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Inhibition of Metabolism and Growth of *Mycobacterium leprae* by Gamma Irradiation[1]

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Mycobacterium leprae, the causative agent of leprosy, is an obligate intracellular pathogen. Although the leprosy bacillus was discovered by Armauer Hansen over 125 years ago, it has yet to be cultured *in vitro* on artificial medium. However, viability of *M. leprae* can be maintained, without multiplication, for a limited time in axenic medium ([7, 21]) and in cultured macrophages, the primary host cells for the bacilli ([8, 16, 17]).

Enormous strides have been made in leprosy research over the past 30 years as researchers have taken advantage of the large numbers of *M. leprae* that can be harvested from the tissues of infected armadillos ([25]) and, to a lesser extent, athymic (nude) mice ([2, 3]). Because of the stigma associated with leprosy in general and an irrational fear of the leprosy bacillus, a Class 2 pathogen, nonviable bacilli are commonly used in research. Various methods have been utilized to kill *M. leprae* before employing it as a research reagent, including formalin fixation, phenol treatment, or heating at 100°C (boiling) or 121°C (autoclaving). With the curious exception that Shepard has shown that autoclaving actually improves the immunogenicity of an *M. leprae* vaccine for mice ([19]), these treatments can cause extensive denaturation and structural damage to the bacilli and, thus, may compromise research goals themselves. Gamma-irradiation (γ-irr) has also been used to kill *M. leprae.* In purification protocols developed in the 1970s, 2.5×10^6 rad appears to have been arbitrarily chosen as a sterilizing dose to render experimentally infected tissues noninfectious, such as the armadillo spleen and liver for the subsequent preparation of *M. leprae* vaccines and purified antigens ([15, 19, 24, 27]).

The purpose of this study was to evaluate the minimum dose of γ-irr required to kill semipurified suspensions of *M. leprae* harvested from the *nu/nu* mouse foot pad. This is a simple enough goal for cultivable microorganisms. But in the case of *M. leprae,* a less direct approach was required. The labor-intensive and tedious mouse foot pad assay ([18]) was employed to address the seemingly simple question of whether the bacilli harvested from the foot pads were

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alive or dead. In addition, the development by Franzblau ([5, 6]) of radiorespirometric assays to measure *M. leprae* metabolic activity, and the adaptation of these assays to bacilli cultured in mammalian cells *in vitro* ([12, 16]), made short-term studies of *M. leprae* feasible. Both methods were employed in the present study to measure the effects of γ-irr on *M. leprae.* Parallel studies employing the cultivable, nonpathogenic *M. lufu* were performed for comparison.

MATERIALS AND METHODS

Mycobacterial cultures

M. leprae are maintained in continuous passage in athymic *nu/nu* mice (Harlan Sprague-Dawley, Inc., Indianapolis, Indiana, U.S.A.) by inoculation of 1×10^8 freshly harvested bacilli into both hind foot pads as previously described ([1]). At 9 months postinfection, foot-pad tissue was aseptically removed and gently disrupted using a hand-held homogenizer (Wheaton Science Products, Millville, New Jersey, U.S.A.). The bacilli were purified by differential centrifugation ([6]), enumerated ([20]), and used within 48 hr. Absence of contamination of the *M. leprae* preparation is ensured by subculture onto blood agar plates, thioglycollate medium, tryptic soy broth, Middlebrook 7H11 medium, and Lowenstein-Jensen medium. The cultivable, environmental mycobacterium, *M. lufu* (obtained from Dr. Francoise Portaels, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium) served as a control. *M. lufu* was cultured in Middlebrook 7H9 medium supplemented with 10% oleic acid-albumin-dextrose-catalase solution (Becton Dickinson, Cockeysville, Maryland, U.S.A.) plus glycerol (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) plus Tween 80 (Sigma), and used while in mid-log phase growth.

γ-Irradiation

M. leprae and *M. lufu* were exposed to varying doses (10^2–10^6 rad) of γ-irradiation (γ-irr) from a ^{60}Co source (Shepherd Model 484 irradiatior; J. L. Shepherd and Associates, San Fernando, California, U.S.A.).

Determination of mycobacterial metabolic activity

M. leprae. The BACTEC 460 system, which measures the catabolic conversion of ^{14}C-palmitic acid to $^{14}CO_2$, was used to determine the effects of γ-irr on mycobacterial metabolic activity. One $\times 10^8$ *M. leprae* in 0.1 ml medium were inoculated into BACTEC 460 vials (5 vials/group). The vials were flushed with a gas mixture consisting of 2.5% O_2/10% CO_2/balance N_2 (Air Liquide America Corp, Houston, Texas, U.S.A.), incubated at 33°C, and read every 3 days for 2 weeks. Because *M. leprae* does not multiply *in vitro,* maximum BACTEC readings are reached early in the assay and then begin to decline. Both the peak growth index (GI) and the rate of decline are dependent on the viability of the organisms ([6]).

M. lufu. *M. lufu* were inoculated into BACTEC 460 vials (5 vials/group) at a concentration of 1×10^6 bacilli per vial. Control vials were inoculated with unirradiated *M. lufu* at 1:1, 1:10 and 1:100 dilutions to represent 0%, 90% and 99% inhibition, respectively. Vials were read daily until the 1:100 dilution gave a GI reading of 30 ([10]).

