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Macrophage Activation and Resistance to Pulmonary Tuberculosis

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Mice were vaccinated with 300 μg of BCG cell walls (BCG-CW) in oil-in-water emulsion intravenously or with a high or low dose of living BCG by inhalation (BCG-HD or BCG-LD, respectively). The consequences of vaccination were evaluated in terms of the growth of BCG in the lungs and spleen, lung and spleen weight, resistance to intravenous and airborne challenge with *Listeria monocytogenes*, airborne challenge with virulent *Mycobacterium tuberculosis* H37Rv, and transfer of adoptive immunity. BCG-CW and BCG-HD mice developed increased lung weight, which was associated with transient, low-level resistance to airborne *L. monocytogenes* and initial resistance to airborne H37Rv. Only BCG-CW mice developed splenomegaly, which was accompanied by high resistance to intravenous challenge with *L. monocytogenes*. The initial resistance of BCG-CW mice to H37Rv was not sustained, whereas that of BCG-HD mice persisted. There was no initial resistance to H37Rv in BCG-LD mice, but immunity was generated later. Overall, BCG-HD mice were most resistant to H37Rv, and BCG-CW and BCG-LD mice were less but equally resistant.

There is ample evidence that animals immunized by the airborne route with BCG and then challenged by the same route with *Mycobacterium tuberculosis* are more resistant than animals that have been immunized by the intravenous (i.v.) and subcutaneous routes (3, 8, 11, 13). A similar situation prevails after mice have been immunized i.v. with BCG cell walls (BCG-CW) suspended in an oil-in-water emulsion (1, 17), a procedure which results in multiple pulmonary lipid emboli and subsequent widespread granuloma formation (2). In both cases, there is a close association between granuloma formation and resistance to airborne challenge with *M. tuberculosis*.

Granulomas are composed of several classes of cells: polymorphonuclear leukocytes, mononuclear leukocytes, lymphocytes, connective tissue cells and products, and vascular elements. As far as immunological processes are concerned, the most important components are the phagocytic and lymphocytic cells. Since polymorphonuclear leukocytes are inconspicuous in the chronic granulomas associated with cell-mediated immunity, the essential immunological constituents of these lesions are macrophages and lymphocytes. Consequently, an attempt was made to determine the contribution that these cell types make to the defense of the lungs against reinfection by the airborne route.

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The role of macrophages was assessed by measuring the resistance of granulomatous lungs to challenge with a heterologous organism, *Listeria monocytogenes*. Numerous studies have shown that this organism is exquisitely susceptible to the enhanced microbicidal activity of macrophages that abound in hypersensitivity granulomas (4, 6, 12, 18). The function of sensitized lymphocytes was measured as adoptive specific antibacterial immunity in syngeneic recipients.

MATERIALS AND METHODS

Animals. Female (C57BL/6 × DBA/2)F_1 inbred mice were introduced into experiments at approximately 8 weeks of age.

Microorganisms. A mouse-virulent strain of *L. monocytogenes* was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C. Strains of mycobacteria, namely, *Mycobacterium bovis* BCG Pasteur (TMC 1011), attenuated *M. tuberculosis* R1Rv (TMC 205), and virulent *M. tuberculosis* H37Rv (TMC 102), were obtained from the Mycobacterial Culture Collection of Trudeau Institute. These cultures were grown in roller bottles containing Proskauer and Beck liquid medium with 2% glycerol and 0.1% Tween 80 by incubation at 37°C for 8 to 10 days. The fully grown cultures were well dispersed and of high viability, approximately 10^9 viable bacilli per ml. For one experiment a very-high-density suspension of BCG was required. A standard culture was allowed to settle until the bacteria had sedimented. Approximately 80% of the supernatant culture medium was aspirated aseptically; the bacteria were resuspended in the remaining medium and found to have a density of 5×10^9 viable units per ml.

