

TEMPORAL DEVELOPMENT OF RESISTANCE TO PULMONARY
TUBERCULOSIS IN SWISS ALBINO MICE*, ‡, §

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In a preceding paper (1) it was shown that protection against the lethal effects of intravenously injected virulent mycobacteria in mice is related to the vaccinal population which develops within an animal's tissue.

Living Calmette-Guérin bacillus (BCG) is also capable of inducing significant antitubercular protection against respiratorily induced tuberculosis (2, 3). But few attempts have been made to correlate the size of the vaccinal population in vivo with the evolution of the immune state.

In the experiments to be described herein, mice were immunized with the Montreal strain of BCG. After vaccination, they were superinfected with virulent human bacilli administered by the aerosol route. At sequential intervals thereafter the numbers of vaccinal or of challenge bacilli present in lungs or spleens were determined. The extent of retardation of the growth of challenge bacilli was used as a measure of immune resistance.

A slight degree of protection was found in the lungs and splanchnic tissues of vaccinated animals. This heightened resistance appeared only after extensive multiplication of the vaccine had occurred, resulting in the accumulation of large numbers of BCG bacilli in the animal's tissues.

Materials and Methods

The materials and methods used in this study have been described (1, 4). 4-6 wk old female mice were used. They were maintained on a commercial diet (D & G pellets, Dietrich & Gambrill Inc., Fredrick, Md.) fed ad libitum.

The H37Rv strain of *Mycobacterium tuberculosis* obtained from The Trudeau Foundation Laboratory, Saranac Lake, N.Y. was cultured in Kirchner's medium containing Tween 80

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and bovine serum albumin. The Montreal strain of BCG was obtained from the Institute de Microbiologie et d'Hygiene de l'Université de Montréal, Montréal, Canada through the courtesy of Dr. Frappier.¹ This strain was supplied as a standardized, commercially available, lyophilized culture containing 112 mg of bacillary material per vial; it was used as such without subculturing.

Respiratory Infection.—Suspensions for nebulization were prepared as follows. Reconstituted suspensions of lyophilized BCG or of young growing H37Rv cultures were homogenized with a Teflon glass homogenizer and filtered through a membrane filter (Millipore Corporation, Bedford, Mass.) of 5 μ pore size to provide a suspension containing predominantly single bacilli. Filtered suspensions were stored at -60°C until use. Mice were infected in a Middlebrook-type aerosol infection chamber (Tri-R Instrument Corporation, Rockville Centre, N.Y.). Infective aerosols were obtained by nebulizing 10 ml of suspension containing approximately 1×10^6 viable units of mycobacteria per ml over a 60 min period.

Enumeration of Mycobacteria in Organs.—At intervals after vaccination or challenge, mice were sacrificed and their lungs and spleens were homogenized in Teflon glass tissue grinders containing 5 ml of 2% albumin water. Samples of homogenate were diluted with distilled water containing 0.01% bovine serum albumin. Suitable portions were pipetted into sterile screw cap tubes and to each tube was added 2 ml of soft agar medium (4). Incubation was at 37°C for 21 days. The number of viable mycobacteria was estimated from the number of colonies obtained.

The organisms derived from the challenge dose were distinguished from BCG bacilli by the differential inhibitor 2-thiophenecarboxylic acid hydrazide. The inhibitor, in water, was sterilized by filtration and added to the soft agar medium in a final concentration of 15 $\mu\text{g}/\text{ml}$.

RESULTS

Growth of BCG in Organs of Mice After Aerosol Vaccination.—Vaccination was readily accomplished by aerosol administration of BCG.

The in vivo growth of the Montreal strain of BCG given in this manner was followed in the first experiment. 6-wk old female mice were placed in the infection basket of the aerosol apparatus. They were infected by exposure to the aerosol obtained by nebulization of a 10 ml suspension containing $10^{5.5}$ viable units of BCG per ml for 60 min. The mice were then randomly recaged in groups of five. At intervals of time after infection a group of mice was sacrificed and the numbers of organisms present in the lungs and spleens determined. The results of this experiment are detailed in Table I.

As seen in Table I, BCG bacilli could be detected in the lung immediately after aerosol infection and increased in numbers for 4–6 wk thereafter. After reaching this maximum the numbers steadily declined and 20–40 wk after infection mycobacteria were present in lungs of occasional animals only.