Determination of mycobacterial growth

M. leprae. *M. leprae* were inoculated into both hind foot pads of BALB/c mice at a concentration of 1×10^4 bacilli per each hind foot pad. The number of bacilli were enumerated 6 months postinfection ([20]). In the titration experiment, control mice were inoculated with 10^2, 10^3, or 10^4 *M. leprae* into each of both hind foot pads. Experimental mice were inoculated with 10^4 irradiated bacilli. The number of *M. leprae* were enumerated at 3, 5, 7 and 12 months postinfection. Experimental groups which yielded counts of *M. leprae* which were less than those obtained from the 10^3 and 10^2 control groups indicated at least a 90% and 99% kill, respectively ([14]).

M. lufu. Irradiated *M. lufu* were serially diluted in Hanks balanced salt solution (HBSS) (GIBCO, Grand Island, New York, U.S.A.) supplemented with 0.05% Tween 80 (Sigma) and spread on Middlebrook 7H11 agar plates. Cultures were incubated at 37°C for 14 days, at which time colony forming units (CFU) were enumerated.

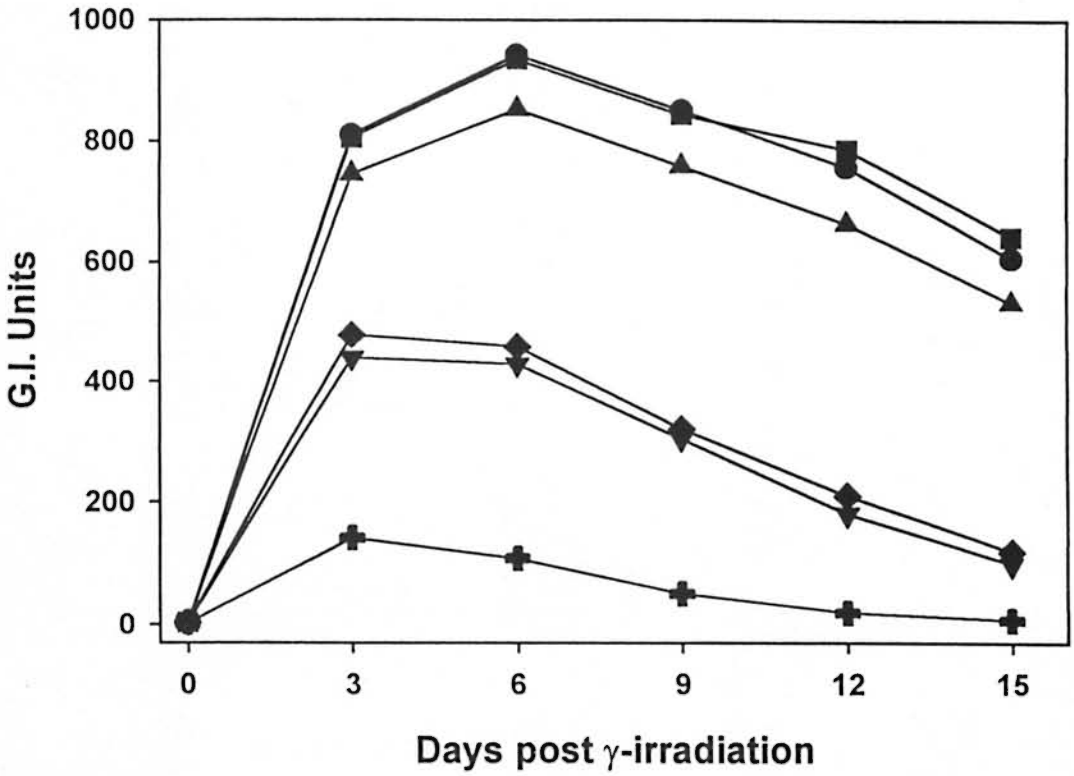

FIG. 1. Effect of γ-irr on the metabolic activity of *M. leprae*. Freshly harvested, *nu/nu* mouse-derived *M. leprae* were exposed to increasing doses of γ-irr (10^2 rad, ■; 10^3 rad, ▲; 10^4 rad, ▼; 10^5 rad, ♦; 10^6 rad, +) from a ^{60}Co source. BACTEC vials were inoculated with 10^8 bacilli and incubated at 33°C. Control vials were inoculated with unirradiated bacilli (●). Vials were read every 3 days. Data points represent means of quintuplicate samples. Error bars are omitted for clarity, but standard deviations were ≤10%.

Scanning electron microscopy (SEM)

M. leprae were adjusted to 1×10^9 bacilli/ml and irradiated with 10^6 rad. Control *M. leprae* received no irradiation. For morphological comparisons, *M. leprae* samples were also heated at 100°C/10 min or were autoclaved at 121°C/15 pounds per square inch/15 min. The bacilli in all treatment groups were washed three times in phosphate buffered saline (PBS) and then fixed in 1.25% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate (CAC) buffer, pH 7.4, for 1 hr at room temperature, washed and resuspended in 0.1 M CAC, and applied to polylysine-coated glass cover slips. After postfixation in 1% OsO_4, pH 7.4, at room temperature for 30 min, they were washed in distilled water dehydrated in ethyl alcohol, critical point dried, mounted with silver paint, sputter coated for 7 min, and viewed on a Cambridge Stereoscan 150 scanning electron microscope.

