COMPLEMENT-FIXATION REACTION IN RABBITS VACCINATED WITH BCG

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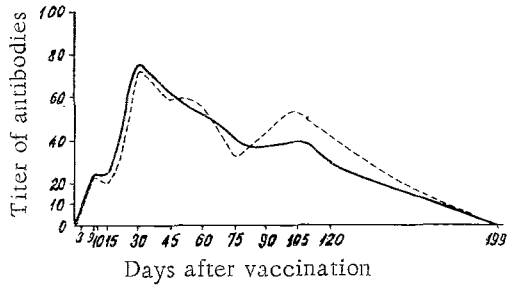
For a deep understanding of immunity to tuberculosis, information concerning the humoral link of the immune mechanisms is highly important. Published reports on this problem are meager and contradictory. In the present work we have studied certain specific humoral immune reactions induced by the BCG vaccine, and have made use of the complement-fixation reaction (CFR).

EXPERIMENTAL METHOD

For the experiment from 25 rabbits 14 were selected weighing 2.5-3 kg, whose serum possessed no nonspecific complement-fixation properties; four of them were used as controls, and 11 were vaccinated with a dry BCG culture. Single intracutaneous injections of 10 mg of the vaccine in 0.2 ml of physiological saline were given. Changes in the titer of the specific antibodies were determined in 3, 9, 15, 30, 45, 60, 75, 90, 105, 120, and 199 days after vaccination. The negative serum of healthy rabbits and of patients with tuberculosis of the lungs giving a positive CFR reaction served as control. On the day before the reaction was carried out the blood for examination was collected from an ear vein of fasting rabbits and was kept in a refrigerator at 4°. The serum was inactivated at 58° for 30 min. The CFR was carried out with the following serum dilutions: 1:10, 1:20, 1:40, 1:60, and 1:80. We used the complement and hemolytic serum (both dry) prepared by the I. I. Mechnikov Moscow Institute of vaccines and sera. As specific antigens in the CFR we used a compound tuberculous antigen of the T. A. Lutsenko Moscow Scientific Research Institute (antigen I), and the dry purified tuberculin of Linnikova [1], which has a high sensitivity when used in the CFR for diagnosis of tuberculosis [2, 4, 5] (series 140 and 147, antigen II).

The dried purified tuberculin is a complex of specific protein (70%), polysaccharide (21-22%), and nucleic acid (8-9%) [2]. By contrast, the complex mixed antigen of Lutsenko (antigen I) contains besides the protein fractions and mixture of polysaccharides an extract of caseous lung totally destroyed by tuberculosis [2].



Changes in the titer of the specific antibodies in the serum of rabbits at various times after vaccination. Continuous line—CFR with antigen I; dotted line—CFR with antigen II.

The antibody titer was determined from the maximum dilution of the serum which gave a weakly-positive CFR, indicated by a +sign.

The results were treated statistically. M represents the mean titer of antibody, and m the standard deviation.

EXPERIMENTAL RESULTS

An analysis of the results showed that the titer of specific antibodies formed during the development of immunity was subject to a regular change (see figure).

In the serum of the experimental rabbits, from the third day after infection with a BDG culture specific antibodies were present,

Changes in the Titer of Antibodies Shown in the CFR in Vaccinated Rabbits

Days after vaccination	Antigen I	Antigen II
	M±m	
3	8,2±1,8	10,0±2,3
9	23,6±4,1	22,7±3,5
15	24,5±6,2	20,0±2,3
30	76,4±6,8	72,7±4,9
45	61,8±5,0	60,0±4,5
60	52,7±4,9	56,4±5,9
75	40,0±4,6	33,6±5,0
90	37,3±4,6	45,4±3,8
105	40,0±6,0	52,7±3,0
120	29,1±3,1	41,8±4,2

although in small amounts. Thus, a weakly-positive CFR (+) diluted 1:10 was found at this time in 5 out of 11 cases after vaccination when the Lutsenko antibody was used, and in 7 out of 11 cases when the reaction was carried out with dried purified tuberculin. The mean antibody titers were 8.2±1.8 and 10.0±2.3 respectively (see table). Subsequently the titer of the complement-fixation antibodies gradually increased. By the 9-15th day after vaccination it already exceeded by $2\frac{1}{2}$-3 times the level reached on the third day after infection with BCG. By the 30th day the titer of specific antibodies had risen still further. A sharply positive CFR, evaluated as +++ or ++++ at a dilution of 1:40 was found on testing with antigen I in 7 out of the 11 rabbits, and with antigen II in 6 out of the 11 rabbits. At this time after vaccination the mean antibody titers were 76.4±6.8 (with antigen I) and 72.7±4.9 (with antigen II (see table).

