

# IL-10 Treatment of Macrophages Bolsters Intracellular Survival of *Mycobacterium leprae*<sup>1, 2</sup>

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## ABSTRACT

In these studies, metabolically active *Mycobacterium leprae* were maintained for as long as 8 weeks in monolayer cultures of mouse peritoneal macrophages (MΦ). Supplemental IL-10, but not TGF-β, bolstered, directly or indirectly, *M. leprae* metabolism in mouse MΦ. In the cell culture system temperature setting is extremely important and 31 to 33°C incubation temperature was more permissive than 37°C. Acid fast staining and transmission electron microscopy (TEM) of intracellular *M. leprae* revealed visible elongation of bacilli cultured under the above ideal conditions.

## RÉSUMÉ

*Mycobacterium leprae* n'a jamais encore été vraiment cultivé sur milieu artificiel. Comme *M. leprae* préfère vivre *in vivo* à l'intérieur de la cellule, nous avons exploré la croissance *in vitro* de *M. leprae* dans des cultures de phagocytes mononucléés ou de macrophages, qui représentent la cellule hôte favorite du bacille de la lèpre. Notre approche expérimentale a tenu compte des faiblesses de ce type d'expérimentation : à savoir la viabilité de l'inoculum et la longue durée de multiplication de *M. leprae*. Le but n'était pas ici de démontrer une augmentation mesurable et significative du nombre de *M. leprae*, mais plutôt le maintien prolongé du métabolisme de *M. leprae*, en mesurant l'oxydation de l'acide palmitique radiomarqué, comme indicateur de viabilité.

Des *M. leprae* ayant un métabolisme actif ont été maintenues jusqu'à 8 semaines dans des cultures mono-couches de macrophages péritonéaux de souris. L'ajout d'IL-10, mais pas de TGF, a augmenté, directement ou indirectement, le métabolisme de *M. leprae* dans les macrophages de souris. Le réglage de la température des cultures cellulaires est extrêmement important et une température d'incubation de 31–33°C était plus favorable qu'une température de 37°C. La coloration acido-alcool-résistante et la microscopie électronique à transmission des *M. leprae* a permis de mettre en évidence des élongations bien visibles parmi les bacilles cultivés dans les conditions idéales mentionnées ci-dessus.

## RESUMEN

*Mycobacterium leprae* todavía no se ha podido cultivar en medios artificiales. Como esta bacteria prefiere una existencia intracelular *in vivo*, en este estudio exploramos el crecimiento *in vitro* de *M. leprae* en cultivos de fagocitos o macrófagos, el huésped preferido del bacilo de la lepra. Reconocemos que nuestro diseño experimental conlleva dos problemas: la viabilidad de *M. leprae* en el inóculo, usualmente baja, y el prolongado tiempo de división de la bacteria. Aunque no esperábamos encontrar un incremento sustancial en los números de *M. leprae*, sí pensamos poder observar cambios en su metabolismo midiendo la oxidación del ácido palmítico radiactivo como un marcador de viabilidad. Encontramos que la bacteria se mantuvo metabólicamente activa hasta por 8 semanas en los cultivos de los macrófagos peritoneales de ratón. La adición de IL-10 pero no de TGF, apoyó, directa- o indirectamente el metabolismo de *M. leprae* en los macrófagos de ratón. Las condiciones de incubación de los cultivos fueron muy importantes y la temperatura de 31–33°C fue más permisiva que la temperatura de 37°C. La tinción para ácido-resistentes y la microscopía electrónica de transmisión revelaron cierto grado de alargamiento de los bacilos bajo las condiciones óptimas de cultivo de los macrófagos.

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In the 130 years since the discovery of *Mycobacterium leprae* as the causative agent of leprosy, a large number of attempts have been made to cultivate this obligate intracellular pathogen in cell-free media<sup>(6,30)</sup>. None of these efforts have fulfilled the criteria for success suggested by John Hanks<sup>(18)</sup>, especially the confirmation of findings by a second laboratory. The inability to culture the leprosy bacillus has undoubtedly hindered almost every aspect of leprosy research, and thus, our understanding of this disease lags behind that of many others of bacterial etiology.

Reasoning that the intracellular milieu would best suit the multiplication of the obligate intracellular leprosy bacillus, as an alternative to culture in axenic medium, a number of attempts have been made to cultivate *M. leprae* in various types of cultured cells. Fieldsteel and McIntosh<sup>(10)</sup> employed a range of rat, mouse, and human tissue but found no evidence of multiplication. The preferred host cell for the leprosy bacillus, however, appears to be the mononuclear phagocyte or macrophage (MΦ) and a number of unsuccessful attempts have been made to grow *M. leprae* in MΦ<sup>(5, 8, 16, 24, 27, 31, 34, 36, 41)</sup>. The present approach also employs MΦ but is novel in that we employed conditions that inhibit innate antimicrobial functions in infected mouse MΦs to bolster the intracellular survival of *M. leprae*. Moreover, we had a number of advantages over the previous attempts by others including: unique resources, previously unavailable to other workers in the form of fresh, highly viable *M. leprae*<sup>(42)</sup>, sensitive techniques for measuring and comparing the metabolic activity of *M. leprae*<sup>(13)</sup> and the extensive experience of our two laboratories in studying the relationship between the MΦ and the leprosy bacillus<sup>(2, 14, 15, 39, 40)</sup>.

