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Abilities of Human Oligodendroglial Cells and Mouse Schwann Cells to Phagocytose *Mycobacterium leprae* and Other Mycobacteria

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Human oligodendroglial KG-1-C cells derived from human cerebral mixed glioma and mouse Schwann cells derived from dorsal root ganglion were studied with respect to their abilities to phagocytose various mycobacteria, especially *Mycobacterium leprae*, and other microorganisms. KG-1-C cells phagocytosed *M. leprae* at a markedly higher rate than BALB/3T3, BHK 21, HeLa S3, mKS-A TU-7, XC, TSV-5, N-18, and Schwann cells but at a lower rate than peritoneal macrophages. Schwann cells also exhibited substantial phagocytic ability against *M. leprae*, and their phagocytic rate against *M. leprae* was much higher than that of N-18 cells, derived from neurons. KG-1-C and Schwann cells phagocytosed mycobacteria other than *M. leprae*, and their phagocytic patterns with various mycobacteria were similar, thereby suggesting that their abilities to phagocytose mycobacteria were based on the same cellular mechanism. The time course of phagocytosis of *M. leprae* by KG-1-C cells markedly differed from that by macrophages, indicating differences in the cellular mechanisms of *M. leprae* phagocytosis. KG-1-C cells also ingested microorganisms other than acid-fast bacilli, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, and *Escherichia coli* but not *Candida albicans*. They also phagocytosed latex beads (0.8-μm diameter) but not sheep erythrocytes. Microscopically, most mycobacterial cells were ingested in the perikaryon of KG-1-C cells and Schwann cells.

During the course of leprosy from a tuberculoid to a lepromatous state, there is peripheral nerve involvement (18). The occurrence of *Mycobacterium leprae* in peripheral nerves has been documented (5). With the electron microscope, *M. leprae* cells have been observed frequently in the cytoplasm of Schwann cells and occasionally within both the myelinated and unmyelinated axons of the nerve fibers (2, 3, 12, 15, 20). Although the intra-axonal bacilli may contribute to the dissemination of leprosy in the nerve system and to nerve fiber destruction (10, 20), the predominant involvement of Schwann cells with *M. leprae* has been noted in peripheral nerves of lepromatous leprosy patients (5). The increased occurrence of segmental demyelination other than axonal destruction, such as Wallerian degeneration, especially in an early phase of leprosy (17), suggests that Schwann cells may be important in the spread of *M. leprae* to the peripheral nerves and in dysfunction and degeneration of nerve fibers. In our preliminary in vitro studies, we found that Schwann cells derived from mouse dorsal root ganglion but not neurons phagocytosed *M. leprae* (unpublished observation). Moreover, it was noted that *Mycobacterium lepraemurium*, which does not cause neuritis of host animals (11), was phagocytosed by Schwann cells at a markedly lower degree than *M. leprae*.

In the present work, we examined the ability of human oligodendroglial KG-1-C cells established from a cerebral mixed glioma (6, 7) to phagocytose various mycobacteria, especially *M. leprae*, and comparisons were made with oligodendroglial cells and mouse Schwann cells with respect to abilities to phagocytose *M. leprae* and other mycobacteria. The imporant role of Schwann cells in nerve involvement in leprosy is discussed.

MATERIALS AND METHODS

Cells. Cells used in this study were as follows: KG-1-C cells (human oligodendroglial cells; from E. Miyake, Kyushu University, Fukuoka, Japan), BALB/3T3 cells (established from a BALB/c mouse; T. Kurimura, Tottori University, Tottori, Japan), HeLa S3 cells (T. Shiomi, Mie University, Mie, Japan), BHK-21 cells (baby hamster kidney cells; T. Shiomi), mKS-A TU-7 cells (simian virus 40-transformed BALB/c mouse tumor cells; T. Kurimura), XC cells (Rous sarcoma virus-transformed rat tumor cells; Y. Takamori, Osaka Prefectural Institute for Radiation, Osaka, Japan), TSV-5 cells (simian virus 40-transformed hamster tumor cells; T. Kurimura), N-18 cells (mouse neuroblastoma cells; T. Amano, Mitsubishi-Kasei Institute of Life Science, Tokyo, Japan). Mouse Schwann cells were derived from mouse (CBA/JN) dorsal root ganglion essentially by the method of Bunge and Wood (1) with some modifications for elimination of neurofibroblasts by the method of Mudge (9). Mouse peritoneal macrophages were prepared from peritoneal cells as described previously (16).