Statistics

Data were analyzed using a two-tailed parametric Student's *t* test. All tests were considered significant at $p < 0.05$.

RESULTS

Effects of γ-irr on mycobacterial metabolic activity

As shown in Figures 1 and 2, γ-irr of both *M. leprae* and *M. lufu* resulted in a dose-dependent inhibition of metabolic activity. γ-Irr of 10^2 rad had virtually no effect on the oxidation of palmitic acid by either organism [$p = 0.7182$ (*M. leprae*), $p = 0.9623$ (*M. lufu*)]. 10^3 Rad induced a small but significant reduction on *M. leprae* metabolism ($p = 0.0099$). However, 10^4–10^5 rad caused an intermediate inhibitory effect

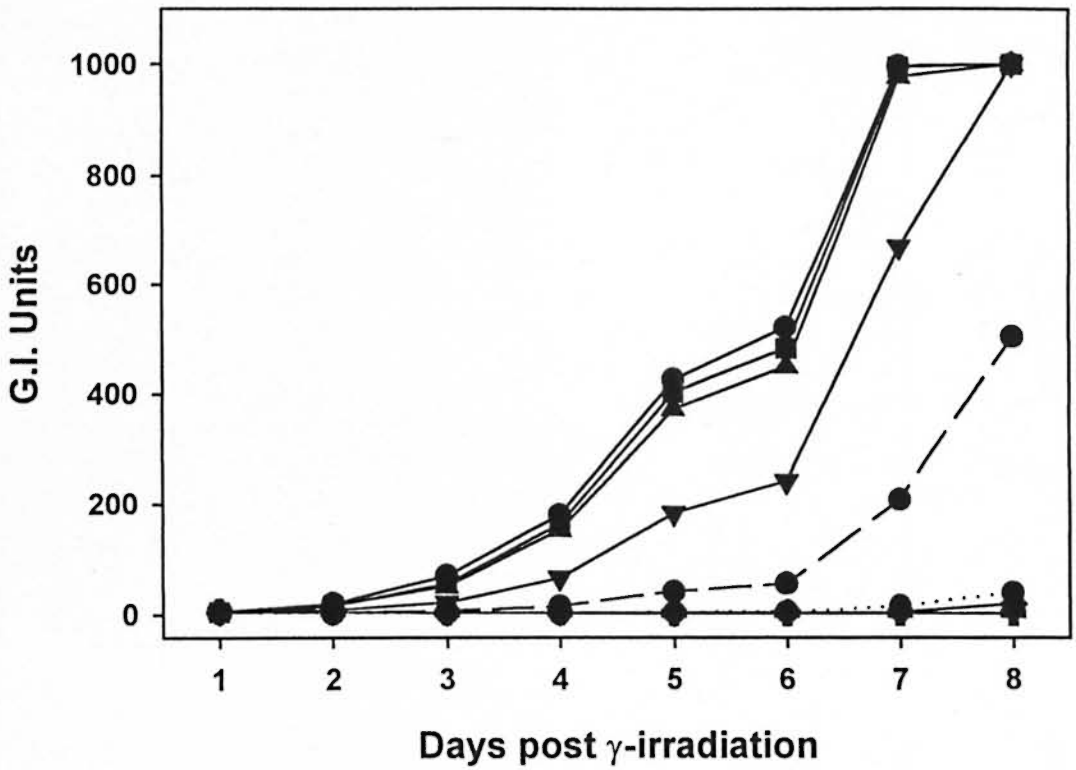

FIG. 2. Effect of γ-irr on the metabolic activity of *M. lufu*. A log phase culture of *M. lufu* was exposed to increasing doses (10^2 rad, ■; 10^3 rad, ▲; 10^4 rad, ▼; 10^5 rad, ♦; 10^6 rad, +) of γ-irr from a ^{60}Co source. BACTEC vials were inoculated with 10^6 bacilli and incubated at 37°C. Controls vials were inoculated with unirradiated bacilli at 10^6(●), 10^5 (--●--) and 10^4 (···●···) organisms to represent 0%, 90% and 99% killing, respectively. Vials were read daily until the 10^4 inoculum gave a growth index (GI) reading of 30. Data points represent means of quintuplicate samples. Error bars are omitted for clarity, but standard deviations were ≤10%.

($p < 0.0001$) on *M. leprae* and 10^6 rad yielded almost total inhibition of metabolic activity ($p < 0.0001$) (Fig. 1). With *M. lufu*, (Fig. 2) 10^4 rad inhibited metabolic activity initially but peak GI readings of 999 were reached by day 8, just one day beyond the unirradiated controls. However, γ-irr of 10^5 rad and above inhibited metabolic activity of *M. lufu* by 99% ($p < 0.0001$).

Effect of γ-irr on mycobacterial growth

The effect of γ-irr on mycobacterial growth was next determined. As shown in Figure 3, the dose of γ-irr was inversely proportional to the number of CFU of *M. lufu* recovered. γ-Irr of up to 10^3 rad did not kill the organisms. In contrast, 10^4 rad caused a 1 log-reduction in the number of CFU of *M. lufu* and 10^5 rad reduced CFU by a 3-log reduction. γ-Irr of 10^6 rad sterilized the culture of *M. lufu* since no CFU were recovered (limits of detection of assay = 10 bacilli).

