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# Lack of *Mycobacterium leprae*-specific Uptake in Schwann Cells[1]

Abdul Hamid Band, Alok Bhattacharya,
and Gursaran P. Talwar[2]

*Mycobacterium leprae* is unique in its predilection for nerves. Barring *M. leprae* and some viruses, no other organisms have been found to establish themselves in peripheral nerves ([19]). Other mycobacteria, such as *M. tuberculosis,* have not been observed to home to cells in the peripheral nerves. Histopathological studies have revealed the presence of *M. leprae* in various cells of the peripheral nerve, such as Schwann cells, endoneurial and perineurial cells, endothelial cells, and infiltrating histiocytic cells ([4, 5, 8, 9, 12–14]). Schwann cells are among the most heavily bacillated. In the paucibacillary forms of leprosy, bacilli, whenever present, are almost invariably found inside Schwann cells ([8, 14]). A study of clinically normal nerves showed that the earliest changes were observed in Schwann cells of the unmyelinated nerve fibers, which also were the earliest to harbor bacilli ([1, 25–27]). It has been suggested ([2, 3]) that these cells may have a mechanism for the specific uptake of *M. leprae.*

Investigations were carried out to test this hypothesis using a rat Schwannoma cell line, 33B. Observations made in this system were subsequently counterchecked, using normal Schwann cells derived from rat sciatic nerves, to exclude the possibility that 33B tumor cells may have been altered in their properties. The results reported here indicate a lack of any selective uptake of *M. leprae* by Schwann cells.

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[2] A. H. Band, M.B.B.S., M.D., Senior Research Officer, National Institute of Immunology, P.O. Box 4922, New Delhi 110029, India. A. Bhattacharya, Ph.D., Scientist, Tata Research Development and Design Center, Pune, India. G. P. Talwar, D.Sc., F.I.C.A., F.A.Sc., F.N.A., Director, National Institute of Immunology, P.O. Box 4922, New Delhi 110029, India.

Reprint requests to Dr. Talwar.

## MATERIALS AND METHODS

**Schwannoma cells.** The 33B rat Schwannoma cell line ([10]) was a kind gift of Professor M. C. Raff, Department of Zoology, University College, London. These cells were grown in Dulbecco's Modified Eagle's medium (DME; GIBCO Laboratories, Grand Island, New York, U.S.A.) supplemented with 10 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid), 1 mM sodium pyruvate, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 40 μg/ml gentamicin sulfate (all Sigma Chemical Company, St. Louis, Missouri, U.S.A.), and 10% heat-inactivated fetal calf serum (FCS; GIBCO) in a humidified atmosphere at 10% $CO_2$ and 90% air. The cells were subcultured every 3 to 5 days. Cell monolayers were removed from flasks by treatment with 0.1% trypsin and 0.02% EDTA in 0.01 M phosphate buffered saline pH 7.4 (PBS) for 5 min at room temperature. After neutralization of trypsin with three volumes of DME containing 10% FCS, the cells were washed once with medium. Viable cells were counted by the trypan blue dye exclusion method and plated.

Tissue culture plasticware was obtained from Costar Co., Cambridge, Massachusetts, U.S.A.

**Schwann cells.** A modification of the method described by Brockes, *et al.* ([6]) was employed. Six-to-ten-week-old Holtzman rats of either sex were killed by cervical dislocation and washed liberally with 70% alcohol. They were pinned on a rat dissecting board inside a laminar flow hood. Sciatic nerves were dissected out and kept in DME containing 25 mM HEPES at room temperature. Nerves from 6 to 9 animals were minced and treated with 3 ml of 0.25% trypsin (1:250; Difco Laboratories, Detroit, Michigan, U.S.A.) and 0.05% collagenase (type IV from *Clostridium histolyticum*; Sigma) in DME + 25 mM HEPES for 75 min

at 37°C. One ml of fresh enzyme cocktail was added at 25 min and at 50 min. At the end of the incubation period, an equal volume of DME + 10% FCS was added to neutralize trypsin. The digested material was passed up and down a Pasteur pipette 10 to 15 times and subsequently through a 21-gauge needle three times. Gross clumps and debris were removed by passage through a 60 μm pore size nylon mesh (Fyltis Co., France), and the unretained cells were collected. These were pelleted at 200 × *g* for 5 min at room temperature, washed once with DME + 10% FCS, and the viable cells were counted.

