Differences in the Interactions of *Nocardia asteroides* with Macrophage, Endothelial, and Astrocytoma Cell Lines

L. BEAMAN AND B. L. BEAMAN*

Department Medical Microbiology and Immunology, University of California
School of Medicine, Davis, California 95616

Received 26 July 1993/Returned for modification 1 September 1993/Accepted 25 February 1994

An *in vitro* model for studying host cell interactions with *Nocardia asteroides* was developed. Thus, macrophage cell lines J774A.1 and P388D1, pulmonary artery endothelium cell line CPAE, rat glial tumor cell line C6, and human astrocytoma cell lines CCF-STTG1 and U-373 MG were infected with either log- or stationary-phase cells of *N. asteroides* GUH-2, and the host cell-nocardia interactions were determined by light microscopy and electron microscopy. Polyclonal antinocardial antibody did not enhance uptake of nocardiae by any of these cell lines; however, log-phase cells of GUH-2 infected a higher percentage of J774A.1 and P388D1 than did stationary-phase organisms. When cells infected with stationary-phase GUH-2 were incubated for 6 h, filaments developed, which indicated that nocardial growth had occurred. In J774A.1 and P388D1, only 31 to 57% of the total stationary-phase coccobacillary cells that were phagocytized formed filaments within 6 h. This indicated that there was some inhibition of growth of the phagocytized nocardiae within these macrophage cell lines; however, the nocardiae grew within the endothelial (>87% filaments) and astrocytoma (100% filaments) cell lines. Microfilament inhibitor cytochalasin B inhibited uptake of GUH-2 by macrophages and other cell lines, except that there was no effect on uptake of nocardial cells by astrocytoma cell line U-373 MG. Scanning and transmission electron microscopy showed phagocytosis of GUH-2 by the different cell lines. In cytochalasin B-treated cells, nocardiae were shown to penetrate through the cell surface and become internalized in a manner distinct from typical phagocytosis, suggesting that filamentous forms of this organism have a phagocytosis-independent invasion factor. The extent of this cytochalasin-resistant cellular penetration by the nocardiae differed in the different cell lines.

Nocardia asteroides GUH-2 has the ability to invade the brains of many mammals and cause central nervous system infection (5). We have shown previously that nocardiae grow in murine macrophages and astrocytes *in vitro* and that brain microglia phagocytizes and inhibits the intracellular growth of GUH-2 (11, 12). Therefore, to understand the mechanisms of nocardial invasion of the brain better, we infected a number of tissue culture cell lines with *N. asteroides* GUH-2 *in vitro*. Since this nocardial strain penetrates the blood-brain barrier and grows perivascularly in neurons and astrocytes, astrocytoma and glial cell lines were selected and their interactions with GUH-2 were compared to those of macrophage cell lines. To grow within a brain, GUH-2 must also be able to leave the capillaries. It was observed that nocardiae penetrate capillary endothelial cells *in vivo* (9); therefore, an endothelial cell line was selected for study.

MATERIALS AND METHODS

Microorganism. *N. asteroides* GUH-2 was a clinical isolate from a patient with disseminated nocardiosis at Georgetown University Hospital, Washington, D.C. *N. asteroides* was grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C on a rotary shaker. Cultures were harvested, and single-cell suspensions were made by differential centrifugation as described previously (2). In the log phase, >99% of the cells were in the filamentous form, and in the early stationary-phase preparation, >99% of the cells were in the

coccobacillary form. Bacterial numbers were determined by direct cell counting with a Neubauer hemocytometer, and then the bacteria were diluted to an appropriate inoculum dose in Dulbecco's modified Eagle's medium (Gibco Laboratories, Grand Island, N.Y.) with 10% calf serum (CS) (Hyclone Laboratories). The actual numbers of viable nocardiae in the inocula were determined by plating dilutions onto agar.

