STIMULATING INFLUENCE OF COMBINED IMMUNIZATION WITH BCG AND OTHER VACCINES ON THE IMMUNOGENESIS OF IRRADIATED AND NONIRRADIATED MICE

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It is known that certain doses of ionizing radiation considerably suppress the immune response, and many investigators have sought for methods of compensating these disturbances. One attempt has been to combine immunization for irradiation with further immunization afterwards [1, 3, 4, 7]; repeated injection of antigen after irradiation has also been tried [6, 7], and so has treatment by injections of homologous spleen or bone-marrow cells, or yeast cultures [8, 12, 13, 14].

We have turned our attention to increasing the effectiveness of immunization by a combination of Gram-positive bacterial antigens, and mycobacterial cells.

It is known that the injection of the BCG vaccine or certain components of tuberculosis bacteria increases nonspecific resistance to infection [2, 9, 15, 16, 17]. The addition of mycobacteria to Freund's "stimulator" [10, 11] is widely used in experiments with tissue antigens to activate immunogenesis. We have found no reports of combined immunization with various vaccines and simultaneous injection of BCG.

The object of the present work has been to study the effectiveness of immunization by vaccines of Gram-positive bacteria and BCG vaccine; the experiments were made on 1,474 irradiated and nonirradiated white male mice weighing 18-20 g.

EXPERIMENTAL METHOD

A single injection was given containing three kinds of antigen: 1) a live culture of B. coli containing 25 or 100 million cells; it was given subcutaneously to 220 mice; 2) a vaccine consisting only of 200 million Breslau paratyphus bacilli cells No. 2503, killed and heated to 56-58° for one hour; the injection was given subcutaneously to 439 mice, of which 80 were used subsequently for tests; 3) the tetravaccine from the Ufimskii Institute of Vaccines and Sera (anti-typhoid, paratyphoid B, and Flexner and Sonne dysenteries) the vaccine contained 2 or 25 million cells in 0.25 ml, and was given subcutaneously to 760 mice, of which 184 were used subsequently for examination.

The BCG vaccine was obtained from the N. F. Gamalei Institute of Epidemiology and Microbiology, AMN SSSR, as a dry preparation. A solution of 1 mg per 0.1 ml was made up with physiological saline, and was added to the vaccine to be tested immediately before injection. The BCG vaccine was used either as supplied, or after it had been autoclaved.

For each experiment, the following groups of mice were used: 1) mice which received one test antigen; 2) mice injected with a mixture of the given antigen with 1 mg of BCG vaccine; 3) mice which received only 1 mg of BCG vaccine; 4) a control group which received an injection of the same volume of physiological saline as was given in the injection.

Irradiation was by x-rays from a RUM-3 apparatus (voltage 180 kv, current 15 ma, filters 1 mm Al+0.5 mm Cu, focusing distance 5 cm, dose 20-22 r/minute); 300 r were administered to 12 mice at a time.

For immunity tests, the animals were infected with bacterial cultures. In the experiments with B. coli, 10 days after injection of the vaccines, the animals received intramuscularly one million bacterial cells, and the percentage of mortality was then determined. In the experiments with pathogenic bacteria, the injection was made after 14 to 28 days with various doses, to determine the LD_{50} by the method of Reed and Mench, and the index of resistance (ratio of the LD_{50} of the experimental group to the LD_{50} of the controls [8]) was determined. In animals which received the tetra-vaccine, immunity only to the typhoid bacillus was determined.

Effectiveness of Immunization of Nonirradiated and Irradiated Animals with Various Vaccines in Combination with the BCG Vaccine

Number of experiment	Vaccine	Nonirradiated			Irradiated			
		number of mice	LD_{50} (in millions)	index of resistance	number of mice	LD_{50} (in millions)	index of resistance with respect to: nonirradiated controls	index of resistance with respect to: irradiated controls
1	Breslau	15	All died from the smallest dose of 1 million		–	–	–	–
	Breslau + BCG	15	1	2.5	20	2.5	7.5	–
	BCG	15	0.1	0.25	20	3	6.2	–
	Control	14	0.4	–	20	Less than 1 million	–	–
2	Breslau	20	30	187.5	20	0.4	2.5	40
	Breslau + BCG	20	60	375	20	2	12.5	200
	BCG	20	10	62.5	20	0.1	0.6	10
	From a live culture of B. coli	20	Less than 1 million	–	20	0.3	0.18	3
	Breslau + living culture of B. coli	20	6	37.5	20	0.1	0.6	10
	Control	20	0.16	–	20	0.01	–	–
1	Tetravaccine	36	225	3.6	36	46	2.1	2.7
	Tetravaccine + BCG	36	359	6.5	36	118	5.6	6.9
	BCG	36	100	1.2	36	19	0.9	1.1
	Control	36	21	–	36	17	–	–
2	Tetravaccine	36	342	3.6	36	141	1.5	4
	Tetravaccine + BCG	36	605	6.5	36	309	3.3	8.8
	BCG	36	115	1.2	36	100	1.0	2.8
	Control	36	93	–	36	35	–	–

Note. Experiments with the vaccine from the Breslau bacillus were carried out with the addition of live BCG vaccine, while those with the tetravaccine were made with the addition of an autoclaved suspension of the BCG vaccine.