## MATERIALS AND METHODS

**Maintenance of a viable *M. leprae* inoculum.** The Thai-53 strain of *M. leprae*<sup>(26)</sup> was maintained in continuous passage in athymic nu/nu mice (Crea Co., Tokyo, Japan) by inoculation of  $1 \times 10^7$  freshly harvested bacilli into both hind footpads. At approximately nine months post, footpads were processed to recover *M. leprae* by Nakamura's method with a slight modification<sup>(28)</sup>. Briefly, tissue was minced and ho-

mogenized with Hanks' balanced salt solution (HBSS) containing 0.05% Tween 80. The homogenate was centrifuged at  $150 \times g$  for 10 min and supernatant of the sample homogenate was treated with 0.05% trypsin at 37°C for 60 min. The suspension was centrifuged at  $4000 \times g$  for 20 min and sediment was re-suspended in HBSS followed by treatment with 1% sodium hydroxide at 37°C for 15 min. The treated material was washed and re-suspended in HBSS at the desired bacillary concentration. Bacillary number in each footpad was enumerated individually according to standard techniques<sup>(37)</sup>.

**Cytokines.** Murine recombinant IL-10 was obtained from Genzyme Corp. T cell growth factor β (TGF-β) was obtained from Kurashiki Bouseki, Kurashiki, Japan). Both cytokines were stored at -80°C until use.

**Mouse MΦ culture.** Mouse peritoneal resident cells (approximately 50% MΦ) were harvested from retired ICR or Swiss White (SW) mice and suspended as previously described<sup>(2)</sup> at a concentration of  $2 \times 10^6$ /ml in RPMI 1640 (GIBCO, Grand Island, NY) + 15% fetal bovine serum (HyClone Laboratories, Logan, UT) + 25 mM hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (GIBCO), 0.2% NaHCO<sub>3</sub> (GIBCO), 2 mM glutamine (Irvine Scientific, Santa Ana, CA), and 100 μg/ml ampicillin (Sigma Chemical Co., St. Louis, MO). 0.5 ml was seeded into 24 well tissue culture plates (Corning) containing 16 mm LUX coverslips (Miles Laboratory, Naperville, IL). After overnight adherence of the cells, MΦ monolayers were obtained after washing non-adherent cells from the coverslip with Hanks Balanced Salt Solution (HBSS) leaving approximately  $1 \times 10^6$  MΦ adhered per coverslip.

**Infection of MΦ with *M. leprae*.** Purified mouse MΦ monolayers were infected with fresh *M. leprae* suspended in 0.5 ml medium at a multiplicity of infection of 20:1. After 4 hr incubation, non-phagocytized bacteria were removed by washing and the cultures reincubated in 1.0 ml media supplemented with the appropriate cytokine in 5% CO<sub>2</sub> at the appropriate experimental temperatures<sup>(2)</sup>. Media was changed and, where appropriate, cytokines replenished at 5 day intervals.

**Radiorespirometry (RR).** The MΦ were lysed with 0.1 N NaOH to release the *M.*

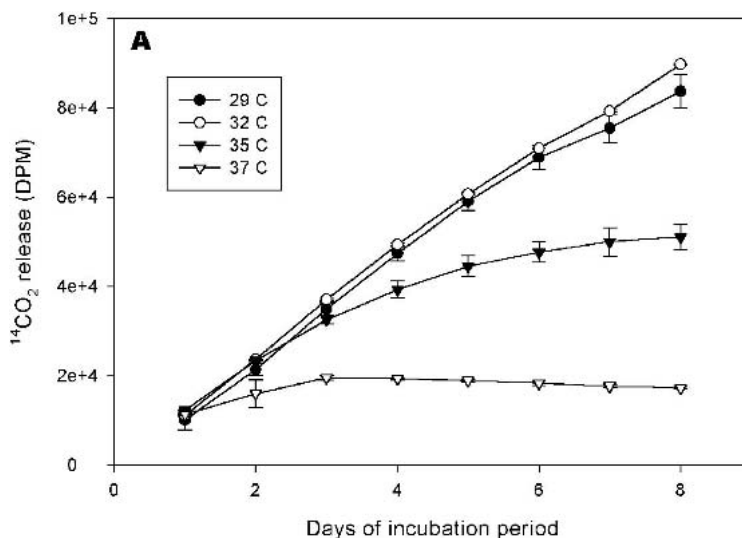


FIG. 1A. Effect of incubation temperature on metabolic activity (RR) of *M. leprae* in axenic media. Each vial was inoculated in triplicate with  $2 \times 10^7$  freshly harvested, nude mouse-derived *M. leprae* and incubated at different temperatures. In the experiment shown, metabolic activity, shown as  $^{14}\text{CO}_2$  release, was monitored every day for 8 days. These findings are representative of dozens of related experiments showing the detrimental effects of  $37^\circ\text{C}$  for *M. leprae*.

