THE PHYSIOLOGICAL MECHANISMS OF IMMUNITY TO TUBERCULOSIS

REPORT 1. THE EFFECT OF VACCINATION AGAINST TUBERCULOSIS ON LEUKOCYTE REACTIONS TO PARENTERAL MILK INJECTION

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Much attention has been paid during recent years to the physiological mechanisms of immunity to tuberculosis resulting from BCG vaccination. A question of particular interest is the role of the changes in nonspecific reactivity in the formation of postvaccinal immunity. It is essential to study, by means of quantitative methods, the nonspecific reactivity of the individual physiological systems or groups of these systems to various accurately graded influences in the course of formation of artificial immunity to tuberculosis.

The object of the present research was to study the changes in the leukocyte composition of the peripheral blood in the course of the development of postvaccinal immunity to tuberculosis. The role of the leukocytes in the protective reactions resulting from the introduction of an infectious agent into the organism is well known. In order to evaluate the changes in the reactivity of the blood leukocytes a chemical stimulus (defatted milk) was used; if injected parenterally this gives rise to the development of an aseptic leukocytosis.

The intramuscular injection of milk leads to regular changes in the leukocyte composition of the blood [1-4, 6, 7]: a marked leukocytosis for 4-5 h and a nuclear shift to the left in the neutrophils. This reaction is reflex in nature: the intramuscular injection of milk into animals anesthetized with ether or barbiturates evoked no regular leukocytic reaction [1-4]. Similar findings have been observed in man [5]. No leukocytosis developed after injection of milk together with a 1% solution of novocain, or injection of milk into a limb deprived of its afferent innervation [7]. A conditioned-reflex leukocytosis has been produced in dogs in response to the parenteral injection of milk [4].

EXPERIMENTAL METHOD

We carried out 142 long-term experiments on ten male gray rabbits weighing 2.5-3 kg. Blood for investigation was taken from the marginal vein of the ear by puncture. As a first step the "spontaneous" variations in the number of leukocytes arising in the experimental environment were determined. For this purpose the rabbits were placed in the room in which the experiment was to be conducted, and the leukocyte count was determined every hour for 4 h. These investigations were repeated until the "orienting" leukocyte reaction had subsided, when control experiments were carried out: for 4 h we studied the effect of an intramuscular injection of 3 ml of warm physiological saline on the number of leukocytes in the peripheral blood. In the first experiments considerable fluctuations were usually observed in the number of leukocytes, much greater than the "spontaneous" variations. When after 2 or 3 successive control experiments the reaction to the puncture and injection of physiological saline was extinguished, the main experiment was carried out with the object of studying the reaction to intramuscular injection of 3 ml of warm defatted milk. After the individual variants of the initial course of the reaction had been established, it was reproduced at intervals of 1.5-2 weeks for a period of 3 months after vaccination. This gave ample time for specific immunity against tuberculosis to develop and become stabilized.

In order to exclude any possible effect of a conditioned reflex to the experimental environment on the leukocyte count, in some animals the injections of milk were alternated with injections of physiological saline.

The rabbits were vaccinated by intradermal injections of 10 mg of a dried BCG culture in 0.2 ml of physiological saline. The efficacy of the vaccination was determined by Mantoux testing on the 10th-15th day after BCG vaccination.

