Mycobacterium bovis BCG-Induced Protection against Cutaneous and Systemic Leishmania major Infections of Mice

A. H. FORTIER,* B. A. MOCK, M. S. MELTZER, AND C. A. NACY
Walter Reed Army Institute of Research, Washington, D.C. 20307-5100

Received 2 February 1987/Accepted 17 April 1987

We examined the protective effects of Mycobacterium bovis bacillus Calmette-Guérin (BCG) administration on Leishmania major infections of BALB/c and P/J mice. There were two treatment protocols. In the first, the footpads of naive animals were inoculated with mixtures of L. major and BCG (viable or heat killed) or the soluble mycobacterial antigen, purified protein derivative. Viable BCG, but not heat-killed BCG or purified protein derivative, inoculated with L. major amastigotes into the footpads of naive BALB/c or P/J mice protected these animals from the metastatic spread of parasites to the viscera and from ensuing lethal systemic infection. This treatment also induced cures of the cutaneous lesions of P/J mice but not of BALB/c mice. In the second protocol, we induced an immune response to BCG before inoculation of L. major. BCG given intraperitoneally 10 days before infection of footpads with leishmania offered protection against the metastatic spread of amastigotes in both P/J and BALB/c mice, regardless of intraperitoneal treatment, and modulated the severity of cutaneous infection by 30 to 50%. Inoculation of a mixture of viable BCG and L. major amastigotes into BCG-immune mice completely protected both BALB/c and P/J strains from cutaneous disease; we recovered no parasites from the inoculated footpads of these animals. Furthermore, each of the nonspecifically protected mice of both the BALB/c and P/J strains developed immunity to rechallenge with viable L. major. Injection of amastigotes at a site remote from the original lesion, the contralateral footpad, resulted in the complete clearance of parasites in the inoculum with no evidence of either cutaneous or systemic disease over an extended observation period.

BALB/c mice develop progressive infections with the protozoan parasite Leishmania major. Inoculation of 10⁶ amastigotes into footpads induces a cutaneous lesion, early metastatic spread of parasites to the viscera, and death of the animal, usually by 12 weeks (2, 12). P/J mice are qualitatively similar to BALB/c mice in susceptibility to L. major. Inoculation of amastigotes into the footpads of P/J mice also leads to cutaneous infection and systemic disease. Parasites metastasize to the spleen and liver and set up foci of infection that ultimately result in death of the animal. The difference in susceptibility between P/J and BALB/c mice is quantitative; 100-fold more parasites are required to induce lethal disease in P/J mice by 12 weeks (7).

Resolution of infection by the obligate intracellular parasite L. major in all mouse strains is dependent upon the development of an effective cell-mediated immunity that leads to intracellular destruction of amastigotes (25). Such protective cell-mediated immune events are presumed to include the generation of soluble products by specifically sensitized lymphocytes (lymphokines [LK]) and consequent activation of macrophages for intracellular killing activities. Susceptibility of BALB/c and P/J mice to L. major infection is the result of defective immune responses by these animals. Macrophages from both BALB/c and P/J mice fail to control intracellular replication of L. major at the site of the cutaneous lesion and within infected viscera. The underlying basis for this defective cellular response is not clear, but several lymphocyte and macrophage defects have been demonstrated in both leishmania-susceptible mouse strains that may contribute to exacerbated disease. (i) BALB/c and P/J mice have defective macrophage responses to LK for induction of potent killing mechanisms in vitro (3, 19, 20). (ii) Adherent cells, phenotypically identified as macrophages, that suppress normal lymphocyte function are observed in spleen cell suspensions from L. major-infected BALB/c mice (28). (iii) BALB/c and P/J lymphocytes have reduced capacity to produce macrophage-activating LK in response to antigens or mitogens in vitro (4, 16, 18, 27). (iv) BALB/c mice develop a unique population of suppressor T lymphocytes during leishmanial disease (13, 30).

