THE INFLUENCE OF LARGE DOSES OF CAFFEINE ON THE FORMATION OF IMMUNITY TO TUBERCULOSIS IN VACCINATED WHITE MICE

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A. E. Perel'man

Experimental Pathology and Therapeutics Laboratory
Tuberculosis Research Institute, Leningrad
(Presented by Academician V. N. Chernigovskii)

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Literature data on the role of the nervous system in the formation of post-vaccination immunity to tuberculosis is extremely limited. The author has investigated the effect of excessive intensification of the stimulatory processes in the cerebral cortex on the development of post-vaccination immunity to tuberculosis in white mice.

Caffeine was used in these experiments, for according to available data [4], prolonged treatment with large doses [5-7 mg per animal) of the substance results in a lowering of excitation in nervous processes, a weakening of internal inhibition and, after a brief intensification of nervous stimulation, a prolonged depression of central nervous system activity.

EXPERIMENTAL METHODS

The experimental white mice were divided into 5 groups with 60 animals in each group. Animals of the 1st and 2nd groups were immunized by subcutaneous injection of 0.5 mg BCG vaccine and the animals of the 3rd and 4th groups by injection of 1 mg vaccine per mouse. At the time of the vaccination and until the infection of the animals, mice of the 1st and 3rd groups received daily subcutaneous injections of caffeine in 5-7 mg doses. The immunized mice of the 2nd and 4th groups did not undergo caffeine treatment and served as controls to their corresponding experimental group. The animals of the 5th group were not vaccinated; they served as controls in evaluation of the vaccination process. On the 34th day after vaccination animals of all groups were infested intravenously with a culture of virulent tuberculosis mycobacteria of the bovine type (strain No 109) at a dosage of 0.1 mg per mouse. In order to evaluate the infectivity of the tuberculosis injection, 10 animals from each group were killed at 15, 25, 35, 46, and 57 day intervals after infection and macroscopic and microscopic examinations of the lungs and spleen were carried out. The weight of the lungs and spleen and total weight of the animals was also determined. In order to conduct a microscopical examination of the lungs and spleen we fixed these organs in 10% formalin and then prepared paraffin sections of the structures. The sections were stained with haematoxylin-eosin and the mycobacteria with Ziel-Nielsen stain. Ten animals in each group were retained for a survival determination.

EXPERIMENTAL RESULTS

Data from microscopical examinations were tabulated.

Animals of the 2nd group during the 1st period of observation were found to exhibit a clearly defined allergic reaction in their lungs: the normal coloration was intensified to a deep red shade. On microscopic observation the foci of tuberculous inflammation consisting of alveolar macrophages, cells of a lymphoidal type and polymorphonuclear leucocytes were found to be surrounded by zones of edematous, hyperemic tissue. In the nonimmunized animals of the 5th group at this time we found only a few tuberculous foci and their surrounding lung tissue had undergone little change. When the allergic reaction had finally subsided among mice of the 2nd group, the general

Comparative Characteristics of Tuberculous Changes in the Lungs of Experimental Animals as Revealed by Microscopic Examination

Day of observation	Expt. (immunization + infection + caffeine)							Control to expt. (immunization + infection)							Control to immunization (infection)						
	Total value	+	++	+++	++++	A	B	Total value	+	++	+++	++++	A	B	Total value	+	++	+++	++++	A	B
	Injected with 0.5 mg vaccine																				
	1st group							2nd group							5th group						
15	10	3	5	2		4	6	9		6	3		1	8	10	10				2	
25	10	1	8	1		4	5	10		9	1			10	9	7	2			3	
35	10	2	3	4	1	3		10		5	4	1	3	3	9		1	4	4		
46	10		3	4	3			10	1	1	3	5	2		10		1	1	8		
57	7		1	4	2			10		3	4	3			10	1			9		
Total	47	6	20	15	6			49	1	24	15	9			48	18	4	5	21		
	Injected with 1 mg vaccine																				
	3rd group							4th group							5th group						
15	8	6	2			3	1	10	10				9	1	10	10				2	
25	10	4	5	1		5		10	6	4			3	7	9	7	2			3	6
35	10	3	3	4				10	5	3	2		2		9		1	4	4		
46	10	2	4	3	1			10	2	2	6				10		1	1	8		
57	10		2	8				10	3	3	4				10	1			9		
Total	48	15	16	16	1			50	26	12	12				48	18	4	5	21		

