THE STIMULATORY EFFECT OF BCG VACCINE ON ANTIBODY PRODUCTION

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N. N. Klemparskaya and G. A. Shal'nova

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The stimulatory effect of a tuberculosis infection on the production of antibodies was established long ago [5, 6] In experiments on the artificial introduction of killed tubercle bacilli 10-12 days before the antigen under investigation or simultaneously with it [7] a significant activation of antibody production was also noted.

Antibody production increased not only upon introduction of whole tubercle bacteria cells to rabbits and pigs but also upon injection of isolated fractions obtained from them [8, 9], during which the authors of these studies observed the development of hyperplasia of the cells of the reticulo-endothelial system.

In experiments on irradiated and nonirradiated mice we found [1] an increase in the effectiveness of immunization and increase antibody production upon joint inoculations of vaccines from Gram positive bacteria and BCG vaccine. A stimulatory effect is noted both during simultaneous introduction of both types of preparations and with their separate use when BCG vaccine was injected 10 days before the primary antigen.

In view of the wide use of vaccination with BCG vaccine among the population of the Soviet Union and other countries we considered it of practical importance to study the effect of this preparation on the immunological reactivity of the organism. It is particularly interesting to determine this effect under conditions of ionizing radiation since it is known (2, 3) that vaccination with BCG vaccine before irradiation increases the survival rate and modifies the clinical manifestations of radiation sickness.

The purpose of the present work was an investigation of the effect of BCG vaccine on antibody production by normal and irradiated rabbits.

EXPERIMENTAL METHOD

We studied the effect of the injection of killed cells of BCG vaccine on antibody genesis with respect to Breslau's paratyphoid bacillus in experiments on 29 rabbits; ten of them were irradiated with a nonlethal dose of Co^{60} gamma rays in an EGO-2 apparatus with equal distribution of the radiation around the whole body at high power (228 R/min). In order to eliminate the possibility of denaturation of the antigenic components of the BCG vaccine during its thermal treatment we selected a method of killing the bacteria with phenol (2% solution, exposed for 48 h at room temperature with three subsequent washings with physiological solution); this method is recommended in several papers on the study of the effect of BCG vaccine on anti-infection resistance of animals [4].

In order to exclude the role of a local depositing effect of BCG vaccine during simultaneous injection of the antigens in the same place we injected this vaccine intraperitoneally or intracutaneously in a 5 mg dose in rabbits weighing 2.8-3 kg, and the Breslau bacillus vaccine in a minimal immunizing dose—25 million bacterial cells—intravenously. Some of the animals were vaccinated with both antigens almost simultaneously: the BCG vaccine was injected first and after 3-5 min intravenous immunization with paratyphoid vaccine was done. Another group of animals were first injected with BCG vaccine and after two weeks—with vaccine from Breslau's bacillus. At the site of the intracutaneous injection of the killed bacteria of the BCG vaccine after 24 h there arose a dense restricted infiltrate with a diameter of 0.5 to 1 cm which disappeared only after 2-3 weeks. From the degree of this local reaction it was possible to judge the effect of the injection of paratyphoid vaccine on the reaction of the organism with respect to BCG vaccine.

