MEASUREMENT OF RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN ALBINO MICE

The Immune Phase*, ‡

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Animals vaccinated with living Calmette-Guérin bacillus (BCG) are known to be significantly more resistant to virulent mycobacteria than are nonvaccinated animals. Nonetheless, despite the unequivocal effectiveness of BCG, little is known about the nature of antitubercular resistance, and widespread controversy exists in almost all practical aspects concerning the induction, development, and persistence of immunity both in experimental animals and in man.

Several lines of experimental evidence gathered in our laboratory over the last several years yield information concerning these matters. Hence, a brief summary of our findings is given in this and subsequent papers. The first line of evidence to be dealt with concerns the comparative effectiveness of living vaccines against different types of experimental tuberculous infections. These studies will be described herein, and in an accompanying paper.

Materials and Methods

Animals.—The NCS strain of Swiss albino mice were used in the majority of experiments in this study (1). COBS[1] strain pathogen-free mice were used in a few experiments. The results obtained with animals of either mouse strain were essentially identical and the results described in this paper will be limited to the findings obtained with NCS mice.

Details of animal husbandry have been previously described (2, 3). Female animals were used in all experiments. Mice were obtained when 4–6 wk of age. Groups of 20 animals were used for mortality experiments and were maintained in cages with wire grid bottoms. Groups of 5 animals were used for all other experiments and were maintained in polycarbonate cages

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with sawdust bedding. Iso-cap® (Lab Cages Inc., Kennett Square, Pa.) barriers were routinely used on these cages. All cages were changed weekly.

Animals were maintained on D & G pellets (Dietrich & Gambrill Inc., Frederick, Md.) unless otherwise stated. Animals were given tap water ad libitum. Water bottles were changed thrice weekly.

Organisms.—The several strains of organisms used in these studies are briefly described below.

BCG.—(a) The Montreal strain of BCG was obtained from the Institute of Hygiene, University of Montreal, Montreal, Canada. In preliminary experiments a culture obtained from the surface of egg medium was transferred into Tween-albumin medium, grown at 37°C for 7 days, and subsequently maintained at 4°C. Subcultures from this stock were made in Tween-albumin medium. (b) Ampules of lyophilized BCG (Montreal strain) containing large quantities of bacilli were generously supplied by Dr. A Frappier of the Institute of Hygiene of Montreal and used in the majority of the experiments. Vials of vaccine (Lot No. 1364-4) contained 112 mg dried organisms per ampule and were reconstituted with albumin water. The vaccine was used without subculture. (c) The Phipps strain of BCG, used in a limited number of experiments, was a stock culture originally described in reference 4. Subcultures were made in Tween-albumin medium and grown for 7 days.

Mycobacterium Tuberculosis.—The H37Rv strain of *M. tuberculosis* was obtained from The Trudeau Foundation Laboratories, Saranac Lake, N.Y. in 1967. The organisms were transferred to Kirchner's medium with Tween albumin (5) on receipt. Cultures were maintained at 4°C. Transfers were made from this stock immediately preceding use. For this purpose, 2 ml of the stock culture was transferred to 20 ml of Kirchner's Tween-albumin medium and incubated at 37°C for 5 days. A second transfer of 2 ml of this growth to 20 m of Kirchner's medium without albumin was made and incubated for a further 5 days. At this time the culture had an optical density of 0.230–0.250 and contained approximately 8×10^6 viable units/ml.

In recent experiments comparative tests have been made with a strain of H37Rv newly obtained from The Trudeau Laboratory (Lot No. TMC102). The results obtained with either subculture were essentially the same.

Vaccination.—Mice were vaccinated by peritoneal injection of the BCG strains previously described. Dilutions of vaccine were made in albumin water. Injections were in a final volume of 0.2 ml.

Challenge Infection.—The mortality test described by Donovick et al. (6) was used with but limited modification. Mice were infected by intravenous injection (caudal vein) of 0.2 ml of the undiluted inoculum of H37Rv grown as described above.

