

RESPONSE OF RABBITS WITH IMPLANTED BROWN-PEARCE TUMOR TO VACCINATION WITH LIVE TULAREMIA VACCINE

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In a previous communication [1] we showed that the formation of immune complement-fixing antibodies in rabbits with an implanted Brown-Pearce tumor was depressed by comparison with the situation in healthy animals. The state of immunogenesis is only one of the indices of the defensive powers of the animal body and does not fully reflect the reactivity of an animal in which a malignant tumor is developing. We know from the literature [3] that post-vaccinal immunity in tularemia is due to the reorganization of the body as a whole and does not depend purely on the reaction of the reticulo-endothelial system or of humoral factors; an important place in the pathogenesis of tularemia is occupied by increased sensitivity to the causative agent of this disease, i. e., by allergy.

In this research we studied the reorganization of the reactive powers of rabbits inoculated with Brown-Pearce tumor and compared them with the response of healthy rabbits, resulting from post-vaccinal immunity against tularemia. The immunity of the rabbits vaccinated with live vaccine was determined by allergic and serological reactions.

METHOD

Experiments were carried out on male chinchilla rabbits of identical weight. Ten rabbits in the first group were inoculated intratesticularly with 0.5 ml each of a 25% suspension of Brown-Pearce tumor cells. A second group, also consisting of 10 rabbits, acted as controls. Six days later, when the development of a tumor could be determined clinically in the experimental group, the rabbits of both groups were vaccinated with a dried live tularemia vaccine, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology (Series No. 492). Vaccination was performed intravenously, once only, each animal receiving an injection of 2×10^9 bacterial cells. The trend of the state of post-vaccinal immunity was studied in accordance with two indices—the allergic test for tularemia and the formation of agglutinins.

Blood was taken for performance of the agglutination test simultaneously with the allergic test to tularin on the 10th, 13th, 18th, 24th, 30th, and 36th day after the animals of the experimental group were inoculated with the tumor. The allergic test was performed as follows: The hair on the rabbit's flank was shaved, the site of the injection was painted with alcohol, and then tularin was injected intradermally through a fine needle in a dose of 0.1 ml (100 million bacterial cells). The reaction was read after 24 and 48 hours and evaluated by plus signs; a reaction of slight hyperemia and edema less than 1 cm in diameter—+, an area of hyperemia and edema 1 cm in diameter—++, and larger than 1 cm in diameter—+++. The second injection of tularin was given on the opposite side of the trunk, and the subsequent injections were made on alternate sides.

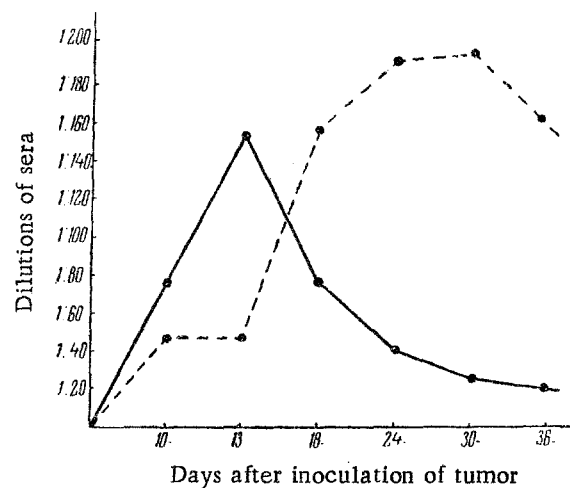


Fig. 1. Changes in agglutinin formation in the sera of rabbits with an implanted tumor and of healthy rabbits after vaccination with tularemia vaccine. Conventional signs: — experimental animals; - - - controls.

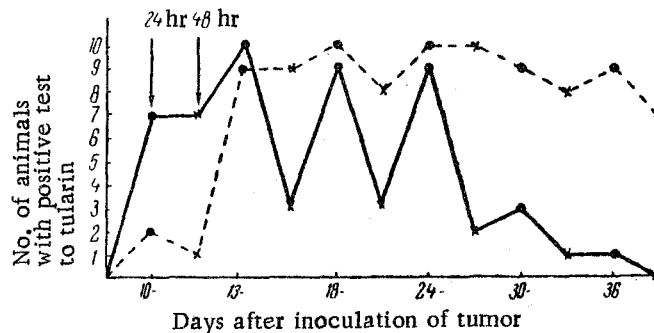


Fig. 2. Changes in the allergic reactions in rabbits with an implanted tumor and in healthy rabbits after immunization with live tularemia vaccine. Conventional signs; — experimental animals; - - - controls; x, — reactions read 48 and 24 hours respectively after injection of tularin.

The agglutination reaction was carried out with tularemia diagnosticum. The sera of the experimental and control groups were tested at the same time. Into each tube was poured 0.5 ml of rabbit serum, diluted by the volume method from 1:10 to 1:1280. Next, to all the tubes was added 0.5 ml of a 10^9 suspension of diagnosticum. The control tubes contained 0.5 ml of antigen and 0.5 ml of physiological saline. The rack with the tubes was shaken and incubated at 37° for 2 hr. The results of the reaction were then read and the tubes were kept at room temperature for 24 hr. The results of the reaction were then read again.