BCG organisms consistently appeared in the spleens of vaccinated animals 5 or 6 wk after aerosol infection. The numbers of organisms recovered from the animals' spleens declined slightly after 10 wk. In contrast to the findings in pulmonary tissue, however, BCG organisms persisted in the animals' spleens for long periods of time.

¹ The authors are indebted to Dr. Frappier of the Institute of Hygiene for making large quantities of vaccine available for this study.

The growth of several other strains of BCG in the organs of mice after aerosol vaccination was tested in subsequent experiments. These results will be described in detail elsewhere.² It may be noted, however, that the *in vivo* growth of other strains of BCG differed from that of the Montreal strain. Phipps strain bacilli multiplied as extensively in pulmonary tissue as did the Montreal strain; however, they declined more rapidly in the lungs than did bacilli of the Montreal strain. Other strains of BCG showed less or no ability to grow in pulmonary tissue. These differences are similar to those reported by other investigators for BCG substrains intravenously injected into mice (5-7).

TABLE I
BCG Organisms in Organs of Mice after Aerosol Vaccination

Time after infection	No. of BCG organisms recovered from organs at indicated time after infection*				
	Lung			Spleen	
<i>wk</i>	<i>log</i>	<i>SD</i>	<i>log</i>	<i>SD</i>	
0	1.6‡	±0.3	0	—	
2	2.7	±0.9	0	—	
3	4.1	±1.2	0	—	
4	5.2	±0.8	0	—	
6	5.8	±0.3	2.4	±0.6	
10	5.0	±0.7	2.5	±0.8	

* Numbers of BCG organisms (expressed as logs) recovered from organs of mice after aerosol vaccination ± standard deviation. Infection was produced by nebulization of 10 ml of suspension containing $10^{5.5}$ BCG bacilli per ml over a 60 min period.

‡ Average of five mice. Values indicate number of bacilli present in entire tissue homogenate.

Effect of BCG Vaccination on the In Vivo Growth of Virulent Human Bacilli.—The numbers of virulent bacilli recovered from organs of BCG-vaccinated mice at intervals after infection is described in the next experiment.

Female mice were vaccinated with living BCG as in the previous experiment. 10 wk after vaccination these, along with comparable untreated animals, were superinfected by aerosol exposure to virulent human bacilli. 10 ml of suspension containing $10^{5.8}$ viable virulent bacilli per ml were nebulized for 60 min. Animals were sacrificed at intervals after infection, their lungs and spleens were removed and homogenized. Dilutions of the homogenate were plated in medium containing 2-thiophenecarboxylic acid hydrazide which prevents the growth of BCG (Table II).

Virulent mycobacteria initially grew at the same rate in lungs of control and of vaccinated mice. 4 wk after challenge, however, significantly fewer bacilli were recovered from lungs of vaccinated mice than from normal animals. The difference between the two groups diminished with time and eventually disappeared.

² Sakurami, T., T. Izumi, and R. Costello. To be published.

Virulent organisms were not obtained from spleens of mice until 4 wk after challenge, that is, at the height of the pulmonary infection. The growth of virulent organisms in the spleens of vaccinated animals was greatly retarded. 10 wk after infection, however, similar numbers of organisms were recovered from the spleens in either group.

Route of Vaccination.—Study was made of the relative effectiveness of immunization achieved in animals given BCG by different routes of administration. It was found that successful immunization could be achieved by the intravenous or intraperitoneal route as well as by aerosol vaccination.

TABLE II
Growth of Virulent Human Bacilli in Organs of BCG-Vaccinated and Unvaccinated Mice

Time after infection	No. of virulent bacilli present in organ at indicated time*							
	Lung				Spleen			
	Control		Vaccinated‡		Control		Vaccinated‡	
wk	log	sd	log	sd	log	sd	log	sd
2	4.7§	±0.6	4.1	±0.5	0	—	0	—
4	7.0	±0.8	5.6	±0.5	4.0	±0.7	2.1	±0.7
6	6.0	±1.0	6.0	±0.8	4.6	±0.6	2.6	±0.7
10	6.4	±0.5	5.8	±0.7	4.6	±0.5	4.0	±1.0

* Numbers of human bacilli (expressed as logs) recovered from organs of mice after aerosol infection ± standard deviation. Infection was produced by nebulization of 10 ml of suspension containing $10^{5.8}$ H37Rv bacilli per ml over a 60 min period.