Organisms. *M. leprae* Kurume-Naha was harvested from the hind footpads of infected BALB/c nude mice and *M. lepraemurium* Hawaiian was obtained from the spleens of infected CBA/JN mice. These cells were purified by the method of Mori et al. (8). *Mycobacterium tuberculosis* $H_{37}Rv$, *Mycobacterium marinum* Shiromaru, *Mycobacterium intracellulare* 31F093TD, *Mycobacterium scrofulaceum* ATCC 19981, and *Mycobacterium chelonae* 481 were cultured in Dubos Tween-albumin medium. *Staphylococcus aureus* Smith, *Listeria monocytogenes* EGD, *Bacillus subtilis* ATCC 6633, and *Escherichia coli* 81 were cultured in tryptic soy broth, and *Candida albicans* NIH A-207 was cultured in Sabouraud broth.

Phagocytosis. The test cells (5×10^4) were plated onto a 15-mm culture well (Corning Glass Works Co., Corning, N.Y.) containing a 14-mm plastic sheet (Wako Pure Chemical Ind., Osaka, Japan) in a 1-ml volume of Dulbecco modified Eagle minimal essential medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (Flow Laboratories, North Ryde, Australia). After 6 h of incubation at 37°C in a CO_2 incubator (5% CO_2

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TABLE 1. Abilities of various cultured cells to phagocytose *M. leprae*[a]

Cells	No. of phagocytosed *M. leprae*/cell[b]	Relative phagocytosis[c]	Phagocytosing cells (%)[d]
Glial KG-1-C	7.17	100	84
Schwann[e]	1.02	14	23
Peritoneal macrophage[e]	11.2	156	94
BALB/3T3[e]	3.16	44	46
BHK-21[e]	1.68	23	38
HeLa S3	2.88	39	44
mKS-A TU-7	1.12	16	30
XC	0.53	7	34
TSV-5	0.51	7	14
N-18	0.32	5	7

[a] Phagocytosis was performed at a multiplicity of infection of 10.
[b] The mean values (n = 2) are indicated. The average variation was 10.4% of each mean value and did not exceed 12.5% in most cases.
[c] The value of phagocytosed *M. leprae* per cell for KG-1-C cells was fixed at 100.
[d] The mean values (n = 2) are indicated, and the average variation was 9.0% of each mean value.
[e] Normal cells; others are transformed cells.

and 95% humidified air), the medium was withdrawn, and 1 ml of Dulbecco modified Eagle minimal essential medium containing 2 or 5% fetal bovine serum and 2.5×10^5 or 5×10^5 test microorganisms was overlaid onto the resulting monolayer cell culture unless otherwise specified. The wells were then incubated at 33°C for 24 h in a CO_2 incubator, and the plastic sheet to which the majority of the test cells were attached was withdrawn, rinsed with Hanks balanced salt solution and phosphate-buffered saline (pH 7.2), fixed with methanol, and stained by the technique of Ziehl-Toda (19) for acid-fast bacilli and with Giemsa solution for microorganisms other than mycobacteria. More than 200 cells on a smear were counted to determine the number of bacteria ingested per cell, and the ratio of cells phagocytosing more than one bacterium (phagocytosing cells) was enumerated by microscopy.

