INFECTION AND IMMUNITY, Dec. 1977, p. 646-653
Vol. 18, No. 3

Printed in U.S.A.

Induction and Expression of Immunity After BCG Immunization

M. J. LEFFORD

Trudeau Institute, Inc., Saranac Lake, New York 12983

Received for publication 11 July 1977

The induction and expression of immunity to *Mycobacterium tuberculosis* after BCG immunization by intravenous, subcutaneous, and pulmonary routes has been investigated in mice. The speed with which protective immunity was engendered was a function of inoculum size; the immunization route was a less influential factor. Tuberculin hypersensitivity varied both with the inoculum size and immunization route, being least after pulmonary immunization. Once immunity was established, a steady state ensued in which the number of sensitized lymphocytes in the spleen was similar, regardless of the route or dose of vaccination. Actively immunized animals controlled intravenous and subcutaneous challenge infections, regardless of the method of vaccination. However, pulmonary challenge was resisted most efficiently by mice immunized by the pulmonary route. Adoptive immunity was well expressed in the spleen only, but the lungs were no more deficient in this regard than the footpad. It is suggested that enhanced immunity in the lungs depends on a population of resident sensitized lymphocytes.

There is ample published evidence that the route of immunization with BCG determines the level of delayed tuberculin hypersensitivity (DTH) and resistance to reinfection with tubercle bacilli. Subcutaneous/intradermal inoculation favors the development of tuberculin DTH, compared with pulmonary (P) and intravenous (i.v.) immunization (14, 16). On the other hand, although animals infected by the P, i.v., or subcutaneous routes are resistant to i.v. challenge with virulent tubercle bacilli, resistance to pulmonary challenge is observed only after P or i.v. immunization (1, 3, 4, 17, 24).

The latter observations in particular are disturbing because they do not conform to the demonstrated efficacy of intradermal immunization with BCG in protecting humans against pulmonary tuberculosis (22). Moreover, since the cells that confer long-term antituberculosis immunity are long-lived, recirculating, small lymphocytes (21), it is puzzling that these cells should be less available to combat pulmonary infection than infection at any other site.

Earlier studies have been limited to the response of actively immunized animals to i.v. or P challenge. The use of actively immunized animals incurs the problem of nonspecific resistance that is found to a greater or lesser extent in animals immunized with BCG (5, 18). In such animals, it is impossible to determine how much of the observed resistance to reinfection is attributable to specific or nonspecific effects. The comparison of the lungs and spleen as sites of tuberculosis infection also creates some difficulties. The spleen is the major central lymphoid organ through which the recirculating lymphocytes traverse (13, 15). The lung parenchyma contain small foci of lymphoid tissue (26) but cannot be regarded as lymphoid organs to which lymphocytes "home." Consequently, it could be argued that the lungs are at a disadvantage to the spleen in their content of immunocompetent cells.

The purpose of this study was to critically reevaluate certain variables that may influence the induction and expression of anti-tuberculosis immunity. These factors include the dose of BCG and its route of administration, and the expression of immunity in the spleen, the lungs, and one other peripheral site, the hind footpad (FP). The problem of nonspecific resistance has been circumvented by testing the ability of spleen cells from immunized mice to confer antituberculosis immunity upon nonimmune recipients (19).

MATERIALS AND METHODS

Animals. Female (C57Bl/6 × DBA/2) F_1 inbred mice (B6D2) were introduced into experiments when 6 weeks old.

Mycobacteria. Strains of mycobacteria were obtained from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y. The organisms were grown in Proskauer and Beck liquid medium (Difco Laboratories, Detroit, Mich.), to which were added Tween

80 and glycerol. Culture vials were stored at −70°C, thawed before use, and diluted appropriately in saline to contain the required number of viable bacilli per milliliter. BCG Pasteur (TMC 1011) and *Mycobacterium tuberculosis* R1Rv (TMC 205) were used for immunization and challenge, respectively.

Immunization. The required numbers of viable BCG in appropriate volumes of saline were injected either i.v. in 0.2 ml or into the left hind footpad (LHFP) in 0.04 ml. For P infection, 10 ml of BCG culture containing approximately 2×10^8 viable bacteria per ml was nebulized in a Middlebrook Airborne Infection Apparatus (Tri-R Instruments, Rockville Centre, N.Y.) in which the mice were held. This procedure implanted approximately 10^4 viable BCG into the lungs. The implantation inoculum was checked by making viable counts of BCG in the lungs of mice immediately after infection.

Challenge. Mice were challenged with R1Rv at doses of 10^5 i.v., 10^4 into the right hind footpad (RHFP), or 10^4 into the lungs as described for BCG immunization above.