Although it is very difficult to distinguish causative factors from epiphenomena in lengthy chronic diseases such as the leishmaniasis, two immunodeficiencies discussed above are shared by both susceptible P/J and BALB/c mice: defective LK production in response to antigens or mitogens for induction of macrophage activation and defective macrophage response to LK for intracellular parasite killing. Intuitively, both of these effector activities of the immune response must be operative for resolution of leishmanial disease. How might one induce these critical activities during an infectious process that characteristically results in a suppressive response in the host? One way to achieve functional augmentation of the immune system is by the use of immunostimulatory agents, such as Mycobacterium bovis BCG and Propionibacterium acnes. The literature is replete with documentation of the immunoenhancing effects of BCG. There are two of particular relevance to this study. (i) BCG infections have systemic effects, as evidenced by an increase in the levels of interferon in serum, a potent LK for activation of macrophages to kill intracellular L. major (33). (ii) BCG inoculation leads to local inflammation, followed by development of an immune response with LK production and induced macrophage activation for killing of intracellular and extracellular targets (22, 24, 26). These unique and potent immunostimulatory properties of BCG have been practically applied in protection studies with a number of infectious and neoplastic agents (8, 23, 34), including several species of leishmania. Treatment of leish-
maniasis with BCG has been only variably successful. Intra-peritoneal (i.p.) or subcutaneous inoculation of C3H mice with BCG did not alter lesion size in Leishmania mexicana-infected animals (9). Intravenous pretreatment of BALB/c mice with BCG was protective against systemic L. major disease but did little to protect these animals from cutaneous infection (32). The cellular functions defective in L. major-susceptible P/J and BALB/c mice, LK production and macrophage activation, are precisely those augmented during BCG infection. In this study we identified BCG treatment protocols that protected mice from both cutaneous and systemic L. major infections. In addition, we demonstrated an adjuvant effect of BCG: animals protected against primary infection were resistant to reinfec tion with L. major inoculated at sites remote from the original infectious area. (This work was conducted by A. H. Fortier in partial fulfillment of the requirements for the Ph.D. degree from Catholic University of America, Washington, D.C.)

MATERIALS AND METHODS

Animals. Six- to eight-week-old male P/J and BALB/c mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The mice were housed in a barrier environment throughout these studies and were tested periodically for apparent infection with common murine viruses. The animals remained serologically negative for these agents.

Parasites. L. major NIH 173 amastigotes were propagated in the footpads of 6- to 8-week-old male BALB/c mice. Parasites were harvested from footpads infected for 3 to 4 weeks, according to the method previously described (21). The amastigote inoculum was adjusted to 5 × 10⁷ parasites per ml, and 0.05 ml was injected into the right hind footpad of each mouse. The parasite inoculum was routinely 50 to 70% viable, as assessed by fluorescein diacetate staining (15).

BCG protection. Mice were inoculated with 0.1 ml of a Mycobacterium bovis BCG suspension that contained 10⁶ CFU/ml (Treadeau Institute, Saranac Lake, N.Y.) or medium i.p. 8 to 10 days before L. major inoculation in the footpad. Fifty microliters of 5 × 10⁷ L. major amastigotes per ml was inoculated simultaneously with medium or with medium containing 10⁶ CFU of BCG, 10 µg of purified protein derivative (PPD) (Connaught Laboratories, Ltd., Willowdale, Ontario, Canada), or 10⁶ heat-killed BCG (viable BCG incubated in a boiling water bath for 1 h). In some studies, BCG-pretreated mice were inoculated with L. major subcutaneously in the footpad and then given 10 µg of PPD intranasally every 72 h for 15 days.

DTH response. Mice were inoculated i.p. with 0.1 ml of 10⁶ BCG CFU per ml and 8 to 10 days later were given 10 µg of PPD or 10⁶ BCG in 50 µl in the left hind footpad. Delayed-type hypersensitivity (DTH) reaction was measured every 24 h for 5 days with Schneltaster calipers (Carobronze Ltd., London, England).

Analysis of cutaneous disease. Development of the cutaneous lesion was monitored by measuring footpad depths with Schneltaster calipers over a 12-week period. Histology of the lesion was analyzed by hematoxylin and eosin staining of a smear from a longitudinal section of infected footpad tissue. Where indicated, a similar smear was prepared, Ziehl-Neelsen stained, and observed for the presence of acid-fast bacilli.