**Peritoneal macrophages.** The peritoneal cavities of 6-to-10-week-old rats were flushed with 15 to 20 ml cold DME. The cells were pelleted and washed twice at 200 × *g* for 5 min at 4°C. The pellet was resuspended in DME + 10% FCS, and the viable cells were counted.

**Mycobacteria.** *M. leprae* were extracted from human leproma biopsies. Skin biopsies obtained from untreated lepromatous leprosy patients attending the Leprosy Clinic of the School of Tropical Medicine, Calcutta, India (courtesy of Dr. S. Chaudhary) were airfreighted from Calcutta in wet ice and received in New Delhi within 6 to 24 hr. The biopsy specimens were washed twice with DME without antibiotics. The epidermis was removed from the skin, and the dermis was minced with scissors. The minced material was homogenized in glass homogenizers with 5 volumes of DME containing 0.1% bovine serum albumin. The supernatant was decanted into a tube, and the residual material was extracted twice more. Pooled supernatant was centrifuged at 50 × *g* for 3 to 4 min to pellet the gross tissue debris. The supernatant was further centrifuged at 1000 × *g* for 30 min at 4°C and washed twice with DME. A final suspension was made in DME without antibiotics and checked for any contamination by inoculation into thioglycollate broth and on nutrient agar plates, and for cultivable mycobacteria by inoculation on Löwenstein-Jensen medium. The bacillary suspensions prepared by this method contained very little tissue debris. Most bacteria were seen as singles or in very small clumps. Bigger clumps, when present, were removed by allowing the bacterial suspension to stand at unit gravity for a few minutes. Only the bacterial suspensions with a high percentage (more than 40%) of solidly staining bacilli were used.

*M. tuberculosis* H37Rv, BCG, and ICRC bacilli were grown in Dubos' medium (Difco) supplemented with 10% normal human serum. "*Mycobacterium w*," *M. vaccae*, *M. nonchromogenicum*, and *M. smegmatis* were grown in Middlebrook 7H9 medium supplemented with 1% glucose and 0.05% Tween 80 (Centron Laboratories, Bombay, India). The ICRC bacillus was obtained from Dr. C. V. Bapat, Indian Cancer Research Institute, Bombay, India. "*Mycobacterium w*" is a cultivable mycobacterium derived from a suspected case of tuberculosis and belongs to Runyon's group IV of anonymous mycobacteria ([23]). Other cultivable mycobacteria were obtained from the Trudeau Institute, Saranac Lake, New York, U.S.A.

Suspensions of cultivable mycobacteria for infection of cells were prepared according to Shepard ([24]). Briefly, cultures in liquid media inoculated 3 to 21 days previously from cultures maintained on Löwenstein-Jensen medium were stirred to suspend the sediment and then allowed to stand for a few minutes to let the larger clumps settle. The supernatant was pipetted off to another tube, pelleted at 1000 × *g* for 30 min, washed three times, and resuspended in DME by repeated pipetting. This resulted in a suspension consisting primarily of single bacilli or small groups. The bacilli were counted by the method of Hanks, *et al.* ([11]).

**Uptake of mycobacteria and latex.** A total of $10^5$ 33B cells or sciatic nerve-derived cells, or $5 \times 10^5$ peritoneal exudate cells in 0.25 ml DME + 10% FCS were plated on each 22-mm diameter glass coverslip placed inside a 35-mm diameter Petri dish. The number of 33B cells per coverslip was adjusted such that at the time of the phagocytosis assay, about 24 hr after plating, a subconfluent monolayer was obtained. For this purpose, $10^5$ 33B cells per coverslip were used. Confluent monolayers were found unsuitable since these tended to float off during the washing procedure. For plating normal Schwann cells, coverslips were precoated with 0.5 mg/ml gelatin (300 Bloom; Sigma)