DTH. Lyophilized human tuberculin-purified protein derivative was obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. The material was brought to a concentration of 125 μg by solution in sterile saline containing 0.05% Tween 80. The thickness of the RHFP was measured with dial-gauge calipers before injection of 5 μg of purified protein derivative (0.04 ml) and was remeasured 24 h later. The difference in FP thickness was expressed in 0.1-mm units. An increase of 2 units (0.2 mm) or more was accepted as a positive result, denoting the presence of DTH.

Irradiation. Before challenge with R1Rv and transfer of spleen cells, all recipient mice and their controls were exposed to 500 rads of total body irradiation from a ^{137}Cs source (19).

Spleen cells. Spleens were removed from donor mice and teased apart, and the dissociated cells were washed in ammonium chloride/tris(hydroxymethyl)aminomethane buffer to lyse erythrocytes (6). The remaining cells were washed in Dulbecco phosphate-buffered saline and suspended in the same solution to a density of 2.5×10^8 leukocytes per ml. Each recipient received 0.2 ml of this suspension, containing 5×10^7 leukocytes, injected i.v. The viability of each spleen cell suspension was checked before injection by trypan blue exclusion. For the entire series of experiments, the cell viabilities were in the range of 60 to 80%.

Viable counts of mycobacteria. Spleens and lungs were homogenized in glass tubes with matching motor-driven Teflon pestles. Feet were severed at the ankle and allowed to soak for 30 min in 0.1% benzalkonium chloride to kill skin surface contaminants. Each foot was then cut into four to five coarse pieces and homogenized in sterile saline with a Virtis grinder (Virtis Co., Gardiner, N.Y.). Appropriate dilutions of homogenate were plated on Middlebrook 7H-10 agar (Difco) and incubated for 14 to 21 days at 37°C; then colony counts were made. The counts were expressed either as geometric means or as an index of immunity that was estimated by subtracting the geometric mean count of a test group from that of the control group.

Statistics. Comparison of group means was made after analysis of variance and application of the *Q* test (32). All DTH and viable-count means were based on groups of five mice.

RESULTS

Induction of immunity after immunization with BCG by the i.v. and LHFP routes. Mice were immunized with either 10^6 BCG i.v., 10^3 BCG i.v., or 10^6 BCG into the LHFP. At intervals thereafter, mice of each group were used for tuberculin tests and as spleen cell donors. Mice immunized with 10^6 BCG i.v. or LHFP developed unequivocal tuberculin hypersensitivity at 3 to 4 weeks, whereas the group receiving 10^3 BCG i.v. never exhibited DTH (Fig. 1). Repeated experiments revealed considerable inter-experimental variation in the development and decay of DTH, but there were certain consistent features (Fig. 1 and 4). Only the larger doses of BCG resulted in perceptible DTH, which was maximal at 3 to 4 weeks and then waned rapidly. LHFP immunization resulted in a biphasic response, and the state of hypersensitivity was sustained for a longer period of time. In all experiments, the level of DTH 3 months after immunization was trivial, regardless of the route and dose of BCG.

The ability of spleen cells of vaccinated mice to confer adoptive immunity upon syngeneic recipients is illustrated in Fig. 2. There was a small but highly significant ($P < 0.01$) transfer of immunity by spleen cells of FP-immunized mice as early as week 1. By week 2, spleen cells of donors given 10^6 BCG i.v. were also effective ($P < 0.01$). Sensitized lymphocytes were detected

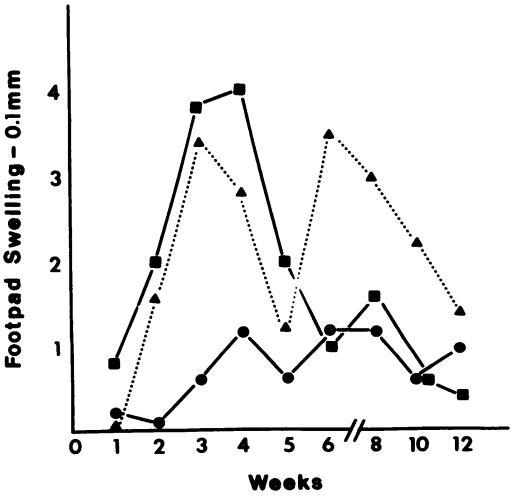

FIG. 1. *DTH after BCG immunization by i.v. and subcutaneous routes. Symbols:* ■, *10^6 BCG i.v.;* ●, *10^3 BCG i.v.;* ▲, *10^6 BCG LHFP.*

after a longer interval in mice vaccinated with 10^3 BCG i.v., but by week 6 the spleen cells of each group conveyed immunity to the same extent, and this capability did not diminish during the remaining 6 weeks of the experiment.

