Mechanism of Action of BCG Vaccine on Neoplastic Proliferation and Host Immune Responses

ANWAR A. HAKIM [x] and NICHOLAS G. GRAND

Abstract □ BCG (Bacillus Calmette–Guerin) vaccine, Tice strain, caused a threefold increase in spleen weight of normal animals and a fourfold increase in spleen weight of sarcoma-bearing mice. In the latter group, the BCG vaccine caused infiltration of the sarcoma cells into the peritoneum and tumor metastasis in the spleen. Spleen lymphocytes from mice immunized with neuraminidase-treated sarcoma or from mice that had overcome an inoculum (100 cells) and a challenge (10^4 cells) of sarcoma P-1798 were cytotoxic against ^{51}Cr- or ^{14}C-2-thymidine-labeled sarcoma cells. The serum of these mice enhanced the cytotoxic activity and inhibited the migration of the syngeneic lymphocytes. These serums also inhibited the migration of peritoneal macrophages from guinea pigs immunized with the sarcoma cells. BCG vaccine enhanced the development and growth of sarcoma P-1798; *i.e.*, 50–100 viable sarcoma cells produced solid tumors in 8% of the untreated animals but in 100% of the BCG-treated animals. The serum of BCG-treated sarcoma-bearing animals inhibited the spleen lymphocyte-mediated cytotoxic action. The spleen lymphocytes from the BCG-treated sarcoma-bearing animals had no effect against ^{51}Cr- or ^{14}C-2-thymidine-labeled sarcoma cells. The data indicate that the serum from BCG-treated sarcoma-bearing animals blocks the spleen lymphocyte-mediated cytotoxic activities directed against proliferation and growth of the sarcoma.

Keyphrases □ Bacillus Calmette–Guerin vaccine—mechanism of action, effect on spleen weight, neoplastic proliferation, mice □ Neoplastic proliferation—effect of BCG vaccine, mice □ Spleen weight—effect of BCG vaccine, mice □ BCG vaccine—mechanism of action, effect on spleen weight, neoplastic proliferation, mice

BCG (Bacillus Calmette–Guerin) vaccine is an attenuated microorganism derived from the bovine tubercle bacillus. Some strains of this mycobacterium act as potent nonspecific stimulators of the reticuloendothelial system. They interfere with the growth of some experimental transplantable neoplasms in laboratory animals and cause regression of established cancers in selected experimental tumor models (1). This form of tumor response occurs in steps which require participation of host immune response (2–4).

Immunological defenses are important in malignant diseases as well as in tuberculosis. The acquired cellular immunity to tuberculosis, whether induced by vaccination or by actual infection, consists of an increased capability of the reticuloendothelial system to prevent intracellular multiplication and both lymphatic and hematogenous spread. Several reports concern the difference between the Tice strain and other Phipps or H 37 Ra strains of the mycobacterium. The Tice strain enhances rather than inhibits tumor growth (5), and more tuberculosis developed among children vaccinated with Tice strain than among the controls (6).

This article reports on the stimulating effect of the BCG Tice strain on tumor growth, namely a transplantable sarcoma P-1798 in BALB/c mice. It also presents *in vitro* evidence of the inhibition of spleen cell- or lymphocyte-mediated response with serum from BCG-treated sarcoma-bearing mice.

EXPERIMENTAL

Cells—Mouse sarcoma P-1798 that had been continuously carried in male BALB/c mice (16–20 g) and lyophilized BCG vaccine, Tice strain[1], were used.

Neuraminidase Treatment—*Vibrio cholera* neuraminidase, 500 units of enzyme/ml, was used. One unit of enzyme activity is equivalent to the release of 1 μg of *N*-acetylneuraminic acid from a glycoprotein substrate at pH 5.5 at 37°. The enzyme itself is not cytolytic (7, 8) and can release sialic acid from both normal and malignant cell surfaces at physiological pH values (9).

The sarcoma cells were incubated with neuraminidase (25 units/5×10^7 cells) at 37° for 1 hr. After incubation, the cells were washed three times in an excess of supplemented Eagle's medium and finally suspended in the same medium.

