the spleens of CBA mice, changes in the weight of the lymphoid organs observed in the present experiments can perhaps be explained by immunological mechanisms. This hypothesis is supported by certain features of the phenomenon observed, resembling the graft versus host reaction. As Tables 1 and 2 show, the increase in the splenic index was accompanied by a progressive decrease in the weight of the thymus; the sum of the indices of the thymus and spleen in these experiments, moreover, was constant [9]. The impression is gained that in this test system there is a "redistribution" of activities of the thymus and spleen.

The characteristic dynamics of the weight of the lymphoid organs observed in CBA mice when infected with oncogenic simian adenovirus during the first day of life (Table 2) was partly due, it seems, to a change in the activity of their spleen cells, for when these cells were transplanted into normal syngeneic newborn recipients similar results were observed: an increase in the splenic index accompanied by a progressive decrease in the index of the thymus. The discovery of cells specifically adsorbed on a monolayer of embryonic fibroblasts and also of cells capable, when transplanted into a syngeneic system, of inducing phenomena resembling the graft versus host reaction in newborn animals in the spleens of CBA mice infected with SA7 (C8) virus, indicates that even in the early stages of carcinogenesis an autoimmune response is formed, evidently by induction by the direct action of the virus on the immunocompetent system.

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MECHANISM OF THE INHIBITORY EFFECT OF BCG VACCINE ON SPECIFIC ANTITUMOR IMMUNITY IN SYRIAN HAMSTERS

L. V. Agibalova

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The duration of the inhibitory effect of BCG vaccine on antitumor immunity induced by SV 40 virus in Syrian hamsters and the possibility of restoring immunity after vaccination were investigated. Specific resistance in animals immunized with SV 40 virus and then inoculated with BCG remained inhibited for 1 year after vaccination. A further injection of SV 40 virus into hamsters previously subjected to combined immunization reinduced specific immunity in the animals to tumors. The results show that the phenomenon of abolition of specific antitumor resistance by BCG vaccine is probably cellular in nature.

KEY WORDS: BCG vaccine; SV 40 virus; antitumor resistance.

A role of increasing importance in experimental studies of immunoprophylaxis and immunotherapy of tumors by means of systemic adjuvants (BCG, etc.) is being played by tests of combined specific immunization and nonspecific immunostimulation. Investigations in the writer's laboratory have shown that injection of BCG

Laboratory of Immunology of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Duration of Inhibitory Action of BCG Vaccine on Resistance

Immunizing material	Number of animals in group	Time between BCG injection and resistance, mos. testing	Resistance to tumor		
			log PD_{50}	log IR	t
—	5	3	2,55	—	—
SV40	5		⩾3,86	⩾1,3	⩾2,2
BCG	5		2,92	0,37	0,9
SV40 followed 14 days later by BCG	5		1,54	—1,0	—1,5
—	10	6	0,87	—	—
SV40	10		2,69	1,82	5,0
BCG	10		1,97	1,1	2,6
SV40 followed 14 days later by BCG	10		1,44	0,97	1,4
—	10	12	2,1	—	—
SV40	10		3,85	1,75	2,8
BCG	10		1,26	—0,84	—1,4
SV40 followed 14 days later by BCG	10		1,68	—0,42	—0,7

vaccine into hamsters immunized with SV 40 virus completely or partially inhibits specific antitumor immunity [1-3]. The inhibitory effect of BCG on resistance extends to antitumor immunity induced by injection of irradiated tumor cells and it is associated with the action of the bacterial, but not the humoral component of the vaccine. The mechanism of this paradoxical action of BCG on specific antitumor immunity is still unknown.

The object of this investigation was to determine the duration of the inhibitory action of BCG vaccine on resistance and to study whether antitumor immunity can be restored after BCG vaccination in Syrian hamsters.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred Syrian hamsters obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Adult hamsters were immunized by a single intraperitoneal injection of 1 ml of a suspension containing simian vacuolizing virus SV 40 (strain No. 128) with an infectious titer of $10^{7.5}$.

To abolish antitumor resistance BCG vaccine was used in the form of an undried bacterial suspension (prepared by the N. F. Gamaleya Institute of Epidemiology and Microbiology). In all cases BCG was injected intradermally in a dose of 0.25 mg in 0.2 ml of Eagle's medium.