‡ Animals were vaccinated by aerosol exposure to Montreal strain BCG (10 ml containing $10^{5.5}$ viable BCG bacilli per ml for 60 min) 10 wk before virulent challenge infection.

§ Average of five mice. Values are for 1 ml of tissue homogenate (out of a total of 5 ml).

|| Indicates values significantly different ($P = < 0.05$) from those of comparable control mice.

In a typical experiment 5-wk old mice were given BCG by different routes. One group of animals was exposed to an aerosol obtained by nebulizing 10 ml of suspension containing $10^{5.5}$ viable units BCG per ml over a 60-min period. A second group of mice received $10^{4.18}$ viable units of BCG by peritoneal injection. Still another group was given $10^{3.18}$ viable units of BCG by the intravenous route.

5 wk after vaccination, representative animals from each group, along with untreated control mice, were challenged with virulent organisms given by the respiratory route. The conditions of the challenge infection were similar to those previously described.

The number of BCG organisms present in animals' spleens and lungs were determined at various intervals after vaccination. In animals exposed to nebulized vaccine, extensive multiplication of BCG occurred initially in the lungs. BCG administered by the peritoneal route was immediately taken up by both spleens and lungs. Little multiplication of BCG occurred in either organ, however. BCG organisms rapidly appeared in both spleens and lungs after intravenous administration of vaccine. They multiplied in both organs.

The number of BCG organisms present in the various organs 5 wk after vaccination is given in Table III. As may be seen in the Table, large numbers of BCG were present in either spleen or lung by the time of challenge infection irrespective of the initial route of vaccination.

4 wk after challenge infection, the number of virulent bacilli present in the lungs and spleens of vaccinated and control animals was determined. As shown in Table III, the number of challenge organisms present in pulmonary tissue at this time were significantly lower in all the vaccinated animals. The degree of depression of growth in pulmonary tissue was relatively similar in all cases.

TABLE III
Virulent Bacilli Recovered from Organs of Mice Vaccinated by Three Routes

Route of vaccine*	BCG present at time of challenge†		No. of virulent bacilli present in organs 4 wk after virulent infection‡, §			
	Lung	Spleen	Lung		Spleen	
	log	log	log	SD	log	SD
Control	—	—	5.9	±0.37	2.4	±0.31
Aerosol	5.4	0.1	4.6	±0.39	1.8	±0.95
Intravenous	3.1	4.3	4.7	±0.33	1.3	±0.71
Intraperitoneal	2.5	1.5	4.3	±0.69	1.9	±0.44

* Animals were vaccinated by aerosol exposure to Montreal strain BCG (10 ml containing $10^{5.5}$ viable BCG bacilli per ml for 60 min), intravenous injection of $10^{3.18}$ BCG bacilli, or intraperitoneal injection of $10^{4.18}$ BCG bacilli.

† Animals were challenged by exposure to virulent aerosol challenge (10 ml containing $10^{5.8}$ H37Rv bacilli per ml for 60 min) 5 wk after vaccination.

§ Average of five mice in each case. Values are for 1 ml of tissue homogenate (out of a total of 5 ml).

|| Indicates values significantly different ($P = < 0.05$) from those of comparable control mice.

Thus, the route of administration of vaccine appeared to have relatively little influence on the development of immunity by pulmonary tissue.

The number of challenge bacilli recovered from splenic tissue was also lower in vaccinated animals than in unvaccinated animals, although only in animals vaccinated by the intravenous route were these differences of statistical significance.

Effect of Vaccine Dose.—The relation between the quantity of BCG administered to animals and the antituberculous resistance attained by their tissues was studied in several experiments.

The procedures were similar to those described in the preceding experiments. Female animals were used. In the experiment illustrated in Table IV, they were 5 wk old when vaccinated. Each group of animals was vaccinated with a serially diluted suspension of freshly reconstituted vaccine given by the intraperitoneal route. 10 wk after vaccination these mice, along with

untreated control animals, were challenged by respiratory infection with virulent human bacilli. They were sacrificed 4 wk after challenge infection and the number of virulent bacilli present in their organs determined.