Effect of BCG Vaccine Dose on Tumor Growth—Various doses of sarcoma cells, from 22 to 2.2×10^7, were inoculated subcutaneously into groups (20 mice/group) of male BALB/c mice (16–20 g). At contralateral sites of the same animal, 4.5 mg of the lyophilized vaccine in 0.1 ml of sterile water was administered by subcutaneous injections. Animals were checked every day to detect appearance of the tumor nodule, and the survival of the animals from the date of appearance of the tumor nodule was noted (Tables I and II).

On the day the mouse expired, the spleens were removed and weighed. When the mouse was observed to be dying, it was sacrificed; the spleen was removed and was used to prepare the lymphocytes and to assess lymphocyte-mediated cytotoxicity. Spleen lymphocytes from normal mice were always prepared fresh and run as controls.

Effect of Various Doses of BCG Vaccine in Tumor Growth by Sarcoma Cells—Inocula of 1.4×10^2 and 1.4×10^4 sarcoma cells were inoculated subcutaneously in groups (20 mice/group) of male BALB/c mice (16–20 g). At contralateral sites of the same animal, the indicated dose of the BCG vaccine in 0.1 ml of sterile water was administered by subcutaneous injections. The animals were checked as described previously.

Preparation of Target Cells—The tumor tissue was cut into 1-mm pieces, gently teased through sieve No. 80 into Eagle's minimum essential medium containing 100 units/ml of both streptomycin and penicillin, and then centrifuged at 100 rpm. The cells were resuspended and washed three times in the Eagle's medium supplemented with 10% heat-inactivated calf serum. Two million sarcoma cells were suspended in 1 ml of the supplemented Eagle's medium, and 0.2 ml of ^{14}C-2-thymidine (specific activity 59 mCi/mole) or 150 μCi ^{51}Cr-sodium chromate (specific activity 200 mCi/mg) was added. After incubation at 37° for 180 min, the mixture was centrifuged at 100 rpm for 5 min. Then the cell pellet was washed with aliquots of the supplemented Eagle's medium until the washings became free of radioactivity.

Lymphoid-Cell Suspension—Lymphoid cell suspensions were prepared from spleens of normal, sarcoma-bearing, and BCG-treated sarcoma-bearing adult BALB/c mice. The spleens were removed aseptically, cut into small pieces, and gently teased through sieve No. 80 into supplemented Eagle's medium. The cell suspension was centrifuged at 1500 rpm for 5 min, and the supernates

[1] Manufactured by the Tuberculosis Research Institute, BCG Laboratories, University of Illinois.

Table I—Effect of Single Dose of BCG Vaccine on Tumor Growth and Spleen Weight by Various Doses of Sarcoma Cells

Inoculum in 0.1 ml Live Cells/ml	Survival[a], days	Average Spleen Weight, mg	Difference, mg (E − C)
2.26×10^7	C: 12–26	C: 324 (280–399)[b]	285
	E: 12–18	E: 619 (387–1124)	
2.26×10^5	C: 18–27	C: 371 (302–424)	326
	E: 18–24	E: 697 (566–1169)	
2.26×10^3	C: 21–25	C: 367 (313–389)	532
	E: 20–25	E: 899 (686–1084)	
22	C: 28–32	C: 359 (308–402)	579
	E: 22–28	E: 938 (755–1399)	
Tumor free[c]		366 (234–673)	

[a] C is the control without BCG treatment, and E is the experimental group that received BCG with tumor cell inoculum. [b] Numbers in parentheses are the range of weights. [c] Group that received only BCG without tumor cell inoculum.

were discarded. Then the cell pellet was resuspended in the Eagle's medium and repelleted at 900 rpm for 60 sec.

The cells in the supernate were recentrifuged at 1500 rpm for 5 min. The resulting cell pellet was washed three times with Eagle's medium, and the cell suspension was evaluated for viability by the trypan blue exclusion reaction. The lymphocytes present in the cell suspension (10^7 cells/spleen) were 90% viable. Morphologically, about 80% of the mononuclear cells present in these suspensions were lymphocytes; the remaining cells were principally macrophages.

Cytolytic Assay—The cytolytic assay used was described by Brunner *et al.* (10). Adult BALB/c mice were injected subcutaneously in the right hindleg with 10^6 sarcoma cells. Twenty-one days later, these animals were sacrificed, the spleens were removed, and a lymphocyte suspension was prepared. This lymphocyte suspension was compared to a lymphocyte suspension of identical cell concentration but obtained from animals (20 mice) inoculated with neuraminidase-treated sarcoma cells.