Development of immunity after i.v., LHFP, and P vaccination with BCG. The next point of interest was whether immunization by the P route was more or less effective than the i.v. or LHFP routes. Groups of mice were immunized with either 10^6 BCG LHFP, 10^3 BCG i.v., or 10^4 BCG P. At 2-week intervals, viable counts of BCG were made from organs into which the inocula had been directly implanted, i.e., the LHFP, spleen, and lungs, respectively (Fig. 3). No apparent growth of BCG occurred in the LHFP, probably because the large inoculum induced a rapid immune response that prevented multiplication (18). As will become evident later, smaller inocula of BCG did multiply in the FP (Fig. 6 to 8). After i.v. infection, BCG multiplied in the spleen for 4 weeks and then slowly decreased in number. Growth of BCG in the lungs after aerosol infection was substantial, and large numbers of viable organisms were present in this organ throughout the course of the experiment.

The development of tuberculin DTH in these mice is shown in Fig. 4. In this experiment, each group of immunized mice exhibited DTH at week 4, but its level varied with the route of immunization. Mice immunized in the LHFP were significantly more sensitive ($P < 0.01$) than P-infected mice, with the remaining group, i.v., occupying an intermediate position.

Transfer of adoptive immunity was not achieved until 4 weeks after immunization, at which point all groups of vaccinated mice were effective donors (Fig. 5). Between 4 and 6 weeks, cells from LHFP-immunized donors were more effective than those from P-immunized mice ($P < 0.01$), but subsequently the difference between the three groups of cell recipients was insignificant.

Expression of active and adoptive immunity at different sites. The above experiments suggested that, regardless of the dose of BCG or its route of administration, the spleen cells of vaccinated mice eventually developed a

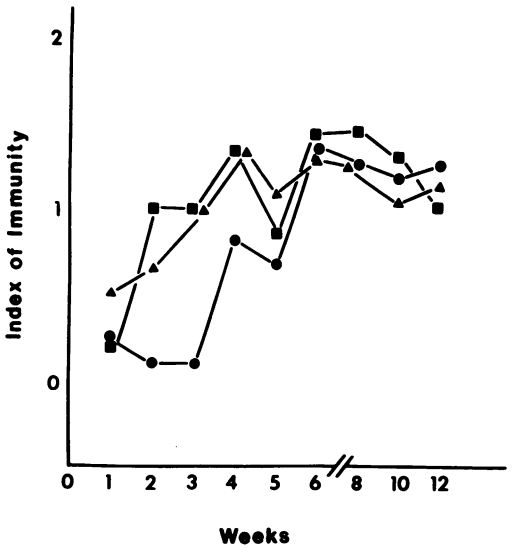

FIG. 2. *Transfer of adoptive immunity to M. tuberculosis with 5 × 10^7 spleen cells at intervals after immunization with either 10^6 BCG i.v. (■), 10^3 BCG i.v. (●), or 10^6 BCG LHFP (▲).*

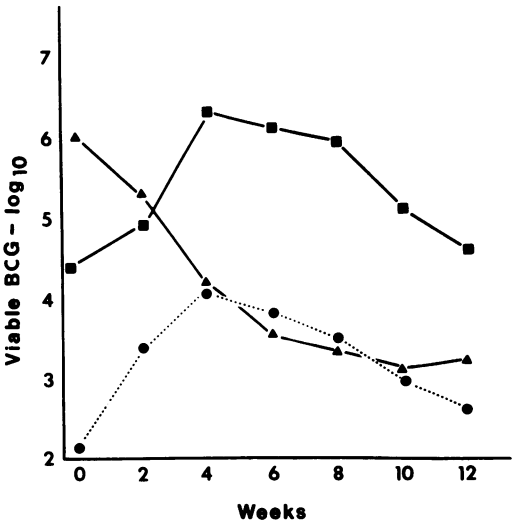

FIG. 3. *BCG was inoculated either i.v., subcutaneously, or into the lungs, and viable counts were made from the spleen (●), FP (▲), and lungs (■), respectively.*