BCG Infection.—The in vivo growth of vaccine bacilli in lungs and spleens of mice was determined follwoing administration by the peritoneal, intravenous, or respiratory route. Dilutions of vaccine were made in albumin water. Peritoneal or caudal vein injections were in a final volume of 0.2 ml.

For respiratory vaccination ampules of lyophilized BCG were utilized. Each ampule was reconstituted with 5 ml of water and the sample was homogenized in a glass Teflon tissue grinder for exactly 1 min. The suspension was filtered through a presterilized Millipore Monitor Filter® (Millipore Corporation, Bedford, Mass.) of 5 μ pore size. 10–15 ml of suspension were passed through one individual filter. Inocula prepared in this manner consisted primarily of single cells suitable for aerosol administration. Material sufficient for several vaccinations was prepared at one time and stored at −60°C until use (7).

Animals were vaccinated in a Middlebrook-type aerosolization chamber (Tri R Instrument Corporation, Rockville Center, N.Y.) (8). 10 ml of undiluted vaccine suspension was nebulized over a 60 min period. The rate of infectious air flow was held constant at 20 liters/

min. Following nebulization of the infective inoculum, the aerosol cloud was allowed to decay for 30 min, and the chamber was decontaminated with UV light for a further 15 min period.

Enumeration of Organisms.—Animals were sacrificed by cervical dislocation or by CO_2 anesthesia. Spleens and lungs were aseptically removed. The organs were homogenized in 2% bovine serum albumin water with the aid of a Teflon-glass tissue grinder. Serial 10-fold dilutions were made in albumin water. Samples of each dilution were transferred to sterile screw cap tubes to which 2 ml of base medium containing 0.2% agar was added. The base medium commonly utilized in these experiments had the following compsition (9): $Na_2HPO_4 \cdot 12H_2O$, 3.0 g; KH_2PO_4 (Anhyd.), 4.0 g; sodium citrate, 1.0 g; ferric ammonium citrate (brown pearls), 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; Bacto asparagine, 2.0 g; Bacto tryptone, 5.0 g; Bacto agar, 2.0 g; glycerin, 10.0 ml, and water to 1000 ml. The following additions were made to each 100 ml of melted medium immediately before use; penicillin (10,000 units/ml), 0.1 ml; lysozyme (0.1% in 0.01 N HCl), 1.0 ml; bovine serum, 10.0 ml. The tubes were incubated at 37°C and the number of colonies developing therein was determined after 3 wk incubation.

RESULTS

The most dramatic evidence for the existence of antitubercular immunity is the marked prolongation of survival of vaccinated animals intravenously challenged with virulent bacilli. Despite the apparent simplicity of such mortality studies, however, almost nothing is known about the factors involved in this type of protective resistance. Indeed, even the relation between the development of the vaccinal population and the length of prolongation of survival has seldom been studied and is poorly understood. This matter is directly investigated in the experiments to be described.

Development of BCG in Organs of Mice.—The intra-organ development of BCG administered by the peritoneal route was initially determined in preliminary experiments.

To this end standardized preparations of lyophilized vaccine were used. Typically, a large number of female mice were obtained and randomly apportioned into several groups. Each group of mice was injected by the peritoneal route with 0.2 ml of a serially diluted sample of freshly reconstituted vaccine. At subsequent intervals thereafter representative animals were sacrificed, their lungs and spleens aseptically removed, and the number of BCG organisms present in these tissues determined.

In the first experiment detailed in Table I, 6-wk old female mice were used. Individual animals in different groups received 0.2 ml of vaccine containing, respectively, $10^{5.6}$, $10^{4.6}$, or $10^{3.6}$ cultivable bacilli. The number of bacilli present in the animals' lungs and spleens were determined 24 hr after infection and at intervals thereafter.

The results of a second similar experiment are also detailed in Table I. In this experiment, a wider range of BCG inocula was used and the animals were observed for longer periods of time. Since the results of the two experiments were essentially the same, they will be discussed together.

As may be seen in Table I, large numbers of BCG organisms were recovered from spleens and lungs of animals 24 hr after vaccination, indicating that vac-

cine organisms were widely distributed throughout the animals' organs immediately after their peritoneal administration. The average number of bacilli recovered from the animals' organs at all times after vaccination was directly related to the size of the vaccinating dose.