The mouse foot pad assay was used to measure the growth of *M. leprae*. Multiplication of unirradiated *M. leprae* peaked at ~10^6 bacilli per foot pad 6 months postinfection (Fig. 4). Up to 10^4 rad had little effect on *M. leprae* growth; however, 10^5 and 10^6 rad resulted in no measurable *M. leprae* growth.

In the mouse foot pad titration experiment (Fig. 5), control mice were inoculated with 10^4, 10^3, and 10^2 *M. leprae* to represent 0%, 90%, and 99% killing, respectively (Fig. 5). Experimental mice were inoculated with 10^4 irradiated bacilli. Peak counts of 10^6 AFB were reached 5 months postinfection in control mice inoculated with 10^4 *M. leprae*. Control mice inoculated with 10^3

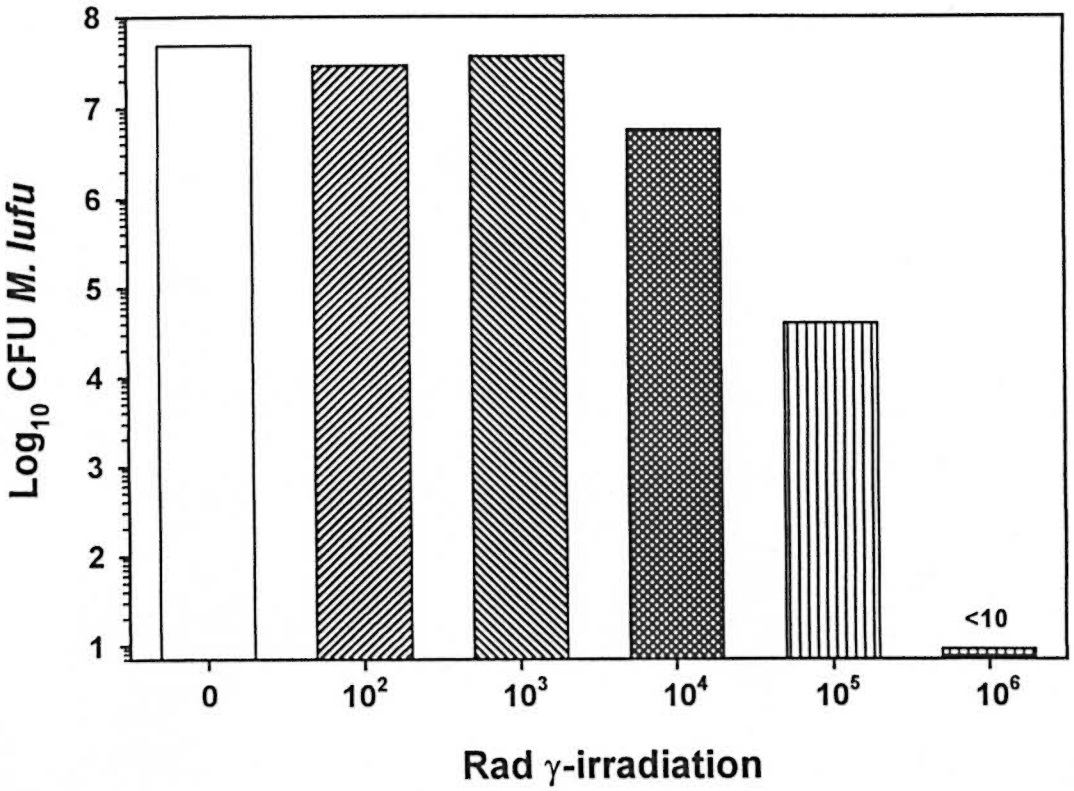

FIG. 3. Effect of γ-irr on the growth of *M. lufu.* A log phase culture of *M. lufu* was exposed to increasing doses of γ-irr from a ^{60}Co source. Bacilli were serially diluted and plated on Middlebrook 7H11 plates. Control plates were inoculated with unirradiated bacilli. Cultures were incubated at 37°C for 14 days and colony forming units (CFU) were enumerated.

and 10^2 *M. leprae* peaked later at 7 months postinfection. Experimental mice which had been infected with 10^4 irradiated *M. leprae* exhibited a dose response dependent upon the level of γ-irr. Inoculation with bacilli which had been irradiated with 10^4 rad showed a growth pattern similar to the 10^3 control, indicating 90% killing of the *M. leprae* with this does of γ-irr. 10^5 Rad killed >99% of the organisms since barely countable numbers were detected 5 months postinfection and no AFB were seen at 7 and 12 months postinfection.

S.E.M.

Control and irradiated *M. leprae,* as well as heat-killed and autoclaved bacilli, were examined by S.E.M. to determine changes in cellular surface morphology (Fig. 6). Control bacilli had a mostly smooth, elongated appearance with some wrinkling and ruffling of the surface. *M. leprae* which had been exposed to 10^6 rad also had a mostly smooth appearance, but with some blebbing and protrusions on the cell surface. Heat-killed organisms and autoclaved bacilli both exhibited extensive denaturation and destruction of the cell integrity.

DISCUSSION

Our results demonstrate that γ-irr is an effective method for killing semipurified suspensions of *M. leprae.* There is no reason to believe that less purified preparations of *M. leprae,* i.e., infected tissues, would be more resistant to γ-irr. Viability was determined by measuring bacterial metabolism and growth of the organisms, and both methods established that γ-irr exerts a deleterious effect on *M. leprae* in a dose-dependent manner. Furthermore, bacterial mortality ensued without extensive structural denaturation to the cell architecture.