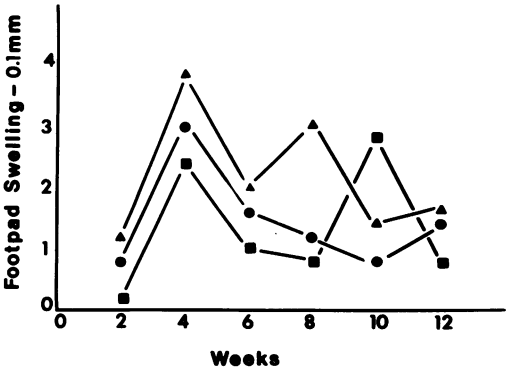

FIG. 4. *Induction of DTH after BCG immunization by FP (▲), i.v. (●), and P (■) routes.*

uniform capacity to transfer anti-tuberculosis immunity within 8 weeks of immunization. In the next series of experiments, mice were vaccinated with BCG, 10^3 i.v. (Fig. 6), 10^6 LHFP (Fig. 7), or 10^4 P (Fig. 8). Eight weeks later, when the ability of spleen cells to transfer immunity

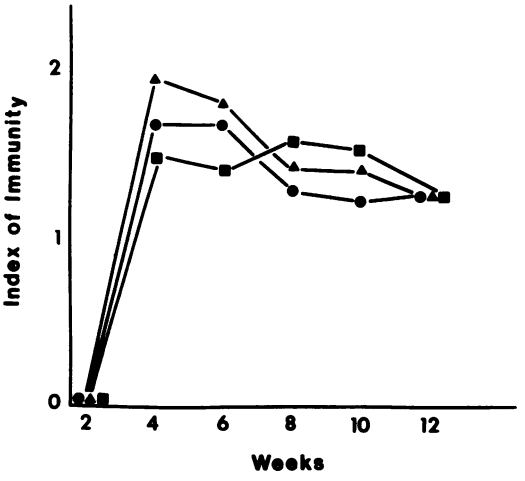

FIG. 5. *Adoptive immunity in recipients of spleen cells from mice immunized by FP (▲), i.v. (●), and P (■) routes.*

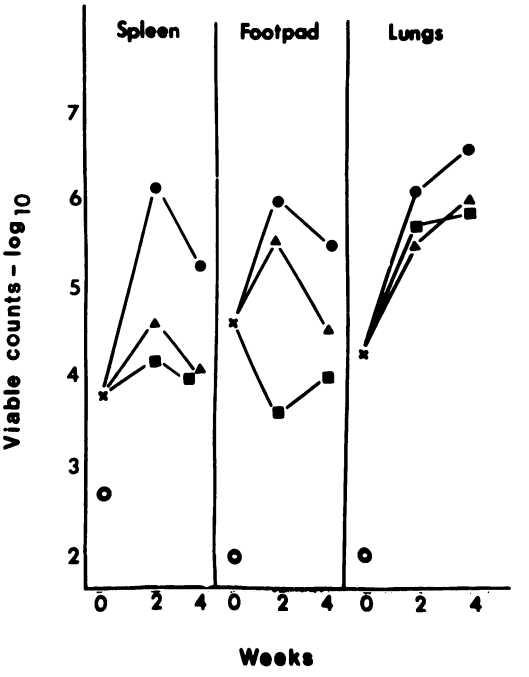

FIG. 7. *Adoptive and active immunity after FP immunization with BCG. Symbols: See legend of Fig. 6.*

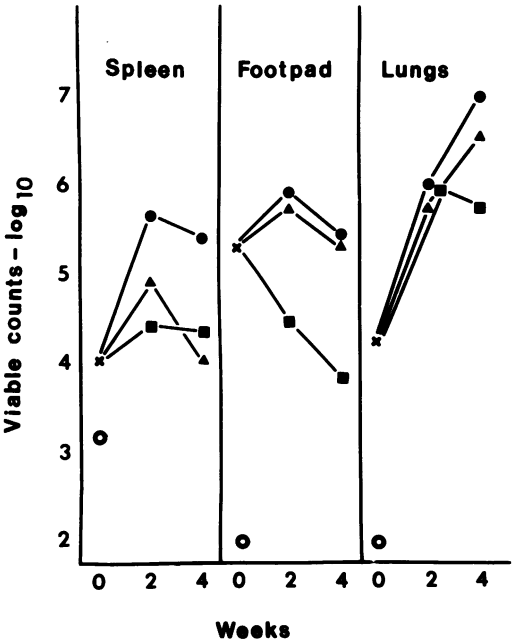

FIG. 6. *Adoptive and active immunity after i.v. immunization with BCG. Symbols: ●, normal controls; ▲, recipients of 5 × 10^7 spleen cells from immunized donors; ■, actively immunized donors; ×, challenge inoculum; ○, residual BCG in donor organs.*