The number of BCG organisms administered to the mice and the vaccinal population present in the animals' organs 6 wk after vaccination are indicated in the first columns of Table IV. As in preceding experiments, the organs of animals receiving the largest intraperitoneal dose of BCG contained the largest

TABLE IV
Virulent Bacilli Recovered from Organs of Intraperitoneally Vaccinated Mice at Indicated Intervals after Infection

Organ	No. of BCG injected (viable units)	No. of vaccine organisms present in tissue*	No. of virulent bacilli recovered at indicated time after challenge infection†					
			2 wk		4 wk		6 wk	
		<i>log</i>	<i>log</i>	SD	<i>log</i>	SD	<i>log</i>	SD
Lung	0	—	4.3	±0.23	7.4	±0.91	6.2	±1.15
	150	0	4.2	±0.38	6.4	±1.33	6.0	±0.85
	1.5×10^4	1.7 ± 0.8	4.3	±0.28	5.1§	±0.91	6.1	±0.80
	1.5×10^6	3.7 ± 2.3	4.1	±0.41	5.2§	±0.91	6.7	±0.60
Spleen	0	—	0	—	3.5	±1.28	4.7	±0.38
	150	3.0 ± 0.7	0	—	2.7	±0.28	4.0	±0.92
	1.5×10^4	5.5 ± 1.0	0	—	2.2§	±0.28	3.5§	±0.69
	1.5×10^6	5.2 ± 1.0	0	—	2.4§	±0.42	3.4§	±0.69

* Number of BCG organisms (expressed in logs) recovered from tissue 6 wk after vaccination ± standard deviation. Average of five mice. All values are for 1 ml of tissue homogenate (out of a total of 5 ml).

† Time between vaccination and challenge was 5 wk. Virulent challenge consisted of exposure to an aerosol produced by nebulizing 10 ml of suspension containing $10^{5.8}$ H37Rv per ml for 60 min.

§ Indicates values significantly different ($P = < 0.05$) from those of comparable control mice.

numbers of vaccine organisms. Both lungs and spleens of these animals were resistant to the growth of virulent bacilli. Mice receiving the next smaller quantity of vaccine had a less extensive BCG infestation. Organs of these mice, however, were also resistant to virulent bacilli. Mice receiving the smallest quantity of BCG showed only a slight vaccinal infection and this was almost entirely limited to splanchnic tissue. They were significantly less resistant to challenge infection.

It should be noted, however, that the degree of resistance attained by animals was not directly related to the size of the vaccinal population within their organs. Thus, the number of challenge organisms present in organs of mice given the highest quantity of BCG were no lower than those recovered from organs of mice given the moderate dose.

Similar results have been obtained in other experiments. When small quantities of BCG were administered by the peritoneal route, the animals often entirely failed to develop pulmonary immunity. After administration of larger quantities of vaccine, they regularly developed resistance. The degree of resistance, as measured by the numbers of virulent bacilli in pulmonary tissue 4 wk after challenge infection, was largely the same irrespective of the size of the vaccinating dose.

TABLE V
Number of Virulent Mycobacteria in Organs of Mice at Different Times After Vaccination

Time after vaccination*	No. of BCG bacilli present in organs at given time after vaccination				No. of virulent bacilli recovered from organs 4 wk after challenge†							
					Lung				Spleen			
	Lung		Spleen		Control		Vaccination		Control		Vaccination	
wk	log	SD	log	SD	log	SD	log	SD	log	SD	log	SD
1	0.8§	±0.21	0	—	6.8	±0.57	7.3	±0.65	4.2	±0.17	4.2	±0.70
2	1.2	±0.35	0	—	—	—	—	—	—	—	—	—
3	3.0	±0.76	0	—	6.5	±0.17	6.0	±0.25	3.8	±0.33	3.0	±0.60
4	5.1	±0.27	0	—	—	—	—	—	—	—	—	—
5	5.4	±0.56	0.1	±0.05	5.9	±0.37	4.6	±0.39	2.4	±0.31	1.8	±0.95
6	6.2	±0.27	1.1	±1.34	—	—	—	—	—	—	—	—
10	3.1	±0.25	3.0	±0.12	5.7	±0.82	4.1	±0.69	3.4	±0.36	2.4	±0.30
20	1.6	±1.15	3.0	±0.28	—	—	—	—	—	—	—	—

* Animals were vaccinated by aerosol exposure to 10 ml containing $10^{5.5}$ viable BCG bacilli per ml nebulized for 60 min.