Analysis of systemic disease. Twelve weeks after L. major inoculation the mice were sacrificed, and the spleens, livers, and popliteal lymph nodes draining the cutaneous infection were removed, weighed, and cut longitudinally to make impression smears. The air-dried organ smears were stained with Wright’s Giemsa (Diff-Quik; Harleco, Philadelphia, Pa.), and the numbers of parasites per 1,000 nucleated cells were determined. This number was multiplied by the organ weight to give an estimate of total parasites for a particular organ, and is expressed as Leishman Donovan Units (LDU) (29).

Rechallenge of protected and unprotected mice. BCG-treated mice and mice that did not receive BCG treatment were rechallenged with 50 µl that contained 5 × 10⁷ L. major amastigotes per ml in the contralateral footpad 6 weeks after primary inoculation with L. major. The course of cutaneous disease was monitored for 12 weeks as outlined above, and systemic disease was analyzed, as before, 12 weeks after rechallenge.

RESULTS

Concurrent infections in naive animals. (i) Effects on cutaneous disease. Mice were inoculated subcutaneously in the hind footpad with L. major either alone or admixed with live BCG, heat-killed BCG, or PPD. Figure 1 shows the development of cutaneous disease in BALB/c and P/J mice over 12 weeks. The combination of L. major amastigotes and live BCG resulted in decreased lesion size in BALB/c mice. No BCG treatment, however, could completely protect these animals from cutaneous disease (Fig. 1A). Microscopic examination of infected footpad tissue showed that lesions of all sizes were characterized by a preponderance of macrophages that were heavily infected with L. major. Concurrent BCG infections in L. major-inoculated P/J mice, on the other hand, completely protected these animals from cutaneous leishmaniasis (Fig. 1B). Microscopic analysis of these inoculated footpad tissues, in contrast to those of BALB/c mice, showed no evidence of L. major. There were, however, numerous acid-fast bacilli, and the cells were characteristic of an inflammatory infiltrate (polymorphonuclear leukocytes, macrophages, and few lymphocytes). Viability of BCG was essential for clearance of the leishmania inoculum, since protection was not observed when P/J mice were treated with heat-killed BCG.

(ii) Effects on systemic disease. Four to eight weeks after cutaneous disease is established in susceptible BALB/c and P/J mice, parasites metastasize to the liver and spleen (7). The resulting systemic infection progresses unchecked in these animals and eventually leads to death of the infected mice in 10 to 18 weeks. We analyzed the extent of systemic disease by quantitation of liver metastases at 12 weeks in BCG-treated and control mice (Fig. 2). The incidence of liver metastases in BCG-treated mice of both strains was markedly different from that of untreated BALB/c and P/J mice. Viable BCG given concurrently with L. major protected P/J mice from lethal systemic disease. Even BALB/c mice, a strain not protected from cutaneous infection by this treatment protocol, showed no evidence of systemic disease by 12 weeks postinfection. In a similar set of studies, BCG-protected mice were observed for up to 20 weeks; no deaths were recorded, and liver and spleen impression smears showed no evidence of systemic disease in these animals. Again, protection was clearly dependent upon viability of BCG. Heat-killed BCG did not prevent systemic spread of L. major and was not effective in rescuing mice from lethal disease. These results confirm the clear distinction between the two major components of L. major infections, cutaneous infection and systemic disease, which are under separate
VOL. 55, DEViations OF FROM FOOTPADS OF GENETIC FIVE MICE.