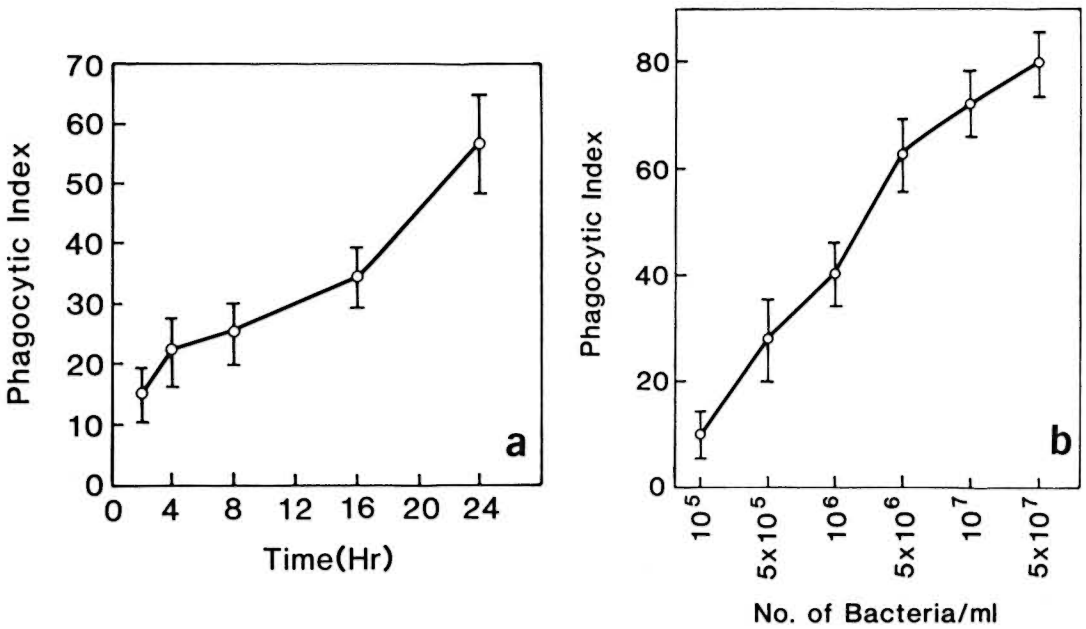


FIG. 1. Time course (**a**) and dose relation (**b**) of the uptake of *M. leprae* by 33B cells. Each point represents the mean ± S.D. of triplicates.

in PBS for 2 hr at room temperature followed by washing three times with PBS. After 6 hr of incubation at 37°C, 1.25 ml of the medium was added. Unattached cells were removed after 24 hr and fresh medium without antibiotics was added. Bacterial inocula or latex (1:5000 final dilution) were added, and the incubation was carried out for the requisite period. Unphagocytosed bacilli or latex particles were removed by washing 4 to 5 times with warm (37°C) DME. Cells were fixed with 10% Formalin in PBS for 30 min and then washed with distilled water. To count the phagocytosed bacilli, coverslips were stained by Ziehl-Neelsen's method, using methylene blue as a counterstain. The latex particles were counted in unstained coverslips.

The percentage of cells containing bacilli or latex particles was expressed as the phagocytic index. At least 200 cells were counted per coverslip. In certain experiments, the number of bacilli or latex particles in each cell was also counted and expressed as a frequency distribution of positive cells containing 1 to 5, 6 to 10, or >10 bacilli or particles per cell.

The statistical significance of the differences between the uptake of various mycobacteria was calculated by Student's *t* test.

## RESULTS

**Uptake of *M. leprae* by 33B Schwannoma cells.** When a constant number ($5 \times 10^6$ ml) of *M. leprae* were incubated with 33B cells ($10^5$/coverslip) for different time periods, the phagocytic index increased with time (Fig. 1a). At 24 hr, 50% of the cells contained bacilli.

When different numbers of *M. leprae* were incubated with the 33B cells for a constant period of 24 hr, phagocytosis increased with inoculum size (Fig. 1b). About 80% of the cells contained bacilli when $5 \times 10^7$ bacilli/ml were used; $5 \times 10^6$ bacilli/ml were considered suitable for comparison of uptake of various mycobacteria since, at this point, higher as well as lower levels of phagocytosis could be discerned by comparison.