TABLE I

BCG Organisms Recovered from Organs of Mice at Various Times after Peritoneal Vaccination

Experiment*	Time after injection	Number of bacilli present in organ‡ (log)					
		$10^{5.6}$ BCG cells injected		$10^{4.6}$ BCG cells injected		$10^{3.6}$ BCG cells injected	
		Spleen	Lung	Spleen	Lung	Spleen	Lung
	days						
1	1	4.9 ± 0.48	1.6 ± 0.02	3.4 ± 0.17	1.3 ± 0.19	2.18 ± 0.03	N.D.
	3	5.0 ± 0.32	2.6 ± 0.42	3.5 ± 0.24	1.4 ± 0.28	2.54 ± 0.10	0.92 ± 0.25
	7	4.3 ± 0.53	2.2 ± 0.28	3.7 ± 0.33	1.1 ± 0.31	2.5 ± 0.65	0.50 ± 0.28
	14	4.0 ± 0.44	1.6 ± 0.41	3.0 ± 0.16	0.7 ± 0.25	2.6 ± 0.36	0.34 ± 0.10
	28	3.8 ± 0.37	1.7 ± 0.17	3.1 ± 0.32	0.8 ± 0.13	2.0 ± 0.28	0.6 (1/5)
	42	2.6 ± 0.49	0.2 (2/5)	2.0 ± 0.36	0.1 (2/5)	N.D.	N.D.
		$10^{5.2}$ BCG cells injected		$10^{3.2}$ BCG cells injected		$10^{1.2}$ BCG cells injected	
		Spleen	Lung	Spleen	Lung	Spleen	Lung
	wk						
2	1	3.9 ± 0.22	1.9 ± 0.38	3.0 ± 0.16	0.7 ± 0.18	0.0 (0/5)	0.0 (0/5)
	4	3.9 ± 0.31	1.2 ± 0.58	3.3 ± 0.34	0.3 (1/5)	0.0 (0/5)	0.3 (1/5)
	10	3.8 ± 0.37	0.3 ± 0.19	3.1 ± 0.52	0.2 (1/5)	0.4 (4/5)	0.5 (1/5)
	20	3.2 ± 0.53	0.1 (1/5)	2.2 ± 0.66	0.0 (0/5)	0.4 (1/5)	0.5 (1/5)

N. D. indicates that values were not determined.

* Mice in experiment No. one were 42 days old when vaccinated with BCG; in experiment No. two they were 35 days old.

‡ No. of bacilli (log) present in 1 ml (out of 5) of organ homogenate at indicated time after injection of Montreal strain BCG ± SE of mean. Values in parentheses refer to number of organs positive out of total number tested. Otherwise values are arithmetical average of five organs.

As shown in Table I, the number of organisms present within splenic tissue 24 hr after vaccination was roughly proportional to the number of bacilli initially injected in an animal's peritoneal cavity. Although fewer organisms were taken up by pulmonary tissue, the initial pulmonary population of BCG was also related to the size of the vaccinating dose.

No significant multiplication of BCG occurred in either tissue. 24 hr after infection, for example, approximately 7 × 10⁴ BCG organisms were recovered

from spleens of animals infected with the largest quantity of BCG. No more than 10×10^4 bacilli were recovered from the spleens of these animals at any subsequent time. Mice receiving smaller numbers of BCG responded in a similar fashion. Thus, spleens of mice injected with $10^{3.6}$ BCG bacilli were found to contain an average of 150 vaccinal organisms in their spleens 24 hr after infection; no more than 400 bacilli were ever recovered from animals in this group.

Somewhat surprisingly, it was found that the splenic population of BCG did not appreciably diminish with time. As shown by the results of experiment 2, the number of vaccinal organisms present 20 wk after vaccination were not strikingly lower than those obtained 1 wk after BCG injection. For example, an average of 8000 BCG were recovered from spleens of animals receiving $10^{5.2}$ BCG organisms 1 wk after infection. 20 wk later spleens of these animals still retained an average of almost 2000 BCG bacilli.