All cultures were distributed in 2-ml vials and stored at −70°C, allowing cultures of uniform, known viability to be used throughout the experiments. Before use, the mycobacterial cultures were exposed to low-intensity ultrasound for a few seconds to disperse bacterial clumps.

BCG-CW. BCG-CW were donated by E. Ribi and were suspended in an oil-in-water emulsion at a concentration of 1,500 μg/ml as described elsewhere (17).

i.v. inoculation. A 0.2-ml amount of the appropriate suspension was injected into a lateral tail vein.

Airborne infection. Mice were placed in a Middlebrook airborne infection apparatus (Tri-R Instruments, Rockville Centre, N.Y.) and exposed to an aerosol generated by nebulizing 10 ml of bacterial suspension. The density of the bacterial suspension was adjusted empirically so as to implant the required number of organisms in the lungs. The duration of nebulization was 30 min; this was followed by a cloud decay period of 30 min and a decontamination time of 15 min. Some of the mice were killed immediately (0 h) after removal from the apparatus to ascertain the implantation inoculum of viable bacteria.

Organ weight. The lungs and sometimes the spleen were removed and weighed on a top-loading balance. The arithmetic mean values for groups of 5 mice were calculated.

Spleen cells. Immunized mice were killed, and their spleens were removed and dissociated in Dulbecco phosphate-buffered saline. The cells were washed twice in phosphate-buffered saline, and total leukocyte counts were made. The viability of the cells was estimated by trypan blue exclusion. The spleen cell suspension was adjusted to 2.5×10^8 leukocytes per ml of phosphate-buffered saline, and 0.2-ml amounts (5×10^7 cells) were injected i.v. into recipient mice.

Adoptive immunity. Recipient mice were exposed to 500 rads from a ^{137}Cs source and then challenged i.v. with 10^5 R1Rv organisms. Groups of 5 mice were subsequently given 5×10^7 spleen cells or no cells i.v. On day 14 after challenge, the mice were killed and viable counts of R1Rv were made from the spleens (10).

Viable counts. The appropriate organs were removed and homogenized in a known volume of sterile saline with a Potter-Elvehjem homogenizer. These suspensions were appropriately diluted and then inoculated on Middlebrook 7H-10 agar or Trypticase soy agar plates (BBL Microbiology Systems) to cultivate mycobacteria or listeria, respectively. The plates were incubated for an appropriate length of time at 37°C, and the colonies were then counted. The geometric mean viable counts from groups of 5 mice were estimated; these results are presented in $\log_{10}$ units either as absolute counts or by subtracting the test group value from that obtained from control mice. The latter values, when positive, denote increased nonspecific resistance when applied to counts of *L. monocytogenes* (see Tables 1, 2, and 5) and specific resistance or immunity when applied to adoptive immunity to R1Rv (see Fig. 3).

Statistics. Data were evaluated by analysis of variance, after which group means were compared with the *Q* test (19).

RESULTS

Induction of specific and nonspecific resistance after immunization with BCG-CW and living BCG. A large number of mice were divided into 4 groups, one of which was set aside as a normal control. The second group was immunized i.v. with 300 μg of BCG-CW in oil-in-water emulsion. The last two groups were immunized by inhalation of large and small inocula of BCG, respectively. The response to immunization was monitored at 2-week intervals for three months as follows: viable counts of BCG in the lungs and spleen; lung and spleen weight; nonspecific resistance to *L. monocytogenes* in the lungs and spleen after airborne and i.v. challenges, respectively; adoptive transfer of antituberculosis immunity; and resistance to airborne challenge with virulent tubercle bacilli.

(i) Growth of BCG in the lungs and spleen. Mice were exposed in the airborne infection apparatus to a total of either 10^{10} or 10^8 viable BCG organisms. This procedure implanted 5×10^4 and 5×10^2 viable BCG organisms into the lungs, respectively. These groups of mice were designated BCG-HD (high dose) and BCG-LD (low dose). The growth of these inocula in the lungs and the subsequent dissemination of BCG to the spleen are recorded in Fig. 1.