Ten million viable lymphocytes from these two groups of mice and from normal mice (control) were mixed with 10^5 ^{14}C-2-thymidine- (or ^{51}Cr-) labeled sarcoma cells in a reaction volume of 1.5 ml of the supplemented Eagle's medium. The interaction between the lymphocytes and the sarcoma cells was allowed to proceed at 37°. After 69 min of incubation, 0.5 ml of tromethamine buffer at pH 8.2, containing 1000 units of trypsin, was added. The cells were centrifuged at 1500 rpm for 5 min, 0.5-ml aliquots of the cell-free supernates were taken, and their radioactivity was assessed.

The percentage cytolysis of the target cells was calculated by subtracting from the radioactivity released in the presence of lymphocytes from sarcoma-treated mice that percentage of radioactivity released in the presence of the same number of normal lymphoid cells.

Cell Migration—Using 10^7 cells/ml suspended in the supplemented Eagle's medium, heparin-coated capillary tubes were filled with the washed spleen cells, lymphocytes, or the washed sarcoma P-1798 cells and placed on the bottom coverslip of a Mackanese-type chamber. The cells were incubated at 37° for 24 hr in the supplemented Eagle's medium in the presence and absence of the factors or serums studied. The area of cell migration was calculated as described previously (11).

Table II—Effect of Various Doses of BCG Vaccine and Sarcoma Cells on Spleen Weight[a]

BCG Vaccine, mg	Tumor Inoculum, viable cells/ml	Viability, days	Average Spleen Weight, mg
4.5	1.4×10^4	26–33	709 (534–993)[b]
	1.4×10^2	27–40	834 (602–1356)
4.5×10^{-2}	1.4×10^4	28–33	338 (273–367)
	1.4×10^2	33–47	453 (130–577)
4.5×10^{-4}	1.4×10^4	29–33	341 (263–445)
	1.4×10^2	41–48	379 (304–444)
4.5×10^{-6}	1.4×10^2	37–41	580 (522–624)
	1.4×10^4	31–32	424 (343–499)
None	1.4×10^4	28–33	445 (210–726)
	1.4×10^2	33–50	200 (180–238)

[a] Groups of 20 mice were used. [b] Numbers in parentheses are the range of weights.

Table III—Spleen Lymphocyte-Mediated Cytotoxicity from Animal Immunized against Sarcoma P-1798 Cells

Spleen Lymphocytes from Animals Treated with	Percent of Radioactivity Released into Cell-Free Supernate	
	^{51}Cr-Sarcoma Cells	^{14}C-Sarcoma Cells
Buffered saline	19.0 ± 1.2[a]	0.86 ± 0.03[a]
Buffered saline, but sarcoma bearing[b]	21.6 ± 1.1	1.14 ± 0.04
Neuraminidase-treated sarcoma cells (10^6)[b]	86.2 ± 4.9	58.17 ± 2.5
100 Sarcoma and then challenged with 10^6 cells[b]	85.8 ± 4.6	57.28 ± 2.4

[a] The data are the averages from 10 experiments ± *SE*. [b] The animals were sacrificed and spleen lymphocytes were obtained 21 days after the last treatment or 21 days after inoculation of the tumor cells.

RESULTS

Effect of BCG Vaccine and Inoculum Size on Tumor Growth—When inoculated subcutaneously in the right hindleg, sarcoma P-1798 grew readily and the animals died between 15 and 20 days, depending on the number of cells inoculated. The sarcoma inoculum varied between 10^6 and 10^2 viable cells/mouse. In over 1000 mice inoculated with the sarcoma, there were no spontaneous regressions.

The spleen wet weight of normal BALB/c mice (16–20 g) varied between 64 and 161 mg (average ± *SE* = 120 ± 10.5 mg). Sarcoma-bearing mice had a spleen wet weight of 150–225 mg (average ± *SE* = 190 ± 36.5 mg). Inoculation of the BCG vaccine into normal mice caused an approximately threefold increase in the spleen wet weight (range, 312–647 mg; average ± *SE*, 462 ± 35 mg). If inoculated into sarcoma-bearing mice, the BCG vaccine produced an approximately fourfold increase in the spleen wet weight (range, 491–993 mg; average ± *SE*, 709 ± 53 mg). A greater increase in the spleen wet weight was produced with the smaller initial sarcoma