Lesions with extensive necrotic tissue. The lesion size in LDU amastigotes. + (BCG simultaneously from metastatic 0 inoculum. and footpads in per group, smears were intralesionally with BCG. BCG HEAT-KILLED BCG BCG + + PPD + PPD + BCG + + BCG + LDU

FIG. 1. Development of cutaneous disease in mice treated intralesionally with BCG. BALB/c (A) and P/J (B) mice were inoculated in the left footpad with 50 µl of a parasite suspension in medium that contained 5 x 10⁶ L. major amastigotes per ml either alone (○) or simultaneously with live BCG (●), heat-killed BCG (△), or PPD (△). Footpad depths were measured every 3 weeks for 12 weeks, and the lesion size was determined by subtracting the uninoculated right footpad depth from the inoculated left footpad depth. Dashed lines indicate lesions with extensive necrotic tissue. The shaded area represents the mean depth of five normal (uninoculated) footpads ± 2 standard deviations of the mean; the upper value of this shaded area is the 95% confidence level for control feet. Each point represents the mean of five mice. This figure shows the results from one of at least three similar experiments.

induced an immune response to BCG before inoculation of L. major into the footpads of BALB/c and P/J mice. Mice inoculated with 10³ CFU of BCG i.p. were tested 8 to 10 days later for DTH to BCG antigens by inoculation of either viable BCG or PPD into the footpad (Fig. 3). All mice inoculated with viable BCG i.p. showed strong, and persistent, DTH responses to both PPD and viable organisms. The footpads of BCG-immune mice, as well as those of untreated controls, were infected with L. major alone or in combination with viable BCG, heat-killed BCG, or PPD. Development of cutaneous lesions was monitored by measuring footpad depths for 12 weeks. Figure 4 demonstrates the dramatic effect of concurrent BCG and L. major infections in BCG-immune animals. Comparison of the lesion size of naive BALB/c mice (Fig. 1A) with that of BCG-immune animals (Fig. 4A) showed a marked modulation of the development of cutaneous disease with prior BCG immunity. The lesion size of most animals pretreated with BCG was 30 to 50% less than that of naive animals. The only treatment protocol that was totally effective in eliminating cutaneous disease in BALB/c mice was the mixture of viable BCG and L. major inoculated into BCG-immune mice. In these mice, the cutaneous lesion completely healed by week 12. Microscopic examination of these healed lesions demonstrated numerous acid-fast bacilli but no amastigotes. Cells present in the tissue were characteristic of an inflammatory infiltrate (polymorphonuclear leukocytes, macrophages, and few lymphocytes). Although decreased in size, lesions did develop in BCG-immune BALB/c mice infected with L. major alone or in combination with heat-killed BCG or soluble PPD. Histologic examination of tissues from these lesions showed numerous amastigotes in macrophages.

The lesions of all BCG-immune P/J mice were substantially smaller (by 50%; Fig. 4B) than the lesions of naive animals (Fig. 1B). However, two of the intrallesional treatment protocols in BCG-immune P/J mice completely protected the animals from development of a cutaneous lesion:

FIG. 2. Development of systemic disease in mice treated intralesionally with BCG. BALB/c (A) and P/J (B) mice, three mice per group, were inoculated with 5 x 10⁶ L. major amastigotes per ml in footpads either alone (LM) or simultaneously with live BCG (BCG + LM) or heat-killed BCG (heat-killed BCG + LM) or PPD (PPD + LM). At 12 weeks, the livers were removed and weighed, and a single lobe was sectioned for impression smears. Stained smears were examined microscopically for the presence of L. major amastigotes. LDU were calculated by the method of Stauber, as described in Materials and Methods. This figure shows the results from one of three similar experiments.

Concurrent infections in BCG-immune animals. (i) Effects on cutaneous disease. In the second treatment protocol, we genetic control (7). Viable BCG present with L. major in the footpads of naive BALB/c or P/J mice protected these animals from metastatic spread of the parasites to the viscera and enabled them to survive an otherwise lethal infectious inoculum.

(ii) Effects on cutaneous disease. In the second treatment protocol, we used an immunized system as described in Materials and Methods. LDU were calculated by the method of Stauber, as described in Materials and Methods. This figure shows the results from one of three similar experiments.
intralesional treatment with viable BCG and intralesional treatment with heat-killed BCG. Histopathology of these tissues confirmed complete clearance of the amastigote inoculum. In BCG-immune P/J mice, then, concurrent infections with BCG and *L. major* were not required for protection. Intralesional heat-killed BCG was as protective as viable BCG.