Tissue culture cell lines. All cell lines were purchased from the American Type Culture Collection (Rockville, Md.). Monocyte-macrophage cell line J774A.1 (ATCC TIB 67) was derived from a tumor in a BALB/c mouse and maintained in Dulbecco's modified Eagle's medium supplemented with 1 mM sodium pyruvate and 10% CS. Monocyte-macrophage cell line P388D1 (ATCC TIB67) was derived from a chemically induced neoplasm in a DBA/2 mouse and cultured in RMPI 1640 supplemented with 1 mM sodium pyruvate and 10% CS. Endothelial cell line CPAE (ATCC CCL209) was derived from a bovine pulmonary artery and maintained in Dulbecco's modified Eagle's medium supplemented with 1 mM sodium pyruvate–20% CS. CCF-STTG1 (ATCC CRL 1718) and U-373 MG (ATCC HTB 17) were derived from human astrocytomas and cultured in Dulbecco's modified Eagle's medium with nonessential amino acids–1 mM sodium pyruvate–10% CS. Glial cell line C6 was derived from a chemically induced rat tumor and cultured in Ham's F-10 medium supplemented with 1 mM sodium pyruvate, 15% horse serum, and 2.5% CS. All cells were cultured at 37°C in an atmosphere of 5% CO₂. Cell monolayers were detached with fresh trypsin (0.25%) and centrifuged at 500 × g, and the cell pellet was suspended in fresh medium. Aliquots containing 10⁵ cells per 0.2 ml of medium were added to Lab-Tek chambered slides (Miles Laboratories, Inc., Naperville, Ill.) and incubated for 24 h prior to infection with nocardiae. This resulted in individually

* Corresponding author. Mailing address: Department Medical Microbiology and Immunology, 3146 MS1A, School of Medicine, University of California, Davis, CA 95616. Phone: (916) 752-9663. Fax: (916) 752-8692.

isolated cells in all but the C6 cell line, which tended to adhere in aggregates.

Infection of cell lines. For phagocytic studies, the cell lines were infected with a single cell suspension of log-phase GUH-2 at a bacterium-cell ratio of 2:1, incubated for 1 h, washed, rinsed, air dried, heat fixed, and stained with the Gram stain. Duplicate samples were fixed in glutaraldehyde and analyzed by scanning electron microscopy (SEM) as described below. The number of gram-positive bacteria per macrophage was quantitated by microscopic counts and compared with the number of bacteria seen on the surface by SEM (see Fig. 1). The same procedures were used with stationary-phase GUH-2. To determine nocardial growth, fresh medium was added to cells infected with stationary-phase GUH-2 for another 6 h of incubation prior to Gram staining. Stationary-phase GUH-2 cells are coccobacillary in morphology. If coccoid nocardiae are capable of growth, then filamentous cells are produced which can be observed and quantitated by using the Gram stain combined with microscopic enumeration (11). Serum was obtained from immunized mice (BALB/c, female) 2 weeks after a booster injection with formalin-killed nocardiae (10 mg of washed bacteria). These mice had been infected with a sublethal intravenous dose of *N. asteroides* GUH-2 8 to 10 months earlier. The immune serum was positive for nocardiae at a dilution of 1:2,048 with an enzyme-linked immunosorbent assay for nocardial cytoplasmic antigens (10). In some experiments, 0.01 or 0.001 mM cytochalasin B or colchicine (Sigma, St. Louis, Mo.) was added to the cell cultures in slide chambers 5 min prior to infection with nocardiae. The stained slides were examined, and the percentages of cells infected and the numbers of coccobacillary nocardiae or nocardial filaments per 600 cells in three separate experiments were quantitated. The results were normalized to reflect a bacterium-tissue culture cell ratio of 2:1 in all experiments. The mean $\pm$ the standard error was determined, and *P* values were calculated by the paired or independent *t* test.

SEM. The infected cells in slide chambers were fixed in 2.5% glutaraldehyde, dehydrated through an ethanol series, critical point dried with CO₂, coated with gold, and examined with a Philips scanning electron microscope at 15 kV as described previously (12).