RESULTS

Abilities of various cultured cells to phagocytose *M. leprae*. Phagocytic ability of oligodendroglial KG-1-C cells was compared with that of various cultured, normal, or transformed cells. KG-1-C cells had a higher ability to phagocytose *M. leprae* than other cells except for peritoneal macrophages (Table 1). The phagocytic ability of Schwann cells was lower than that of KG-1-C cells but much higher than that of neuroblastoma N-18 cells. The normal cells showed a tendency toward a higher phagocytic ability than the transformed cells. Figure 1 shows the distribution patterns of various types of cultured cells in the number of *M. leprae* phagocytosed per cell. KG-1-C cells had a distribution curve similar to that of the peritoneal macrophages, with peaks at 1 to 3 bacilli per cell (KG-1-C cells) and 4 to 6 bacilli per cell (macrophages). The other cells so far tested showed no such peak in the distribution curves. This may indicate

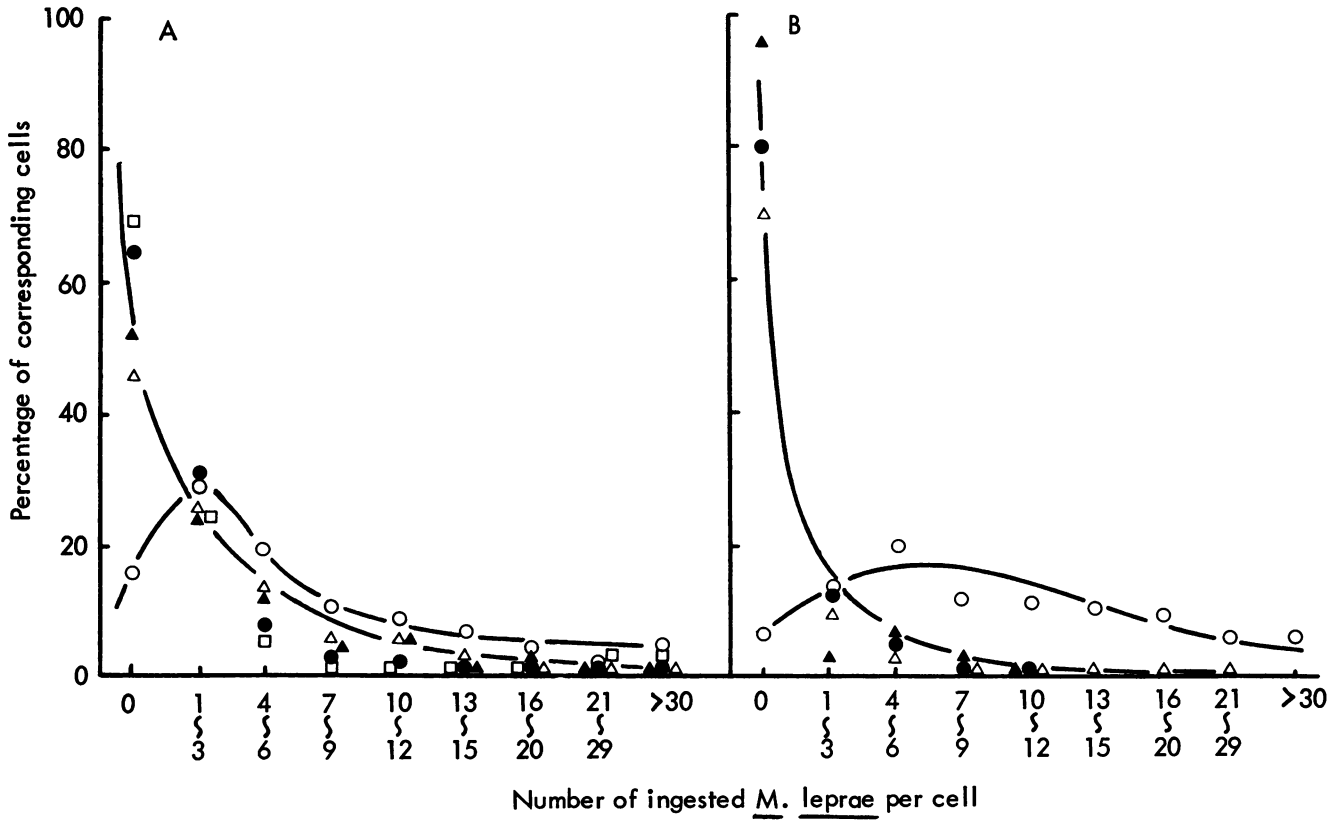

FIG. 1. Distribution patterns of various cultured cells, including glial KG-1-C cells, with respect to the number of phagocytosed *M. leprae* per cell. Phagocytosis was performed at a multiplicity of infection of 10. (A) Symbols: ○, glial KG-1-C cells; ●, BHK-21 cells; △, HeLa S3 cells; ▲, BALB/3T3 cells; □, mKS-A TU-7 cells. (B) Symbols: ○, macrophages; ●, XC cells; △, TSV-5 cells; ▲, N-18 cells.