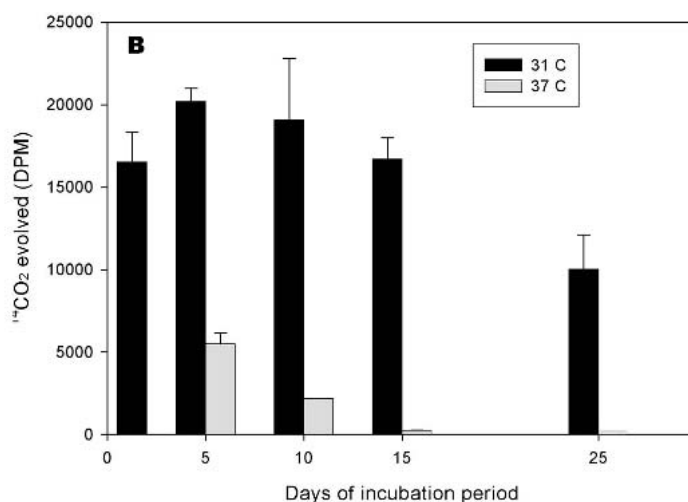


FIG. 1B. Metabolic activity of *M. leprae* in MΦ cultured at  $31^\circ\text{C}$  or  $37^\circ\text{C}$ . *M. leprae* were released from infected MΦ on the days shown (in quadruplicate) and inoculated into RR vials. The data shown represent RR data obtained after 7 days.

*leprae*, and the viability of the bacilli was determined by evaluating the oxidation of  $^{14}\text{C}$ -palmitic acid to  $^{14}\text{CO}_2$  by radiorespirometry as described previously (<sup>13</sup>). Total isotope release was usually analyzed after one week of incubation at  $31^\circ\text{C}$  (<sup>2</sup>).

**Staining of *M. leprae*-infected MΦ.** Coverslips of *M. leprae*-infected adherent MΦs were prefixed with absolute methanol, and

acid-fast stained Photomicrographs were taken using a Nikon Optiphot microscope using an oil immersion Plan APO 100 lens.

**Transmission electron microscopy (TEM).** MΦ monolayers on coverslips were pre-fixed in 2% glutaraldehyde/0.1 M Na-cacodylate buffer followed by post-fixation with osmium tetroxide/K-CN and endblock staining with uranyl acetate. The

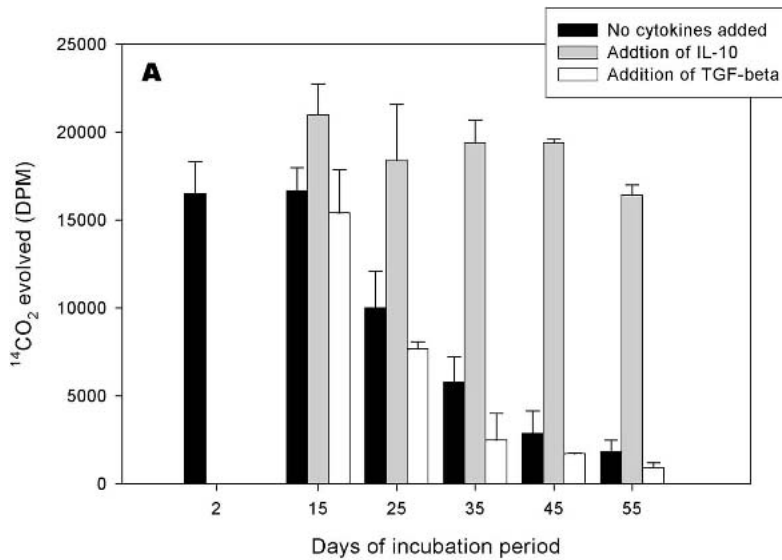


FIG. 2A. Metabolic activity of *M. leprae* in MΦ cultured in the presence of IL-10 or TGF-β. *M. leprae* infected MΦ were incubated at 31°C in the presence or absence of 2 U/ml IL-10 or 10 ng/ml TGF-β and bacilli were released from infected MΦ on the days shown (in triplicate) and inoculated into RR vials. The data shown represent RR data obtained after 7 days.