The effect of the inoculum size on the growth of BCG in the lungs after airborne infection was similar to that observed in the liver and spleen after i.v. infection, in that the larger the inocu-

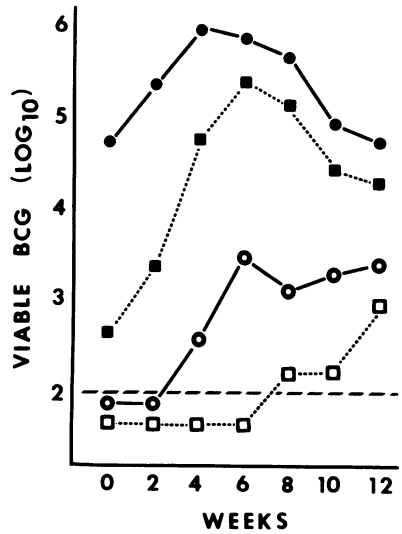

FIG. 1. *Multiplication of two inocula of BCG after implantation in the lungs. Symbols:* ● *and* ■, *BCG in lungs;* ○ *and* □, *BCG in the spleen;* ● *and* ○, *high dose;* ■ *and* □, *low dose.*

lum, the shorter the period of rapid multiplication (9). Consequently, although the original inocula differed by 100-fold, by 6 weeks the difference between these two groups of mice with respect to viable BCG in the lungs was only 3-fold. Several weeks elapsed before BCG appeared in the spleen, and this period was inversely related to the inoculum size.

(ii) Lung and spleen weight. The normal weight of the lungs of B6D2 mice is approximately 200 mg. There was an increase in the lung weight of immunized mice, beginning at week 4 in BCG-HD mice and week 6 in BCG-CW and BCG-LD mice (Fig. 2). The maximum lung weight was observed at week 6. Thereafter, the weight of the lungs diminished in all three groups of immunized mice. However, the lungs remained heavier than normal in BCG-HD and BCG-CW mice. Although BCG infection spread to the spleens of BCG-HD and BCG-LD mice, it did not cause an increase in spleen weight. On the other hand, the spleens of BCG-CW mice enlarged rapidly between weeks 4 and 6, and splenomegaly persisted thereafter.

(iii) Nonspecific resistance to *L. monocytogenes*. Separate batches of mice of each group were challenged either i.v. with 10^5 or by aerosol with 2×10^8 *L. monocytogenes* organisms. Immediately after airborne challenge, 5 normal mice were killed, the lungs were removed, and viable *L. monocytogenes* organisms enumerated. For the entire experiment, the implantation inocula of *L. monocytogenes* in the lungs were similar, with a mean value of 4.26 $\log_{10}$ units (standard deviation, 0.19). The remaining mice were killed 24 h after challenge, and viable counts of *L. monocytogenes* were made from the spleen or lungs, depending on the route of challenge.

The levels of nonspecific resistance in the spleen are shown in Table 1. Significant resistance ($P < 0.01$) was observed only in BCG-CW mice that were challenged 4 and 6 weeks after immunization. This resistance coincided with the phase of rapid enlargement of the spleen (Fig. 2). It is notable that, although splenomegaly persisted, nonspecific resistance did not.

Nonspecific resistance to *L. monocytogenes* in the lungs is recorded in Table 2. At only one time point was significant resistance observed in BCG-CW and BCG-HD mice, namely, 6 weeks after immunization, when their lung weights were maximal. The level of nonspecific immunity in the lungs was much lower than that in the spleen.