† Virulent challenge occurred at indicated times after vaccination. Infection was produced by nebulization of 10 ml containing $10^{5.8}$ H37Rv per ml for 60 min. Animals were sacrificed 4 wk later; figures indicate the number of organisms recovered from their organs.

§ Average of five mice. Values are for 1 ml of tissue homogenate (out of a total of 5 ml).

|| Indicates values significantly different ($P = < 0.05$) from those of comparable control mice.

Rate of Appearance of Acquired Resistance.—In the next experiment mice were again vaccinated by exposure to nebulized BCG. At intervals thereafter they were sacrificed and the vaccinal populations in their tissues ascertained. At intervals of times, groups of animals, along with comparable nonvaccinated mice, were challenged with virulent bacilli by the respiratory route. The relative resistance was assessed by determining the numbers of virulent organisms present in lung and spleen 4 wk after challenge. The results are shown in Table V.

As may be seen, the appearance of immunity was closely related to the phase of development of the vaccinating process. Increasing numbers of BCG bacilli were recovered 4 wk after aerosol vaccination. No heightened resistance could be detected, however, until the 5th wk after BCG administration when vaccinated animals appeared to be able to retard the pulmonary growth of challenge bacilli. No retardation of growth, however, was found in the spleens of

immunized mice. 6 wk after vaccination BCG organisms were first recovered from the splenic tissue of treated animals; in animals challenged after this period, an increased antitubercular resistance of both splenic and pulmonary tissue was found.

Duration of Immunity in Vaccinated Mice.—The persistence of pulmonary immunity in BCG-vaccinated animals was determined in the next experiment.

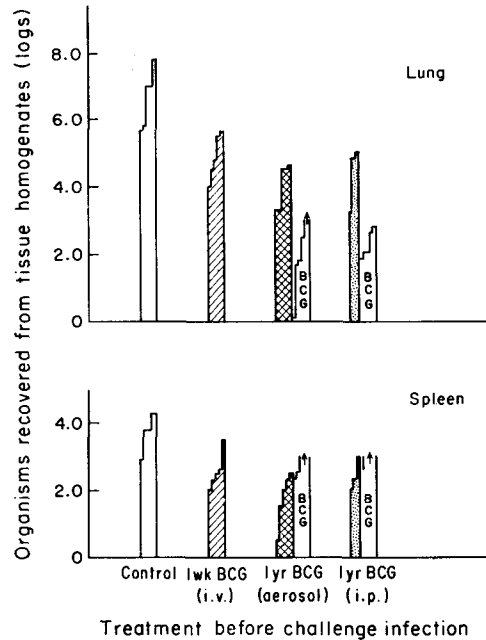


FIG. 1. Number of virulent human bacilli in organ of control and vaccinated mice. Bars refer to organisms recovered from five individual animals 4 wk after aerosol challenge with 10 ml of suspension containing $10^{5.8}$ viable H37Rv per ml, nebulized for 60 min. Animals were vaccinated with BCG 1 wk (i.v.) or 1 yr (aerosol and i.p.) before challenge. Number of BCG organisms present in organs of these latter animals is also indicated in figure.

Several litters of day old mice were obtained and the babies were randomly mixed. One group of baby animals was vaccinated by aerosol infection with BCG. The newborn animals were placed in the infection basket and 10 ml of suspension containing $10^{5.5}$ viable BCG were nebulized over a 60 min period. A second group of babies were intraperitoneally injected³ with

³ Intraperitoneal injection of baby mice was accomplished by the following technique. The animal was inverted and held with its tail away from the operator. A 1/4 ml syringe with a 30 gauge 3/4 inch needle was used. Subcutaneous entry was made at the level of the upper vertebrae. The needle was directed towards the sternum, being carefully kept between the animal's skin and its rib cage. When the diaphragm was reached the needle was depressed slightly, puncture of the diaphragm made, and the peritoneal cavity entered. After injection of the inoculum the needle was slowly withdrawn.