Table IV—Lysis of ^{51}Cr- or ^{14}C-Labeled Sarcoma Cells: Spleen Cell-Mediated Cytotoxic Activity

Spleen Cell Source	Percent of Radioactivity Released into Cell-Free Supernate	
	^{51}Cr-Sarcoma Cells	^{14}C-Sarcoma Cells
Normal mice	27 ± 2.6	0.66 ± 0.02
Sarcoma-bearing mice		
1st week	37.5 ± 1.7	62.9 ± 1.9
2nd week	30.6 ± 1.3	29.5 ± 1.1
3rd week	21.3 ± 1.06	0.93 ± 0.04
BCG vaccine-treated[a] sarcoma-bearing mice		
1st week	30.4 ± 1.6	10.06 ± 0.94
2nd week	11.5 ± 1.5	6.06 ± 2.35
3rd week	9.6 ± 0.67	5.88 ± 0.09
BCG vaccine-treated[b] sarcoma-bearing mice		
1st week	68.5 ± 2.4	94.33 ± 4.7
2nd week	47.7 ± 2.9	65.60 ± 3.7
3rd week	32.3 ± 1.2	41.16 ± 2.1
Immune mice[c]	85.9 ± 4.7	96.23 ± 2.4

[a] Mice were treated with 4.5 mg/mouse of the lyophilized BCG vaccine. The data are the averages of 10 experiments ± *SE*. [b] Mice were treated with 4.5×10^{-6} mg/mouse of lyophilized BCG vaccine. [c] Mice were immunized with the neuraminidase-treated sarcoma cells. The data are the averages of 10 experiments ± *SE*.

Table V—Effect of Serum on Spleen Cell-Mediated Cytoxic Activities

Serum	Spleen Cells: Normal Mice	Sarcoma-Bearing Mice	Immune Mice	BCG Vaccine-Treated Sarcoma-Bearing Mice
Normal mice	32 ± 3[a]	2278 ± 132[a]	2260 ± 113[a]	184 ± 12[a]
Sarcoma-bearing mice				
1st week	572 ± 39	4125 ± 179	4067 ± 169	4265 ± 183
2nd week	109 ± 8	3397 ± 116	3845 ± 145	1983 ± 74
3rd week	47 ± 3	3918 ± 137	4257 ± 173	49 ± 4
Immune mice[b]	73 ± 4	4689 ± 192	4985 ± 207	76 ± 5
BCG vaccine-treated sarcoma-bearing mice[c]				
1st week	986 ± 97	3671 ± 167	3578 ± 143	4329 ± 191
2nd week	95 ± 8	732 ± 67	895 ± 83	3194 ± 157
3rd week	75 ± 6	77 ± 36	236 ± 36	531 ± 72
O Inactivated	37 ± 3	2530 ± 143	4124 ± 173	92 ± 7
BCG vaccine-treated sarcoma-bearing mice[d]				
1st week	375 ± 27	4123 ± 180	4429 ± 213	4135 ± 185
2nd week	125 ± 8	3915 ± 162	4215 ± 198	3876 ± 157
3rd week	115 ± 6	3761 ± 153	4198 ± 180	3625 ± 132

[a] The data are in disintegrations per minute, the average of 10 experiments ± *SE*. [b] Immune mice are the mice immunized with neuraminidase-treated sarcoma cells. [c] Mice were treated with 4.5 mg/mouse of the lyophilized Tice BCG. [d] Mice were treated with 4.5 × 10^{-6} mg/mouse of the lyophilized Tice BCG.

inoculum of less than 10^2 viable cells. In this experiment, 20 mice/group were used.

The data summarized in Table I suggest that, if administered subcutaneously to mice inoculated simultaneously with sarcoma cells and 4.5 mg of the lyophilized BCG vaccine, the vaccine increased the spleen wet weight in a magnitude that varied inversely with the number of the sarcoma cells in the inoculum.

Effect of BCG Dose on Tumor Growth Induced by Sarcoma Cells—Table II summarizes the results obtained from the effect of various doses of the BCG vaccine administered to mice inoculated with 1.4 × 10^2 and 1.4 × 10^4 sarcoma cells. All groups treated with the vaccine showed hyperplasia of the spleen. The greatest increase in the spleen wet weight was obtained with the highest dose of the BCG vaccine in mice inoculated with 10^2 viable sarcoma cells.