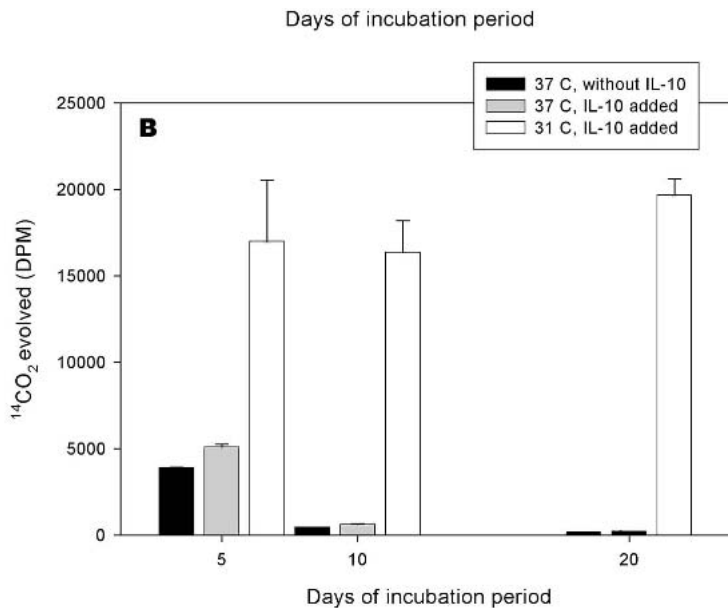


FIG. 2B. Comparison of metabolic activity of *M. leprae* in MΦ cultured at 31°C and 37°C in the presence of IL-10. *M. leprae* infected MΦ were incubated at 31°C or 37°C in the presence or absence of 2 U/ml IL-10 and bacilli were released from infected MΦ on the days shown (in triplicate) and inoculated into RR vials. The data shown represent RR data obtained after 7 days.

specimen was then dehydrated with ethanol, embedded in Epon-Araldite, sectioned at 90 nm, stained with uranyl acetate-lead citrate and viewed with a Philips 410 TEM as previously described<sup>(39)</sup>.

## RESULTS

***In vitro* temperature preferences of *M. leprae*.** *M. leprae* clearly prefers cooler incubation temperatures. As shown in Figure

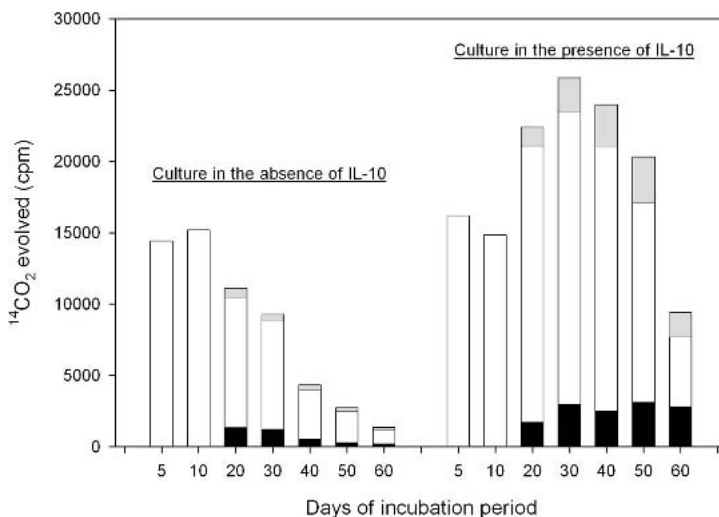


FIG. 3. Metabolic activity of *M. leprae* in MΦ cultured in the presence of IL-10. *M. leprae* infected MΦ were incubated at 31°C in the presence or absence of 2 U/ml IL-10. On the days shown (starting on day 20) we accounted for the metabolic activity of all bacilli in each well (free bacilli or detached infected cells in supernatant plus bacilli present in attached MΦ). The middle open portion of each bar represents RR activity of bacilli released from adherent MΦ. The upper shaded portion of each stacked bar represents RR activity of bacilli found in the supernatant when the media was changed 5 days previously (day 15, 25, 35, etc.). The lower black portion of each bar represent RR of bacilli in the supernatant on the day of the harvest.

1A, in axenic culture, it was apparent that 37°C was not an ideal temperature to demonstrate sustained viability. Incubation at 35°C was more supportive than 37°C, and results at 29°C and 32°C were indistinguishable but even more ideal.

Similarly, intracellular *M. leprae* thrives better at cooler temperatures. Mouse MΦ appeared to function normally at 33°C and even 31°C, as judged by attachment to plastic and phagocytic capacity, although they did not spread as well at these lower temperatures as they do at 37°C. In the experiment depicted in Figure 1B, infected MΦs were incubated at either 31°C or 37°C and at 5 day intervals released bacilli were studied by RR for an additional 7 days. The detrimental effects of incubation at 37°C on *M. leprae* metabolism were apparent by day 5. In marked contrast, *M. leprae* cultured in MΦ at 31°C thrived for at least 15 days and retained most of its viability after 25 days in MΦ maintained at the lower temperature.