0.05 ml of a suspension of vaccine containing $10^{6.2}$ organisms. In both cases, animals were returned to randomly selected foster mothers after vaccination. 8 animals were placed with each nursing female. At 21 days of age they were weaned, segregated by sex, and recaged in groups of 20 animals. All animals, including untreated control mice, were maintained without further treatment for the subsequent year.

At this time one half of the untreated animals were given an intravenous injection of $10^{6.2}$ viable units of BCG. The following wk animals from each vaccinated group, along with the remaining untreated control mice, were challenged by superinfection with virulent human bacilli administered by the respiratory route. At the same time the numbers of BCG present in lungs and spleens of animals vaccinated the preceding year were ascertained. 4 wk after challenge infection, all animals were sacrificed and the number of virulent bacilli present in their lungs and spleens determined. The results are given below and in Fig. 1.

All vaccinated animals were significantly protected against challenge infection. Moreover, the degree of protection was approximately the same in each case. Thus, tissues of animals having received vaccine a year previously were as able to retard the growth of virulent bacilli as organs of animals vaccinated 1 wk before challenge.

As seen in Fig. 1, organs of animals vaccinated the preceding year still contained significant quantities of vaccinal organisms at the time of challenge infection. The numbers of BCG were approximately the same in organs in animals vaccinated by either the peritoneal or respiratory route.

Histological Observations.—The histological development of pulmonary lesions was followed in several of the experiments described above.

Animals were sacrificed at intervals after respiratory vaccination or challenge infection. Gross observation of pulmonary tissue was made *in situ*. Tissues were subsequently fixed in 10% formalin and sections stained with hematoxylin and eosin or with Ziehl-Neelsen acid fast stain.

The typical pulmonary lesion in animals challenged with virulent bacilli was a well defined granulomatous process as described by other investigators. Lesions were well organized and consisted primarily of epithelioid cells and lymphocytes. Giant cells and foam cells were rare. Caseation necrosis did not occur. Acid fast bacilli were rare within the organized lesions.

Similar lesions occurred in vaccinated animals. Visible lesions occurred at generally the same time in vaccinated or control animals. No significant difference in numbers of lesions were found in animals of the two groups. Lesions in vaccinated animals were not appreciably smaller than in untreated mice.

On the other hand, animals vaccinated with BCG by aerosol but not challenged with virulent organisms also developed pulmonary lesions within 6 wk after vaccination. By 10 wk after vaccination, lesions attained a size of 1–2 mm and became slightly larger, reaching a maximum size by the 12th wk. Beyond this period the size of the lesion slowly decreased. Appreciable resolution occurred by the 4th month. The BCG lesions had largely healed by the 6th–9th month after infection.

Similar lesions appeared in mice given aerosol vaccination with Phipps organisms. Only small lesions, if any, occurred in animals vaccinated with other strains of BCG.

On microscopic observation the BCG lesions were characteristic of a well organized granulomatous process. The lung showed striking lymphoid infiltration of perivascular areas. Lesions consisted of epithelioid cells and large numbers of deeply staining lymphoid cells. Few giant or foam cells were present. No necrosis occurred. Extremely few BCG bacilli were

found. Undamaged parenchymal tissue of the animals appeared to have an increased cellularity; many of the additional cells were structurally compatible with mononuclear phagocytic cells.

DISCUSSION

Mice vaccinated with living BCG are significantly more resistant to respiratory infection than are unvaccinated control mice. Larson and Wicht have reported that the appearance of grossly visible pulmonary lesions in animals challenged with virulent mycobacteria by the respiratory route is greatly retarded in mice vaccinated with large doses of H37Ra, BCG, or atypical mycobacteria by the intravenous or respiratory route (2, 8). These investigators also noted that the numbers of mycobacteria present in the lungs of vaccinated animals were significantly lower than in control mice. Ribi et al. confirmed these findings, adding that significant pulmonary protection, greater than that obtained with living BCG, was also achieved by intravenous injection of oil in water emulsions of mycobacterial cell walls (9, 10). Smith et al. found little difference in the response of BCG-vaccinated and unvaccinated mice to virulent human respiratory infection, although the numbers of bacilli recovered from lungs and spleens in immunized mice were smaller at certain intervals of time after infection (11).