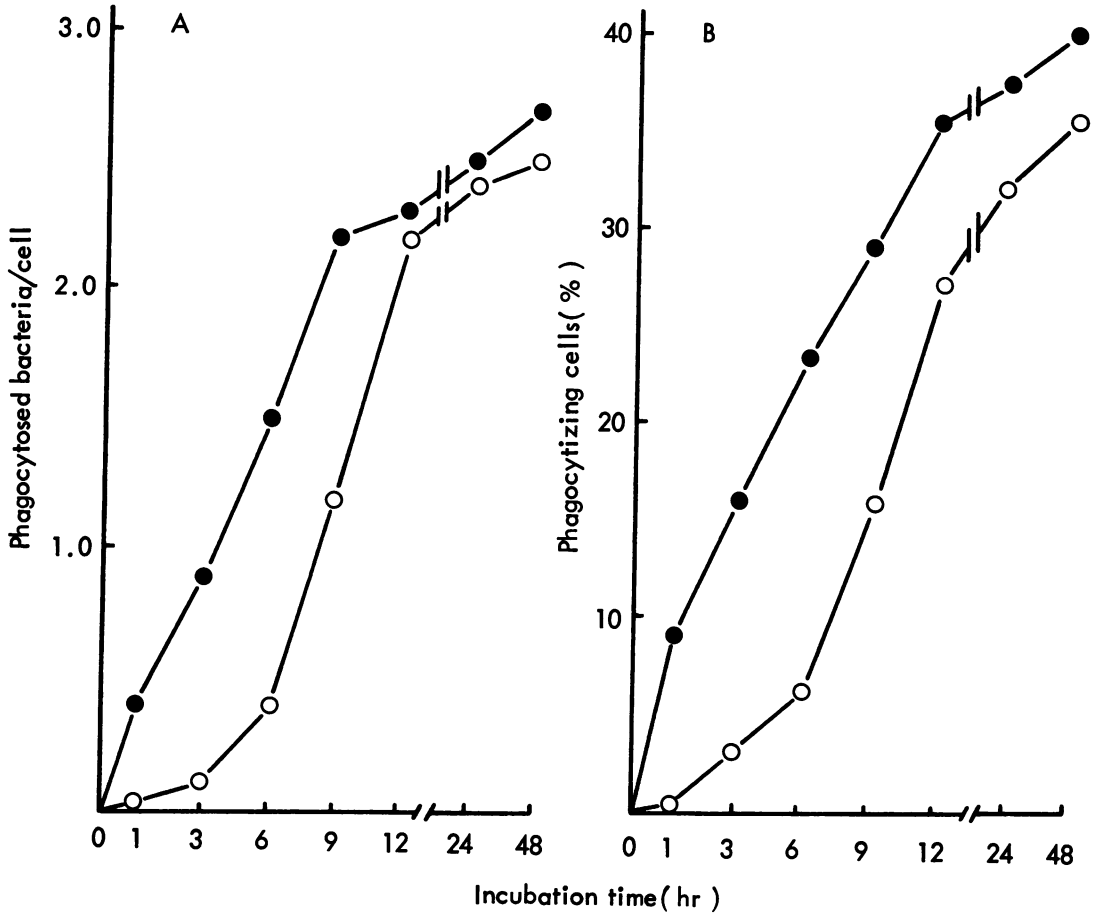

FIG. 2. Time course of phagocytosis of *M. leprae* by glial KG-1-C cells (○) and macrophages (●). Phagocytosis was performed at a multiplicity of infection of 5.

some similarity between the phagocytic function of KG-1-C cells and macrophages. However, a marked discrepancy was noted between KG-1-C cells and macrophages with respect to their phagocytic nature. Figure 2 shows the kinetics of phagocytosis of *M. leprae* for these cells. The rate of phagocytosis of *M. leprae* by KG-1-C cells was much lower than that by macrophages during the first 6 h and thereafter increased to nearly the same level as that by macrophages. The sigmoidal curve observed in the case of KG-1-C cells, with respect to the time course of *M. leprae* phagocytosis, may indicate the importance of a rigid interaction or binding between the cells and *M. leprae* which requires at least 3 to 6 h to be established before onset of invasion of bacilli into the cytoplasm of KG-1-C cells.

Phagocytosis of various microorganisms by glial KG-1-C cells and Schwann cells. Table 2 compares abilities of KG-1-C and Schwann cells to phagocytose acid-fast bacilli. Although KG-1-C cells had considerably greater phagocytic ability than Schwann cells, the patterns of phagocytosis of test mycobacteria in the two nerve cell lines were the same except for the following situations. First, KG-1-C cells phagocytosed *M. tuberculosis* at a somewhat higher rate than *M. leprae*, whereas