This persistence of vaccinal organisms extended over long periods of time. In several experiments it was found that BCG bacilli were still present in splenic tissue of mice 1 yr after peritoneal injection of vaccine.

A similar lack of vaccine multiplication was found in pulmonary tissues. In contrast to the findings with splenic tissue, however, vaccine organisms were slowly eliminated from pulmonary tissue and eventually this organ became free of cultivable bacilli.

Similar results have been found in a large number of experiments performed under a variety of experimental conditions over a period of several years. The results of such experiments can be briefly summarized as follows. Montreal strain BCG administered by the peritoneal route consistently initiated a persistent splenic infection from the outset of administration of the vaccine. The number of bacilli present in the spleens were maintained without appreciable multiplication or destruction for significant periods of time. Furthermore, the size of the splenic infection was proportional to and partially determined by the size of the vaccinal dose.

Thus it appears that BCG injected into mice by the intraperitoneal route behaved much as a living but nonmultiplying antimycobacterial vaccine.

The conditions affecting the acquisition of vaccinal dormancy by intraperitoneally injected BCG were studied more closely in other experiments. The results of these studies are briefly detailed in the next sections.

Comparative Behavior of Peritoneally Injected Phipps Strain BCG.—BCG strains are known to differ in their ability to multiply in vivo. It was therefore of interest to determine whether the ability to persist in the dormant state was peculiar to the Montreal strain of BCG. To this end, a study was made of the development of the Phipps strain of BCG in splenic tissue in comparison with that of the Montreal strain.

6-wk old female NCS mice were obtained and divided into different groups. Actively growing cultures of the two strains of BCG were used in this experiment. In either case, individual

animals received 0.2 ml of undiluted 7 day Tween-albumin culture or the same volume of culture diluted 100-fold. The experimental findings are illustrated in Fig. 1.

As may be seen, the results obtained with the Phipps strain vaccine were similar to those with the Montreal strain. The Phipps strain of BCG rapidly developed a stable bacterial population in splenic tissue, and the size of the ensuing population was determined by the size of the peritoneal inoculum.

The original metabolic condition of the vaccine, i.e. whether in a state of active growth or not, had little noticeable effect on the results. As illustrated in Fig. 1, the development of freshly cultivated Montreal vaccine in the spleens of mice was essentially the same as that shown for lyophilized organisms in Table I.
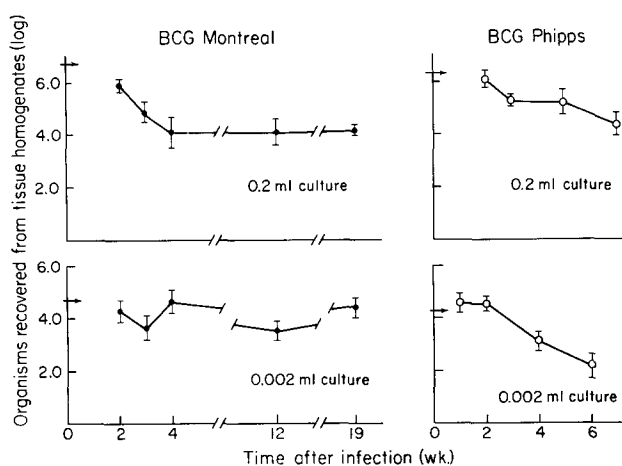

FIG. 1. BCG organisms recovered from spleens of mice after peritoneal infection. Horizontal lines indicate SE of mean.

Comparative Fate of BCG Organisms Injected by Different Routes.—The failure of peritoneally injected BCG to multiply within an animal's organs was studied in other experiments. Experiments comparing the effect of the route of vaccine administration on the ability of BCG to multiply in vivo are of particular interest in this respect.