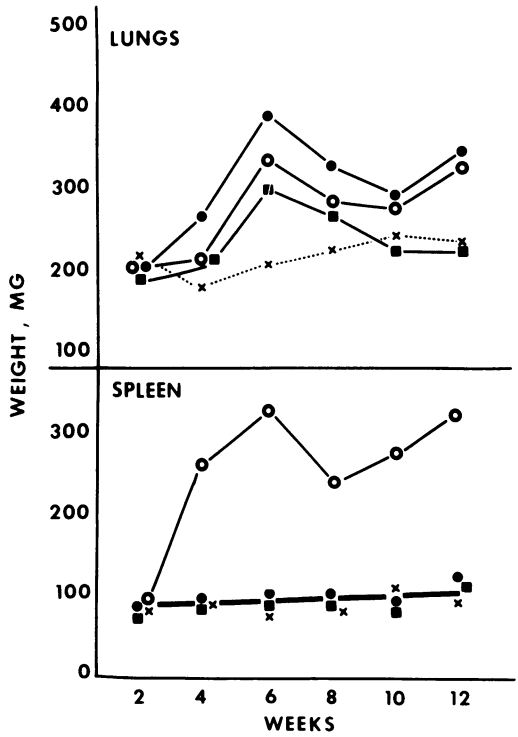

FIG. 2. *Weight of lungs and spleen after immunization with either 300 μg of BCG-CW i.v.* (○) *or inhalation of* 5×10^4 *BCG organisms* (●) *or* 5×10^2 *BCG organisms* (■). *Normal controls* (×).

TABLE 1. *Nonspecific resistance in the spleen to L. monocytogenes after i.v. challenge*

Week	Resistance to *L. monocytogenes* ($\log_{10}$)		
	BCG-CW	BCG-HD	BCG-LD
2	0.12	0.14	0.06
4	2.11[a]	0.22	0.11
6	1.00[a]	0.46	0.19
8	0.42	0.20	0.19
10	0.39	0.16	0.24
12	0.42	0.12	0.36

[a] Differs significantly from normal controls ($P < 0.01$).

TABLE 2. *Nonspecific resistance in the lungs to L. monocytogenes after airborne challenge*

Week	Resistance to *L. monocytogenes* ($\log_{10}$)		
	BCG-CW	BCG-HD	BCG-LD
2	0.17	0.10	0.37
4	−0.24	−0.11	−0.26
6	0.50[a]	0.63[b]	0.27
8	−0.01	0.17	0.17
10	0.21	0.37	−0.01
12	−0.24	0.00	0.14

[a] Differs significantly from normal controls ($P < 0.05$).

[b] Differs significantly from normal controls ($P < 0.01$).

(iv) Transfer of adoptive immunity to tuberculosis. As early as 2 weeks after immunization, significant adoptive immunity was conferred by spleen cells from BCG-CW ($P < 0.01$), BCG-HD ($P < 0.0l$), and BCG-LD ($P < 0.05$) mice (Fig. 3). At subsequent time points, even higher levels of immunity were transferred. Throughout the course of the experiment, spleen cells from BCG-HD mice conferred much higher levels of specific immunity ($P < 0.01$) than did cells from BCG-CW mice (Fig. 3). This result may be misleading because the BCG-CW mouse spleens were much larger (Fig. 2); therefore, the number of sensitized lymphocytes per spleen might well be similar in BCG-CW and BCG-HD mice.

At the earlier time points, weeks 2 and 4, greater protection ($P < 0.01$) was conferred by spleen cells of BCG-HD mice than by those of BCG-LD mice. Subsequently, however, their cells were of equal activity, in conformity with earlier studies (11).

(v) Resistance to challenge with virulent *M. tuberculosis* H37Rv. The results of the experiment on resistance to challenge with virulent *M. tuberculosis* H37Rv organisms are shown in Fig. 4. It was hoped to implant approximately 10^2 H37Rv organisms in the lungs. This was achieved, except at week 2, when the implantation inoculum was low: 1.33 $\log_{10}$ units per mouse. The growth of H37Rv in the lungs of control mice was similar for each infection, amounting to approximately 5 $\log_{10}$ units in 4 weeks.