Transmission electron microscopy. Cells in slide chambers (untreated and cytochalasin B treated) were infected with nocardiae as described above, fixed overnight with cold 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), and postfixed for 1 h in 1% osmium tetroxide. The cells were scraped from the slides, treated with 0.5% uranyl acetate for 30 min, dehydrated through an ethanol series to 100% propylene oxide, and embedded in med-cast epoxy resin (Ted Pella, Inc., Redding, Calif.). Gold to silver sections were cut with a diamond knife, and the sections were placed on copper Athene grids, stained for 30 min with 0.5% uranyl acetate in 50% methanol-water, washed, and stained for 5 min in 0.1% lead citrate. The sections were washed in deionized water, dried, and visualized with a Philips 400 electron microscope operated at 80 kV.

FIG. 2. Percentage of tissue culture cells infected with log-phase (16 h) or stationary-phase (48 h) *N. asteroides* 1 h postinfection. Means $\pm$ the standard errors for 600 cells in three experiments are shown.

RESULTS

Monocyte-macrophage cell lines. Monocyte-macrophage cell lines J774A.1 and P388D1 were infected with log- and stationary-phase strain GUH-2 cells. In tissue, the filamentous form of the organism predominates during the early, acute phase of infection, whereas coccobacillary cells are more dominant during the later, more chronic stages of disease (2, 4, 8). Figure 1 demonstrates that log-phase cells of *N. asteroides* GUH-2 efficiently infected macrophage cell line J774A.1. Comparison of the Gram stain reactivity (Fig. 1A and B) with the SEM analysis (Fig. 1C and D) showed that about 95% of the cell-associated bacteria became internalized within 1 h of contact. As shown in Fig. 2, the log-phase culture of GUH-2 infected a higher percentage of J774A.1 macrophages ($58\% \pm 8\%$) than did the stationary-phase culture ($8\% \pm 2\%$) within 1 h postinfection ($P < 0.001$). Addition of immune serum to the log-phase culture of GUH-2 did not enhance uptake ($63\% \pm 5\%$) compared with normal serum ($63\% \pm 5\%$) ($P > 0.05$). With the same bacterium-to-cell ratio as with J774A.1, fewer bacteria infected P388D1 macrophages ($18\% \pm 5\%$) ($P < 0.001$) than J774A.1 cells and antibody addition did not enhance nocardial infectivity ($13\% \pm 4\%$) ($P > 0.05$) (Fig. 2). When J774A.1 and P388D1 cells were infected with coccobacillary GUH-2 (stationary phase) and incubated for 6 h, filaments formed, indicating that nocardial growth had occurred. However, as shown in Fig. 3, with J774A.1 cells, only $57\% \pm 15\%$ of the total nocardiae taken up in the presence of normal serum formed filaments and $31\% \pm 7\%$ of the total nocardiae taken up in the presence of immune serum formed filaments, indicating that some inhibition of nocardial growth had occurred. Similar results are shown for P388D1 in Fig. 3. Treatment of macrophage cell lines with cytochalasin B, an inhibitor of microfilaments (26), inhibited uptake of nocardiae (Fig. 4). The higher dose of the microtubule inhibitor (25)

FIG. 1. Phagocytic uptake of log-phase *N. asteroides* GUH-2 cells in macrophage cell line J774A.1 as determined by Gram stain and SEM analysis. (A and B) Gram-stained preparations of two different samples of J774A.1 1 h after exposure to a suspension of single log-phase *N. asteroides* GUH-2 cells (bacterium-macrophage ratio, 2:1). Note that a total of 33 macrophages are shown containing 60 bacteria in 26 cells, resulting in an infection rate of 2.3 bacteria per macrophage; 78.8% of the macrophages contained nocardiae. (C and D) Scanning electron micrographs of duplicate samples from each experimental preparation (A and C; B and D). Note that three bacteria (n) are visible in panel D, whereas no bacteria are detectable in panel C. A total of 34 macrophages are visualized in panels C and D. Since panels C and D are from the samples shown in panels A and B, it was concluded that 95% of the bacteria visualized in panels A and B resided entirely within the macrophages. Bars, 10 μ m.