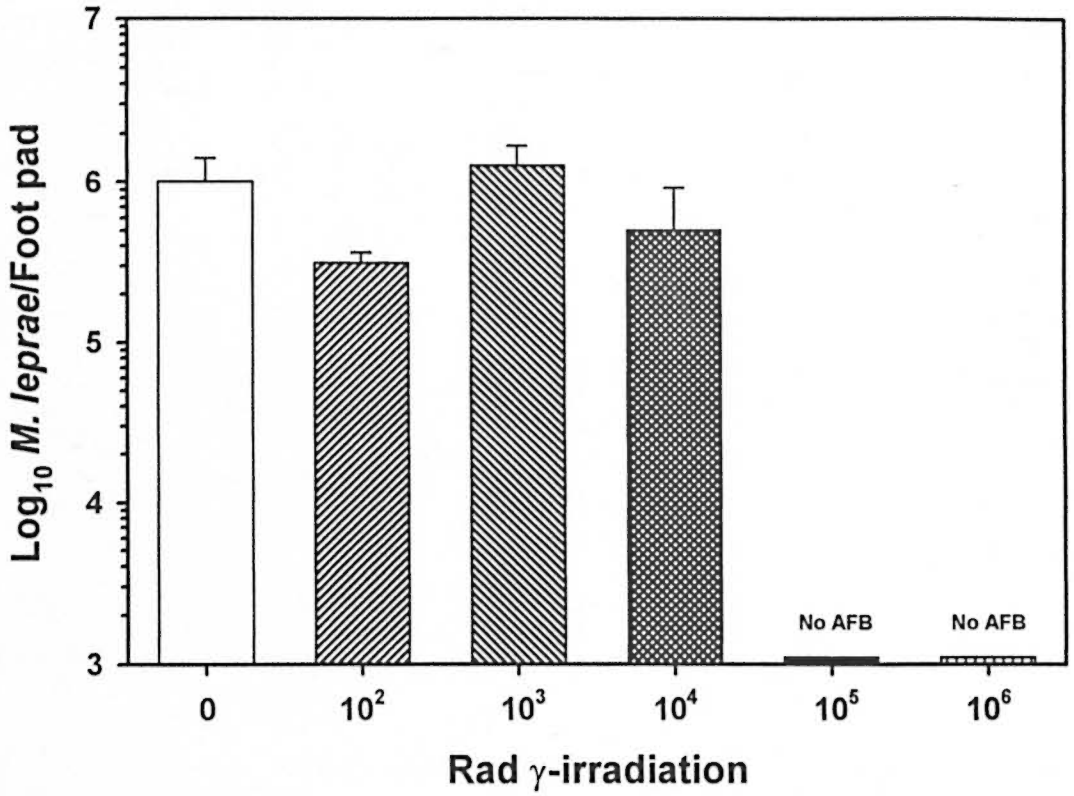

FIG. 4. Effect of γ-irr on the growth of *M. leprae*. Freshly harvested, *nu/nu* mouse-derived *M. leprae* were exposed to increasing doses of γ-irr from a ^{60}Co source. Bacilli were diluted in PBS, and BALB/c mice were inoculated with 5×10^3 *M. leprae* in each hind foot pad. Control mice were inoculated with unirradiated bacilli. Foot pads were harvested 6 months postinfection and the bacilli enumerated. Results are mean ± S.D.

Exposure of bacteria to ionizing radiation results in numerous types of DNA lesions, including single- and double-strand breaks, crosslinks, and base modifications ([11]). The hydroxyl radical is the major reactive species generated in aerated aqueous solutions following γ-irr, and it is highly reactive toward the 5–6 double bond of thymine residues ([9]). In addition to modifying the pyrimidine bases, hydroxyl radical also forms purine adducts ([4, 13, 26]). If left unrepaired, these forms of base damage are read incorrectly by the replicative polymerase, resulting in base substitution mutations. It is generally believed that the double-strand break is the most biologically significant of the lesions formed ([11]), and few prokaryotes have the ability to survive more than a few such lesions. A pair of double-strand breaks can result in the loss of all genetic material lying between the break points. A sufficiently large deletion is likely to remove an essential gene, killing the organism.

Two indicators of bacterial viability were determined. The first, bacterial metabolism, was assayed by inoculation of each species into the BACTEC 460 system. This procedure measures the oxidation of ^{14}C-palmitic acid to $^{14}CO_2$. γ-Irr of both *M. leprae* and *M. lufu* resulted in a dose-dependent inhibition of metabolic activity. Up to 10^3 rad had little effect on either organism, 10^4–10^5 rad yielded intermediate inhibitory effects, and 10^6 rad essentially inhibited metabolic activity of both organisms entirely. While measurement of palmitic acid oxidation in the BACTEC 460 system is an excellent assessment of mycobacterial viability, growth of the organisms is the ultimate indicator of viability. Therefore, a second method, bacterial multiplication, was evaluated for *M.*