Female mice were used; they were 5–6 wk old when vaccinated. Vaccine was administered by the peritoneal, intravenous, or respiratory route. The same lot of lyophilized BCG (Montreal) was used in all cases. Typically, animals were intraperitoneally injected with 0.2 ml of suspension containing $10^{4.8}$ BCG organisms, intravenously with 0.2 ml of suspension containing $10^{3.7}$ bacilli, or by exposure to an aerosol prepared by nebulizing $10^{5.5}$ organisms per ml over a 60 min period. The number of organisms present in the spleens and lungs of animals in each case was determined at intervals after infection. The results are given in Table II.

As seen in Table II, BCG multiplied extensively in organs of animals given vaccine by the intravenous route. An average of $10^{1.6}$ bacilli was recovered from

animals' spleens 24 hr after intravenous infection; the lungs retained even fewer organisms. By the following fortnight, however, the number of bacilli had increased significantly in the spleens and in pulmonary tissue. Multiplication continued in both organs through the 4th wk after infection. At this time, large numbers of bacilli were present in both organs. 6 wk after infection the number of BCG present in the animals' spleens and lungs had begun to recede.

Vaccine organisms administered by aerosol also multiplied extensively in vivo.

TABLE II

Multiplication of BCG in Organs of Mice Vaccinated by Different Routes

Route of vaccine administration	Dose (Viable units per mouse)	Time after infection	Number of bacilli present in organ*			
			Spleen		Lung	
		day	*log*	SD	*log*	SD
Intravenous	$10^{3.7}$	1	1.6	±0.46	1.1	±0.16
		14	3.0	±1.22	1.4	±0.64
		28	5.4	±0.89	3.5	±0.38
		42	4.1	±0.45	3.0	±1.01
Aerosol	—‡	1§	0	—	0.7	±0.41
		14	0	—	1.6	±0.72
		28	0	—	4.3	±0.48
		42	1.7	±0.71	5.1	±0.43
Intraperitoneal	$10^{4.8}$	1	3.4	±0.38	1.3	±0.44
		14	3.0	±0.36	0.7	±0.51
		28	3.1	±0.57	0.8	±0.40
		42	2.2	±0.40	0.1 (1/5)	

* No. of bacilli (log) present in 1 ml (out of total 5 ml) of organ homogenate at different times after infection with Montreal strain BCG. Values in parenthesis refer to number of organs positive out of total number tested. Otherwise values are arithmetical average for five organs.

‡ Mice were exposed to the aerosol obtained by nebulizing 10 ml of suspension containing $10^{5.5}$ BCG/ml over a 60 min period.

§ Time of sacrifice 60 min instead of 24 hr.

Organisms were first recovered from pulmonary tissue in which the vaccinal organisms grew profusely. They were also recovered from the spleen but only at a later interval. The kinetics of BCG growth after respiratory infection is given more fully in the following paper and will not be described in great detail here.

The numbers of cultivable BCG organisms recovered from spleens and lungs of mice vaccinated by the peritoneal route did not increase significantly with time. Thus, as in the previous experiments, large numbers of bacilli were initially found in the animals' spleens, and fewer in the lungs. No significant change oc-

curred in the vaccinal population of splenic tissue thereafter. The population of BCG present in pulmonary tissue, which was never large, steadily declined throughout the experiment.

Effect of Age and Diet.—The in vivo multiplication of intraperitoneally injected BCG was studied in mice of different ages maintained on several nutritional regimes.

Adult female mice were used in all cases. The age of the mice at the time of infection of vaccine varied from 5 to 40 wk. In other experiments, 5-wk old mice were fed either whole corn diets without supplementation, adequate or inadequate synthetic diets, or commercial pellet rations. These diets were used for 3 wk before the administration of BCG. The number of BCG bacilli present in the animals' spleens and lungs at intervals of time after infection was determined.

The behavior of BCG was essentially the same in the organs of mice of the various groups studied. As in the experiments previously described, vaccine bacilli were rapidly taken up by the animals' spleens and lungs after peritoneal injection. They persisted in splenic tissue without overt multiplication and slowly declined in pulmonary tissue. Neither the animals' age nor its dietary history significantly altered the course of the vaccinating infection.