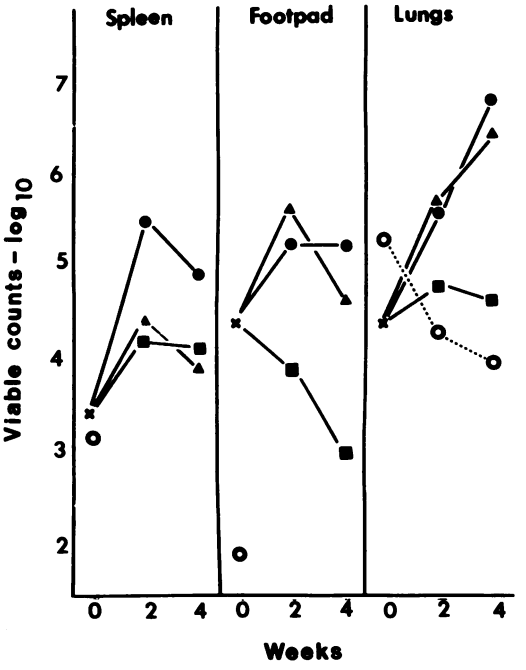

FIG. 8. *Adoptive and active immunity after P immunization with BCG. Symbols: See legend of Fig. 6.*

was established, either the actively immunized mice were challenged with R1Rv, or their spleen cells were transferred to syngeneic recipients that were also challenged, together with irradiated controls. Three types of challenge were evaluated: 10^5 i.v., a procedure that deposited approximately 10^4 bacilli in the spleen; 10^4 RHFP; and 10^4 P. Viable counts were made from the appropriate organs (spleen, RHFP, and lungs, respectively) 1 h after challenge and 2 and 4 weeks later (Fig. 6 to 8).

The effects of spleen-cell transfer will be considered first. As in earlier experiments, spleen cells from the different groups of immunized donors provided excellent protection ($P < 0.01$) against i.v. infection with R1Rv, and immunity was manifest within 2 weeks of challenge. The extent of adoptive immunity in the spleen was usually less than that in actively immunized donors ($P < 0.01$) at 2 weeks (Fig. 6 and 7), but at 4 weeks there was no difference between the counts in the two types of mice (Fig. 6 to 8).

The expression of adoptive immunity in the RHFP and lungs was defective as compared with the spleen. Spleen cells from i.v.- and P-vaccinated mice did not confer resistance in either of these sites at 2 weeks after challenge or in the RHFP at 4 weeks (Fig. 6 and 8). A modest degree of protection, 0.41 $\log_{10}$ unit ($P < 0.05$), was expressed in the lungs at 4 weeks in recipients of cells from i.v.-immunized mice (Fig. 6). Spleen cells from LHFP-immunized mice were more effective. They conferred resistance in the RHFP at 2 weeks ($P < 0.05$) and 4 weeks ($P < 0.01$) and in the lungs at 4 weeks ($P < 0.01$) (Fig. 7). It is notable that this particular batch of cells also conferred the highest level of adoptive immunity in the spleen (Fig. 6 to 8).

Before reinfection with R1Rv, donor mice of each group were killed, and viable counts of residual BCG from the immunizing inoculum were made in the spleen, RHFP, and lungs (Fig. 6 to 8). Each group of donor spleens contained approximately 10^3 viable BCG at the time of challenge and an undetectably low ($<10^2$) number of organisms in the RHFP. Less than 10^2 viable BCG were isolated from the lungs of LHFP- or i.v.-immunized mice, but approximately 10^5 BCG were present in the lungs of P-immunized mice. This population of BCG was approximately 10-fold greater than the challenge inoculum of R1Rv (Fig. 8).

Turning now to the expression of active immunity, the anti-mycobacterial mechanism was most effective in the RHFP, where growth of the organisms was completely suppressed and there was a net decrease of viable organisms by 2 weeks (Fig. 6 to 8). There was limited growth of R1Rv in the spleens during the first 2 weeks after challenge, but the inhibition of growth was highly significant ($P < 0.01$) relative to normal mice. The control of challenge infection in the RHFP and spleen was closely similar in all groups of vaccinated mice. On the other hand, the ability to control P infection varied with the route of immunization. Only mice vaccinated by the P route inhibited the growth of R1Rv in the lungs ($P < 0.01$) within 2 weeks of reinfection (Fig. 8), but all donor mice controlled the infection ($P < 0.01$) by 4 weeks (Fig. 6 to 8).

DISCUSSION

An examination of the induction of immunity after BCG vaccination has been made by the method of adoptive transfer of resistance with lymphoid cells. This system has been shown to depend on an immunologically specific population of T-lymphocytes and circumvents the problem of nonspecific resistance that exists in the actively immunized donors (19, 20). It was found that the rapidity of onset of immunity was a function of the size of the immunizing inoculum, a conclusion that had been inferred in earlier work (18). The influence of inoculation route was less apparent, but it appeared that immunity appeared first after FP, then i.v., and finally P vaccination. More impressive than the rate of induction of immunity was the consistent, stable level at which the numbers of sensitized lymphocytes in the spleen achieved equilibrium. Within 2 months of immunization, this equilibrium was such that the transfer of adoptive immunity with 5×10^7 spleen cells was closely similar regardless of the dose and route of vaccination. This steady state reflects a homeostatic mechanism that compensates for a 1,000-fold variation in inoculum size, and is the more remarkable because the initial lymphoproliferative response to living BCG is very much dependent on the dose (21).