EXPERIMENTAL RESULTS

The combined immunization was well tolerated by the nonirradiated animals. They gained as much weight as those which had not been vaccinated (7-9% of the initial weight gained in 14 days), their appearance was good, and they ate all the food given. There was no death from immunization itself. Of the irradiated mice, all of which received the vaccines 24 hours after irradiation, there was a 5-10% mortality among both the animals receiving a single antigen, and in those immunized with a mixture of the antigen with the BCG vaccine. The gain in weight was less in the animals receiving the BCG vaccine alone or together with the preparations tested.

In all experiments the results obtained were uniform and indicated a well-marked stimulating effect from the addition of BCG cells to the vaccines received by both the nonirradiated and the irradiated groups.

In the experiments with vaccines of pathogenic bacteria, before the main experiments were carried out, 246 white mice were used for the selection of the optimal doses and methods of injection. The table shows the results of the main experiments on the nonirradiated and irradiated mice (935 mice) immunized with two kinds of vaccine, given either separately, or in combination with the BCG vaccine.

As judged from the LD_{50} of the index of resistance, in all the experiments the effectiveness of immunization of the combined vaccine was twice as great or more as that due to the injection of the normal vaccines. Particularly striking results were obtained in the irradiated groups of animals. Stimulation of immunogenesis was shown not only in the increased resistance of the animals to infection, but also in the 2- to 3-fold increase in the titer of the agglutinins obtained from the animals receiving the combined vaccine, as compared with those receiving normal immunization; the sera were taken from 3-5 mice together of each group.

It is interesting that in our experiments, and in agreement with published reports, the injection of the BCG vaccine alone also induced a nonspecific increase of the resistance of mice to pathogens, and this effect was particularly well shown in nonirradiated animals receiving intraperitoneal BCG injections.

Besides the BCG vaccine, we also attempted to use a living culture of B. coli as a stimulator (see table). However, this combination did not increase, but actually reduced the effectiveness of the immunity.

It was found that the stimulating effect of the addition of the BCG vaccine was not restricted to vaccines of pathogenic bacteria.

In experiments on 220 mice, we tried the effect of immunization with nothing but a suspension of cells of a 24-hour agar culture of B. coli M_{17}, and with the same culture in combination with an autoclaved suspension of BCG vaccine. Of the noninoculated animals, 10-13% survived; of those which received the injection of B. coli, 41-45% survived; and of the animals treated with the combined vaccine, 72-86% remained alive. It is interesting that the stimulating action was observed also with separate immunization, when the BCG vaccine was injected 10 days before the B. coli culture.

N. N. Klemparskaya and V. M. Zemskov immunized 55 mice against tetanus with a combined antitoxin and an autoclaved BCG vaccine, and found an increased immunity to the toxin. After intramuscular injection with a live tetanus culture diluted 1:50 with physiological saline mixed with calcium chloride, all the control animals died. In the group which had received antitoxin only, 20% died, and marked local tetanus occurred in half of the survivors. In the group receiving the combined injection, not a single mouse died, and in only 16% of the animals were there slight signs of tetanus poisoning.

Therefore, the addition of a suspension of mycobacterial cells of a BCG vaccine to vaccines prepared from Gram-negative bacteria, or to anatoxins, greatly increased the effectiveness of immunization of both nonirradiated and irradiated animals, a result which is of theoretical and practical importance. We shall carry out further investigations on various aspects of the mechanism of this influence.

SUMMARY

Experiments on 1474 white mice showed that the addition of live or killed cells of the BCG vaccine to antigens from Gram-negative bacteria (B. coli, Breslau bacilli, and typhoid bacilli) considerably increased the effectiveness of immunization both of animals irradiated with 300 r, and of nonirradiated animals.

The inoculations were made 24 hours after irradiation. The stimulating effect of the BCG vaccine was also marked when the two immunizations were made separately; in this case the BCG vaccine was given first, and the other vaccine 3-10 days later.

As a result of the combined immunization, not only did more of the animals infected with living bacteria survive, but, in addition, antibody formation was augmented.

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