Massive Intravenous Challenge.—The results just mentioned indicate that the number of viable bacilli present in the lungs and spleens of mice injected intraperitoneally with BCG is largely determined by the size of the vaccinating dose. It seemed useful to test whether the degree of resistance acquired by mice after intraperitoneal injection of BCG was also determined by the amount of vaccine administered.

For these experiments the simple survival test described by Donovick et al. was utilized (6). A typical experiment will serve to illustrate the techniques involved with this test.

4-wk old female mice were randomly divided into groups of 20 mice. Representative groups of animals were vaccinated by peritoneal injection of BCG. To this end, the Montreal strain of BCG was subcultured in Tween-albumin medium for 7 days. Serial dilutions of growth were made in albumin water and individual animals received 0.2 ml of diluted culture. 27 days later these animals, and also comparable untreated mice, were challenged by superinfection of 0.2 ml of virulent human bacilli administered intravenously. The results are given in Table III.

Unvaccinated animals began to die 11 days after challenge infection. All animals in this group succumbed to the infection within a fortnight. The average survival time was 15 days.

All vaccinated animals exhibited some protection against the lethal effects of challenge. The degree of protection, however, was largely dependent on the amount of vaccine that the animals had received.

Animals receiving the largest quantity of vaccine were most highly protected.

Only a few of them died during the first 30 days after challenge; deaths occurred infrequently thereafter and one animal of this group was still alive when the experiment was terminated after 120 days. In contrast, the majority of mice receiving the lowest dose of vaccine died within the first 30 days after challenge infection. The susceptibility of these mice was little different from that of untreated mice. A few animals in this group, however, exhibited significant resistance against the lethal effects of the challenge infection. The life of these protected animals was prolonged almost as much as for the majority of mice receiving the larger immunizing dose. Animals receiving intermediate doses of vaccine attained a higher degree of protection than animals receiving the lowest quantity of vaccine but a lower degree of protection than those receiving the

TABLE III

Effect of Vaccination on the Survival Time of Swiss Albino Mice Intravenously Challenged with H37Rv

Vaccine	Amount of vaccine injected*	No. of mice	No. of deaths‡							Average survival time
			before 20	20–30	30–40	40–50	50–75	75–100	>100	
	ml									*days*
None	diluent	17	15	2	—	—	—	—	—	15
BCG	0.2×10^{-4}	17	7	2	1	4	2	0	1	21
	0.2×10^{-3}	17	4	3	1	3	4	0	2	31
	0.2×10^{-2}	18	4	1	0	6	3	4	—	41
	0.2×10^{-1}	16	1	2	0	5	6	1	1	47

* Montreal strain of BCG administered by intraperitoneal injection.

‡ Mice dead in period of time (days) after intravenous injection of 0.2 ml of undiluted culture of *M. turberculosis*.

largest amount of BCG. This relative immunity is shown in Figs. 2 *a* and 2 *b* where the mortality data are graphically plotted.

Fig. 2 *a* shows the percentage of animals that died of tuberculous disease at given intervals after challenge. Actual graph points of individual animals are given only for the control group and for the group receiving the largest quantity of vaccine. Fig. 2 *b* presents the same data as a probit graph. The latter graph shows that not only was the average survival time of mice prolonged by vaccination, but also that the kinetic rate of the mortality reaction was significantly altered. This is especially noticeable for mice receiving suboptimal quantities of vaccine.

It should be noted, however, that the differences are due almost entirely to the fact that many of the mice in the latter groups were unprotected. Thus, 53% of the animals receiving the smallest quantity of vaccine died in an acute manner (10) before the 30th day of infection and therefore can be considered

to be nonimmune. Of the animals which received the next three dilutions of BCG, 41, 28, and 19% were also apparently not immunized.