**Effects of cytokines on viability of *M. leprae* in MΦ.** Supplementation of the infected MΦ culture medium with 2 U/ml murine IL-10 was clearly associated with sustained viability of intracellular *M. leprae*. In the more prolonged experiments depicted in Figure 2A and 2B, *M. leprae*

steadily lost viability in control MΦ at 31°C and 37°C. In contrast, in MΦ incubated in the presence of IL-10, *M. leprae* maintained their viability, but only at the permissive temperature of 31°C. (Figure 2A and 2B). As shown in Figure 2A, addition of TGF-β to the infected MΦ had no effect on the viability of *M. leprae*.

Experiments were run to account for all of the *M. leprae* in the long term cultures, assuming that during prolonged culture some infected MΦ may detach or lyse, releasing their bacilli. In the experiment depicted in Fig. 3, media was changed as usual every 5 days and data points recorded every 10 days. In order to account for bacilli released from MΦ or bacilli in "detached" MΦ we collected and saved the "old" media at 4°C at the time it was changed (midpoints of the 10 day time points plotted at 20, 30, 40 days, etc.). The viability of bacilli in the individual MΦ monolayers and in the MΦ detached from the monolayers are shown separately and as a total. These data show that only a few *M. leprae* were released or infected cells detached into the supernatant media, and the cumulative radio-respirometry (RR) results from individual wells confirmed the ability of IL-10 treatment to sustain intracellular viability of *M. leprae*.

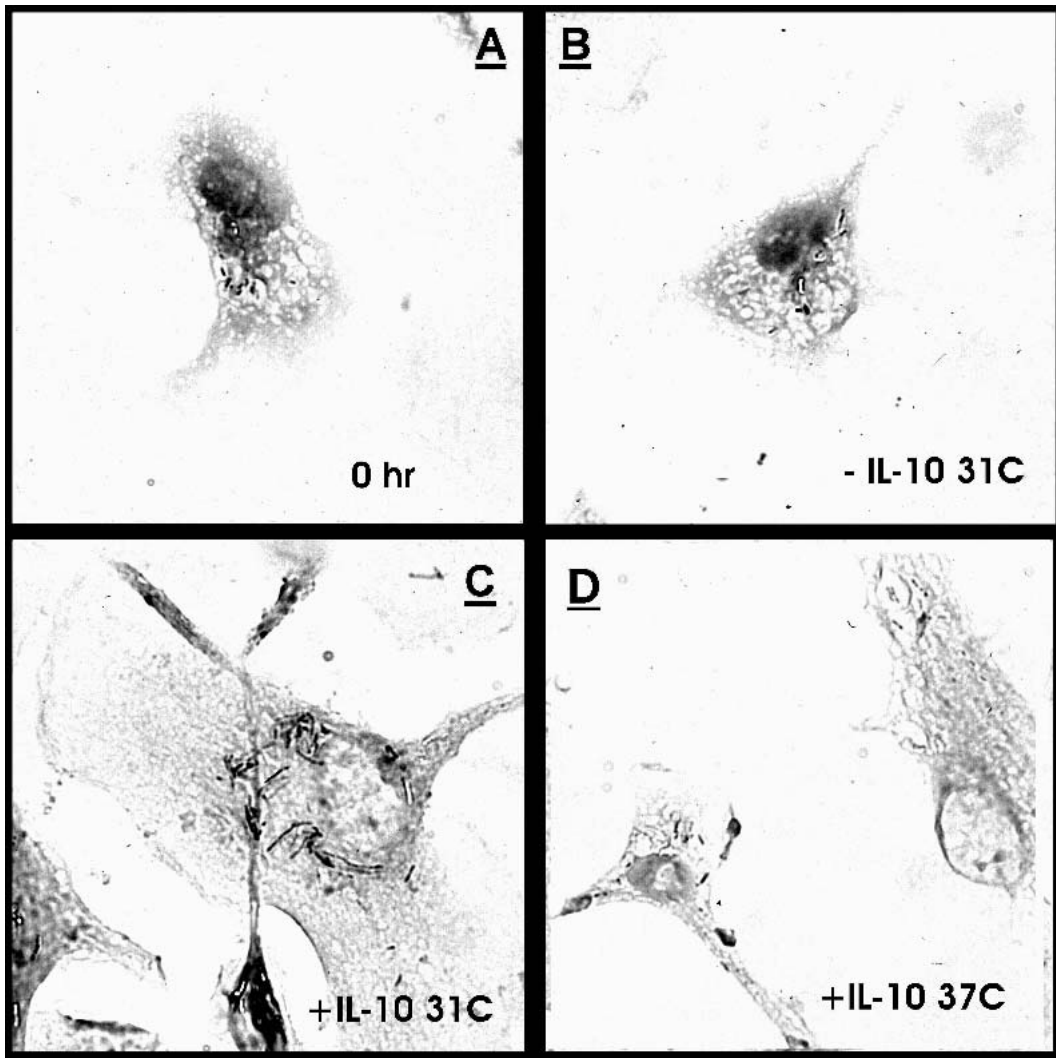


FIG. 4. Elongation of *M. leprae* in mouse M $\Phi$  cultured in the presence of IL-10 at 31°C or 37°C. The cells were acid fast stained and observed under light microscopy with magnification at  $\times 1000$ . Panel A = 0 hr (31°C). B = 4 wks at 31°C without IL-10. C = 4 wks at 31°C + IL-10. D = 4 wks at 37°C + IL-10. Figures are representative of observations from 2 experiments.