An agglutination reaction in which the liquid was completely transparent and no conglomerate developed on shaking was denoted by +++. In the presence of two or three large clumps of agglutinate and slight opalescence of the liquid above the precipitate, the reaction was counted as +++. When the liquid above the precipitate was perceptibly cloudy and the precipitate was composed of loose floccules, the reaction was denoted by ++. A reaction of + was one in which turbidity was observed above the precipitate and characteristic floccules spread all over the liquid after shaking. In the discussion of the results of the agglutination reaction and the allergic test to tularin, we took reactions of ++ and above to be positive.

We took note of the time of death of the rabbits from metastases during the experiments and of the post-mortem findings.

RESULTS

In Fig. 1 we show the results of investigation of the agglutinin titer in the sera of the experimental and control groups of rabbits after immunization of these animals with live tularemia vaccine. Along the axis of abscissas is denoted the day after inoculation of the Brown-Pearce tumor on which blood was taken for obtaining serum and the agglutination reaction was performed; along the axis of ordinates are plotted the titers of the sera. The curve in the form of a continuous line shows the mean titers of the sera obtained in the agglutination reaction in the experimental group (animals with an implanted Brown-Pearce tumor). The equivalent data for the control animals (healthy rabbits) are shown by the broken line.

It is clear from Fig. 1 that the titers of immune agglutinins in the experimental group reached their maximum value of 1:150 on the 13th day after inoculation of the tumor. With the further development of the tumor, the antibody titers began to fall sharply, whereas in the control rabbits they gradually increased. On the 30th day the titers of the sera of the experimental rabbits fell to 1:24, whereas the titers of the control sera amounted to 1:200.

It is interesting that in the first period of development of the tumor, the reactivity of the experimental animals was higher than that of the controls, for immunogenesis took place more intensively in the former; this is shown by the higher peak of the antibodies in their sera than in the controls (10th-13th day after inoculation of the tumor). These indices are in agreement with the findings obtained from a study of the allergic response of the experimental and control animals.

In Fig. 2 we show the results of experiments with the diagnostic test to tularin. Along the axis of abscissas are denoted the days after inoculation of the Brown-Pearce tumor on which the intradermal tularin test was carried out; along the axis of ordinates is plotted the number of rabbits in which a positive reaction to the intradermal injection of tularin was observed. The mean results of the allergic test to tularin in the experimental group are shown by a continuous line, and those in the control group by a broken line. A black circle on the curves indicates an allergic test carried out 24 hr after intradermal injection of tularin, and a cross—a test 48 hr after the injection.

Examination of the results given in Fig. 2 shows the following feature, which confirms the experiments on the study of immunogenesis.

On the 10th day after inoculation of the tumor the allergic test to tularin was positive in seven experimental rabbits, whereas it was positive in only two controls. Subsequently, until the 30th day, positive tests to tularin when read 24 hr after injection were observed in roughly the same number of animals in the experimental and control groups. Starting on the 13th day, however, sharp differences in the extinction of the allergic reaction were found.

After 48 hr, in the rabbits with an implanted tumor, the tularin test either became negative or was assessed at +, whereas in the control group it maintained its intensity even for 72 hr in the majority of animals. This index was so regular that we later used it to establish the prognosis regarding death of the rabbits with an implanted tumor, and it was always found to be right.

When most of the animals' organs were affected by metastases (30th-36th day), the tularin test in fact became negative when read after 24 hr in nearly all the rabbits with an implanted tumor.

These findings are in agreement with the observations described by other authors [2, 4]; they demonstrate that the tumor itself is a protein sensitizer, modifying the reactivity of the animal. In the first period of development of the tumor the reactivity of the animals is therefore increased, which accounts for the stimulation of immunogenesis and the allergic reactions on the 10th-13th day after inoculation. Subsequently, as metastases become widespread, allergy is depressed and may even proceed as far as its irreversible form—*anergy*.

In our experiments vaccination with live tularemia organisms induced additional sensitization. In consequence of this, the rabbits with an implanted tumor became hypersensitized, which was shown by a considerable depression of their reactivity. At the end of their life a state of total *anergy* developed and they lost the power both of immunogenesis and of manifestation of allergic reactions.

SUMMARY

The reactivity of rabbits with an implanted Brown-Pearce tumor was studied following their vaccination with live tularemia vaccine. Formation of immune agglutinins and allergic reactions were investigated both in healthy rabbits and in animals with an implanted tumor. The study of these reactions demonstrated an intensified reactivity of the experimental animals during the first stage of tumor development; its marked depression up to the time marking the development of anergy was noted during metastasis.

LITERATURE CITED

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* Original Russian pagination. See C. B. translation.