**Uptake of different mycobacteria by 33B cells.** Uptake by the 33B cells of *M. leprae* and seven other mycobacteria, which do not cause nerve damage, was determined. The bacilli were taken at $5 \times 10^6$/ml and incubated with $10^5$ 33B cells/coverslip for 24 hr. Contrary to expectations, uptake by these cells of *M. nonchromogenicum, M. vaccae, M. tuberculosis,* BCG, and "*Mycobacterium w*" was distinctly higher ($p < 0.001$) than that of *M. leprae* (Table 1). The phagocytic

TABLE 1. *Uptake of different mycobacteria by 33B cells.*[a]

| Bacteria | Phagocytic index (Mean ± S.E.M.) | % of positive cells with bacterial no. of | | |
|---|---|---|---|---|
| | | 1 to 5 | 6 to 10 | >10 |
| *M. leprae* | 51.2 ± 2.9 | 82.6 | 11.2 | 6.2 |
| "*Mycobacterium w*" | 82.6 ± 4.2 | 39.4 | 29.0 | 31.6 |
| BCG | 89.2 ± 3.7 | 51.7 | 25.5 | 22.8 |
| *M. tuberculosis* | 87.7 ± 3.9 | 46.4 | 30.4 | 23.2 |
| *M. nonchromogenicum* | 97.7 ± 2.5 | 11.3 | 20.9 | 67.8 |
| ICRC bacillus | 50.0 ± 3.1 | 67.3 | 21.2 | 11.5 |
| *M. smegmatis* | 11.6 ± 4.0 | 92.2 | 6.8 | 1.0 |

[a] $10^5$ 33B Cells per coverslip were plated overnight, washed, and then incubated with $5 \times 10^6$ bacteria/ml for 24 hr. Results represent mean ± S.E.M. of 3 to 5 experiments each in triplicate.

TABLE 2. *Uptake of live versus autoclaved mycobacteria by 33B cells.*[a]

| Bacteria | Phagocytic index (Mean ± S.E.M.) | % of positive cells with bacterial no. of | | |
|---|---|---|---|---|
| | | 1 to 5 | 6 to 10 | >10 |
| *M. leprae* (live) | 51.2 ± 2.9 | 82.6 | 11.2 | 6.2 |
| *M. leprae* (autoclaved) | 52.0 ± 3.5 | 77.7 | 14.8 | 7.5 |
| "*Mycobacterium w*" (live) | 82.6 ± 4.2 | 39.4 | 29.0 | 31.6 |
| "*Mycobacterium w*" (autoclaved) | 83.1 ± 5.0 | 40.9 | 34.3 | 25.8 |

[a] Bacteria were autoclaved for 15 min at 15 pounds pressure. Results represent mean ± S.E.M. of 5 experiments each in triplicate.

index for the ICRC bacillus was similar to that for *M. leprae*. Uptake of *M. smegmatis* was the lowest, with a phagocytic index of 11.6% ($p < 0.001$ compared to *M. leprae*).

Bacillary density was highest for *M. nonchromogenicum*, with over 67% of the cells showing more than 10 bacilli. Uptake of *M. leprae* was moderate with about 83% of the cells showing 1 to 5 bacilli per cell.

These experiments indicated the a) ability of Schwannoma cells to phagocytose mycobacteria, b) lack of discrimination for uptake of pathogenic and nonpathogenic mycobacteria, and c) lack of specificity for *M. leprae* regarding entry and accumualation in these cells. The degree of uptake of mycobacteria by Schwannoma cells was not dependent on the viability of the bacilli; both live and autoclaved bacilli were picked up to a similar extent with a similar degree of bacterial density (Table 2).

**Adherence versus true uptake.** To distinguish adherence from uptake, 33B cells were treated, at the end of incubation with mycobacteria, with 0.1% trypsin/0.02% EDTA in PBS for 30 min at room temperature and then washed three times. The cells were resuspended in tissue culture medium, allowed to attach to coverslips for 6 hr, and then stained for acid-fast bacilli. As seen in Table 3, treatment with trypsin/EDTA, which removed adhering bacilli, led to a decrease of the phagocytic index in all cases investigated. A proportion of bacilli, however, remained within the cells, confirming their true uptake. Thus, the overall phagocytosis represented both adherence and ingestion.