Tuberculin hypersensitivity after immunization with BCG was a function of dose and route of administration. The highest levels of DTH were obtained with 10^6 LHFP, followed by 10^6 i.v., 10^3 i.v., and lastly 10^4 P. The levels of DTH varied considerably between experiments. It is worth noting that the fluctuation in DTH is in marked contrast to the consistency with which spleen cells conferred immunity.

This study has confirmed the observations of others that P immunization with living BCG engenders a greater resistance to subsequent P reinfection than does i.v. or subcutaneous vaccination (1, 3, 4, 17, 24). Admittedly, the i.v. immunization procedure of Ribi et al. (30) also produces strong resistance to P challenge, but this is due to the vehicle in which the antigen

is injected, an oil-in-water emulsion. The oil droplets, into which antigen is incorporated, localize in the lungs due to embolism of the pulmonary capillaries and induce extensive granulomatosis (1, 29). Similarly, the system of Myrvik (25) also induces chronic pulmonary granulomatosis. By contrast, i.v. injection of living BCG in saline deposits 90% of the inoculum in the liver, 10% in the spleen, and only 1% in the lungs (10, 11). Hence, the i.v. injection of mycobacterial antigens in oil and aerosol infection with BCG both constitute vaccination by the P route.

A number of explanations for the association between P immunization and pulmonary resistance will now be considered. Ribi and co-workers have shown that the protective action of his nonliving vaccines is a function of the granulomatosis that is produced, measured as increase in lung weight (2, 23). The implication of this observation is that the increased resistance to P infection is due to nonspecific resistance exerted by the macrophages participating in the pulmonary granulomas. An analogous situation exists in animals that have been immunized with living BCG, in whom resistance to reinfection at a particular site is associated with the persistence of live vaccinating organisms at that site (17). These observations are supported by this study, in which maximal resistance to reinfection in the lung was expressed in mice carrying a substantial number of BCG ($>10^5$) in that organ at the time of challenge. The association between resistance at a given site and persistence of immunizing organisms is not invariable. In this study, resistance to reinfection was highest in the RHFP, from which BCG was never isolated. Whereas it is not contended that activated macrophages in the lungs or other organs do not restrain the growth of challenge organisms, it is doubtful whether such action fully accounts for the experimental results. The growth of R1Rv in the lungs of i.v.- and FP-immunized mice is unimpeded for at least 14 days (Fig. 6 and 7), whereas the effective duration of nonspecific resistance is measured in as many hours (28). It is therefore probable that some other component of the granulomata, namely lymphocytes, makes a potent contribution.

Clearly, if aerosol immunization resulted in a larger or more efficient population of specifically sensitized lymphocytes than i.v. or FP inoculation, increased resistance to reinfection at every site would logically ensue. This conjecture is untenable, because resistance to reinfection in the spleen and the RHFP was no better after P than i.v. or FP immunization, and the same holds true for adoptive immunity. Another possibility is that, due to the adjuvant properties of mycobacteria, immunization with BCG induces not only an immune response to mycobacterial antigens but an autoimmune response to tissues at the site of infection (7, 9). If this were so, P and FP infection might be expected to generate lymphocytes that produced better protection at the homologous than the heterologous site. The results of the cell transfer experiments provide no support for this notion. Cells from P-immunized mice did not confer preferential protection upon the lungs of recipients. Paradoxically, cells from i.v.- and FP-immunized mice were more effective in this regard. Moreover, actively immunized mice combated i.v. and FP challenge with equal facility, regardless of the immunization route.

It is proposed that the sheer proximity of sensitized lymphocytes to the implanted, homologous, challenge organisms results in a brisker anamnestic response, because time is gained that would otherwise be spent in recruiting a sufficient number of sensitized lymphocytes from the circulation into the site of challenge. Such tissue lymphocytes may correspond to the sessile memory cells of humoral immunity (12, 31) and may share identity with the lung cells that can produce migration-inhibiting factor after immunization via the respiratory tract (8, 14, 27, 33). The rapid expression of cell-mediated immunity in the spleen is attributable in part to the migration through that organ of recirculating lymphocytes in which anti-tuberculosis immunity is vested (21), obviating the need for further recruitment. Moreover, regardless of the route of immunization, a significant number of living BCG eventually reach the spleen, where they generate tuberculoid granulomas.