in the same amount of time, Schwann cells phagocytosed half as many of the former bacilli as the latter bacilli. Second, a similar discrepancy was noted with phagocytosis of *M. intracellulare*. Table 3 shows the ability of KG-1-C cells to phagocytose bacteria other than acid-fast bacilli and particles such as latex beads and sheep erythrocytes. KG-1-C cells ingested bacteria, especially *B. subtilis*, at nearly the same level as they ingested *M. leprae*. On the contrary, *C. albicans* was hardly phagocytosed. Although latex beads (0.8-μm diameter) were phagocytosed to some extent, sheep erythrocytes could not be

TABLE 2. Phagocytosis of various mycobacteria by glial KG-1-C cells and Schwann cells[a]

Organisms	No. of bacteria phagocytosed/cell[b]		Relative phagocytosis[c]		Phagocytosing cells (%)[b]	
	Glial[d]	Schwann[e]	Glial	Schwann	Glial[f]	Schwann[g]
M. leprae	2.77	0.63	100	100	51	19
M. lepraemurium	0.19	0.05	7	8	14	0.2
M. tuberculosis	4.72	0.32	170	51	75	10
M. marinum	2.19	0.39	79	62	35	12
M. scrofulaceum	1.38	0.27	50	43	56	7
M. intracellulare	1.51	0.08	55	13	32	6
M. chelonae	0.51	ND[h]	18	ND	29	ND

[a] Phagocytosis was performed at a multiplicity of infection of 5.
[b] The means (n = 2) are indicated.
[c] Phagocytosis against *M. leprae* was fixed at 100.
[d] Average variation from the mean was 6.5%.
[e] Average variation from the mean was 25%.
[f] Average variation from the mean was 6.7%.
[g] Average variation from the mean was 24%.
[h] ND, Not determined.