Spleen Lymphocyte-Mediated Cytotoxic Activity of Animals Immunized against Sarcoma P-1798—Mice that were immunized with three successive doses of nonviable sarcoma cells or with neuraminidase-treated viable sarcoma cells successfully overcame a tumor inoculum of 100 viable cells/mouse and survived a second challenging dose, 10^4 viable sarcoma cells, given a month later. During this time, mice from each of these groups (initially 40 mice/group) were sacrificed at various periods to establish the general pattern of the development of cellular immunity.

Four days after the second challenge, six spleens from each group were removed and examined for lymphocyte-mediated cytotoxic activity using the ^{51}Cr- and the ^{14}C-2-thymidine-labeled sarcoma cells. The data summarized in Table III show that low levels of cytotoxic activity against the ^{51}Cr-labeled sarcoma cells were found in the spleen lymphocytes of normal mice. High levels of cytolytic activity against the labeled cells were found in the spleens of mice that were immunized with either nonviable sarcoma cells or neuraminidase-treated sarcoma cells and survived the challenging doses of viable sarcoma cells.

Spleen Lymphocyte-Mediated Cytotoxic Activity of Animals Treated with BCG Vaccine—The same techniques were used to compare the properties of the spleen cells derived from immune mice [*i.e.*, mice inoculated with a low number (60–100) of viable sarcoma cells but tumors failed to grow], sarcoma-bearing, and BCG-treated sarcoma-bearing mice. One group of mice was treated with 0.1 ml of 4.5 mg/ml of BCG vaccine, and a second group was treated with 0.1 ml of 4.5 × 10^{-6} mg/ml. Seven days later, the mice were challenged with 10^4 viable sarcoma cells. In this experiment, 20 BALB/c mice were used per group. The results (Table IV) indicate that pretreatment with BCG vaccine (0.45 mg/ml) suppresses the cellular immune response against the sarcoma challenge. In spite of their depressed cytolytic potentials, the spleens from BCG-treated animals were hyperplastic and histological examination revealed viable sarcoma cells.

Effect of Serum on Spleen Cell-Mediated Cytolytic Activities—The cytolytic activities of spleen cells mixed with serums of normal, sarcoma-bearing, immunized, and BCG-treated sarcoma-bearing mice are presented in Table V. The data indicate that the spleen cell-mediated cytolytic activity against the ^{14}C-2-thymidine-labeled sarcoma cells in the sarcoma-bearing mice decreased as the tumor progressively grew. Similarly, the spleen cell-mediated cytolytic activity against ^{14}C-2-thymidine-labeled sarcoma cells in the BCG-treated tumor-bearing mice also decreased as the tumor grew. The magnitude of the spleen cell-mediated cytolytic activity varied with the dose of BCG vaccine administered. Ani-

Table VI—Cytotoxic Activities of Spleen Cells and Serum

	Spleen Cells: Disintegrations per Minute	Spleen Cells: Disintegrations per Minute Released, %	Serum: Disintegrations per Minute	Serum: Disintegrations per Minute Released, %
None	4501 ± 124	0	4501 ± 124	0
Normal mice	30 ± 6	0.66	35 ± 7	0.78
Sarcoma-bearing mice				
1st week	2837 ± 74	62.9	1218 ± 32	27.1
2nd week	1327 ± 37	29.5	539 ± 17	12.8
3rd week	42 ± 5	0.93	32 ± 5	0.93
BCG vaccine-treated sarcoma-bearing mice[b]				
1st week	453 ± 11	10.1	2347 ± 71	52.14
2nd week	273 ± 8	6.06	2167 ± 63	48.14
3rd week	265 ± 7	5.88	658 ± 21	14.62
Immune mice	2531 ± 82	56.23[c]	1656 ± 47	36.79
BCG vaccine-treated sarcoma-bearing mice[d]				
1st week	4249 ± 120	94.33	3785 ± 103	84.09
2nd week	2953 ± 80	65.60	3156 ± 95	70.11
3rd week	1873 ± 57	41.61	275 ± 80	60.98

[a] The data are in disintegrations per minute, the average of 10 experiments ± *SE*. [b] Mice were treated with 4.5 mg/mouse of the lyophilized Tice BCG. [c] Immune mice are the mice immunized with neuraminidase-treated sarcoma cells. [d] Mice were treated with 4.5 × 10^{-6} mg/mouse of the lyophilized Tice BCG.