The immunity displayed by immunized mice appeared to be of two types: low and high. Low-level immunity was manifested by a significant inhibition of growth of H37Rv at 28 days but not at 14 days. Such immunity was seen in BCG-CW ($P < 0.05$) and BCG-HD ($P < 0.01$) mice after the 2-week challenge and in BCG-LD mice after the 4-week ($P < 0.05$) and subsequent ($P < 0.01$) challenges. High-level immunity was denoted by an inhibition of growth of H37Rv within 14 days of challenge. Such immunity was observed only in BCG-CW and BCG-HD mice from the 4-week challenge onward ($P < 0.01$). A corollary of these findings is that the 14-day counts from BCG-CW and BCG-HD mice were significantly lower ($P < 0.05$ or $P < 0.01$) than those obtained from BCG-LD mice. At 28 days, the BCG-HD counts were significantly lower ($P < 0.05$ or $P < 0.01$) than those obtained from BCG-CW or BCG-LD mice.

A comparison of the BCG-CW and BCG-LD counts of H37Rv revealed a paradoxical situation. The BCG-LD counts were much lower at 14 days ($P < 0.05$ or $P < 0.01$), but closely similar to the BCG-CW counts at day 28. This point is made clear in Table 3, in which the total growth of H37Rv in the lungs has been separated into that which occurs in the first and second half of

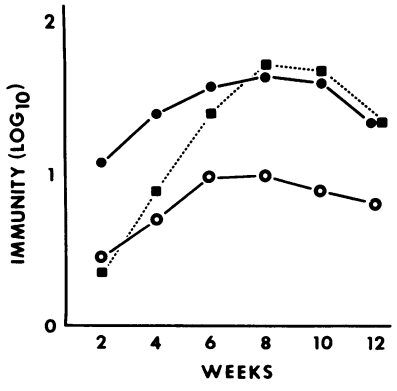

FIG. 3. *Adoptive immunity in recipients of 5 × 10^7 spleen cells from BCG-CW (○), BCG-HD (●), and BCG-LD (■) donors.*

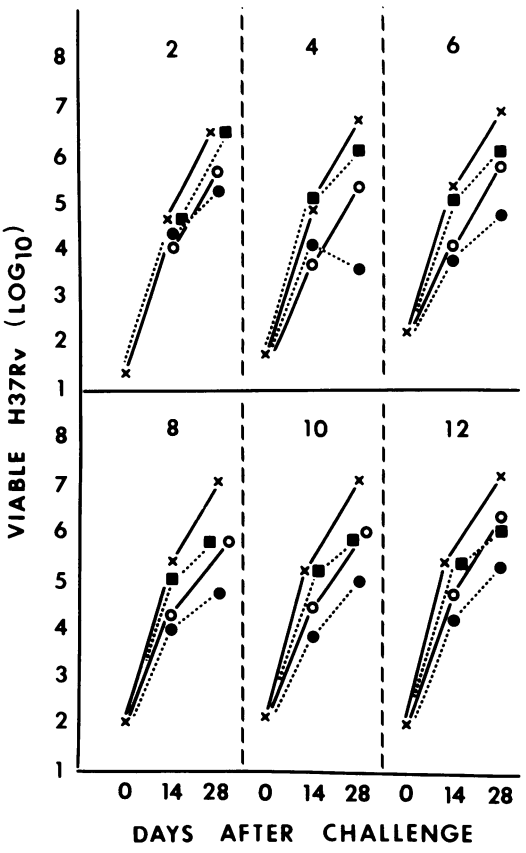

FIG. 4. *Growth of M. tuberculosis H37Rv in the lungs of normal (×), BCG-CW (○), BCG-HD (●), and BCG-LD (■) mice at indicated number of weeks between immunization and challenge.*