That the soluble mycobacterial antigen, PPD, was totally ineffective in inducing protection against *L. major* in BCG-immune mice was somewhat of a surprise. These animals clearly demonstrated DTH to PPD, although the magnitude of the DTH response was less than that observed with viable organisms (Fig. 3). However, Liew et al. (17) have clearly dissociated the DTH response to leishmanial infection from the BCG model. An alternative explanation for this lack of protection might lie in the soluble nature of the PPD antigen versus the persistence of whole mycobacteria. Since *L. major* induces a chronic infection, the long-term presence of a local immune response might be required for complete clearance of the parasite. To test this hypothesis, we pretreated mice with BCG i.p. and infected them with *L. major* plus PPD. Every 72 h thereafter, for 15 days, the mice were injected with 10 μg of PPD into the infected foot (Fig. 5). In both BALB/c and P/J mice, intercurrent inoculation of PPD exacerbated leishmanial disease and resulted, particularly in the P/J mice, in substantially larger lesions than the lesions in mice inoculated with *L. major* alone. Stained impression smears from these infected footpads at 12 weeks showed numerous intracellular amastigotes. It is possible that the increased trauma induced by intermittent inoculation of PPD contributed to exacerbation of the disease (11). Indeed, Titus et al. (31) also observed exacerbation of leishmanial disease with PPD. Thus, BCG qualitatively alters the immune response, and PPD does not.

(ii) Effects on systemic disease. Impression smears of liver tissue at 12 weeks from BCG-immune mice inoculated with *L. major* alone or in combination with viable BCG, heat-killed BCG, or PPD were examined for the presence of amastigotes (Fig. 6). Markedly fewer amastigotes were present in the livers of all the BCG-immune animals, and in several of the groups no amastigotes were observed at all (Fig. 6). The exact pattern of few to no amastigotes was observed in splenic impression smears of these same BCG-immune mice (data not shown). Thus, systemic disease did not develop in mice pretreated i.p. with BCG, regardless of subsequent intralesional treatment. It was not clear from these studies whether systemic infections that occur in naive animals (Fig. 2) were controlled by BCG in protected animals at the site of metastasis from the cutaneous lesion or within the liver and spleen. To address this point, we examined the popliteal lymph nodes draining the cutaneous lesions of BALB/c mice pretreated with medium or BCG and infected with *L. major* (Fig. 7). In all cases, the number of amastigotes in lymph nodes of BCG-treated mice was substantially less than that of untreated mice.

**Adjuvant effects of BCG-induced protection in development of immunity to *L. major***. Six weeks after the initial infection, we inoculated nonspecifically protected BALB/c and P/J mice in the contralateral footpad with *L. major* alone (no further treatment with BCG). All nonspecifically protected animals rechallenged with *L. major* alone were completely protected from development of cutaneous and systemic disease (Fig. 8). Inoculated footpads, draining lymph nodes, livers, and spleens demonstrated no parasites 12 weeks after the rechallenge with inoculum of *L. major*. In contrast, deaths were observed in the unprotected groups. Five of five BALB/c mice and two of five P/J mice died, and tissues from the surviving mice had numerous infected macrophages at all these anatomical sites. It is interesting to note that footpad swelling, usually a hallmark of cutaneous disease, was completely absent in the rechallenged foot of unprotected P/J mice, despite the presence of numerous parasites in tissues and the fact that two of five animals had died of systemic infection (Fig. 8B). Primary infection in naive BALB/c mice was much more complicated. Six weeks after initial infection in BALB/c mice, metastasis of amastigotes occurred in the contralateral foot that resulted in an initial footpad measurement greater than base line at the time of rechallenge. It was impossible to determine the fate of the second parasite inoculum in these mice, since metastatic lesions had already developed. In any case, lesions persisted and grew larger with time in BALB/c mice that had not been pretreated with BCG.