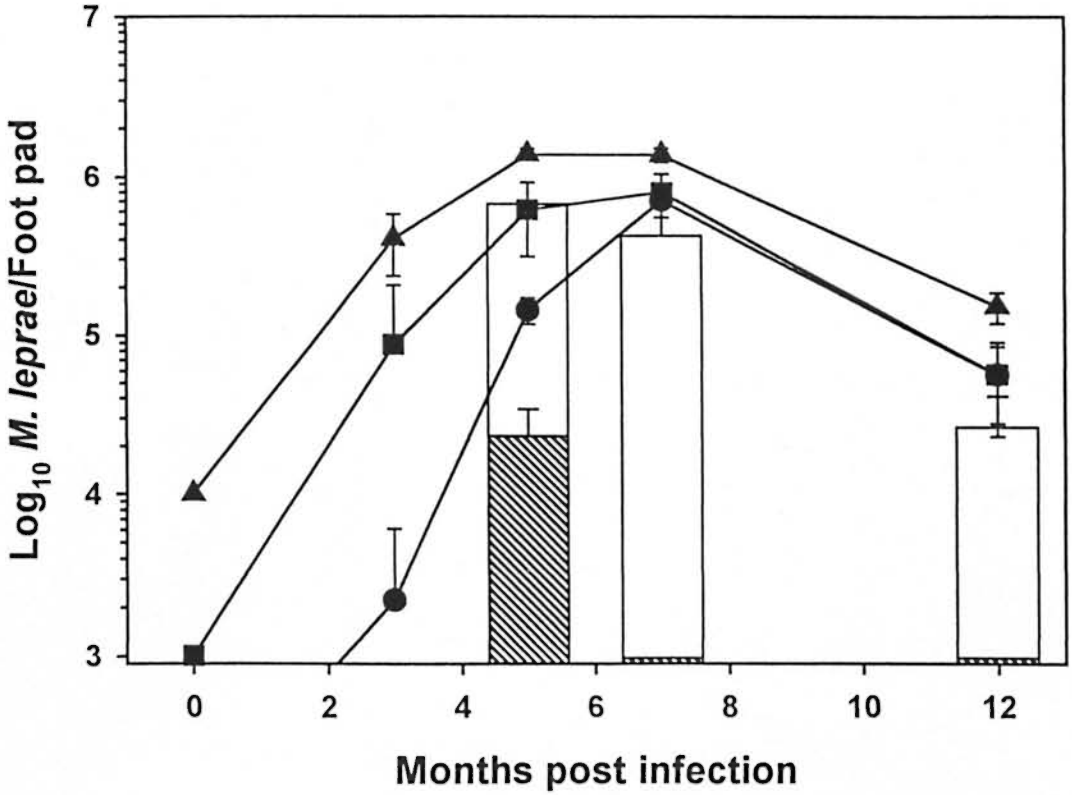

FIG. 5. Titration of γ-irr *M. leprae* in the mouse foot pad. Freshly harvested, *nu/nu* mouse-derived *M. leprae* were exposed to 10^4 rad (open bar) and 10^5 rad (hatched bar) of γ-irr from a ^{60}Co source. Bacilli were diluted in PBS, and BALB/c mice were inoculated with 10^4 *M. leprae* in each hind foot pad. Control mice were inoculated with unirradiated *M. leprae* at 10^4 (▲), 10^3 (■), and 10^2 (●) bacilli per each hind foot pad. Foot pads were harvested 3, 5, 7, and 12 months postinfection. Results are mean ± S.D. (Zero time counts are theoretical).

leprae using the mouse foot pad assay and for *M. lufu* by plating serial dilutions on 7H11 agar plates for enumeration of CFU. Again, a dose response was observed and 10^6 rad inhibited growth of both organisms completely.

Of interest is the slight disparity between the comparative effects of 10^5 rad on the viability of *M. lufu* and *M. leprae* where a 3-$\log_{10}$ decrease in CFU was seen in *M. lufu* compared with the apparent complete loss of viability of *M. leprae* in the foot pad assay. These findings could represent the relative insensitivity of the *in vivo M. leprae* assay in comparison to the *in vitro M. lufu* CFU assay. Alternatively, unlike *M. lufu* growth on 7H11 agar, intracellular growth in the foot pad tissues is not only a manifestation of *M. leprae* viability, but also of its pathogenicity and the innate and induced host defenses of the mouse. Even slight damage to the pathogen by γ-irr could tip the survival balance in favor of the host.

Examination of the surface morphology of *M. leprae* using S.E.M. revealed that γ-irr appeared to be a less damaging method for killing the bacilli than either heating at 100°C or autoclaving. Morphologically, irradiated organisms exhibited a generally smooth appearance, similar to that observed in unirradiated bacilli, although a blebbing on the cell surface was observed with increased frequency. In contrast, irradiated *M. leprae* did not exhibit the blatant denaturation observed in the boiled and autoclaved samples.

