AN EXPERIMENT TO DETERMINE THE PHYSIOLOGICAL NATURE OF THE ANAMNESTIC REACTION

V. I. Luk'yanenko

Department of Immunology (Head – Active Member AMN SSSR N. N. Zhukov-Verezhnikov), Institute of Experimental Biology (Director – I. N. Maiskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 54, No. 12, pp. 71-74, December, 1962
Original article submitted February 12, 1962

To study the physiology and laws of immunogenesis it is particularly important to make a many-sided approach to the so-called anamnestic reaction, i.e., to the phenomenon of the nonspecific stimulation of the production of antibodies by a wide range of stimuli which include both antigens and nonantigens [4, 5, 10-14, 16, 17].

According to published reports, the phenomenon of the anamnestic reaction is associated with the active and renewed production of antibodies which occurs without the injection of a specific antigen, and which is of great importance in connection with the hypothesis of the indirect formative influence of the antigen on the specificity of antibodies [8, 9, 15].

At the same time, the physiological mechanisms of this reaction remain unexplained. In many recent reports [1-3] attention has been drawn to the great importance of conditioned reflex mechanisms, and to the laws of the dynamic stereotype in determining the physiological basis of the anamnestic reaction.

We have attempted to reveal the physiological mechanisms and the conditions under which the anamnestic reaction develops. The method we used was to elicit the anamnestic reaction under conditions in which the immunogenesis produced by the initial injections was strongly inhibited by the dynamic stereotype already established.

EXPERIMENTAL METHODS

The experiments were performed on 18 chinchilla rabbits weighing 3.5-4.5 kg, which were divided into three equal groups, two experimental and one control. As antigens we used formol vaccines of B. coli com. 1094, Flexner's dysentery bacillus 170 "C," and the typhoid bacillus (Salmonella typhi Ty 2.4446). The animals of the first group (Nos. 11, 12, 13, 17, 18, 19), and those of the control group (Nos. 23, 25, 26) were vaccinated with a combined B. coli and Flexner bacillus vaccine; animals of the second group (Nos. 14, 15, 16, 20, 21, 22) and of the control group (Nos. 24, 27, 28) received a combined typhoid and dysentery antigen. Each animal received from two to four injections of 1 billion bacterial cells.

The initial inhibition of the immune response was made by establishing in the rabbits a stabilized fixed dynamic stereotype induced by 60-90 applications of a complex set of stimuli given together with a daily injection of physiological saline. We have previously given an account of the method of formation of the dynamic stereotype [6, 7]. Here it is important to point out the principal condition in the formation of such a stereotype is the strictly constant quantitative constitution of the measures used, consisting of combined remote and contiguous stimuli applied in a regular sequence; it is also important to maintain a strict constancy with regard to the time and place of the sequence of stimuli.

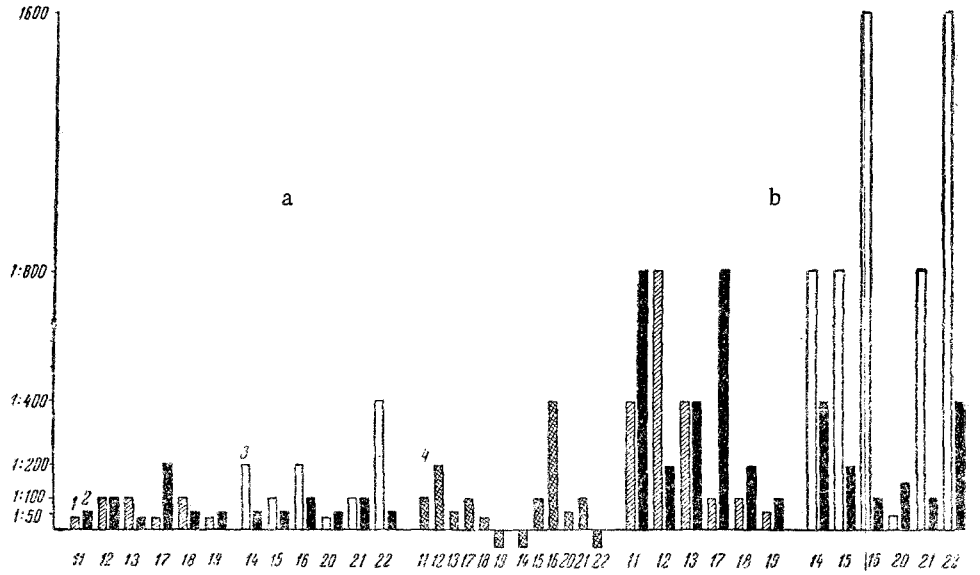
One month after the last injection of the combined antigens described above, the whole experimental group was inoculated with a heterologous antigen consisting of 2 billion paratyphus bacteria (S. paratyphi B 24).