EXPERIMENTAL RESULTS

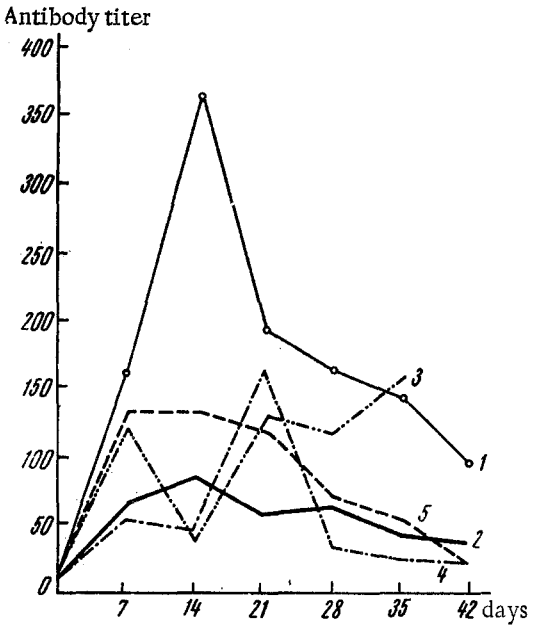

Fig. 1. Dynamics of the production of agglutinins to Breslau's bacillus by nonirradiated rabbits vaccinated with BCG vaccine and paratyphoid vaccine, and vaccinated only with paratyphoid vaccine. 1) Intraperitoneal injection of BCG vaccine two weeks before intravenous immunization with paratyphoid vaccine (5 rabbits); 2) intraperitoneal injection of BCG vaccine 3-5 min before intravenous injection of paratyphoid vaccine (3 rabbits); 3) intravenous injection of BCG vaccine two weeks before intravenous injection of paratyphoid vaccine (3 rabbits); 4) intracutaneous injection of BCG vaccine 3-5 min before intravenous immunization with vaccine of Breslau's bacillus (3 rabbits); 5) control—injection of only paratyphoid vaccine (5 rabbits).

The first series of experiments was carried out on nonirradiated rabbits (Fig. 1).

As shown in Fig. 1 clear stimulation of antibody production was shown only in group 1 * which was injected with BCG vaccine two weeks before the primary antigen. In spite of the significant difference in the level of the curves of experimental group 1 and the control group (curve 5), plotted from average data, statistical treatment using Student's criterion showed a statistically reliable difference in both groups only on the 28th day after vaccination ($P < 0.01$). This is explained by the presence of large variations in the individual titers of experimental group 1 on the 7th, 14th, and 21st day which may be judged also from the value of the mean error (m) which varied during these period from ± 27 to ± 48.5. In the control group m varied from ± 4 to ± 13 which indicated the great uniformity of the indices obtained.

Apparently in the first three weeks after vaccination significant individual variations in the stimulatory effect preliminary vaccination with BCG vaccine are noted. These differences level off by the 28th day when the value of the individual deviations from the means are decreased which makes it possible to establish statistically reliable differences between the experimental and control groups.

A quite different result is obtained in group 2 (the interval between the injection of both antigens here was a few minutes). Combined immunization led to a decrease in antibody production with respect to the second antigen (see Fig. 1), that is, a concrete effect of BCG vaccine was expressed, which up until now, other authors have not reported in their papers.

With simultaneous immunization of these two types of antigens, apparently there occurs inhibition of the reaction not only to paratyphoid vaccine but also to BCG vaccine. We could judge this from the local reaction to BCG vaccine upon its intracutaneous injection. In group 3 intracutaneous immunization was carried out two weeks before vaccination with Breslau bacillus vaccine. The development of an infiltrate in the skin of the animals of this group showed up clearly. In group 4 (simultaneous immunization) on the first days it was generally impossible to note the occurrences of inflammation at the site of the vaccination and even at later periods the development of only a slight induration of the skin was observed. In the case of intracutaneous injection of BCG vaccine some stimulatory effect at later periods was noted only upon separate immunization (group 3).

In the second series of experiments two weeks before irradiation 5 rabbits were vaccinated intraperitoneally with 5 mg of BCG vaccine and 24 h after irradiation with a dose of 600 R vaccinated intravenously with Breslau vaccine. The same number of animals, the control group, were only irradiated and vaccinated 24 h afterwards.

The radiation dose which we used was usually nonlethal for the rabbits, but they showed in symptoms of radiation sickness, that is, leukopenia, loss of weight, disturbance of active immunogenesis and a change in the quality and quantity of the microbial autoflora. Combination of irradiation with immunization significantly complicated the course of the radiation injury: of the 5 control rabbits 4 died (on the 5th, 8th, 10th and 14th days). The average duration of their lives was 9.2 days. Thus, we were only able to observe the dynamics of agglutinin buildup in the one surviving animal.