To test the level of antitumor immunity the transplantation test in vivo was used in its most sensitive modification [4]. The logarithm of the dose of cells injected, which caused tumors to develop in 50% of animals (log PD_{50}), was determined by the method of Reed and Muench [6], and the difference between PD_{50} in the groups of experimental and control hamsters gave the index of resistance (IR). The significance of differences in IR was determined by Student's t-test.

EXPERIMENTAL RESULTS

The object of the present experiment was to determine how long after injection of BCG vaccine its inhibitory effect on specific antitumor immunity lasted. For this purpose hamsters inoculated with SV 40 virus, BCG, or SV 40 virus and 14 days later with BCG, as well as control uninoculated animals, were divided into three subgroups, the first of which contained 20 animals, but the other two, because of the long duration of the experiment, contained twice that number of hamsters. The level of resistance to transplantation of cells of the test tumor (SV 40) was determined in the first subgroup three months, and in the second and third subgroups 6 and 12 months respectively after injection of BCG. The inhibitory action of BCG vaccine on resistance was clearly manifested at all the above times. Meanwhile the preparation of BCG used was unable to induce nonspecific antitumor resistance per se in any of the subgroups (Table 1).

To investigate the possibility of restoring resistance in simian hamsters when induced by the virus, experiments were carried out on five groups of animals, which were immunized either with SV 40 virus or with BCG virus or with a combination of both, the order of administration of the components being varied. One group of hamsters, inoculated with SV virus and, 14 days later, with BCG vaccine, received a further injection

TABLE 2. Effect of Reinoculation of SV 40 Virus on Resistance of Hamsters Inoculated with SV 40 and BCG

Immunizing material	No. of animals in group	Interval between immunizations	Resistance to tumor		
			log PD_{50}	log IR	t
—	5	—	2,62	—	—
SV40	5	—	>4,25	>1,63	>2,6
BCG	5	—	≧2,68	≧0,06	≧0,1
SV40+BCG	5	14 days	2,25	−0,37	−0,5
SV40+BCG+SV40	5	14 »	>3,96	>1,34	>2,2
BCG +SV40	5	14 »	>4,25	>1,63	>2,6

of SV 40 virus 14 days after the BCG. Uninoculated animals served as the control. The results summarized in Table 2 show that reimmunization of the hamsters with SV 40 virus two weeks before transplantation of tumor cells neutralized the inhibitory action of the vaccine on resistance and reinduced antitumor immunity in the animals of this group.

A high level of specific antitumor resistance also was observed in the animals inoculated with BCG vaccine and 14 days later with SV 40 virus, confirming previous observations [1-3]. The preparation of BCG vaccine used in this case, just as in the first experiment, was itself unable to induce resistance.

To explain the phenomenon of inhibition of specific resistance by BCG vaccine it has been suggested that the lymphocytes responsible for resistance induced by SV 40 virus are a target for BCG, administration of which may cause them to undergo intensive proliferation of the blast-transformation type [1, 2]. Another mechanism of this phenomenon could be selective stimulation of the cells which neutralize the action of specific immune lymphocytes, possibly by preventing their contact with the target cell or by neutralizing lymphocytes secreted by them. The existence of such T-lymphocytes in the spleen of animals immunized against tumors has been reported by Klein [5]. It can tentatively be suggested that these suppressor cells are more sensitive to the stimulating effect of BCG than T-killers and, as a result of combined immunization, the resultant response of the recipient is directed toward a lowering of antitumor resistance.

Reimmunization of the BCG-vaccinated hamsters with virus caused the formation of new specifically sensitized lymphocytes, and a high level of specific antitumor immunity was restored in these animals.

The mechanism of the inhibitory action of BCG vaccine on resistance thus still remains unexplained. Preservation of the inhibitory effect of the vaccine on specific antitumor immunity for a long period of time and the possibility of reinduction of resistance by means of SV 40 virus indicate that this phenomenon in all probability is cellular in nature. Adaptive transfer experiments may perhaps help to pinpoint the mechanism of abolition of antitumor resistance by BCG vaccine.

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