TABLE 3. Ability of glial KG-1-C cells to phagocytose various bacteria and particles[a]

Phagocytosis subject	No. of phagocytosed bacteria or objects/cell[b]	Relative phagocytosis[c]	Phagocytosing cells (%)[d]
Organism			
M. leprae	1.68	100	30
S. aureus[e]	0.74	44	35
L. monocytogenes[e]	0.26	16	13
B. subtilis[e]	1.41	84	53
E. coli[e]	0.17	10	11
C. albicans	0.01	1	1
Object			
Latex beads	0.43	24	24
Sheep erythrocytes	0	0	0

[a] Phagocytosis was performed at a multiplicity of infection of 5.
[b] The means (n = 2) are indicated. The average variation was 20% of each mean value.
[c] Phagocytosis against *M. leprae* was fixed at 100.
[d] The means (n = 2) are indicated. The average variation was 12% of each mean value.
[e] Bacteria were UV irradiated (15-W UV lamp, at a distance of 15 cm for 15 min).

ingested by KG-1-C cells. Heating (100°C for 30 min) and UV irradiation did not affect the affinity of mycobacteria, such as *M. leprae*, *M. tuberculosis*, and *M. intracellulare*, for KG-1-C cells except in the case of *M. lepraemurium*, the affinity of which was augmented threefold by heating (data not shown).

Microscopic observations on phagocytosis of *M. leprae* by glial KG-1-C cells and Schwann cells. Figure 3 shows the light micrographs of KG-1-C and Schwann cells which phagocytosed *M. leprae* or *M. tuberculosis* (Fig. 3A to D). A majority of the organisms were found in the perikaryon (Fig. 3A, B, and D), but in some cases, a small number of bacilli were also present in the processes (Fig. 3C). Frequently, the bacilli internalized into the cytoplasm of the perikaryon were surrounded by a halo, indicating the formation of a phagosome (Fig. 3A and D). Bipolar KG-1-C cells phagocytosed *M. leprae* to almost the same degree as the multipolar cells did (data not shown). Electron micrographs of the KG-1-C cells phagocytosing *M. leprae* show that *M. leprae* cells were ingested into the cytoplasm of the KG-1-C cell (Fig. 4). Figure 4B (at arrow with asterisk) shows the formation of a phagosome surrounding *M. leprae*. No particular change was noted in the organelle after phagocytosis of *M. leprae*.

DISCUSSION

The ability of human oligodendroglial KG-1-C cells to phagocytose *M. leprae* was compared with that of mouse Schwann cells derived from dorsal root ganglion. KG-1-C cells showed a higher ability to phagocytose *M. leprae* and most other mycobacteria. In contrast, neuroblastoma N-18 cells phagocytosed *M. leprae* markedly less than those cell lines with a myelinating ability. This indicates that glial and Schwann cells have a higher affinity than do neurons for *M. leprae*, based on the evidence of phagocytosis. This finding is noteworthy for the following reasons. First, although the