Note: + a few scattered foci; ++ single compact foci; +++ a moderate number of compact foci; ++++ a large number of compact foci; A = slight perifocal reaction; B = strong perifocal reaction.

picture was one of more heavy infection of the lungs than in animals of the 5th, control, group (cf. table). Furthermore, as the allergic inflammation subsided the relative weight of the lungs among immunized mice fell, whereas among the control mice it steadily increased (Fig. 1). The number of mycobacteria in the lungs of the 5th group was greater than in those of the 2nd group, but the number of epithelioid tubercles in the spleen was less. What were left of the immunized mice outlived the control animals by a mean value of 126 days.

Hence it is clear that the immunization of white mice with 0.5 mg of BCG vaccine was effective and led to a milder form of the tuberculous condition. At the same time, an acute allergic reaction was observed in the lungs of the mice during the initial period of observation. It should be noted that this allergic reaction in the lungs of immunized white mice following their infection with tuberculosis mycobacteria has been observed by other authors [5].

Only a moderate perifocal reaction (in the form of edema and hyperemia of lung tissue around the tuberculous foci) developed during the initial period of observation in the lungs of animals immunized with 1 mg of BCG vaccine (4th group). The allergic inflammation disappeared on the 35th day after infection and a very slow increase in the number of foci was found to take place, whereas in animals of the 5th group (the control) the increase was a rapid one. The spleen of immunized animals became free from epithelioid tubercles and from mycobacteria as the infection ran its course, whereas the appearance of the infected spleen in non-immunized mice suffered no ameliorative change (Fig. 2). The duration of life among immunized mice of the 4th group exceeded that of the control animals by a mean of 277 days.

Thus, immunization of mice with 1 mg BCG vaccine leads to the course of tuberculous infection being much milder and is accompanied by a moderate allergic reaction in the lungs. Our experiments have shown that the effectiveness of immunizing white mice with a dose of 1 mg BCG is greater than that with a dose of 0.5 mg BCG. The allergic reaction was less intense, however, in the 1st case.

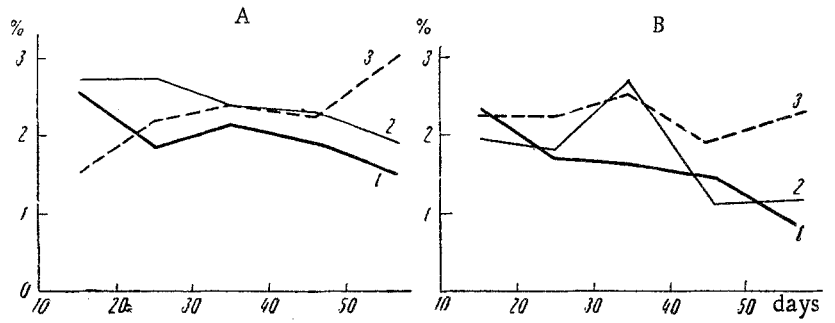

Fig. 1. Changes in the relative weight of the lungs (A) and the spleens (B) of white mice immunized with 0.5 mg of BCG vaccine. 1) First group of animals (expmnt); 2) second group (controls to expmnt); 3) fifth group (controls to immunization).