**Morphological evaluation of *M. leprae* with sustained metabolic activity in M $\Phi$ .** The morphological characteristics of *M. leprae* maintained in prolonged culture in mouse peritoneal M $\Phi$  were observed with light and electron microscopy. Elongated *M. leprae* were only observed under conditions where infected M $\Phi$  were maintained at 31°C in the presence of IL-10. As shown in Fig. 4, acid fast staining of infected M $\Phi$  at 4 weeks revealed that at 31°C in the presence of IL-10, many of the intracellular *M. leprae* were clearly elongated in compari-

son to those seen at 0 time or in M $\Phi$  maintained at 31°C without IL-10 (Figure 4A, B, C). At 37°C elongation of bacilli was not observed regardless of the presence of IL-10 (Fig. 4D). Under the transmission electron microscope (TEM), elongation was even more apparent. Not all bacilli in the 31°C, IL-10 group were observed to be elongated, as this required all bacilli to be sectioned through their long axis; but examination of dozens of infected cells in 2 experiments revealed elongated cells (8 to 10  $\mu$ ) only in the 31°C, IL-10 group. *M. leprae*

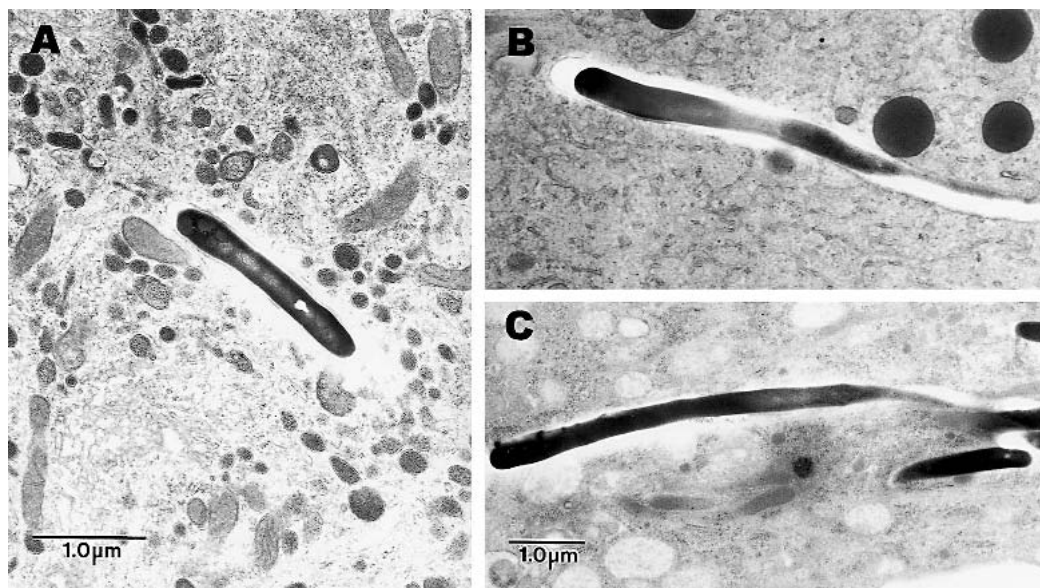


FIG. 5. Elongation of *M. leprae* in macrophages cultured *in vitro*. Mouse macrophages were infected with *M. leprae* followed by washing and incubation in the presence or absence of IL-10 at 31°C for 4 weeks. The cells were fixed, cut and observed under transmission electron microscopy. A, control (without IL-10); B, C, +IL-10. Bars in each panel = 2 µm.

in the control group were consistently 2 to 4µ in length (Fig. 5).

### DISCUSSION

Our goals in this study were limited. Convincing evidence of actual intracellular multiplication of *M. leprae* would require at least a 10-fold increase in bacillary numbers. With a calculated multiplication cycle of 12.8 days in the mouse footpad model<sup>(37)</sup>, this minimally acceptable increase in numbers would be difficult to demonstrate in a few weeks of MΦ tissue culture. However, the present study did show that the metabolism, and presumably the viability<sup>(42)</sup>, of *M. leprae* could be sustained under culture conditions which also appeared to support the intracellular elongation of the leprosy bacillus.