**Latex uptake by 33B cells.** Since all of the mycobacteria tested were taken up by 33B cells, it argued for a generalized property of phagocytosis. As seen in Table 4, these cells also phagocytosed latex particles very avidly; nearly all of the cells contained latex particles at the end of 1 hr.

**Uptake of mycobacteria by macrophages.** In view of the low uptake of *M. smegmatis* by Schwannoma cells, uptake of some mycobacteria by a nondiscriminatory phagocytic cell, the peritoneal macrophage, was studied. The uptake of *M. smegmatis* by these cells was also apparently much lower than that of *M. nonchromogenicum*, with the phagocytic index for *M. leprae* of the same order as for Schwannoma cells (Table 5). We have now identified this apparently lower uptake to be due to the quick loss of acid-fastness of *M. smegmatis*, resulting in their being stained the same color as the cells harboring them, thus making their morphological identification difficult (unpublished observations).

**Uptake of mycobacteria by sciatic nerve-derived Schwann cells.** *M. leprae*, *M. tuberculosis*, and "*Mycobacterium w*" were selected for these experiments. All three mycobacteria were phagocytosed by both the Schwann cells and the fibroblasts present in these cultures (Table 6). Uptake by fibroblastic cells was quantitatively higher.

TABLE 3. *Effect of trypsin/EDTA on the uptake of mycobacteria by 33B cells.*[a]

| Bacteria | Trypsin/ EDTA | Phagocytic index (Mean ± S.D.) | % of positive cells with bacterial no. of | | |
|---|---|---|---|---|---|
| | | | 1 to 5 | 6 to 10 | >10 |
| *M. leprae* | − | 54.0 ± 5.7 | 75.2 | 15.3 | 9.5 |
| | + | 42.3 ± 4.8 | 84.5 | 11.6 | 3.9 |
| "*Mycobacterium w*" | − | 80.8 ± 6.1 | 40.0 | 31.6 | 28.4 |
| | + | 60.5 ± 5.4 | 83.3 | 11.7 | 5.0 |
| BCG | − | 97.0 ± 3.1 | 37.4 | 32.3 | 30.3 |
| | + | 58.0 ± 7.3 | 60.0 | 35.0 | 5.0 |
| *M. nonchromogenicum* | − | 97.7 ± 1.9 | 19.2 | 27.7 | 53.1 |
| | + | 68.6 ± 8.1 | 67.1 | 24.3 | 8.6 |

[a] After 24 hr phagocytosis, cells were washed three times with medium and treated with 0.1% trypsin/0.02% EDTA for 30 min. After washing three times and reattachment to coverslips, the cells were fixed, stained, and counted. Control coverslips were fixed immediately after removal of unphagocytosed bacteria. Values represent mean ± S.D. of triplicates.

**Latex uptake by sciatic nerve-derived Schwann cells.** Both cell types (Schwann cells and fibroblasts) showed uptake of latex particles with a time-dependent increase in the phagocytic index (Table 7). However, the rate of phagocytosis was slower compared to that in the 33B cells. Also, the number of particles per cell was less compared to 33B cells.

The results with normal Schwann cells indicate that the nonspecific uptake of mycobacteria by 33B cells represents a true Schwann cell property and not an altered behavior due to the neoplastic nature of 33B cells.

## DISCUSSION

The possibility of a selective uptake of *M. leprae* by Schwann cells was tested using the rat Schwannoma cell line 33B. However, the neoplastic nature of these cells makes it necessary to interpret the observations with caution. That tumor cell lines may behave differently from their normal counterparts is evident from their differential response to chemical modulators. For example, cyclic AMP is mitogenic for normal Schwann cells ([20]) but inhibits the proliferation of Schwannoma cells ([7]). Lest the phagocytic properties of these cells be a reflection of their malignant state, the uptake of bacilli was also studied in Schwann cells derived from sciatic nerves with essentially similar results.

Avid phagocytosis of *M. leprae* by 33B cells is consonant with the results obtained with human acoustic neuroma-derived Schwannoma cells ([15, 16]). Both live and autoclaved mycobacteria were picked up by these cells. This is in accord with the *in vivo* behavior of rat ([18]) and human ([21]) Schwann cells toward killed mycobacteria, which were avidly phagocytosed. However, it is contrary to the report that autoclaved *M. leprae* are not taken up by mouse Schwann cells in culture ([17]).