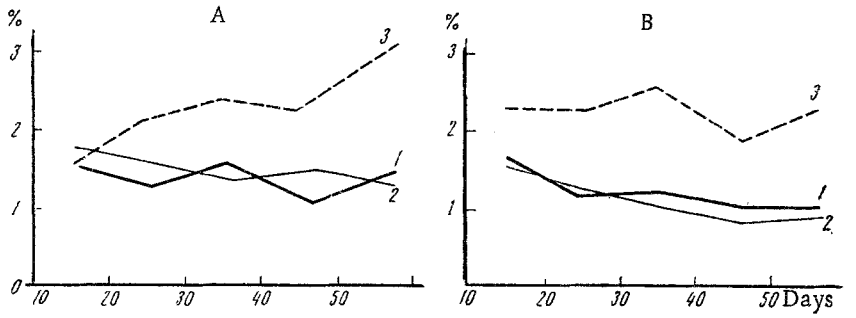

Fig. 2. Changes in the relative weights of lungs (A) and spleens (B) of white mice immunized with 1 mg BCG vaccine. 1) Third group (expmnt); 2) fourth group (control to expmnt); 3) fifth group (controls to immunization).

Comparing the course of the tuberculosis infection in mice immunized with 0.5 BCG and treated with caffeine up to the time of infection (first group) with that of mice not given caffeine (second group), the following observations can be made. Allergic inflammation occurred in the lungs of mice belonging to both groups during the initial period after infection. In mice of the first group, the edema and hyperemia of the lung tissue was less intense, disappeared earlier and was noticed in fewer cases than in mice of the second group. In the first group of animals fewer tuberculous foci were to be observed and those present contained less mycobacteria (cf. table). The infection of the spleen and its mycobacterial content was rather less among animals of the first group than among those of the second. On average the mice of the first group outlived those of the second group by a period of 52 days.

It follows, therefore, that prolonged caffeine treatment during the period when post-vaccination immunity to tuberculosis is developing leads to a marked reduction in the hyperergic inflammation of animals immunized with 0.5 mg BCG vaccine. In addition, there is some resultant amelioration of the subsequent course of the disease. We would dissociate ourselves from the opinion of many authors [2, 7] who state that the ability of the organism to respond to infection by the development of an allergic inflammatory reaction (an ability acquired under the influence of immunization) is a definite attempt at protection against superinfection. Indeed, though part of a specific protection mechanism, a top vigorous allergic reaction may actually lead to damage of the organism [1, 6]. It would seem that the violent allergic reaction in the lungs of mice belonging to the second group goes beyond the bounds of a purely protective reaction. Hence its weakening in animals subjected to caffeine actually boosts the effect of the original immunization.

In animals of the third group, which were immunized with 1 mg BCG vaccine and also received caffeine treatment, allergic inflammation of the lungs was observed on rare occasions and was less intense than among animals of the fourth group which, although immunized with the same dose of BCG, received no caffeine. Ultimately, however, the mice of group 3 developed a serious infection of their lungs more often than did those of the second group. The gradual freeing of the lungs from epithelial tubercles and their associated mycobacteria was expressed in a more pronounced manner among animals of the fourth group. The duration of life among animals of group 3 was on average

78 days less than those of group 4. Thus, applying large doses of caffeine to white mice immunized with 1 mg BCG leads to an initial intensification of the allergic inflammation of the lungs and ultimately to a more serious affection with the tuberculosis disease. The more moderate allergic inflammation of the lungs in animals immunized with 1 mg of vaccine is evidently an important link in the complex immunobiological reorganization of the organism. Hence, its almost complete suppression in animals treated with caffeine is accompanied by a deterioration of the tuberculous process.

We interpret the suppression of the allergic reaction in the lungs of immunized animals and the diminution in intensity of the post-vaccination immunity which accompanies it as an expression of a general reduced reactivity of the organism caused by a prolonged overstimulation of the excitatory processes in the cerebral cortex. The results of the experiments together with those obtained earlier [3], indicate the immunological reorganization of the organism evoked by BCG vaccination is dependent on the functional state of the higher centers in the central nervous system.

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