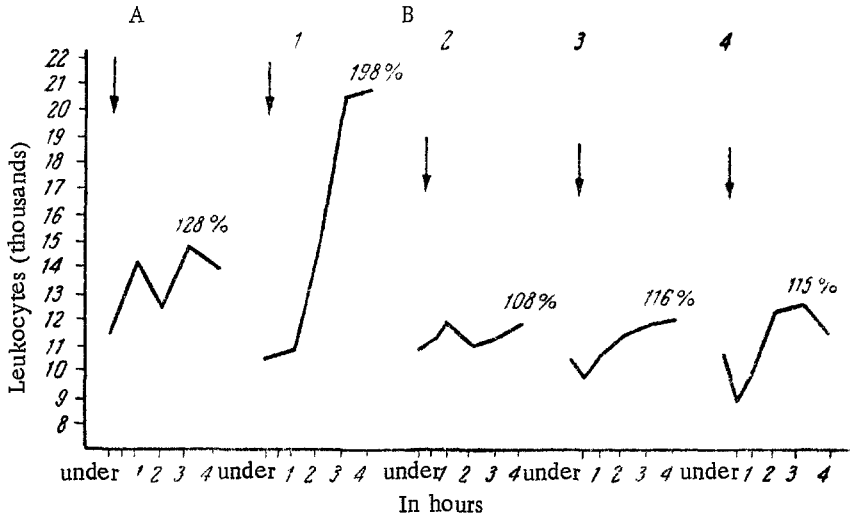

Fig. 1. Changes in the character and magnitude of the leukocyte reaction in a rabbit in response to the intramuscular injection (marked by an arrow) of 3 ml of milk before (A) and after (B) vaccination on the 13th (1), 32nd (2), 66th (3), and 90th (4) day.

EXPERIMENTAL RESULTS

Before vaccination, the intramuscular injection of 3 ml of defatted milk caused a regular and marked leukocytosis to develop in all the experimental animals, with an increase in the leukocyte count to 1550-3650 /mm^3 of blood (by 13.5-35.7%). The average number of leukocytes rose by 2830/mm^3 of blood (by 27%).

After injection of the BCG culture considerable changes took place in the magnitude of the leukocyte reaction to intramuscular injection of milk (Fig. 1). In 6 of the 10 rabbits during the first 10-20 days after vaccination the magnitude of the reaction was twice that in the same animals before vaccination. In four animals injection of milk also caused an increase in the number of leukocytes, although this was smaller than before vaccination.

During this period after vaccination the absolute number of leukocytes increased by 1350-10,350 (by 12.9-108.9%). The average increase in the leukocyte count was 4730 (42.8% of the original level), i.e., 15.8% more than the corresponding increase in the control animals before vaccination (Fig. 2).

From the 30th to the 75th day after vaccination, the magnitude of the leukocyte reaction caused by injection of milk had a tendency in most experiments to return to its original level.

Between the 30th and 50th days after injection of the BCG culture, the magnitude of the leukocyte reaction in 6 animals was smaller than before vaccination. In three rabbits it came close to the initial level, and in one it exceeded this figure. The injection of milk during this period caused an increase of 400-6700 in the leukocyte count (by 3.5-62.5%), with an average figure of 2557 (23.6% of the initial level). These summarized figures indicate that between the 30th and 50th days the leukocyte reaction to injection of milk was rather less intensive than before vaccination (by 3.4%).

Between the 60th and 75th days the leukocyte reaction in 5 of 9 rabbits was smaller than in the controls, in two animals it was close to the original value, and in the remaining two it was greater than the reaction before vaccination and also than the corresponding reaction during the first 10-20 days after injection of the BCG culture. The leukocyte count increased by 300-7000/mm^3 (by 9.5-69.1%), and the average increase was 3240 (30.5% of the original figure). Hence, between the 60th and 75th days after vaccination, the mean value of the leukocyte reaction to injection of milk was more marked than between the 30th and 50th day, but at the same time it was close to its original (pre-vaccination) level, which it exceeded by only 3.5%.

On the 90th day after BCG vaccination the magnitude of the leukocyte reaction and, in some animals, its character underwent considerable change. In 6 of the 8 rabbits surviving until this time of observation, the injection of milk caused an increase in the leukocyte count, although much smaller in degree than in the same animals before vaccination, amounting to only 13.9%. In two rabbits the response reaction became leukopenic, with a

decrease of 21% in the leukocyte count. On the 90th day after vaccination the injection of milk led to an average increase of 530 (5.3%) in the number of leukocytes in the peripheral blood, indicating a sharp decrease in the magnitude of the reaction compared with its level before the experiment began (by 27%).