*In vivo* *M. leprae* is able to enter and survive in a wide variety of tissues and cell types<sup>(24)</sup>. Attempts to culture *M. leprae* in tissue culture have included the use of numerous cell lines derived from humans, rats, and mouse tissue with no evidence of multiplication<sup>(10, 27)</sup>. The MΦ, the preferred host cell for the leprosy bacillus, offers an advantage over tissue culture cell lines since MΦ actively phagocytize *M. leprae*

and, unlike cell lines, MΦ in culture are non-dividing adherent cells. Consequently the intracellular status of *M. leprae* over time is not confounded by an increase in host cell numbers. Chang and Neikirk<sup>(5)</sup> demonstrated the long term infection of mouse MΦ cultures with *M. leprae*, and a report of success in culturing *M. leprae* in MΦ was made by Garbutt<sup>(16)</sup>, but was not confirmed by McRae and Shepard<sup>(27)</sup>. Others reported limited, questionable, and unconfirmed success at detecting multiplication of the organism in MΦ cultures<sup>(8, 31, 34, 41)</sup>. An exhaustive but unsuccessful attempt to cultivate *M. leprae* in tissue culture was made by Sharp and Banerjee<sup>(36)</sup> who employed MΦ from conventional mice and rats, nu/nu mice and rats and armadillos, rather than dividing cells and cell lines. Their *M. leprae* inocula was derived from 3 sources (human leproma, nu/nu mouse footpad and frozen infected armadillo tissue). Incubation temperature was varied from 31°C to 35°C and infected cells were maintained for up to 200 days. They rigorously evaluated any increase in leprosy bacilli and concluded that no significant multiplication occurred.

Our studies provide groundwork for fu-

ture rational attempts to cultivate *M. leprae* with notable advantages to our approach. Most importantly, our starting inoculum of *M. leprae* was freshly obtained for each experiment from infected nu/nu mice maintained under conditions designed to maximize *M. leprae* viability<sup>(42)</sup>. We also were able to rapidly quantify the metabolic activity of *M. leprae* using the RR technique adapted by Franzblau<sup>(13)</sup>. This assay can readily detect activity from as few as 10<sup>6</sup> bacilli with the results available in 1 wk (compared to 6 to 12 months when titrated in mouse footpads). RR data correlates well with other *in vitro* systems<sup>(13)</sup> but, more importantly, as shown in a recent series of 36 separate experiments, RR metabolic data correlated well with "viability" studied in the so-called "gold standard" mouse footpad system<sup>(42)</sup>.

Whether intracellular or extracellular, *M. leprae* clearly prefers temperatures cooler than normal human body temperature, lending experimental credence to the clinical observations of generations of leprologists regarding the distribution of *M. leprae* in the cooler, permissive sites in human disease, the skin and mucous membranes of the upper respiratory tract. These studies also confirm and extend the results of dozens of other *in vitro* experiments<sup>(42)</sup> where 37°C appeared to be highly detrimental to *M. leprae* viability.

Under conditions of an effective cellular immune (CMI) response, MΦ are likely a major anti-microbial effector cell in host capacity to cope with the leprosy bacillus. We have previously shown that mouse MΦ, activated by interferon (IFN-γ), kill or inhibit a wide variety of intracellular pathogens<sup>(38)</sup>, including *M. leprae*<sup>(32)</sup>. Enhanced fusion of secondary lysosomes with *M. leprae*-containing phagosomes occurs in activated MΦ<sup>(39)</sup> and is accompanied by the enhanced production of reactive oxygen (ROI) and nitrogen intermediates (RNI) as potent anti-microbial mechanisms<sup>(2)</sup>. We have subsequently shown *in vivo* in transgenic knock-out mice and *in vitro* in MΦ from these mice that ROI are relatively ineffective in comparison to the potent effects of RNI in host defense against *M. leprae*<sup>(1,3)</sup>. However, although the enhanced production of RNI by activated MΦ is likely a principal antimicrobial mechanism, in each

of our studies, normal MΦ produced a measurable baseline level of RNI, insufficient to rapidly kill bacilli but perhaps sufficient to inhibit the long term viability of the fastidious, intracellular leprosy bacillus.

In choosing TGF and IL-10 as the cytokines that might bolster the intracellular survival of *M. leprae*, we were attempting to down regulate any innate ability of the normal MΦ to cope with the organism. TGF-β is produced by activated MΦ and other inflammatory cells and has a broad array of modulatory functions on the immune response. TGF-β has been shown to interfere with MΦ antimicrobial mechanisms including generation of ROI<sup>(43)</sup> and RNI<sup>(7)</sup>, and has been shown to enhance the intracellular growth of *M. tuberculosis* in human monocytes<sup>(21)</sup>. However, as employed in the present studies with mouse MΦ exogenous TGF-β had no detectable effect on sustaining intracellular *M. leprae* viability, a finding perhaps attributable to the enhanced innate antimicrobial ability of human monocytes in comparison to human monocyte derived MΦ<sup>(44)</sup> which are more akin to resident mouse peritoneal MΦ.