For comparative purposes, these animals were removed from the experimental data and the survival curve for the remaining vaccinated mice (those
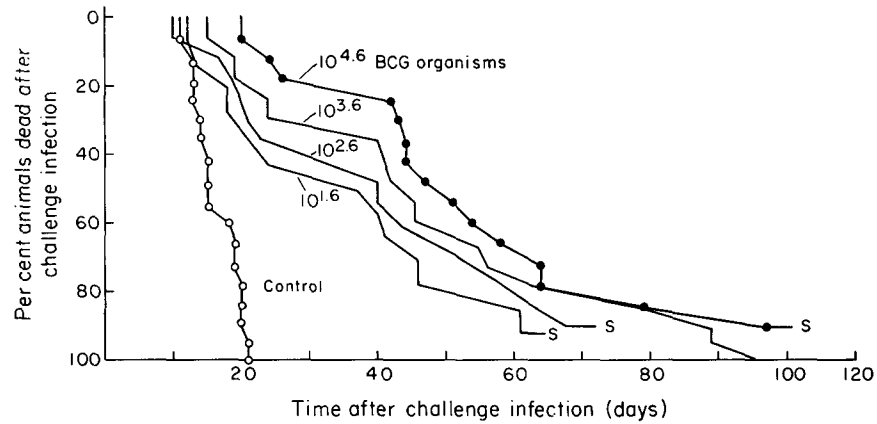

FIG. 2 a. Deaths of vaccinated and control NCS mice after intravenous infection with virulent human bacilli.
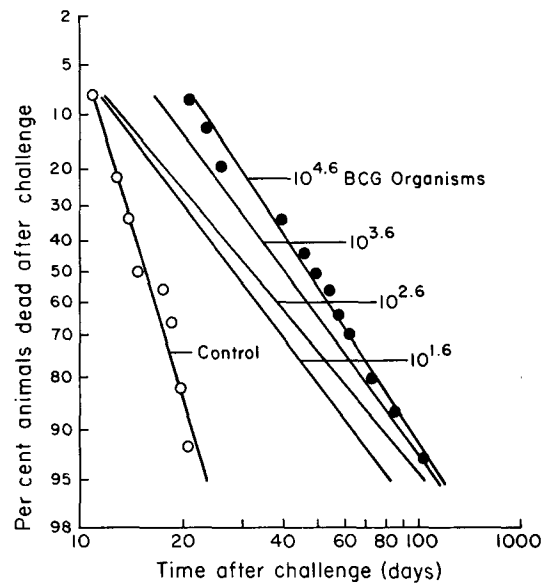

FIG. 2 b. Deaths of vaccinated and control NCS mice after intravenous challenge infection recorded as logarithmic probability.

that died after the 30th day) was replotted. This plot is shown in Fig. 2 c, which shows that there was little apparent difference in the average survival time of such immune animals.

Vaccination Time.—The length of time between vaccination and challenge was increased from 27 to 56 days to allow for any effect due to the in vivo multiplication of the vaccine bacilli. This change, however, did not affect the results. The degree of protection afforded by vaccination was again found to be directly proportional to the quantity of BCG initially injected. As in the preceding experiment, a relatively larger percentage of mice given the larger amounts of vaccine were protected. There were no large differences in the mean survival time of protected animals within the different groups.
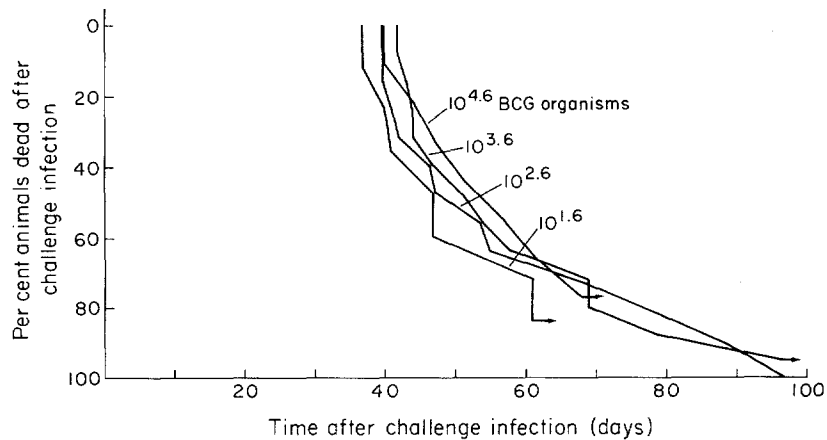

FIG. 2 c. Deaths of immune mice after intravenous challenge infection. See text for explanation.