In the experiments described here, successful vaccination was attained after aerosol exposure of mice to BCG as well as by intravenous or intraperitoneal injection of the vaccine.

BCG organisms multiplied extensively in pulmonary tissue after inhalation. Growth of the vaccine in the lung continued unabated until 6 wk after exposure. After this time, the numbers of BCG bacilli in the animals' lungs declined. At this same time, BCG organisms were first recovered from spleens. Vaccinal multiplication continued in this organ for several wk, after which time the numbers of BCG slightly declined, persisting at these values thereafter.

The development of immune resistance was closely related to the multiplication of the vaccinal bacilli. Thus, animals first became resistant to the growth of virulent challenge bacilli when the BCG population in pulmonary tissues had reached its height and had begun to decline. Similarly, splenic tissue did not become consistently resistant to the human organisms until the animals' spleens contained large numbers of vaccinal organisms.

It is worth noting that the development of pulmonary resistance was also correlated with the appearance of significant histological changes in the lungs of BCG-vaccinated mice. 6–10 wk after aerosol vaccination lungs of mice developed a significant granulomatous reaction characterized by large infiltrations of macrophages and other lymphoid cells. At the same time, animals first demonstrated pulmonary resistance to challenge infection.

The development of discrete macroscopic lesions in lungs of mice infected with certain strains of BCG has been noted by others (12), although their

occurrence has rarely been considered in studies concerned with antitubercular immunity. Youmans and Youmans (13) and Barclay et al. (14) have described in detail the granulomatous response developing in mice after intravenous injection of living or dead mycobacterial cells. Both groups of investigators noted a correlation between the magnitude of the macrophage response and the degree of resistance against virulent challenge.

At least two lines of evidence reported in this paper tend to speak against a simple correlation between pulmonary resistance to challenge organisms and the occurrence of granulomatous pulmonary tissue. First, growth of challenge bacilli was not immediately depressed in the lungs of vaccinated animals as it would be if the inhibitory response was due solely to the presence of an inimical tissue environment. In fact, organisms grew at the same rate in either vaccinated or unvaccinated animals for 2 wk after infection and only during the following fortnight was their growth severely depressed. Secondly, mice vaccinated by intraperitoneal administration of BCG developed as large a degree of resistance as animals vaccinated by the intravenous or respiratory route. Relatively few BCG bacilli reached pulmonary parenchyma following intraperitoneal injection of vaccine. Moreover, gross pulmonary lesions rarely developed in mice given vaccine by this route.

It is of interest to compare the ability of mice to repress virulent mycobacteria administered by the respiratory route with the protection attained against the lethal effects of infection such as that described in the preceding paper. Resistance of pulmonary tissue developed only several wk after respiratory or peritoneal vaccination. Mortality protection is much more rapidly attained, at least within 7 days and perhaps within 3 (15). Protection against mortality is effective against a limited range of challenge doses and is readily overwhelmed (16). Resistance of pulmonary tissue is equally exerted against both heavy and light challenge infections (4). Both mortality and pulmonary resistance depend on the presence within the animal of a large population of vaccinal organisms. The degree of resistance attained against mortality protection, however, is related to the total quantity of vaccinal organisms present with the tissues, i.e. is dose dependent, whereas pulmonary resistance was present at relatively the same level in all successfully vaccinated animals. Mortality resistance is readily induced by either living or dead mycobacterial preparations; pulmonary resistance is difficult to induce with dead mycobacteria, requiring relatively large quantities of vaccine administered by the intravenous route.⁴

SUMMARY

The course of pulmonary tuberculosis was followed in control and BCG-vaccinated mice. Resistance was evaluated by determining the numbers of

⁴ Costello, R., and T. Izumi. Data to be published.

virulent mycobacteria recovered from the organs at various intervals after airborne infection.

A limited but significant retardation of growth of challenge bacilli was observed in both pulmonary and splenic tissues of successfully vaccinated animals. This retardation was manifested chiefly during the early stages of the infection. However, the numbers of virulent organisms recovered from the organs of either vaccinated or control animals were much the same at later stages of the infection.

The appearance of pulmonary immunity was related to the stage of development *in vivo* of the vaccinal population. Only after large numbers of vaccinal bacilli could be recovered from the tissues was heightened resistance observed.

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