Therefore, γ-irr at 10^6 rad is a highly effective means to kill suspensions of *M. leprae*. These findings are applicable to leprosy research in several areas. Our own re-

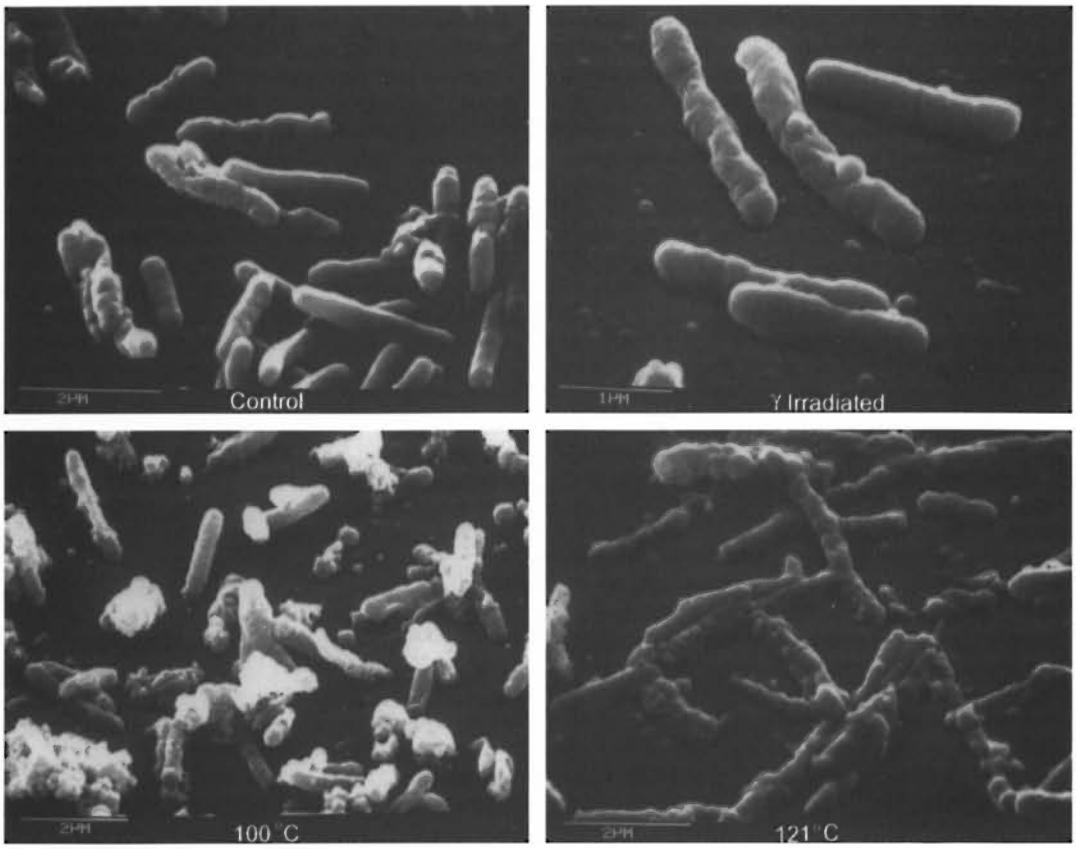

FIG. 6. S.E.M. of *M. leprae*. Effects of various methods of killing on the morphology of *M. leprae*. Control bacilli (A) were smooth and elongated, with some wrinkling of the cell surface. γ-Irr *M. leprae* (10^6 rad) (B) showed a similar appearance to the control bacilli, but had an increased incidence of small protrusions on the cell surface. Extensive denaturation was observed in the boiled (C) and autoclaved (D) *M. leprae* preparations.

search has focused on the relationship between the leprosy bacillus and its preferred host cell, the macrophage ([12]), and marked differences between the effects of live and killed bacilli have been reported, especially in the study of phagosome-lysosome fusion ([22]) and the effects of live and killed *M. leprae* on macrophage afferent and efferent effector functions ([23]). But the harsh methods used to kill the organisms may have unduly affected the bacilli. Because of its complete inhibition of bacterial metabolism and growth, yet mild morphological changes, γ-irr may be the preferred technique when comparing cellular responses to live versus dead bacilli *in vitro* and *in vivo*. γ-Irr should also become the preferred method to kill *M. leprae* that will be shipped internationally under the mandated new, stricter guidelines for transporting infectious agents (IATA Dangerous Goods Regulations 1.3.3.1).

SUMMARY

Mycobacterium leprae is uncultivable on artificial medium, but viability can be maintained without multiplication for a limited time *in vitro*. In this study, we evaluated gamma-irradiation (γ-irr) as a means to kill this slowly growing organism. Freshly harvested, viable, athymic, *nu/nu* mouse-derived *M. leprae* were exposed to varying doses of γ-irr from a ^{60}Co source. Two indicators of bacterial viability were determined: metabolism, measured by oxidation of ^{14}C-palmitic acid to $^{14}CO_2$ in the BACTEC 460 system, and multiplication, measured by titration in the mouse foot pad. γ-Irr of both *M. leprae* and *M. lufu*, a cultivable control mycobacterium, resulted in

a dose-dependent inhibition of viability. γ-Irr of up to 10^3 rad had little effect on the metabolic activity of either organism. For *M. leprae,* 10^4–10^5 rad caused an intermediate inhibitory effect; whereas 10^6 rad yielded almost total inhibition. In the mouse foot pad assay, up to 10^4 rad had little effect on *M. leprae* growth; however, 10^5 rad resulted in at least a 2-log reduction in the number of bacilli recovered and no *M. leprae* growth was measurable after exposure to 10^6 rad. With *M. lufu,* 10^5 rad inhibited metabolic activity by 99% and caused ≥2-log reduction in the number of colony forming units (CFU). No CFU of *M. lufu* were recovered after exposure to 10^6 rad. Scanning electron microscopy revealed the presence of some aberrant protrusions on the cell surface of lethally irradiated *M. leprae;* whereas boiling and autoclaving caused obvious morphological denaturation. These data suggest that γ-irr is an effective way to kill *M. leprae* without causing extensive damage to the cell architecture. Killing *M. leprae* by γ-irr may be preferable when comparing cellular responses to live versus dead bacilli *in vitro* and *in vivo.*