TABLE 4. *Uptake of latex particles by 33B cells.*[a]

| Incubation period (hr) | Phagocytic index (Mean ± S.D.) | % of positive cells with particle no. of | | |
|---|---|---|---|---|
| | | 1 to 5 | 6 to 10 | >10 |
| 1 | 100 ± 0 | 1.1 | 2.3 | 96.6 |
| 3 | 100 ± 0 | 0 | 1.4 | 98.6 |

[a] Cells were incubated with latex particle suspension (1.1 μm diameter) at a final dilution of 1:5000. After washing and fixing, unstained coverslips were examined and particles inside the cells were counted. Values represent mean ± S.D. of triplicates.

TABLE 5. *Uptake of mycobacteria by peritoneal macrophages.*[a]

| Bacteria | Phagocytic index (Mean ± S.D.) | % of positive cells with bacterial no. of | | |
|---|---|---|---|---|
| | | 1 to 5 | 6 to 10 | >10 |
| *M. leprae* | 53.1 ± 5.5 | 67.0 | 19.1 | 13.9 |
| "*Mycobacterium w*" | 89.1 ± 6.8 | 35.7 | 21.7 | 42.6 |
| *M. nonchromogenicum* | 100.0 ± 0 | 3.8 | 5.1 | 91.1 |
| *M. smegmatis* | 32.0 ± 6.9 | 84.3 | 5.9 | 9.8 |

[a] $5 \times 10^5$ Peritoneal exudate cells were plated per coverslip, washed after 24 hr, and then incubated with $5 \times 10^6$ bacteria/ml for 24 hr. Each value represents mean ± S.D. of triplicates.

TABLE 6. *Uptake of* M. leprae, M. tuberculosis, *and a cultivable nonpathogenic mycobacterium,* "Mycobacterium w," *by sciatic nerve-derived cells.*[a]

| Bacteria | Cell type | Phagocytic index (Mean ± S.D.) | % of positive cells with bacterial no. of | | |
|---|---|---|---|---|---|
| | | | 1 to 5 | 6 to 10 | >10 |
| *M. leprae* | Schwann cells | 43.5 ± 5.5 | 81.5 | 12.3 | 6.2 |
| | Fibroblasts | 48.6 ± 5.0 | 62.7 | 22.9 | 14.4 |
| *M. tuberculosis* | Schwann cells | 51.9 ± 6.1 | 66.4 | 18.6 | 15.0 |
| | Fibroblasts | 56.9 ± 6.9 | 59.4 | 28.9 | 11.7 |
| "*Mycobacterium w*" | Schwann cells | 40.5 ± 4.3 | 72.6 | 19.3 | 8.1 |
| | Fibroblasts | 43.1 ± 6.4 | 56.5 | 30.8 | 12.7 |

[a] $10^5$ Sciatic nerve-derived cells were plated per coverslip, washed after 24 hr, and then incubated with $5 \times 10^6$ bacteria/ml for 24 hr. Each value represents mean ± S.D. of triplicates.

All of the mycobacteria tested (with the exception of *M. smegmatis*), irrespective of whether or not they cause nerve involvement, were avidly phagocytosed by both Schwannoma cells and sciatic nerve-derived normal Schwann cells. This suggested the lack of any *M. leprae*-specific uptake mechanism in Schwann cells. Both of the cell types in the sciatic nerve cultures, i.e., Schwann cells and fibroblasts, phagocytosed mycobacteria and latex particles. Similar uptake of *M. leprae* by both of these cell types in the dorsal root ganglion cultures was reported by Mukherjee, *et al.* ([17]). Relatively lower phagocytosis of *M. tuberculosis* compared to that of *M. leprae* by Schwann cells was reported by Saito and Watanabe ([22]). However, no such differences were observed in the present study and, in fact, *M. tuberculosis* was phagocytosed more avidly compared to *M. leprae.* Experiments with latex uptake further strengthen the conclusion that Schwannoma, as well as normal Schwann cells, possess nonspecific phagocytic properties and do not show any specific uptake of *M. leprae.*