FIG. 3. Percentage of total nocardial cells that are filaments at 6 h postinfection. Mean $\pm$ the standard errors for cells in three experiments are shown.

colchicine (0.01 mM) also affected nocardial uptake; however, this dose had a toxic effect on the macrophage monolayer, as shown by the one-fourth reduction in the average number of tissue culture cells per field compared with untreated control monolayers (data not shown). Cytochalasin B (0.01 mM) significantly inhibited uptake of nocardiae more than did 0.01 mM colchicine ($P < 0.01$ for both cell lines). As shown in Fig. 5A and B, J774A.1 macrophages phagocytized nocardiae. However, nocardiae were seen adhering to the surface of J774A.1 cells after treatment with cytochalasin B (Fig. 5C) and several nocardiae appeared to infect macrophages by penetrating the surface of J774A.1 or P388D1 cells treated with cytochalasin B (Fig. 5D and 6C and D). However, the primary mechanism of nocardial infection of these macrophage-like cell lines appeared to be typical phagocytosis (Fig. 6A) or zipper phagocytosis (20) of nocardiae by P388D1 (Fig. 6B), because cytochalasin B reduced the number of internalized bacteria (Fig. 4).

Transmission electron microscopy revealed that J774A.1 cells rapidly internalized log-phase cells of *N. asteroides* GUH-2 (Fig. 7A), and few bacteria remained surface associated (Fig. 1). Most of the internalized bacteria were tightly surrounded by the phagosomal membrane; however, some of the bacteria appeared to reside freely within the cytoplasm of J774A.1 cells (Fig. 7B, arrows). In contrast, fewer bacteria resided inside cytochalasin B-treated J774A.1 cells (Fig. 7C and 8); instead, bacterial filaments attached to the J774A.1 cell membrane and penetrated into the cells (Fig. 7C, arrows). The host cell membrane did not surround the penetrating bacteria as tightly in cytochalasin B-treated cells (Fig. 7C) as in untreated controls (Fig. 7A). Furthermore, nocardiae penetrated cytochalasin B-treated cells only by the bacterial apex (Fig. 7C) and bacteria that adhered along the side of the filament (longitudinally) could not penetrate into treated

FIG. 4. Relative percent inhibition of (cell-associated) infection of tissue culture cells infected with log-phase strain GUH-2 cells and treated with cytochalasin B or colchicine compared with that of untreated controls. Relative percent inhibition was calculated as follows: $100 - [(\% \text{ of treated cells with associated nocardiae} / \% \text{ of untreated cells with associated nocardiae}) \times 100]$. The average variation in the control values for 600 to 800 cells was $\pm 6\%$.

J774A.1 cells (Fig. 7D). Even though Gram-stained preparations revealed that cytochalasin B inhibited uptake of nocardial cells, electron microscopy showed that it did not prevent penetration and internalization completely (Fig. 8A and B).

Pulmonary endothelial cell line. CPAE cells did not appear to be as phagocytic for nocardiae as the macrophage cell line J774A.1, as shown in Fig. 2 and confirmed by electron microscopy. On the other hand, nocardiae associated equally well with CPAE cells and P388 macrophages (Fig. 2). When coccobacillary nocardiae infected CPAE cells, $87\% \pm 8\%$ of the ingested bacteria formed filaments within 6 h of incubation, as shown in Fig. 3, which indicated that significantly more nocardial growth occurred in these cells than with either J774A.1 ($57\% \pm 15\%$; $P < 0.05$) or P388D1 ($39\% \pm 5\%$; $P < 0.001$) cells. Cytochalasin B reduced nocardial uptake by CPAE cells, as shown in Fig. 4 and confirmed by electron

FIG. 5. Nocardial interactions with macrophage cell line J774A.1. (A and B) Log-phase *N. asteroides* GUH-2 cells being phagocytized by untreated J774A.1 cells. Arrows indicated the leading edge of the phagocytic membrane engulfing the nocardial cell (N). (C) A nocardial cell (N) adhering (arrow) to the surface of a J774A.1 cell treated with cytochalasin B. (D) A nocardial cell (N) penetrating the surface (arrow) of a J774A.1 cell after treatment with cytochalasin B (0.001 mM). Bars, 1 μ m.

FIG. 6. Nocardial interactions with macrophage cell line P388D1. (A) Nocardial filament (N) being actively engulfed by a phagocyte. The arrow points to the leading edge of the pseudopod engulfing the filament. (B) Branching nocardial filament (N) being phagocytized by zipper phagocytosis (arrows) in an untreated P388D1 cell. (C and D) Nocardial interactions with P388D1 cells treated with 0.001 mM cytochalasin. Each arrow points to a location where the nocardial filament (N) penetrates the cell surface. Bars, 1 μ m.

