharply reduced.

Dependence of the intensity of the secondary immune response on the dose of antigen injected during reimmunization, in the form of a solution or in the immobilized form, was next studied.

Intravenous injection of only 0.1 μg of EGG in the form of a solution into mice previously immunized with the SOC-EGG complex, during reimmunization induced intensive antibody forma-

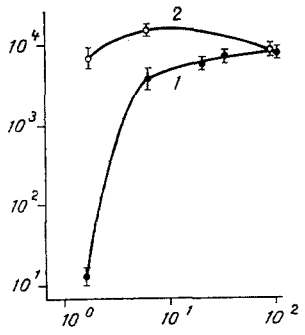

Fig. 1

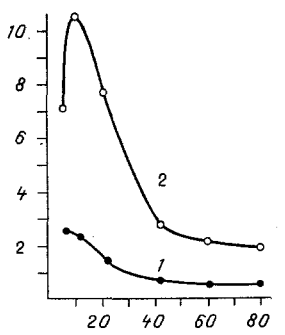

Fig. 2

Fig. 1. Intensity of secondary immune response depending on dose of immobilized antigen injected on primary immunization with protein-cellulose complex as a single injection (1) or fractionally (2). Abscissa, dose of SOC-EGG (in μg of immobilized protein) on primary immunization; ordinate, number of AFC per 10^6 splenocytes. Primary immunization of mice with SOC-EGG complex, injected as a single dose or fractionally (three injections at intervals of 1 week). Two months later 10 μg of EGG in solution was injected intravenously and the number of AFC determined in the spleens on the 4th day. Data shown in the form $M_g \pm m$.

Fig. 2. Determination of absolute content of antibodies in course of secondary immune response in mice immunized primarily with protein-cellulose complex. Abscissa, time after secondary immunization (in days); ordinate, absolute contents of antibodies (in mg/ml) in sera. On primary immunization a single injection of 100 g of immobilized antigen in the form of the SOC-EGG complex was given. The mice were reimmunized 2.5 months later, when 10 g of EGG in solution was injected intravenously into the animals of one group, and animals of the other group received 10 μg of EGG in solution intravenously together with 4 mg of immobilized EGG, in the form of the SOC-EGG complex, intraperitoneally (2). Antibody titers determined in pooled sera from 10 animals in a group.

TABLE 3. Injection of Protein-Cellulose Complex during Reimmunization ($M_a \pm m$)

Reimmunization		7th Day		Number of AFC in spleen		
				4th day	7th day	
EGG intravenously	SOC-EGG intraperitoneally	weight of spleen, mg	no. of cells in spleen, millions	per 10^6 splenocytes	per 10^6 splenocytes	per organ, millions
+	−	204±10	164±10	12 812±1824	3 683±655	0,6
+	+	459±20	306±16	39 324±4970	55 127±7805	16,9
−	+	457±36	287±17	18 650±2729	32 589±4822	9,4

Legend. On primary immunization a single injection of 100 μg of immobilized protein in the composition of the SOG-EGG complex was given. At reimmunization 2 months later 10 μg EGG in solution was injected intravenously and 1600 μg of immobilized EGG in the composition of the SOC-EGG complex was injected intraperitoneally.

tion (Table 2). The level of the secondary immune response rose with an increase in the dose of antigen. After intravenous injection of a solution containing 100 μg EGG or more, some of the mice died. To estimate the effect of a further increase in the dose of antigen, on reimmunization we injected the EGG solution intravenously (10 μg) and intraperitoneally (1600 μg) simultaneously. Under these circumstances the intensity of the secondary immune response was much stronger than after intravenous injection of 10 μg EGG only (Table 2).

Intraperitoneal injection of a large quantity of immobilized antigen in the composition of the protein-cellulose complex during reimmunization was more effective still. With a combination of intravenous injection of 10 μg EGG in solution with intraperitoneal injection of 1600 μg of immobilized EGG in the composition of the SOC-EGG complex, not only could extremely intensive antibody formation be induced, but it could also be prolonged. This becomes particularly clear if it is recalled that, besides an increase in the number of AFC per 10^6 splenocytes, there was also a marked increase in the weight of the spleen and the number of cells in it. As a result, the total number of AFC in the spleens reached $2 \cdot 10^7$ (Table 3). On the 7th day on average there were 55,000 AFC per 10^6 splenocytes. In individual animals the number of AFC exceeded 100,000 and could even reach 200,000 per 10^6 spleen cells.

The higher than usual intensity of antibody formation in these mice could be judged not only by the number of AFC in the spleen, but also by the appearance of an unusually large quantity of specific antibodies in the serum, in which their concentration on the 11th day after reimmunization reached 10-12 mg/ml (Fig. 2). These large quantities of immunoglobulins were discovered only in the spleens of animals with myeloma. The normal concentration of total immunoglobulins in the serum of mice of this strain is 5.5 mg/ml [8].

As a result of intraperitoneal injection of the protein-cellulose complex on reimmunization, white growths were formed on the surface of the spleen in the form of firmly attached films, and white formations appeared alongside the spleen. Both in the films and in these white formations many AFC were present. Structures of this kind were not formed after injection of the protein-cellulose complex into unimmunized mice. They are at present being studied.

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