It has been argued that adoptive immunity cannot be expressed in the lungs (34), the implication being that there is defective migration of sensitized lymphocytes into this organ. The data in Fig. 7 refute this contention. Admittedly, adoptive immunity is poorly expressed in the lungs as compared with the spleen. But the lungs are not unique in this respect, because the FP is similarly affected. Furthermore, in some experiments (Fig. 6 and 7) there was significant adoptive immunity in the lungs. It is pertinent that the number of cells transferred, 5×10^7, was relatively modest, and there is a strong possibility that more substantial effects would result from the use of larger doses of cells. Although adoptive immunity is expressed to a similar extent in the lungs and FP, active immunity is inexplicably more effective at the latter site.

In conclusion, it appears that there is a wide variation in intrinsic resistance to tuberculosis

TABLE 1. *Expression of adoptive and active immunity to tuberculosis at different sites*

Type of immunity	Level of resistance in:		
	Spleen	FP	Lungs
Adoptive	Moderate	Low	Low
Active	Moderate	High	Variable[a]

[a] Low after i.v. or LHFP immunization; moderate after P immunization.

in different organs (Table 1). There appears to be a rational explanation for the relatively good resistance of the spleen. The intrinsic susceptibility of the lungs and FP is high and low, respectively, and currently there is no good reason for this discrepancy. The resistance of a susceptible organ like the lung can be increased by appropriate immunization, but only at the cost of inducing substantial ganulomatous disease. There was no association between antituberculosis immunity, active or adoptive, and the level of tuberculin hypersensitivity.

ACKNOWLEDGMENTS

I thank Robert J. Emerson and Dwight Milne for excellent technical assistance.

This work was supported by Public Health Service grants AI-12404 and AI-07015 and contract NO1 AI-52529 from the National Institute of Allergy and Infectious Diseases, and by Public Health Service grant 5SO7 RR05705 from the Division of Research Resources.