FIG. 7. Transmission electron microscopy of nocardial interactions with macrophage cell line J774A.1. (A) Thin section of an untreated cell which has internalized at least five bacterial cells (arrows). (B) High-magnification insert from panel A (arrow). The curved arrow points to an intact membrane (probably phagosomal), and the straight arrow points to the outer layer of the bacterial cell envelope with a disappearance of the phagosomal membrane. (C) High magnification of a thin section of a cell treated with 0.001 mM cytochalasin B showing apical penetration of the cell by a nocardial filament. The arrow points to a site of attachment between the bacterial cell wall and the membrane of the macrophage. (D) High-magnification view of a nocardial filament attached longitudinally to the surface of a cytochalasin B-treated cell (as in panel C). The arrow points to the site of attachment. Note that there is no evidence of penetration of the host cell surface. Bars in panels B to D, 1 μm.

FIG. 8. Microscopy of nocardial uptake by macrophage cell line J774A.1 after treatment with cytochalasin B. (A) Gram stain of cytochalasin B-treated J774A.1 cells 1 h after exposure to log-phase *N. asteroides* GUH-2 cells (cf. Fig. 1A and B). The arrow indicates nocardial filaments (probably inside the cell). (B) Electron micrograph of a thin section of the cells shown in panel A demonstrating the intracellular location and nature of the nocardiae in cytochalasin B-treated cells. Note the loose phagosomal compartment surrounding the cells (cf. Fig. 7A and B). The arrows indicate nocardiae inside cells.

microscopy (Fig. 9). In Fig. 9A, a CPAE cell surface membrane is shown surrounding a nocardial filament, and in Fig. 9B, nocardial filaments are shown adhering to the surface of CPAE cells. Figure 9B shows that the bacterial surface appeared either to merge or be tightly associated with the CPAE cell membrane and the bacteria became internalized by a sinking-like or penetration phenomenon that appeared to be different from the process observed in any of the other cell lines studied (compare Fig. 9 with Fig. 5, 6, 7, and 10). In the presence of cytochalasin B, a greater percentage of cell-associated nocardiae appeared to penetrate CPAE cells than macrophage cell lines, as shown in Fig. 9C and D.

Astrocytoma and glial cell lines. Only $15\% \pm 5\%$ of the C6 glial cells had log-phase bacteria after infection, compared with $58\% \pm 8\%$ of J774A.1 cells. Furthermore, when coccobacillary nocardiae were added to C6 glial cells, $96\% \pm 8\%$ of the cell-associated bacteria formed filaments within 6 h of incubation, which indicated that *N. asteroides* GUH-2 grew readily within these glial cells.

Astrocytoma cell line U-373 MG was not as phagocytic (confirmed by electron microscopic analysis) as J774A.1 cells ($17\% \pm 6\%$ versus $58\% \pm 8\%$), even though its uptake of nocardiae was similar to that observed with macrophage cell line P388D1 (Fig. 2). There was no difference in GUH-2 uptake by U-373 MG cells in the presence or absence of immune serum. Nearly 100% of the nocardiae taken up by these astrocytoma cells formed filaments, indicating that nocardial growth had occurred (Fig. 3). Similarly, nocardial cells were associated with $13\% \pm 3\%$ of astrocytoma CCF-STTG1 cells versus $58\% \pm 8\%$ of J774A.1 cells. Cytochalasin B inhibited uptake of nocardiae by glial cells and astrocytoma CCF-STTG1 cells (Fig. 4), while in contrast, there was no inhibition of uptake by astrocytoma cell line U-373 MG (Fig. 4). SEM showed phagocytosis of nocardial filaments by control CCF-STTG1 cells (Fig. 10A and B); however, the nocardiae actively penetrated cytochalasin B-treated CCF-STTG1 cells (Fig. 10C and D). In addition, the nocardiae were seen penetrating cytochalasin B-treated U-373 MG cells (data not shown) in a manner that appeared to be the same as that observed with CCF-STTG1 cells (Fig. 10C and D).