TABLE 3. *Multiplication of H37Rv in the lungs of normal and immunized mice*

Week of challenge	Increase in viable H37Rv (log_{10})							
	0 to 14 days				15 to 28 days			
	Control	BCG-CW	BCG-HD	BCG-LD	Control	BCG-CW	BCG-HD	BCG-LD
2	3.26	2.61	2.88	3.08	1.76	1.66	0.94	1.98
4	3.14	1.93	2.29	3.36	1.90	1.66	−0.47	0.93
6	3.00	1.83	1.51	2.91	1.61	1.59	1.12	0.84
8	3.36	2.16	2.02	2.98	1.62	1.59	0.67	0.79
10	3.17	2.32	1.75	3.15	1.88	1.58	1.18	0.71
12	3.38	2.67	2.26	3.31	1.72	1.56	0.75	0.75
Mean	3.22	2.25	2.10	3.13	1.75	1.61	0.75	1.00

the growth period. In all groups of mice, the growth during the first 14 days after challenge greatly exceeded that in the later period. It is apparent that during the first 14 days the growth of H37Rv in control and BCG-LD mice was closely similar, but growth was substantially inhibited in the BCG-CW and BCG-HD mice. Much more interesting was the growth of H37Rv in the 15- to 28-day interval after infection. During this period, the growth of H37Rv was closely similar in the control and BCG-CW mice, whereas multiplication of the organisms was substantially inhibited to a remarkably similar extent in the BCG-HD and BCG-LD mice from week 4 onward.

The pattern of resistance to H37Rv indicated that, from 4 to 12 weeks after immunization, BCG-CW and BCG-HD mice were able to inhibit the growth of the tubercle bacilli from the time of their implantation. This, in turn, suggested that such rapid expression of antibacterial resistance was due to the presence of lung macrophages, both alveolar and interstitial, with increased microbicidal activity (activated macrophages). These cells would be expected to express their microbicidal activity indiscriminately against *L. monocytogenes* and against H37Rv; yet, paradoxically, nonspecific resistance in the lungs was of relatively low degree and transient. It was thought that this apparent anomaly might be due to technical problems. Among the factors considered were the following: implantation of *Listeria* into the lungs might vary among the experimental groups; the challenge inoculum of *Listeria* might be too small; and the interval between challenge and sacrifice might have been inappropriate.

Fate of *L. monocytogenes* shortly after implantation into the lungs. Other studies have shown that after i.v. infection with *L. monocytogenes* the resident macrophages in the liver of normal mice have the ability to kill 95% of the implanted organisms (16). The lungs appear to have a more limited capability in this respect (21), but it was considered advisable to confirm the earlier observation.

Thirty mice were exposed to an aerosol containing 4×10^{10} viable *L. monocytogenes* organisms. Groups of 5 mice were killed immediately after exposure (0 h) and 2, 4, 6, 8, and 24 h later. Viable counts of *L. monocytogenes* were made from the lungs (Fig. 5). There was an apparent reduction in viable *L. monocytogenes* in the lungs between 0 and 2 h. However, this difference was not statistically significant. For that matter, the counts obtained at 4 and 6 h also did not differ from those at 0 h. At subsequent time points there was a highly significant ($P < 0.01$) increase in the number of *L. monocytogenes*.

This experiment suggested that there might be a small, initial kill of implanted *Listeria* which occurred in the 0 to 2 h interval.

Nonspecific resistance in the lungs after BCG-CW and BCG-HD immunizations. Another attempt was made to demonstrate macrophage activation in the lungs, but this experiment differed from the earlier one in several ways. The mice were exposed to a very high dose of BCG to implant a large number of mycobacteria in the lungs because it was thought that this would favor the development of nonspecific resistance, which is dependent on the inoculum size (5, 9). The challenge inoculum of *L. monocytogenes* was also raised twofold in the hope of increasing the sensitivity of the assay, and viable counts of *L. monocytogenes* were made from each group of mice at 0, 2, and 24 h postchallenge.

Groups of mice were immunized either i.v. with 300 μg of BCG-CW or by exposure to an aerosol of 5×10^9 viable BCG organisms per ml. Normal mice were set aside as controls. At 2-week intervals for 12 weeks, separate batches of mice from each group were used to determine resistance to *L. monocytogenes* after exposure to an aerosol of 4×10^{10} organisms.