* From this point on numbers of the groups of experimental animals correspond to the numbers of the curves.

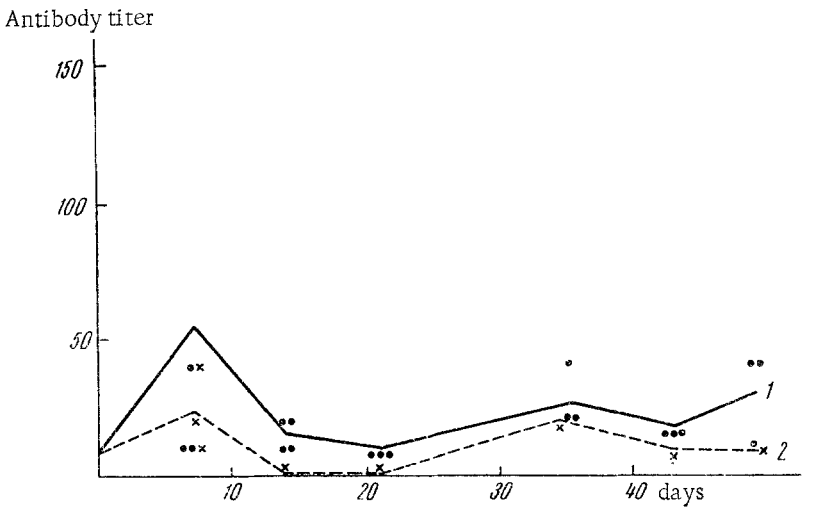

Fig. 2. Dynamics of agglutinin production by irradiated rabbits vaccinated with BCG vaccine and by control animals. 1) Irradiated animals vaccinated with BCG vaccine; 2) control animals. Circles—individual data of animals vaccinated with BCG vaccine, and crosses —controls.

After a small rise on the 7th day the antibody level in the course of two weeks fell to zero and only from the 35th day again increased a little (Fig. 2). Before the death of the rabbits in this group a deeper than usual leukopenia was noted, the weight decreased more and the number of bacteria on the skin and in the mouth increased sharply.

The favorable effect of BCG vaccine on the course of radiation sickness known from our earlier work and other data in the literature [2,3] showed up also in this experiment. Of five rabbits vaccinated with BCG vaccine we could observe the dynamics of antibody production in four (one died immediately after irradiation from shock). In these four rabbits, as seen from Fig. 2, there was the most reaction after 7 days, then the antibody titer decreased but did not disappear completely, as in the control group, and from the 35th day again increased. The leukopenia was less deep and the weight decreased less. On the 15th day one of these rabbits died from pneumonia.

The protective effect of preliminary vaccination with BCG vaccine was also expressed in fewer changes in the mouth and skin autoflora of the experimental rabbits in comparison with the controls. Thus, of 10 analyses of the mouth flora of the control animals (3rd-5th day) in 8 cases we observed the appearance of a large number of E. coli (in 3 of them—before uniform growth) while in the experimental rabbits of 8 analyses there were only three such cases (in only one of them uniform growth was observed). In rabbits of the experimental group cases of uniform growth at the inoculation sites from the surface of the skin were not recorded while in the control animals of ten inoculations on the 3rd-5th day there were two such cases. From the tenth day there was observed in the animals of the experimental group complete normalization of the composition of the mouth and skin autoflora, while in the one control (surviving) rabbit normalization occurred ten days later.

Thus, preliminary (two weeks before irradiation) vaccination with BCG vaccine decreased the disturbance of the immunological reactivity of the animals usually surviving after irradiation. Consequently, under certain conditions (depending on the place of injection and the length of the interval) the use of BCG vaccine in nonirradiated and irradiated rabbits may promote the activation of antibody production with respect to the injection of other antigens. However, further investigations are necessary to clarify the nature of this effect.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