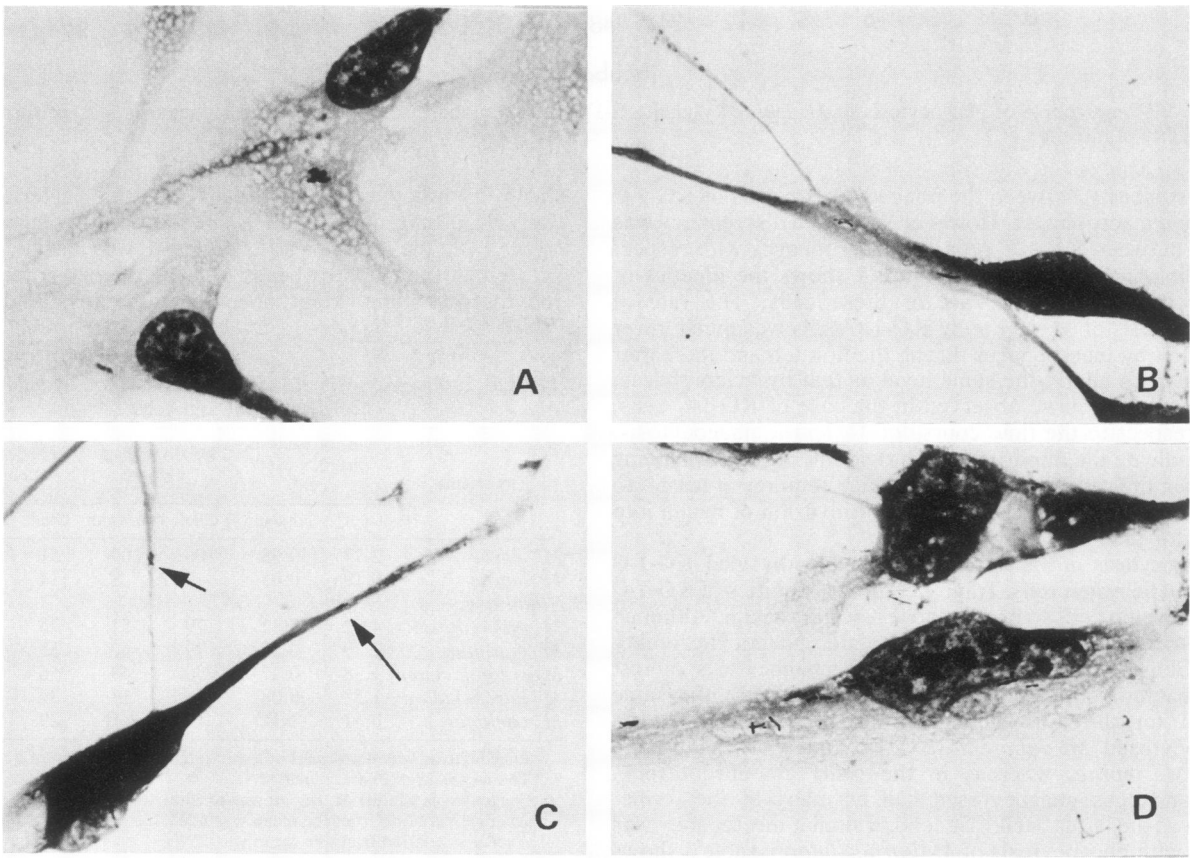

FIG. 3. Light micrographs of glial KG-1-C (A) and Schwann (B) cells phagocytosing *M. leprae* and KG-1-C cells phagocytosing *M. tuberculosis* (D) in their perikaryons. Panel C shows a KG-1-C cell phagocytosing *M. leprae* in its process (arrow). Magnification, ×2,400.