Table VII—Migration Inhibition or Enhancing Factor

Cells	Serum[a] from Mice			
	Normal Mice	Immune Mice	BCG-Treated Sarcoma-Bearing Mice	Sarcoma-Bearing Mice
Spleen cells from mice				
Normal	100 ± 4.5[b]	99 ± 4.4[b]	100 ± 5.2[b]	98 ± 4.3[b]
Sarcoma bearing	96 ± 4.3	65 ± 3.1	153 ± 6.4	71 ± 3.7
BCG-treated sarcoma bearing	115 ± 5.3	87 ± 3.9	92 ± 4.1	95 ± 4.2
BCG-treated normal	99 ± 4.4	98 ± 4.4	69 ± 3.2	99 ± 4.4
Immune[c]	98 ± 4.3	8 ± 0.8	141 ± 6.0	56 ± 3.2
Macrophages from guinea pigs				
Normal	93 ± 4.1	94 ± 4.2	105 ± 5.1	98 ± 4.3
Immune	95 ± 4.3	6 ± 0.7	93 ± 4.1	51 ± 3.1
BCG-treated normal	92 ± 4.0	98 ± 4.5	73 ± 3.3	101 ± 4.7
BCG-treated sarcoma-cell inoculated	97 ± 4.5	143 ± 6.5	154 ± 7.1	99 ± 4.4
Sarcoma cells	100 ± 4.6	2 ± 0.0	127 ± 6.0	85 ± 4.2

[a] The serum was drawn from animals 7 days after appearance of the tumor. [b] The data are in percent of the control and represent the average of four separate experiments ± *SE*. [c] Immune animals are mice immunized with three subcutaneous injections of neuraminidase-treated sarcoma cells.

mals receiving higher doses of the vaccine had lower spleen cell-mediated cytotoxic activity.

These experiments suggest that higher doses of the vaccine cause the release of "blocking factors" which block the cytolytic effects of the spleen cells. Serum was drawn from normal, sarcoma-bearing, BCG-treated sarcoma-bearing, and immune mice. Each serum was mixed with a fixed number of viable cytotoxic lymphocytes from immune mice, *i.e.*, from mice that had survived at least one challenge of viable sarcoma cells. The lymphocyte-mediated cytolytic activities were then assessed. The data in Table V indicate that the serum from normal mice had no effect but that the serum from the BCG-treated (4.5 mg/mouse) sarcoma-bearing mice inhibited the spleen lymphocyte-mediated cytotoxic activity of spleens from sarcoma-bearing and immune mice; it had no effect on spleen lymphocytes from normal or BCG-treated sarcoma-bearing mice.

The data in Table VI summarize the cytotoxic activity of spleen cells and of serum from the various groups, each examined separately. The data indicate differences in the cytotoxic activities of spleen cells and serum from BCG-treated sarcoma-bearing mice. In mice treated with 4.5×10^{-6} mg of BCG, the cytotoxic activity of both spleen cells and the serum was enhanced. Higher doses of BCG vaccine inhibited the cytotoxic activity of the spleen cells and of the serum.

Migration of Spleen Cells, Guinea Pig Macrophages, and Sarcoma Cells—In absence of mouse serum and in Eagle's essential medium, migration of spleen cells from capillaries results in three zones: dense, medium, and light. The data in Table VII indicate that if 0.05 ml of serum from normal mice was incorporated into the 1.0 ml of the medium, normal spleen cells migrated in dense and medium zones. The migration pattern indicates that the serum from tumor-bearing mice had no apparent inhibiting effect on the migration of spleen cells from normal mice. On the other hand, the serum from tumor-bearing mice inhibited the migration of spleen cells from tumor-bearing animals. The serum from BCG vaccine-treated tumor-bearing mice enhanced the migration of spleen cells from the tumor-bearing and immune mice but had no effect on the migration of spleen cells from normal mice. Therefore, these observations suggest that the effect of the serum from tumor-bearing mice is specific or related to the tumor. This effect disappeared if the tumor-bearing animals were treated with BCG vaccine, Tice strain. If migration inhibition is caused by the release of a "migration inhibition factor," the serum from the BCG-treated sarcoma-bearing mice causes the release of a "migration enhancing factor."