**DISCUSSION**

Although both BALB/c and P/J mice die from the lethal effects of *L. major* infection, these strains differ in their...
relative susceptibilities to the parasite. Despite this difference, we were able to induce protection against chronic cutaneous disease, as well as lethal systemic infections, in both mouse strains by using the classic macrophage activation agent, BCG. The protection achieved was, in many respects, similar to the Mackaness model of nonspecific immunity; induction of immunity to one infectious organism protects against infection with a second, antigenically unrelated microorganism. The difference in our protection model was primarily one of approach and was based on the natural history of leishmanial disease. *L. major*, an obligate intracellular parasite of macrophages, initiates a chronic intradermal infection after the bite of its sandfly vector or subcutaneous inoculation into susceptible experimental animals. From this peripheral site, parasites metastasize to the regional draining lymph nodes and then to the viscera, and the animals die of systemic disease. The challenge was to focus the developing immune response to BCG at the dermal site of amastigote replication, in this case the footpad. By simply

**FIG. 4.** Development of cutaneous disease in BCG-immune mice. BALB/c (A) and P/J (B) mice, five mice per group, were given $10^7$ viable BCG i.p. Eight to ten days later, mice were inoculated with $5 \times 10^7$ *L. major* amastigotes per ml in footpads either alone (○) or simultaneously with live BCG (●), heat-killed BCG (▲), or PPD (△). Footpad depths were measured as described in the legend to Fig. 1.

**FIG. 5.** Development of cutaneous disease in BCG-immune animals treated with PPD. BALB/c (A) and P/J (B) mice, five mice per group, were given $10^7$ viable BCG i.p. Eight to ten days later, mice were inoculated with $5 \times 10^7$ *L. major* amastigotes per ml in footpads either with one 10-μg dose of PPD simultaneously (○) or with a 10-μg dose of PPD simultaneously and a 10-μg dose of PPD every 72 h for 15 days (●). Footpad depths were measured as described in the legend to Fig. 1. This figure shows the results from one of two similar experiments.

**FIG. 6.** Development of systemic disease in BCG-immune mice. BALB/c (A) and P/J (B) mice, three mice per group, were given $10^7$ viable BCG or medium i.p. (IP). Eight to ten days later, mice were inoculated with $5 \times 10^7$ *L. major* amastigotes per ml (LM) in footpads either alone or simultaneously with live BCG (BCG + LM) or heat-killed BCG (heat-killed BCG + LM) or PPD (PPD + LM). At 12 weeks, the livers were processed as described in the legend to Fig. 2. This figure shows the results from one of three similar experiments.
admixing *L. major* and viable BCG in the infectious inoculum, we were able to rescue both mouse strains from lethal systemic infections. This treatment also initiated resolution of cutaneous disease in P/J mice, but BALB/c mice did not fare as well under this treatment protocol.

To protect BALB/c mice from cutaneous disease, we had to establish immunity to BCG before *L. major* infection and then focus a second immune response at the site of amastigote inoculation. Under these circumstances, we were able not only to protect the mice from lethal systemic disease, but also to prevent chronic cutaneous infections in both BALB/c and P/J mice. The most dramatic effect was observed in P/J mice. These mice cleared the amastigote inoculum completely and never had any evidence of a lesion.

Thus, development of an immune response in the local environment of the leishmanial inoculum was sufficient to prevent the establishment of a focus of infection, and mice that normally die from *L. major* infections behaved like animals naturally resistant to the parasite. The relative susceptibilities of the two mouse strains were reflected in the different treatment protocols required for protection. P/J mice did not have to be immune to BCG before leishmania inoculation for protection from cutaneous disease to occur. The BALB/c mice, a more susceptible strain, required a rigorous treatment protocol to induce protection. Indeed, other attempts to completely protect BALB/c mice from leishmania infection by systemic treatment with BCG have failed (32). Our results suggest that intravenous BCG inoculation was not protective in BALB/c mice because the immune response generated by BCG must be focused at the cutaneous lesion site. The success of Bloom (unpublished) in human leishmaniasis may also be attributable to the intraleisional (focused) BCG treatment.