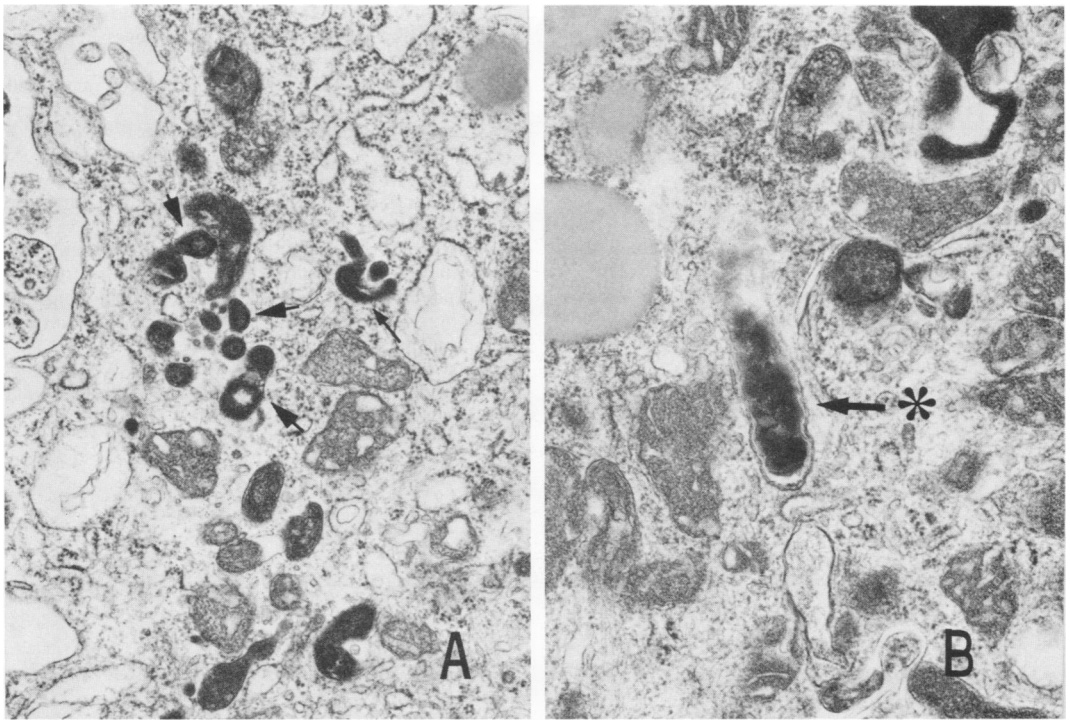

FIG. 4. Electron micrographs of glial KG-1-C cells phagocytosing *M. leprae* in their cytoplasms. Arrows indicate *M. leprae*. The asterisk (B) indicates the formation of a phagosome surrounding *M. leprae*. For transmission electron microscopy, glial KG-1-C cells phagocytosing *M. leprae* (multiplicity of infection of 400) were fixed with glutaraldehyde-paraformaldehyde and then with osmium tetroxide in phosphate buffer (pH 7.3), dehydrated, and embedded in Epon 812 resin. The ultrathin sections were stained with uranyl acetate-lead citrate before observation under a LEOL JEM-200CX electron microscope. (A) Magnification, ×22,000; (B) magnification, ×31,000.

presence of *M. leprae* in peripheral nerves is evidence of the progress of leprosy (5), the exact location of bacilli and sequence of events leading to nerve damage, such as segmental demyelination and Wallerian degeneration, remain controversial. Nishiura and colleagues (12, 13) and Khanolkar (4) proposed the concept that, in peripheral nerves, *M. leprae* is initially phagocytosed by regenerating growth cones of neurons and disseminates into the axoplasm. Nishimura et al. (12, 13), Yoshizumi and Asbury (20), and more recently Mukoyama and Sasaki (10) reported intraaxonal bacilli in peripheral nerves of leprosy patients. On the contrary, the propensity of Schwann cells to be the initial and primary target of invading *M. leprae* has been proposed. Lumsden (5) reported a high capacity of Schwann cells to phagocytose and harbor *M. leprae*. Rees et al. (15) and Dastur et al. (2) noted the essential role of Schwann cells as a target of invasion and dissemination of *M. leprae* into the nervous system, on the basis of their histological studies on nerves from patients with lepromatous leprosy. Swift (18) found widespread segmental demyelination but not Wallerian degeneration in biopsied nerves from patients with dimorphous or lepromatous leprosy, thereby indicating the predominant Schwann cell involvement by *M. leprae*. Our present observation supports the latter hypothesis that Schwann cells are the primary target of *M. leprae* invasion into the peripheral nerves, since Schwann cells could phagocytose *M. leprae* at a much higher rate than could neuronal N-18 cells. Actually, no neuron ingested *M. leprae* in our Schwann cell culture (unpublished observation). However, the finding that glial KG-1-C cells derived from central nerve cells had a more marked ability to phagocytose *M. leprae* remains to be explained, although this might be due simply to difference in some inherent properties between human (a natural host for *M. leprae*) and mouse (not a natural host) (14) cells. Moreover, further studies are needed to elucidate the meaning of the present results regarding in vitro phagocytosis of *M. leprae* by the nerve cells in the interaction of the organisms with the host nerve cells in vivo.

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