The immunological response to the antigens was studied in terms of the agglutination reaction. Ten days after each inoculation the antibody titer was determined with respect to each antigen separately. After one month the titer of the specific agglutinins was made also with respect to the antibodies to the paratyphoid antigen. As antigen

in the agglutination reaction we used the same vaccine which was employed to produce immunity. The reaction was estimated in terms of the limiting dilution of this serum.

EXPERIMENTAL RESULTS

One month after the end of the experiments in which the combined antigens were injected, under the standard conditions of the previous experiment all the experimental animals received an intraperitoneal injection of a suspension of 2 billion cells of a 24 h culture of paratyphoid antigen grown on agar; these had not previously been used for vaccination. After two weeks, the antibody titer to all the previously used antigen and to the new antigen was as follows: (see figure).



Part played by the "new" antigen in the manifestation of a stereotype of immune responses inhibited by the mechanism of the dynamic stereotype. Abscissa – number of animals; ordinate – titer of antibodies: 1) to the B. coli antigen; 2) to the dysenteric antigen; 3) to typhoid antigen; 4) to paratyphoid antigen; a) before; b) after inoculation with paratyphoid antigen.

In a considerable proportion of the animals, the new antigen caused a marked increase in the titer of antibodies to the previous antigens or to one of them, whereas the elaboration of the antibodies of the Hertner antigen were considerably reduced, or absent altogether.

It is important to note that in 5 out of 12 rabbits (Nos. 11, 13, 14, 15, and 22) the titer of the antibody to the previously used antigens increased 4-16 times above its original value. In some other animals the antibody titer increased appreciably for only one previously used antigen. For example, in rabbits Nos. 16 and 21, the titer of agglutinins to the typhoid antigen increased eight times, but there was no change in the titer to the dysentery antigen. On the other hand, in rabbit No. 20, the antibody titer to the dysentery antigen increased four times, but there was no change with respect to typhoid. In rabbit No. 18 the injection of paratyphoid antigen caused a 4-fold increase in the antibody titer to the dysentery antigen only; the antibody titer to B. coli remained at the previous level. At the same time, in rabbit No. 12 the antibody titer of the B. coli antigen increased 8-fold, but increased only 2-fold for the dysentery antigen.

Thus, when immunity had been established through the development of a stereotype of specific antigens, in some cases (Nos. 11, 13, 14, 15, and 22) a new antigen injection reflexly activated the previous set of immune responses to both antigens by the mechanism of the dynamic stereotype. In other animals (Nos. 12, 16, 17, 21) there was a considerable increase in the antibody titer to one of the antigens of the original group. Only in three of the rabbits of the experimental group (Nos. 18, 19, 20) did the antibody titer increase insignificantly or not at all.

The figure also shows that the production of antibodies to Hertner's antigen in rabbits Nos. 14, 19, 22 was completely suppressed. In rabbits Nos. 13, 18, 20 the response to Hertner's antigen was indefinite and insignificant, and in certain animals (Nos. 12, 16) the antibody titer reached a value of 1:200-1:400.