RESUMEN

Mycobacterium leprae no es cultivable en medios artificiales pero puede mantenerse viable, sin replicarse, por periodos limitados de tiempo. En este estudio se evaluó el efecto de la irradiación gamma (γ-irr) sobre la viabilidad de este microorganismo de lento crecimiento. Se expusieron suspensiones frescas de *M. leprae* obtenidas de la almohadilla plantar del ratón desnudo al efecto de varias dosis de γ-irr emitida por una fuente de ^{60}Co. Como indicadores de viabilidad de los bacilos se midieron su capacidad para oxidar al ^{14}C-ácido palmítico en el sistema BACTEC 460 y su habilidad para replicarse en la almohadilla plantar del ratón. La irradiación gamma de *M. leprae* y de *M. lufu,* una micobacteria cultivable usada como control, redujo la viabilidad de ambos microorganismos en un forma que fue dependiente de dosis. La γ-irr con hasta 10^3 rad tuvo poco efecto sobre la actividad metabólica de los dos microorganismos. Para *M. leprae,* 10^4–10^5 rad tuvieron un efecto inhibitorio intermedio, mientras que 10^6 rad causaron una inhibición casi total. En el ensayo de la almohadilla plantar, hasta 10^4 rad tuvieron poco efecto sobre la viabilidad de *M.leprae;* sin embargo, 10^5 rad disminuyeron cuando menos en 2 log el número de bacilos recuperados y no hubo crecimiento medible cuando los bacilos se expusieron a 10^6 rad. Con *M. lufu,* 10^5 rad inhibieron su actividad metabólica en un 99% y causaron una reducción mayor a 2 log en el número de unidades formadoras de colonias (UFC). No hubieron UFC de *M. lufu* cuando se expusieron a 10^6 rad. La microscopía electrónica de barrido reveló la presencia de estructuras aberrantes en la superficie de *M. leprae* sujeto a irradiación letal, mientras que su calentamiento a ebullición y al autoclave causó una obvia desnaturalización morfológica. Estos datos sugieren que la γ-irr es una forma efectiva para matar a *M. leprae* sin causar daño extenso en su arquitectura celular. Esta forma de matar a *M. leprae* podría ser la preferida cuando se comparan las respuestas celulares hacia bacilos vivos y/o muertos, tanto *in vivo* como *in vitro.*

RÉSUMÉ

Mycobacterium leprae n'est pas cultivable sur milieu artificiel. Cependant, une certaine viabilité peut être maintenue sans multiplication pendant un temps limité *in vitro.* Dans cette étude, nous avons évalué l'irradiation aux rayons gammas (γ-irr) comme outil pour inactiver cet organisme à croissance lente. Des *M. leprae,* récemment récoltées de souris nues athymiques *nu/nu,* furent exposées à des doses croissantes de γ-irr provenant d'une source de ^{60}Co. Deux indices de viabilité bacillaire furent déterminés: le métabolisme, estimé en mesurant la quantité d'oxydation du ^{14}C présent dans l'acide palmitique en $^{14}CO_2$ à l'aide du système BACTEC460, et la multiplication, mesurée par la titration bacillaire des pattes de souris inoculées. γ-Irr a permis d'obtenir une inhibition de viabilité dose-dépendante à la fois de *M. leprae* et de *M. lufu,* une mycobactérie contrôle cultivable. Jusqu'à 10^3 rads, γ-irr n'a eu qu'un effet très limité sur l'activité métabolique des 2 organismes testés. Un effet inhibiteur intermédiaire a été observé pour *M. leprae* lors d'une exposition à 10^4–10^5 rads, tandis que 10^6 rads a causé une inhibition métabolique presque complête. Le test d'inoculation dans la plante des pieds de la souris a révélé qu'il n'y avait que peu d'effets sur la croissance de *M. leprae* à des doses inférieures ou égales à 10^4; cependant, 10^5 rads ont résulté en une réduction de 2 unités logarythmiques de base 10 du nombre de bacilles récupérés et il n'y avait pas de croissance mesurable de *M. leprae* après une irradiation de 10^6 rads. Concernant *M. lufu,* 10^5 rads ont provoqué une inhibition à 99% de l'activité métabolique et causé une réduction de 2 unités logarythmiques de base 10 du nombre d'unités capable de former des colonies (CFU). L'exposition à 10^6 rads a inhibé toute capacité à former des colonies (absence de CFU) chez *M. lufu.* L'examen au microscope électronique à balayage a révélé la présence de quelques protrusions anormales de la surface bacillaire lorsque *M. leprae* fut irradiée à des doses léthales, tandis que l'inactivation par la chaleur humide ou l'eau bouillante a provoqué une dénaturation évidente à l'examen morphologique. Ces données suggèrent que γ-irr est une façon afficace de tuer *M. leprae* qui évite de provoquer des altérations profondes de l'architecture cellulaire. L'inactivation de *M. leprae* par γ-irr est probablement préférable d'emploi lorsque l'on veut comparer les

réponses cellulaires aux bacilles vivants ou morts *in vitro* ou *in vivo.*

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