DISCUSSION

Animals vaccinated with living BCG by the peritoneal route were significantly more resistant to the lethal effects of intravenously injected mycobacteria than were untreated animals. Moreover, the degree of resistance elicited by vaccination was directly related to the quantity of vaccine administered to the animal.

Previous studies from this laboratory have revealed that when the amount of BCG injected intravenously into mice is small, the induction of antitubercular immunity depends upon the multiplication of the vaccine in vivo (11-13). In contrast, when the vaccinal dose injected intravenously is very large, the amount of bacterial protoplasm injected provides outright an antigenic mass sufficient to elicit a high level of antitubercular resistance.

The experimental results reported in the present paper extend these findings. It was found that the ultimate vaccinal population which develops within the organs of mice given BCG by the intraperitoneal route is directly proportional to the initial size of the vaccinating dose. Animals intraperitoneally vaccinated

with large quantities of BCG develop a large population of BCG in their organs and are highly protected against tubercular mortality; in contrast, animals given smaller quantities of vaccine exhibit but limited numbers of BCG in their organs and develop little antitubercular resistance.

It must be emphasized, however, that these findings apply only with regard to the level of protection achieved by each group of mice taken as a whole. Individual animals vaccinated with suboptimal quantities of BCG respond to, the challenge infection in two separate ways. On the one hand, some animals exhibit significant resistance to the lethal effects of the challenge infection, on the other hand, some exhibit little or no protective response.

It should be noted in this regard that Swiss strain mice are not highly inbred. Therefore, it is possible that some variation may occur in the relative sensitivity of different animals to immunization. Nonetheless, it seems unlikely that this explanation can totally account for the results reported here.

The failure of BCG to multiply within the animal's organs after its peritoneal administration is of especial interest and deserves comment. It is well known that strains of BCG differ widely in their ability to grow within an animal's tissues (14, 15). However, the two strains of BCG utilized in this study, namely the Montreal and the Phipps strain, have consistently been found to multiply entensively in vivo. Moreover, as reported here, both strains grow extensively within an animal's tissues when the vaccine is administered by the intravenous or the respiratory route. There is no way at present to account for the tuberculostasis which occurs in splenic tissue following peritoneal injection. Material injected into the peritoneum is rapidly removed via the diaphragmatic lymphatics (16) and reaches the animal's bloodstream within minutes after its administration. Moreover, BCG is as rapidly taken up by the animal's organs after peritoneal injection as it is after intravenous administration. Yet BCG organisms, given by the peritoneal route, fail to multiply in either spleen or lung tissue, whereas the same material injected directly into the bloodstream multiplies extensively in both organs.

Other investigators have noted that large doses of intravenously administered BCG fail to multiply within an animal's tissues, whereas smaller quantities of vaccine grow profusely (17). It is probable that, after administration of large quantities of BCG, a state of acquired resistance is rapidly initiated and that subsequent multiplication of the vaccinating organisms is thereby limited. Whether a similar type of infection immunity is initiated after the peritoneal injection of smaller quantities of BCG cannot be said. It is worth mentioning in this respect, however, that the immune resistance of splenic tissue has been found to be rapidly established.[2] It would appear that the rapid vaccinal dormancy achieved by peritoneally injected mycobacteria might be a useful tool in elucidating the theoretical and practical basis of infection or premunition immunity.

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[2] Costello, R., and S. C. Slats. Data to be published.

SUMMARY

Enhanced resistance against experimental tuberculosis was acquired by albino mice after peritoneal vaccination with living BCG.

Prolongation of life was used as a measure of resistance against massive intravenous challenge infection. The average degree of prolongation of life was found to be directly related to the size of the vaccinating dose. The ultimate numbers of BCG bacilli recovered from the animal's organs were also directly related to the quantity of vaccine initially administered. It appears therefore that the relative degree of antitubercular resistance achieved by immunized mice is closely related to the vaccinal population present in the animal's organs.

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