In contrast, supplementing media with IL-10 clearly affected the long term viability of *M. leprae* in mouse MΦ. IL-10 is produced in TH1 responses by T cells, B cells and MΦ<sup>(11,29)</sup>. IL-10 has been shown to be a potent down-regulator of CMI to intracellular pathogens<sup>(33)</sup>. *In vivo*, endogenous IL-10 dampened the CMI response to avirulent mycobacterial infection<sup>(35)</sup> and appeared to lead to loss of control of *M. tuberculosis* infection with widespread dissemination<sup>(9)</sup>. IL-10 functions in part at the level of the macrophage by attenuating inducible nitric oxide synthase (iNOS) mRNA expression, iNOS activity and, by inference, NO production<sup>(22)</sup>. *In vitro*, exogenous IL-10 inhibited production of nitric oxide (NO) in MΦ infected with *Babesia* merozoites<sup>(17)</sup>. In our own studies, IL-10 did markedly inhibit the production of NO by IFN-activated MΦs but any inhibition by IL-10 of baseline production of NO by normal MΦs was below the limits of detection of the NO<sub>2</sub> assay (data not shown). Exogenous IL-10 also interferes with IFN-induced antimycobacterial MΦ activities as shown in studies with *M. bovis*<sup>(12)</sup>.

In addition to sustained metabolism of *M.*



*leprae*, we also explored a morphological parameter of *M. leprae* vitality within cultured macrophages, bacillary elongation. Nakamura, *et al.* reported the elongation of *M. lepraemurium* in culture medium<sup>(28)</sup>, and in 1969 Chang and Anderson<sup>(4)</sup> evaluated intracellular growth of *M. lepraemurium* in cultured mouse peritoneal MΦ over a 12 to 17 week period and observed marked elongation of bacilli well before the appearance of bacillary multiplication. In our studies, in addition to sustained or enhanced metabolism, intracellular elongation of individual bacilli was observed after 4 weeks culture in murine MΦ maintained with IL-10. Elongation was first observed with the light microscope and subsequently confirmed with the transmission electron microscope. A drawback to the use of the TEM for such observations is that unlike light microscopy which revealed the bacilli at their full intracellular length, processing for the TEM required sectioning infected MΦ with no certainty that the full length of each bacillus was cut. Nevertheless sufficient numbers of clearly elongated bacilli were seen to confirm the light microscopy findings. Similarly, in preliminary studies with armadillo peripheral blood monocyte derived MΦ maintained at 33°C for 4 weeks, intracellular *M. leprae* were predominantly elongated (data not shown).

Septal formation has been described in TEM studies from human leprosy biopsies by Hirata<sup>(20)</sup> and was occasionally observed by us in individual bacilli under the TEM after 4 weeks of incubation of infected mouse MΦ in the presence of IL-10. The small sample size precluded quantification but septa appeared to be observed far less frequently in bacilli in the control MΦ. Septal formation in *M. leprae murium* in the mouse model has been reported to indicate dividing stage of the bacillus, and Hart and Rees<sup>(19)</sup> concluded that elongation *in vitro* was an inherent feature of *M. leprae murium* that distinguishes it from *M. leprae* although it is likely that the *M. leprae* inocula employed were of very low viability. Experiments are currently underway to study *M. leprae* in our cell culture system employing an environmental scanning electron microscope which by passes the need for critical point drying and its attendant ar-

tifacts and will permit quantitation of elongation.

Further work with infected armadillo MΦ is also clearly warranted. Other than having a core temperature of ~33°C, little is known about the unique characteristics of *Dasyus novemcinctus*, the nine-banded armadillo, that render it as a permissive host for the leprosy bacillus<sup>(25)</sup>. *In vivo*, mononuclear phagocytes in virtually every organ of the natural or experimentally infected armadillo become heavily parasitized with propagating *M. leprae*<sup>(24)</sup>.

The inability to culture *M. leprae* has undoubtedly hindered almost every aspect of leprosy research, and thus, our understanding of this disease lags behind that of many others of bacterial etiology. These promising results represent only preliminary findings, but suggest that this approach of inhibiting the innate anti-microbial properties of the MΦ to bolster the intracellular survival of *M. leprae* may ultimately provide clues allowing the long sought-after cultivation of the leprosy bacillus. *In vitro* cultivation of *M. leprae* could make available for the first time, large quantities of pure bacilli produced inexpensively under defined conditions. Thus, large amounts of purified antigens would be available for basic and applied immunological studies, including the development of specific skin test antigens and vaccine preparations. The time and cost of screening new drugs and susceptibility testing of clinical isolates would also be greatly reduced. Our understanding of leprosy epidemiology might increase by determining the existence of human carriers, non-human reservoirs, or environmental sources of *M. leprae*. Phenotypic variation among cultured worldwide isolates could become feasible as might the generation and characterization of mutants. Finally, cultivation of *M. leprae*, in concert with the genome project, would clearly enhance our understanding of the physiology of this fastidious pathogen, including elucidation of metabolic pathways, studies of virulence mechanisms, drug resistance and the factors underlying "persistence."

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