DISCUSSION

Macrophages obtained from normal mice, rabbits, and humans are not able to prevent intracellular growth of virulent cells of *N. asteroides* (3, 4, 14, 18). Strain GUH-2 has been shown to affect phagocyte function by inhibiting phagosome-lysosome fusion (17), neutralizing oxygen-dependent microbicidal activity (7), altering lysosomal enzymes (6, 14–16), and utilizing acid phosphatase to enhance intracellular growth (13). However, activated macrophages are capable of killing nocardiae (19).

It has been shown that monocyte-macrophage cell line J774A.1 synthesizes large amounts of lysozyme and exhibits antibody-dependent cytotoxicity, although less than the P388D1 cell line (28). The level of cytolysis appears to vary inversely with the phagocytic activity of J774A.1 cells (28). Both macrophage cell lines have levels of β -glucuronidase and acid

phosphatase similar to those observed in activated macrophages (28). The phagocytic activity of J774A.1 cells has been reported to be two- to fourfold higher than that of P388D1 cells (28). Similarly, J774A.1 macrophages phagocytized more nocardiae than did P388D1 cells and there appeared to be more inhibition of nocardial growth by J774A.1 cells than by P388D1 cells. Therefore, many of the GUH-2 cells in these macrophage cell lines remained in the coccobacillary form after incubation, as was previously observed with activated macrophages (18). However, some of the bacteria appeared to be able to escape from the phagosome by dissolution of the host cell membrane that had become tightly associated with the nocardial cell wall (Fig. 7B). These nocardial cells then appeared to grow within the cytoplasm of macrophages (data not shown).

Rat glial cell tumor cell line C6 has been shown to phagocytize latex beads, and this phagocytosis was inhibited by treatment with cytochalasin B (27). This cell line resembles primary astrocytes; however, mononuclear cells may be present in these cultures (27). Astrocytoma line CCF-STTG1 exhibits acid phosphatase activity, and immunocytochemical tests indicate the presence of glial fibrillary acidic protein characteristic of astrocytes in this cell line (1). Human astrocytoma cell line U-373 MG stains positive for glial fibrillary acidic protein (24). U-373 MG cells proliferate upon exposure to interleukin 1 or tumor necrosis factor in vitro (24) and produce granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in response to interleukin 1 (29). The nocardiae interacted with these cells differently than with the macrophage cell lines. Professional phagocytic cells, such as macrophages, have specific cell receptors which bind bacteria prior to engulfment, such as receptors for immunoglobulin (Fc) and complement (C3). In addition, fibronectin has been shown to enhance phagocytosis of bacteria by P388D1 cells and macrophages (21, 23).

In the murine brain, log-phase *N. asteroides* GUH-2 cells attach to capillary endothelial cells and then rapidly penetrate through these cells to enter the parenchyma without disruption of the blood-brain barrier (9). Figure 8C shows a nocardial cell with one end entering an artery endothelial cell (cell line CPAE; lower arrow) while its distal end appears to be against the inner surface of the plasma membrane, perhaps in an attempt to exit at this point (Fig. 8C, upper arrow). This extension through the endothelial cell might explain how nocardiae travel across the capillary endothelium in vivo (9). Of course, additional information obtained with polarized cells in vitro should clarify this interpretation.

Rickettsiae enter human endothelial cells through induced phagocytosis (30), and the enteric pathogen *Yersinia pseudotuberculosis* produces a bacterial surface protein (invasin) which interacts with the integrin receptors of host nonprofessional phagocytic cells (22). It is not clear how strain GUH-2 binds to or enters nonprofessional phagocytic cells; however, since immune serum and antibody had no effect, it is unlikely that Fc receptors play a significant role in these interactions. *N. asteroides* GUH-2 secretes a protein that has a molecular mass of 31 kDa on sodium dodecyl sulfate-gel electrophoresis (10). This protein is also bound to the nocardial cell surface (10). A

FIG. 9. Nocardial interactions with bovine artery endothelial cell line CPAE. (A to C) Different nocardial filaments (N) interacting with untreated CPAE cells. (A) Typical phagocytic process (arrow). Panels B and C show that the nocardial cells (N) melt (fuse) with surface sinking into the cell. (D) Nocardial cell (N) penetrating the surface (arrow) of a CPAE cell treated with 0.001 mM cytochalasin B. Bars, 1 μ M.