The antibody titer in the blood with respect to all the antigens mentioned was studied also in the control animals (Nos. 23, 24, 25, 26, 27, 28) receiving no paratyphoid antigen; the object was to study the range of "physiological" variations in the antibody titer of the antigens previously used. No marked "spontaneous" variations in antibody titer were observed at these times, or else they were included within the limits of a single dilution.

The experiments showed that it is possible in principle to elicit the anamnestic reaction in rabbits immunized simultaneously with more than one bacterial vaccine, by subsequent injection of an antigen of the same (bacterial) type.

The principal feature of our experiment is that the anamnestic reaction is produced also in the case where the previous immunization caused no active or marked formation of antibodies, i.e., when there was a "symptomless" specific immunologic reorganization of the organism.

Our experiments and others which have been reported lead us to suppose that the physiological basis of anamnestic reactions is the conditioned-reflex stimulation of an immunogenesis brought about by the mechanisms of the fixed dynamic stereotype, representing a functional combination of conditioned and unconditioned reflexes.

SUMMARY

This paper deals with experiments conducted for assessing the physiological mechanisms of the anamnestic reaction. As demonstrated experimentally, subsequent injection of a new antigen (S. paratyphi B. 24) to rabbits following their simultaneous immunization with a complex of microbial vaccines (two variants): 1) B. coli com. 1094 + B. dys. Flexner, 170 "C"; 2) S. typhi Ty2.4446 + B. dys. Flexner, 170 "C" led to the production of the anamnestic reaction. An important specific feature of the aforementioned experiment is that the anamnestic reaction was also reproduced if the preceding immunization provoked no marked antibody production, i.e., as if against the background of "asymptomatic" reorganization of the organism. On the basis of experiments and literature data the author believes that conditioned reflex immunogenesis stimulation serves as the physiological basis of the anamnestic reactions. This stimulation is effected by the dynamic stereotype mechanisms representing the functional merger of conditioned and unconditioned reflexes.

LITERATURE CITED

1. D. V. Berezhnykh, Byull. éksper. biol., No. 8 (1955), p. 49.
2. A. O. Dolin and V. N. Krylov, Zh. vyssh. nervn. deyat., No. 4 (1952), p. 547.
3. A. O. Dolin, V. N. Drylov, V. I. Luk'yanenko, et al., Zh. vyssh. nervn. deyat., No. 6 (1960), p. 832.
4. P. F. Zdrodovskii, The Problem of Reactivity in a Study of Infection and Immunity [in Russian] (Moscow, 1950).
5. V. I. Ioffe and L. P. Kopytovskaya, In book: Experimental and Clinical Immunology [in Russian], Leningrad (1959), p. 175.
6. V. I. Luk'yanenko, Byull. éksper. biol., No. 2 (1961), p. 82.
7. V. I. Luk'yanenko, Folia biol (Praha), Vol. 7, No. 6 (1961), p. 379.
8. F. Burnet and F. Fenner, The Production of Antibodies, Melbourne (1949).
9. F. Burnet, Aust. J. Sci., Vol. 20 (1957), p. 67.
10. H. Conradi and R. Bieling, Dtsch. med. Wschr., Bd. 42, S. 1280 (1916).
11. G. Dreyer and E. Walker, J. Path. Bact., Vol. 14 (1909), p. 28.
12. R. Fleckseder, Wien. klin. Wschr., Bd. 29, S. 637 (1916).
13. W. Morgan, J. Immunol., Vol. 8 (1923), p. 449.
14. F. Obermayer and E. Pick, Wien. klin. Wschr., Bd. 17, S. 265 (1904).
15. W. Taliaferro and D. Talmage, J. Infect. Dis., Vol. 97 (1955), p. 88.
16. W. Weichardt and E. Schräder, Münch. med. Wschr., Bd. 66, S. 289 (1919).