## SUMMARY

Among mycobacteria, *Mycobacterium leprae* have a unique property to infect peripheral nerves, which is the cause of a variety of debilities seen in leprosy. The possibility of selective uptake of *M. leprae* by Schwann cells was studied using a rat Schwannoma cell line 33B and rat sciatic nerve-derived Schwann cells. *M. leprae* were phagocytosed by 33B cells but so also were seven other mycobacteria ("*Mycobacterium w,*" BCG, *M. tuberculosis* H37Rv, *M. nonchromogenicum, M. vaccae,* ICRC bacillus, and *M. smegmatis*) which do not involve peripheral nerves. All three mycobacteria tested (*M. leprae, M. tuberculosis* and "*Mycobacterium w*") were phagocytosed by sciatic nerve-derived Schwann cells. Both Schwannoma and Schwann cells phagocytosed even inert latex particles. These results fail to demonstrate any *M. leprae*-specific uptake system in Schwann cells.

## RESUMEN

Entre las micobacterias, el *Mycobacterium leprae* tiene la característica propiedad de infectar a los nervios

TABLE 7. *Uptake of latex particles by sciatic nerve-derived cells.*[a]

| Cell type | Incubation period (hr) | Phagocytic index (Mean ± S.D.) | % of positive cells with particle no. of | | |
|---|---|---|---|---|---|
| | | | 1 to 5 | 6 to 10 | >10 |
| Schwann cells | 1 | 37.0 ± 3.6 | 60.3 | 16.2 | 23.5 |
| | 3 | 83.5 ± 7.4 | 36.9 | 21.7 | 41.4 |
| Fibroblasts | 1 | 57.7 ± 4.8 | 45.1 | 26.3 | 28.6 |
| | 3 | 90.0 ± 6.4 | 18.5 | 14.9 | 66.6 |

[a] Cells were incubated with latex particle suspension at a dilution of 1:5000. Each value represents mean ± S.D. of triplicates.

periféricos. Esto causa una serie de debilitamientos en los pacientes con lepra. En este estudio se investigó la posibilidad de que las células de Schwann fagocitaran selectivamente al *M. leprae*. Se usaron células de la línea 33B derivadas de un Schwannoma de la rata y células de Schwann derivadas del nervio sciático de la rata. El *M. leprae* fue fagocitado por las células 33B pero también lo fueron otras 7 micobacterias ("*Mycobacterium w*," BCG, *M. tuberculosis* H37Rv, *M. nonchromogenicum*, *M. vaccae*, el bacilo ICRC, y *M. smegmatis*) las cuales no afectan a los nervios periféricos. Las 3 micobacterias probadas (*M. leprae*, *M. tuberculosis* y "*Mycobacterium w*") fueron igualmente fagocitadas por las células de Schwann derivadas del nervio sciático. Tanto las células de Schwann como las del Schwannoma fagocitaron también partículas inertes (látex). Estos resultados no demuestran ningún consumo específico del *M. leprae* por parte de las células de Schwann.

## RÉSUMÉ

*Mycobacterium leprae* a une propriété unique parmi les mycobactéries, à savoir celle d'infecter les nerfs périphériques. Ceci produit une variété de troubles nerveux tels qu'on les observe dans la lèpre. La possibilité d'une capture sélective de *M. leprae* par les cellules de Schwann a été étudiée au moyen d'une lignée cellulaire d'un Schwannôme de rat, 33B, et de cellules de Schwann obtenues à partir du nerf sciatique de rats. *M. leprae* était phagocyté par les cellules 33B, mais il en était de même pour sept autres mycobactéries à savoir : "*Mycobacterium w*," BCG, *M. tuberculosis* H37Rv, *M. nonchromogenicum*, *M. vaccae*, le bacille ICRC, et *M. smegmatis*. Ces bacilles, pourtant, n'affectent pas les nerfs périphériques. Les trois mycobactéries qui ont été étudiées (*M. leprae*, *M. tuberculosis*, et "*Mycobacterium w*,") étaient phagocytées par les cellules de Schwann dérivées du nerf sciatique. Les cellules de Schwannôme, de même que les cellules de Schwann, phagocytaient même des particules de latex inertes. Ces résultats n'ont pas permis de démontrer une capture spécifique de *M. leprae* par les cellules de Schwann.

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