An important consideration in this experiment was the possible variation in the implantation

and growth of *L. monocytogenes* in the lungs of normal mice. However, it is evident from Table 4 that the implantation inocula of *L. monocytogenes* were closely similar. There was a consistent loss of viable *L. monocytogenes* between 0 and 2 h, which, taking the experiment as a whole, amounted to 0.32 $\log_{10}$ viable bacilli, a small but significant ($P < 0.01$) difference. Between 2 and 24 h, there was a multiplication of *L. monocytogenes* to the extent of approximately 1.5 $\log_{10}$ units.

The results from BCG-CW and BCG-HD mice are shown in Table 5, in which the viable counts are expressed relative to those obtained from the control mice. Considering first the question of implantation inocula (0-h counts), these did not differ significantly from the controls, except for the BCG-CW mice at week 2. This difference might have been due either to a decreased pulmonary ventilation resulting in low implantation or to normal implantation associated with a rapid kill of the bacteria. Low counts at 2 h were observed only in BCG-CW mice at week 2 and BCG-HD mice at week 4. In the former case, the low 2-h counts may simply reflect a lower implantation inoculum. Substantial nonspecific resistance at 24 h was observed

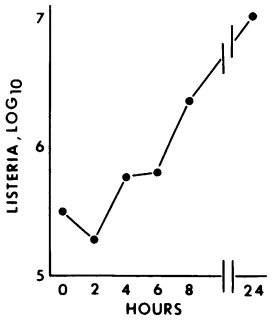

FIG. 5. *Fate of L. monocytogenes in the lungs of normal mice during the 24 h after implantation.*

TABLE 4. *Growth of L. monocytogenes in the lungs of normal mice*

Week	Inoculum implanted ($\log_{10}$)	Growth of *L. monocytogenes* ($\log_{10}$)	
		0 to 2 h	2 to 24 h
2	5.32	−0.53	1.66
4	5.31	−0.22	1.61
6	5.57	−0.31	1.30
8	5.42	−0.21	1.39
10	5.59	−0.33	1.51
12	5.63	−0.35	1.35
Mean	5.47	−0.32	1.47
SD[a]	0.14	0.12	0.15

[a] SD, Standard deviation.

TABLE 5. *Nonspecific resistance in the lungs of mice immunized with BCG-CW and live BCG*

Week	Resistance to *L. monocytogenes* ($\log_{10}$)					
	BCG-CW			BCG-HD		
	0 h	2 h	24 h	0 h	2 h	24 h
2	0.45[a]	0.48[a]	0.66[a]	0.03	0.06	0.04
4	0.07	0.19	0.54[a]	0.18	0.34[b]	0.89[a]
6	0.16	−0.03	−0.20	0.05	0.25	0.26[b]
8	−0.02	0.06	0.06	0.00	0.10	0.48[b]
10	0.03	0.02	−0.06	0.18	0.06	0.34[b]
12	−0.02	0.00	−0.15	0.00	0.05	0.01

[a] Differs significantly from normal controls ($P < 0.01$).

[b] Differs significantly from normal controls ($P < 0.05$).

in BCG-CW mice at 2 and 4 weeks only, but was also present to some extent in BCG-HD mice from 4 through 10 weeks. It should be emphasized that the levels of nonspecific resistance were trivial compared with those seen in the liver and spleen after i.v. infection (5, 9). The nonspecific resistance observed in BCG-HD mice at 6, 8, and 10 weeks was very low. Such small differences are often not statistically significant, but happen to be so in this experiment due to unusually low intragroup variation.

DISCUSSION

The purpose of this study was to identify the relative contribution of activated macrophages and sensitized lymphocytes to the defense of the lungs against airborne infection with *M. tuberculosis*. To this end, mice were immunized by the pulmonary route either by inhalation of living BCG or by i.v. injection of BCG-CW in oil-in-water emulsion. These procedures produced tuberculoid pulmonary granulomatosis, whose extent was assessed by lung weight. After BCG infection, the lung weight appeared to be a function of the size of the implantation inoculum and was unaccompanied by splenomegaly. On the other hand, BCG-CW produced substantial splenomegaly, presumably because many of the oil droplets containing cell wall fragments passed through the pulmonary capillary bed and thence to the systemic circulation (22). Splenomegaly was associated with a transient but substantial rise in nonspecific resistance to i.v. challenge with *L. monocytogenes*. No such increase was observed in BCG-infected mice.