Next, from the 45th day onwards, the concentration of specific antibodies in the serum of the vaccinated animals fell gradually. However, up to the 60th day the antibody titer remained quite high and was the same for both antigens, and equal to 54.5±5.4. At this time a positive CFR reaction, evaluated as ++ was found in 4 out of the 11 rabbits when tested against antigen I, and in 5 out of the 11 with antigen II. After 4 months, by the 120th day after vaccination, the antibody titer had fallen markedly, but nevertheless it still remained quite high. Complete disappearance of the specific antituberculous antibodies from the serum of the vaccinated rabbits was not recorded until the 199th day after vaccination.

A comparison of the results obtained with the CFR based on antigens I and II shows that the rate of accumulation and the decrease of concentration of antibodies in the serum in the two cases were closely similar (see figure). We need only point out that the CFR was somewhat more sensitive when carried out with antigen I during the 45 days following vaccination. At later times, from the 90th day onward,. the CFR was more sensitive when carried out with antigen II. This difference was statistically significant only at the 120th day after vaccination ($P < 0.05$).

The reason for the differences found is in all probability as follows. The development of specific antituberculous immunity was associated with a gradual increase in the blood of specific antibodies, and therefore, the reaction with pure antigen (dried purified tuberculin) during the second period of observation, when there were no pathological changes in the body induced by BCG, showed up to a greater extent than it did in the reaction with complex mixed antigens containing tissue antigens.

The somewhat greater sensitivity of the CFR when it was carried out with antigen I, as found in the early period after vaccination is most likely to be associated with the presence in this antigen of tissue antigens which were present in addition to the specific protein bacterial fractions. Possibly the former substances react at this period with the corresponding antibodies. Such auto-immune bodies may be formed in conjunction with the damaging action of BCG on the tissues, an effect which is shown chiefly in the first 2-4 weeks after vaccination.

The results we have obtained lead us to disagree with the opinion of T. A. Lutsenko that the complex mixed antigen is overall three times more sensitive than dried purified tuberculin. Our observations indicate that a CFR may be carried out with equal success either with Lutsenko antibodies or with the dried purified tuberculin of Linnikova.

Thus, the formation of specific antituberculous post-vaccination immunity is associated with the accumulation in the blood of specific antituberculous antibodies. To judge from the content of antibodies shown in the CFR the greatest strength of the humoral link in the immunity is reached by the 30th day after infection with a BCG culture. It is at this time that the highest concentration of specific antibodies in the serum of the vaccinated animals is reached. At later times after vaccination the amount of antibodies falls, but they do not disappear completely even by the fourth month. This result agrees with the observation that after vaccination microbial BCG cells penetrate to the lymphatic system, even after 2-3 days, and disappear from it after 3-4 months, or sometimes after 12 months.

Therefore, according to our observations, the CFR is a sensitive serological reaction giving a certain quantitative determination of changes in immunity to tuberculosis after vaccination. The final evaluation of its significance will be possible when a careful comparison has been made of the CFR with the rate of change of other indices giving direct or indirect information on the specific and nonspecific factors concerned in antituberculous immunity following vaccination.

SUMMARY

A study was made of the complement-fixation reaction in rabbits after vaccination against tuberculosis. As a specific antigen dried purified Linnikova tuberculin and Lutsenko complex-mixed antigen were used; apart from proteins, polysaccharides, and lipids, the latter contained an extract from caseous lung destroyed by tuberculosis. There was a gradual increase of specific antibodies in the rabbit serum after BCG vaccination. The immunity attained a maximum by the 30th day. Subsequently the antibody titer declined and no specific antibodies were detectable by the 199th day. Both of the antigens used were found to have practically the same sensitivity and specifity in the complement-fixation reaction.

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