One effector function manifest during BCG infection is LK-induced macrophage activation for killing mechanisms. Such activated macrophages are nonspecific in their action; they kill intracellular leishmania just as well as BCG (24). How this mechanism plays a protective role in either BALB/c or P/J mice is difficult to envisage, since both strains have defective macrophage responses to LK in vitro and in vitro-defined LK defects (18, 19). Two pieces of information from the P/J mouse suggest that observed in vitro defects may be overcome during an infectious process. (i) While macrophages from BCG-treated mice are unable to kill intracellular leishmania, they do appear to be cytostatic for the parasite (A. H. Fortier, M. S. Meltzer, and C. A. Nacy, Abstr. Int. Congr. Immunol., 5.22.33, p. 626, 1986). (ii) Macrophages from leishmania-infected mice have a lowered threshold level of signal required for activation and can respond to certain LK for intracellular killing (A. H. Fortier and C. A. Nacy, Fed. Proc. 41:583, 1982). It is conceivable, then, that during BCG-leishmania coinfections, macrophages are better able to control parasite replication and more responsive to LK generated during an immune response to BCG. Thus, the two infectious processes act in concert to effect the curing of *L. major* disease.

Not only is intraleisional BCG treatment of BCG-immune mice protective against cutaneous and systemic disease, but these mice are also resistant to infection with *L. major* even when rechallenged without BCG (Fig. 8). Concomitant im-

![FIG. 7. Development of lymph node metastases in *L. major*-infected BALB/c mice. BALB/c mice, five mice per group, were given medium (A) or 10^7 viable BCG (B) i.p. Eight to ten days later, animals were inoculated with 5 × 10^7 *L. major* amastigotes per ml (LM) into footpads either alone or simultaneously with live BCG (BCG + LM) or heat-killed BCG (heat-killed BCG + LM) or PPD (PPD + LM). Twelve weeks later, draining popliteal lymph nodes were removed and cross-sectioned for impression smears. Smears were fixed, stained, and examined for the presence of parasites. The results are expressed as the number of amastigotes observed per 1,000 lymph node cells seen in approximately 100 random fields. This figure shows the results from one of two similar experiments.](image)

![FIG. 8. BCG-protected animals rechallenged with *L. major*. BALB/c (A) and P/J (B) mice, five mice per group, were either given medium i.p. and 8 to 10 days later inoculated with 5 × 10^7 *L. major* amastigotes per ml into the left hind footpad or were given 10^5 viable BCG i.p. and 8 to 10 days later inoculated with 5 × 10^7 *L. major* amastigotes per ml simultaneously with viable BCG into the left hind footpad. Both groups were rechallenged with 5 × 10^5 *L. major* amastigotes per ml in the right hind footpad 6 weeks after the first footpad inoculation. Lesion size was calculated as described in the legends for the previous figures for the rechallenged (right) footpad. In panel B, the solid triangle represents footpad depths of unprotected animals inoculated with *L. major*. In panel A, the asterisk indicates that at the time of rechallenge right hind footpad lesion size was increased to 2.2 mm as a result of metastatic infection from the primary lesion. The dagger (†) indicates deaths observed within the group: five of five BALB/c mice and two of five P/J mice died. Lesion size was calculated by subtracting the mean footpad depth of normal animals from that of inoculated footpad. This figure shows the results from one of two similar experiments.](image)
munity to leishmania induced during BCG infection is an important coincidence of the protection we observe and may be due to the adjuvant effects of BCG (1). In the past, BCG has been a useful adjunct to poorly immunogenic vaccines. Two recent human studies of note have implicated BCG as both a potent immunopotentiator and an adjuvant for chronic diseases. Intraleison BCG treatment of patients with disseminated cutaneous leishmaniasis has led to regression of lesions, and simultaneous inoculation of lepromat in BCG led to clearance of lepromatous lesions despite documented anergy to lepromat (5, 6). Collectively, these results suggest that BCG has potential as an adjuvant for leishmanial vaccines, which thus far have not been suitable immunogens (10, 14).

ACKNOWLEDGMENTS

We thank Tamara Turner for secretarial assistance in manuscript preparation. We also gratefully acknowledge John Lawson and Earl Wilson for excellent animal husbandry.

LITERATURE CITED


