Assessment of the immunologic activity of lymph nodes regional with respect to the tumor is thus an important stage in the analysis of the patient's immunologic status.

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IMMUNOLOGIC TOLERANCE AND IMMUNITY TO TUBERCULOSIS

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The intensity of immunity to tuberculosis and resistance to the disease in the case of infection with the virulent strain Mycobacterium tuberculosis $H_{37}Rv$ were studied in a model of immunologic tolerance induced in CBA mice by injection of a large dose of BCG polyantigen and the immunodepressant cyclophosphamide. Cellular immunity to tuberculosis (tested by the blast-transformation and tuberculin skin tests) was shown not to develop in tolerant animals after BCG vaccination, and vaccination had no protective effect under these circumstances in the group of tolerant mice.

KEY WORDS: immunologic tolerance; immunity to tuberculosis.

The study of the mechanisms of immunologic tolerance (areactivity) as one form of immune response has been the subject of much research [1-3, 7, 8]. This phenomenon is known to have both a positive and a negative effect on the course of various experimental infections [4-6]. However, it is not clear what is the role of tolerance in tuberculosis.

In this investigation the effect of induced tolerance was studied on the character of formation of immunity to tuberculosis after BCG vaccination and its role in resistance to infection with tuberculosis.

EXPERIMENTAL METHOD

Experiments were carried out on 615 male CBA mice weighing 16-18 g. Tolerance was induced by intraperitoneal injection of a combined antigen obtained by disintegrating BCG vaccine in a ball mill (in a dose of 20 mg), followed after 48 h by intraperitoneal injection of cyclophosphamide (CP) in a dose of 100 mg/kg.

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TABLE 1. Intensity of Reactions of Cellular and Humoral Immunity in Tolerant and Control Animals after Vaccination and Infection (M ± m)

Group of animals	1 month after vaccination				2 months after vaccination			
	incorporation of thymidine-^{3}H, thousands of cpm		results of tuberculin tests, mm	log antibody titer	incorp. of thymidine-^{3}H, thousands of cpm		results of tuberculin tests, mm	log antibody titer
	control (without PPD)	experiment (with PPD)			control (without PPD)	experiment (with PPD)		
1	1,2±0,47	1,5±0,31	0,11±0,029	4,8±0,47	0,9±0,49	2,7±0,21	0,08±0,02	7,2±0,33
2	1,6±0,66	6,8±0,53*	0,36±0,045*	8,6±0,28*	1,3±0,48	5,9±0,48*	0,40±0,03*	8,8±0,59*
3	1,7±0,83	3,7±0,39*	0,28±0,038*	6,6±0,22*	1,1±0,49	4,5±0,39*	0,33±0,04*	7,6±0,53
4	1,4±0,78	4,0±0,50*	0,30±0,04*	6,8±0,48*	1,2±0,72	4,6±0,39*	0,39±0,04*	7,5±0,29

*Difference compared with values for animals of group 1 (tolerant) is statistically significant.

TABLE 2. Mean Duration of Survival of Tolerant and Control Vaccinated Animals after Infection (M ± m)

Group of animals	Number of animals	Mean duration of survival of tested animals, days	Significant
1	36	23,1±0,42	$P_{1-2} < 0,05$
2	35	51,2±0,28	$P_{1-3} < 0,05$
3	38	38,1±0,30	$P_{1-4} < 0,05$
4	36	42,2±0,25	$P_{1-5} < 0,05$
5	37	28,3±0,24	

Legend. Intact infected animals constituted group 5.

All the animals receiving BCG polyantigen and CP (group 1), together with controls (groups 2, 3, and 4), were vaccinated 7 days later with a museum strain of a living culture of BCG subcutaneously at several points in a dose of 1 mg. Animals receiving BCG polyantigen (group 2) or CP (group 3) alone, and intact vaccinated mice (group 4) served as the control.