We may conclude from the analysis of the results that the development of immunity to tuberculosis after vaccination with a BCG culture is accompanied by fluctuating changes in the nonspecific reactivity of the organism. This was demonstrated by changes in the leukocyte reaction to intramuscular injection of milk. This reaction increased sharply in intensity for the first 20 days, and then for the next 2.5 months it corresponded approximately to the initial value; towards the end of the observations, on the 90th day, it decreased considerably and, in some cases, it was actually reversed. This pattern of the change in the reactivity of the leukocytes of the peripheral blood is readily seen if the mean values of the leukocyte reaction as percentages of the initial value are plotted graphically (Fig. 2).

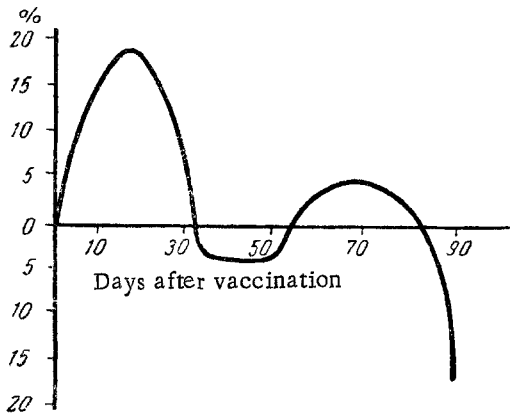

Fig. 2. Changes in the mean value of the leukocyte reaction to intramuscular injection of 3 ml of milk. The reaction before vaccination is evaluated as zero.

We also noted that during the first 3 weeks after injection of the BCG culture, although there was a considerable general increase in the magnitude of the leukocyte reaction, the range of the individual differences in its magnitude was wider than in the control experiments before vaccination: in the first case the number of leukocytes rose after injection of milk by 12.9-108.9%, and in the second by 13.5-35.7%. This demonstrates the lability and instability, and also the high excitability of the mechanisms responsible for the reactivity of the blood leukocytes during this period. This is confirmed by the fact that during the first 3 weeks of the development of immunity the leukocytosis following injection of milk appears more quickly. For instance, whereas before vaccination the leukocytosis reached its peak 3-4 h after the injection of milk, after vaccination the latent period of the reaction was usually shortened, and the peak of the leukocytosis was reached 2-3 h after the injection of milk. Little change took place in the character of the reaction at this period. The changes in the reactivity of the blood leukocytes described above were accompanied by stabilization of the underlying leukocyte count. For instance, in 6 of 10 rabbits the variations in the initial leukocyte count after vaccination were actually smaller than in the control experiments, in 3 animals they were identical, and only in one animal were they greater.

One further conclusion may be drawn from our results and from those described in the literature. The changes in nonspecific reactivity of the leukocyte count, arising as a series of phases during the development of postvaccinal immunity to tuberculosis, are primarily due to changes in the phase of reactivity of the nervous mechanisms regulating the composition of the blood. Consequently, there is reason to suppose that vaccination against tuberculosis modifies the functional state of the nervous mechanisms responsible for the phenomena of nonspecific reactivity.

SUMMARY

As established in this work, the development of postvaccinal antituberculosis immunity, caused by the BCG injection, was associated with the phased changes of nonspecific body reactivity. This is the result of the phased changes of the nervous mechanism reactivity. This was demonstrated by the experimental study of the leukocyte reaction occurring in response to the intramuscular injection of defatted milk (2 ml). The reaction studied underwent the following changes after the vaccination: during the first 20 days its value increased greatly, and then within the subsequent 2.5 months it approached the initial level and often showed a considerable reduction by the end of the observation period (by the 90th day). The range of individual differences in the response to leukocytic reactions also altered considerably. At the same time the initial background composition of leukocytes became stabilized after the vaccination.

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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.