Increased resistance of the lungs to airborne challenge with *L. monocytogenes* was not observed in BCG-LD mice and was rather unimpressive, even though statistically significant, in BCG-CW and BCG-HD mice, despite their substantial increase in lung weight. Similar results

have been observed repeatedly. It was thought that the presence of nonspecific resistance in the lungs might be concealed by variations in the implantation inocula of *L. monocytogenes* at different time points or in the different experimental groups or both, but in practice, only trivial variations were encountered. In experiments not reported here, smaller challenge inocula of *L. monocytogenes* were tested, the growth of *L. monocytogenes* in the lungs was followed for longer periods (24 to 72 h), and a different target organism, *Yersinia enterocolitica*, was used, with similar results. It therefore appears that enhanced nonspecific microbicidal activity of the alveolar macrophages is not an important component of the host defense mechanism in the lungs of tuberculous mice. I had expected to witness a substantially greater increase in the microbicidal activity of alveolar macrophages, despite the realization that these cells, being intra-alveolar, were not a constituent part of the granulomas, which are interstitial. This belief was supported by reports of the participation of alveolar macrophages in cell-mediated immunity reactions (14), including the expression of enhanced microbicidal activity by these cells (7, 20). Such changes are presumably brought about by lymphokines released from neighboring granulomas.

Resistance to airborne H37Rv was clearly the highest in BCG-HD mice, in which there was inhibition of bacterial multiplication during the 0- to 14- and 15- to 28-day periods. The BCG-LD mice did not impede the multiplication of H37Rv in the earlier period, but did in the later period. In this respect, the BCG-LD mice resembled mice that have been immunized by the i.v. or subcutaneous routes (11). Thus, high resistance to airborne challenge with *M. tuberculosis* is achieved by vaccination with airborne BCG only when the implantation inoculum is relatively high and consequent granulomatosis is extensive. The growth of H37Rv in BCG-CW mice differed from that of the other groups in that multiplication was substantially inhibited in the 0- to 14-day period, resembling BCG-HD mice, but growth was not inhibited in the 15- to 28-day period. This suggests that specific sensitization to *M. tuberculosis* was suboptimal in BCG-CW mice, a notion that is supported by the observation that spleen cells of BCG-CW conferred less adoptive immunity than did spleen cells of BCG-HD and BCG-LD mice.

Resistance of actively immunized mice to airborne H37Rv is the result of two cell-mediated effector mechanisms. First, upon implantation, the tubercle bacilli are ingested by the resident alveolar macrophages. Should those cells be activated, they would exert their microbicidal effects against the challenge organisms from the moment of ingestion. Second, sensitized lymphocytes migrate from the circulation into the lungs and mount a secondary cell-mediated immune response with a consequent influx of blood-borne monocytes, which in turn become activated and limit bacterial multiplication. The lymphocyte-mediated mechanism requires time for its generation; therefore its effects become manifest only after an interval during which the tubercle bacilli may be free to replicate (11).

The results with BCG-LD mice confirm that the component of specific immunity that is mediated by recirculating sensitized lymphocytes is usually not expressed in the lungs until several weeks after challenge. However, an explanation for the rapidly expressed immunity in BCG-HD and BCG-CW mice is in order. It appears improbable that such bacteriostasis was mediated by alveolar macrophages because they were relatively ineffective against *Listeria*. It is more likely that H37Rv excited a specific cell-mediated immune response more rapidly in the lungs of BCG-HD and BCG-CW mice than in BCG-LD mice. Such an event is analogous to the acute granulomatous response that can be evoked in the lungs of BCG-immunized rabbits (15). That response also depends on the presence of preexisting lung granulomas.

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