The animals of all groups were infected with a virulent strain of Mycobacterium tuberculosis H_{37} Rv in a dose of 0.05 mg into the caudal vein 30 days after vaccination.

On the 30th day after vaccination and 2 weeks after infection the intensity of immunity to tuberculosis was studied in the animals of all groups by means of the following tests: Blast-transformation of lymphocytes, the delayed skin test, and aggregate agglutination. In all tests tuberculin (PPD) was used as the antigen.

The immunologic tests were carried out on 36 mice at each time, with 12 mice in each group in three similar experiments; at the same times blood was taken for setting up control cultures (without PPD) from 18 mice in each group (six animals from each experiment of the same kind). Blood was taken from each mouse with sterile precautions from the orbital sinus in a volume of 1.1-1.2 ml. Each sample taken for the blast-transformation test contained 3 ml whole blood (1 ml from each mouse). For the aggregate-agglutination test, each sample contained blood from three mice – 0.6 ml (0.2 ml from each mouse). The blast-transformation test with lymphocytes and the circulating antibody titer were studied in 12 samples at each time in all groups.

Blood for the blast-transformation test was collected in test tubes containing 25 units heparin/ml. The blood of each sample, diluted 1:2, was layered on a solution of Ficoll and Urotrast (density 1.077) and centrifuged at 400g for 25-35 min. The layer of lymphocytes (a narrow ring) was then transferred to clean tubes and washed twice in medium No. 199 (1000 rpm for 10 min). After removal of the supernatant the cells were resuspended in medium RPMI-1640 and their total number and the number of viable cells (stained with trypan blue) counted; on average 1×10^6 lymphocytes with a viability of over 80% were collected from each mouse. After counting, the cell suspension was cultured in flasks containing embryonic calf serum, glutamine, HEPES, medium RPMI-1640, and antibiotics for 6 days with PPD (30 μg/ml) or without it (control) at 37°C.

Thymidine-^{3}H (1 μCi/ml) was added to the flasks 12 h before the end of incubation. Pulses were counted on the SVS-1 counter.

For the aggregate-agglutination test blood from three mice (0.2 ml from each) was mixed and serum separated. An 8% concentration of group O(I) Rh erythrocytes, stabilized and activated with glutaraldehyde,

was used for the test. The erthyrocyte suspension was sensitized with tuberculin PPD-L as antigen (4 mg/ml erythrocytes). The sera for testing were inactivated, after which serial dilutions of the sera were prepared, and sensitized erythrocytes were added to each. The reaction was read first after 2 h and finally after 24 h.

Parallel with the blast-transformation and aggregate-agglutination tests, the intensity of the skin reaction to tuberculin was studied. Under superficial ether anesthesia 0.05 ml of Koch's old tuberculin in a dilution of 1 : 10 was injected into a footpad of the mice, and physiological saline (reaction control) was injected into the footpad of a second limb. The thickness of the pads was measured before and 24 h after injection of the antigen.

The protective effect of vaccination was assessed from the mean duration of survival of the test groups after infection.

The numerical results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Values reflecting the intensity of cellular and humoral immunity after vaccination and subsequent infection of the tolerant and control animals are given in Table 1.

In the "experimental" group reactions of cellular immunity to injection of BCG vaccine did not develop and humoral immunity was less marked than in the control group of intact vaccinated animals. Reactions of cellular and humoral immunity in the control groups were well marked.

The reactions of cellular and humoral immunity still persisted 2 weeks after infection in the animals of the control groups. In the group of tolerant animals, despite the presence of humoral immunity (a high titer of specific hemagglutinins), reactions of cellular immunity to the specific antigen were absent.

The duration of survival after infection (Table 2) was much shorter for tolerant animals than for mice of the control groups (including the group of animals which were infected without preliminary vaccination).

It can be concluded from these results that an induced state of tolerance to BCG polyantigen prevents the formation of immunity to tuberculosis during BCG vaccination and reduces the resistance of the animal to infection with tuberculosis.

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