LITERATURE CITED

1. **Anacker, R. L., W. R. Barclay, W. Brehmer, G. Goode, R. H. List, E. Ribi, and D. F. Tarmina.** 1969. Effectiveness of cell walls of *Mycobacterium bovis* strain BCG administered by various routes and in different adjuvants in protecting mice against airborne infection with *Mycobacterium tuberculosis* strain H37Rv. Am. Rev. Respir. Dis. **99**:242-248.
2. **Anacker, R. L., W. R. Barclay, W. Brehmer, C. L. Larson, and E. Ribi.** 1967. Duration of immunity to tuberculosis in mice vaccinated intravenously with oil-treated cell walls of *Mycobacterium bovis* strain BCG. J. Immunol. **98**:1265-1273.
3. **Anacker, R. L., E. Ribi, D. F. Tarmina, L. Fadness, and R. E. Mann.** 1969. Relationship of footpad sensitivity to purified protein derivatives and resistance to airborne infection with *Mycobacterium tuberculosis* of mice vaccinated with mycobacterial cell walls. J. Bacteriol. **100**:51-57.
4. **Barclay, W. R., W. M. Busey, D. W. Dalgard, R. C. Good, B. W. Janicki, J. E. Kasick, E. Ribi, C. E. Ulrich, and E. Wolinsky.** 1973. Protection of monkeys against airborne tuberculosis by aerosol vaccination with Bacillus Calmette-Guérin. Am. Rev. Respir. Dis. **107**:351-358.
5. **Blanden, R. V., M. J. Lefford, and G. B. Mackaness.** 1969. The host response to Calmette-Guérin Bacillus infection in mice. J. Exp. Med. **129**:1079-1107.
6. **Boyle, W.** 1968. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. Transplantation **6**:761-764.
7. **Burell, R. G., and M. S. Rheins.** 1958. Production of anti-lung substances in rabbits by homologous tuberculous tissue antigens. Am. Rev. Respir. Dis. **78**:259-267.
8. **Cantey, J. R., and W. L. Hand.** 1974. Cell-mediated immunity after bacterial infection of the lower respiratory tract. J. Clin. Invest. **54**:1125-1134.
9. **Cate, C. C., and R. Burrell.** 1974. Lung antigen induced cell-mediated immune injury in chronic respiratory disease. Am. Rev. Respir. Dis. **109**:114-123.
10. **Collins, F. M., and T. E. Miller.** 1969. Growth of a drug-resistant strain of *Mycobacterium bovis* (BCG) in normal and immunized mice. J. Infect. Dis. **120**:517-533.
11. **Collins, F. M., L. G. Wayne, and V. Montalbine.** 1974. The effect of cultural conditions on the distribution of *Mycobacterium tuberculosis* in the spleens and lungs of specific pathogen-free mice. Am. Rev. Respir. Dis. **110**:147-156.
12. **Ellis, S. T., and J. L. Gowans.** 1973. The role of lymphocytes in antibody formation. V. Transfer of immunological memory to tetanus toxoid: the origin of plasma cells from small lymphocytes, stimulation of memory cells *in vitro* and persistence of memory. Proc. R. Soc. London Ser. B **183**:125-139.
13. **Ford, W. L.** 1969. The kinetics of lymphocyte recirculation within the rat spleen. Cell Tissue Kinet. **2**:171-191.
14. **Galindo, B., and Q. N. Myrvik.** 1970. Migratory response of granulomatous alveolar cells from BCG-sensitized rabbits. J. Immunol. **105**:227-237.
15. **Gowans, J. L., and E. J. Knight.** 1964. The route of recirculation of lymphocytes in the rat. Proc. R. Soc. London Ser. B **159**:257-282.
16. **Kawata, H., Q. N. Myrvik, and E. S. Leake.** 1964. Dissociation of tuberculin hypersensitivity as mediator for an accelerated pulmonary granulomatous response in rabbits. J. Immunol. **93**:433-438.
17. **Larson, C. L., and W. C. Wicht.** 1962. Studies of resistance to experimental tuberculosis in mice vaccinated with living attenuated tubercle bacilli and challenged with virulent organisms. Am. Rev. Respir. Dis. **85**:833-846.
18. **Lefford, M. J.** 1971. The effect of inoculum size on the immune response to BCG infection in mice. Immunology **21**:369-381.
19. **Lefford, M. J.** 1975. Transfer of adoptive immunity to tuberculosis in mice. Infect. Immun. **11**:1174-1181.
20. **Lefford, M. J., D. D. McGregor, and G. B. Mackaness.** 1973. Immune response to *Mycobacterium tuberculosis* in rats. Infect. Immun. **8**:182-189.
21. **Lefford, M. J., D. D. McGregor, and G. B. Mackaness.** 1973. Properties of lymphocytes which confer adoptive immunity to tuberculosis in rats. Immunology **25**:703-715.
22. **Medical Research Council.** 1972. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life. Bull. W.H.O. **46**:371-385.
23. **Meyer, T. J., E. Ribi, and I. Azuma.** 1975. Biologically active components from mycobacterial cell walls. V. Granuloma formation in mouse lungs and guinea pig skin. Cell. Immunol. **16**:11-24.
24. **Middlebrook, G.** 1961. Immunological aspects of airborne infection: reactions to inhaled antigens. Bacteriol. Rev. **25**:331-346.
25. **Myrvik, Q. N., E. S. Leake, and S. Oshima.** 1962. A study of macrophages and epithelioid-like cells from granulomatous (BCG-induced) lungs of rabbits. J. Immunol. **89**:745-751.
26. **Nagaishi, C.** 1972. Functional anatomy and histology of the lung. University Park Press, Baltimore.
27. **Nash, D. R., and B. Holle.** 1973. Local and systemic cellular immune responses in guinea-pigs given antigen parenterally or directly into the lower respiratory tract. Clin. Exp. Immunol. **13**:573-583.
28. **North, R. J.** 1974. T cell dependence of macrophage activation and mobilization during infection with *Mycobacterium tuberculosis*. Infect. Immun. **10**:66-71.
29. **Ribi, E., R. L. Anacker, W. R. Barclay, W. Brehmer, G. Middlebrook, K. C. Milner, and D. F. Tarmina.**

1968. Structure and biological functions of mycobacteria. Ann. N.Y. Acad. Sci. **154**:41–57.

30. **Ribi, E., C. Larson, W. Wicht, R. List, and G. Goode.** 1966. Effective nonliving vaccine against experimental tuberculosis in mice. J. Bacteriol **91**:975–983.
31. **Smith, J. B., A. J. Cunningham, K. J. Lafferty, and B. Morris.** 1970. The role of the lymphatic system and lymphoid cells in the establishment of immunological memory. Aust. J. Exp. Biol. Med. Sci. **48**:57–70.
32. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames.
33. **Spencer, J. C., R. H. Waldman, and J. E. Johnson.** 1974. Local and systemic cell-mediated immunity after immunization of guinea pigs with live or killed *M. tuberculosis* by various routes. J. Immunol. **112**:1322–1328.
34. **Truitt, G. L., and G. B. Mackaness.** 1971. Cell-mediated resistance to aerogenic infection of the lung. Am. Rev. Respir. Dis. **104**:829–843.