Migration of sarcoma cells was inhibited by serum from immune and sarcoma-bearing mice, but it was enhanced by serum from BCG-treated sarcoma-bearing mice.

DISCUSSION

The observed changes in the wet weight of the spleens could be the result of several reactions. One of the earliest events in the initiation of humoral immune response is the recruitment of peripheral lymphocytes (12–17), and Zatz and Lance (14,15) showed that lymphocyte "trapping" exhibits immunological specificity and demonstrated the retention of circulating lymphocytes in lymph nodes and spleen after challenge with conventional antigens and skin grafts (15, 18). This effect is thought to lead to the recruitment of increased numbers of antigen-sensitive cells during initiation of an immune response (19). Aberration in lymphocyte circulation patterns also occurs in response to tumor growth (20). More recently, Zatz *et al.* (21) demonstrated the trapping of ^{51}Cr-labeled Moloney sarcoma in the lymph nodes and spleens of tumor-bearing mice.

Freund's adjuvant, complete or incomplete, modified the immune response of mice to an immunizing dose of tumor antigen, and the tumor was able to grow and kill the animals. Pretreatment with complete Freund's adjuvant alone also led to enhanced tumor growth (22).

Animals immunized with normal or neoplastic tissues and individuals bearing spontaneous tumors carry humoral antibodies and cytotoxic lymphocytes which compete for the same antigens on the target cell surface (10, 23–27). Lymphocyte-mediated cytolysis is a complex multistage phenomenon separable into binding followed by a lytic phase (28–32). Therefore, the present studies represent an attempt to expose the mechanisms allowing enhanced tumor growth in mice treated with BCG vaccine, Tice strain. Two phenomena were investigated.

The cytotoxic activities of the spleen cells from tumor-bearing animals are in line with the results of other investigators using a variety of tumor systems. Individuals carrying progressively growing neoplasms have lymphoid cells with a specific cytotoxic effect on cultured tumor cells from the same individual (33–36). The magnitude of the spleen cells cytotoxic effect varied from one group to another and from one period to another. These variations are to be expected since the serums of tumor-bearing individuals have been shown to prevent tumor cell destruction by immune lymphocytes of the spleen cells *in vitro* (34, 37–39) and since this serum blocking activity appears early in primary tumor development (37, 39).

Differences are to be expected in the magnitude of the blocking or enhancing activities of the serum on the spleen cell cytotoxic potency. At any time, a serum may show complete blocking by one method but only partial by another and even a reverse effect against some target cells (40). Even with the same tumor system, the level of blocking detectable may vary under different experimental conditions. For example, by adding serum and lymphocytes to mammary tumor target cells at the same time, Heppner (41) detected only partial blocking whereas Blair and Lane (40) observed complete blocking.

An alternative possibility is that animals with progressively growing tumors have depressed immunological systems. Studies have demonstrated the immunosuppressive properties of various carcinogens (42–52). Based on these observations, the enhancing growth by the BCG vaccine, Tice strain, could be associated with a

BCG-mediated immunosuppression. Thus the vaccine might be acting similarly to a carcinogen.

CONCLUSIONS

Aberrations in lymphocyte circulation patterns cause infiltration of the sarcoma cells into the lymphatic system and their trapping in lymphoid tissue. Catalysis of these aberrations by BCG vaccine, Tice strain, resulted in the hyperplasia of the spleen and metastasis of the tumor in the spleen and the peritoneum.

BCG vaccine also modified the immune response of mice to an immunizing dose of neoplastic cells, so as to enable the tumor to grow and kill the animals. The significance of this effect is complicated by its dependence on several factors: the dose of the mycobacterium and the immune status of the host. At doses higher than 4.5×10^{-6} mg of the lyophilized vaccine, BCG caused suppression of the host cellular immunity.

The serum of progressively growing sarcoma-bearing or BCG-treated sarcoma-bearing mice contains inhibitor(s) of lymphocyte-mediated cytotoxic activities. Since there is no reason to assume that immune responses in any tumor system are directed against a single antigen and since a serum of a tumor donor may contain not only different levels of free antigen, antigen–antibody complexes, and free antibody but also antigens and antibodies of different specificities at different times in the development and growth of the tumor, BCG vaccine, Tice strain, must have catalyzed the formation of humoral complexes that blocked the cellular immunity directed against neoplasms, in this case sarcoma P-1798.

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[x] To whom inquiries should by directed.