FIG. 10. Nocardial interactions with human astrocytoma cell line CCF-STTG1. (A and B) Nocardial filaments (N) being actively phagocytized by pseudopod extensions (arrow) of untreated CCF-STTG1 cells. (C and D) Nocardial cells (N) penetrating through the surface (arrow) of CCF-STTG1 cells treated with 0.001 mM cytochalasin B. Bars, 1 μ M.

similar 31-kDa protein has been reported on the surface of *Mycobacterium tuberculosis*, and it was shown that this 31-kDa protein is derived from a class of fibronectin-binding proteins (31). Fibronectin-binding proteins are believed to be important for mediating attachment of whole bacteria to fibronectin-coated surfaces (31). Further work needs to be done to elucidate the differential binding of nocardiae to various types of cells.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant RO1-AI-20900 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

- Barna, B. P., S. M. Chou, B. Jacobs, R. M. Ransohoff, J. R. Hahn, and J. W. Bay. 1985. Enhanced DNA synthesis of human glial cells exposed to human leukocyte products. *J. Neuroimmunol.* **10**:151–158.
- Beaman, B. L. 1975. Structural and biochemical alterations of *Nocardia asteroides* cell walls during its growth cycle. *J. Bacteriol.* **123**:1235–1253.
- Beaman, B. L. 1977. In vitro response of rabbit alveolar macrophages to infection with *Nocardia asteroides*. *Infect. Immun.* **15**:925–937.
- Beaman, B. L. 1979. Interaction of *Nocardia asteroides* at different phases of growth with in vitro-maintained macrophages obtained from the lungs of normal and immunized rabbits. *Infect. Immun.* **26**:355–361.
- Beaman, B. L. 1992. *Nocardia*: an environmental bacterium possibly associated with neurodegenerative diseases in humans, p. 147–166. In R. L. Isaacson and K. F. Jensen (ed.), *The vulnerable brain and environmental risks*, vol. 2. Toxins in food. Plenum Press, New York.
- Beaman, B. L., and C. Black. 1985. Interaction of *Nocardia asteroides* in BALB/c mice: modulation of macrophage function, enzyme activity and the induction of immunologically specific T-cell bactericidal activity. *Curr. Top. Microbiol. Immunol.* **122**:138–147.
- Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman. 1985. Role of superoxide dismutase and catalase as determinants of pathogenicity of *Nocardia asteroides*: importance in resistance to microbicidal activities of human polymorphonuclear neutrophils. *Infect. Immun.* **47**:135–141.
- Beaman, B. L., and S. Maslan. 1978. Virulence of *Nocardia asteroides* during its growth cycle. *Infect. Immun.* **20**:290–295.
- Beaman, B. L., and S. A. Ogata. 1993. Ultrastructural analysis of attachment to and penetration of capillaries in the murine pons, midbrain, thalamus, and hypothalamus by *Nocardia asteroides*. *Infect. Immun.* **61**:955–965.
- Beaman, L., and B. L. Beaman. 1990. Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of *Nocardia asteroides* within the intact host. *Infect. Immun.* **58**:3122–3128.
- Beaman, L., and B. L. Beaman. 1992. The timing of exposure of mononuclear phagocytes to recombinant interferon γ and recombinant tumor necrosis factor α alters interactions with *Nocardia asteroides*. *J. Leukocyte Biol.* **51**:276–281.
- Beaman, L., and B. L. Beaman. 1993. Interactions of *Nocardia asteroides* with murine glia cells in culture. *Infect. Immun.* **61**:343–347.
- Beaman, L., M. Paliescheskey, and B. L. Beaman. 1988. Acid phosphatase stimulation of the growth of *Nocardia asteroides* and its possible relationship to the modification of lysosomal enzymes in macrophages. *Infect. Immun.* **56**:1652–1654.
- Black, C. M., B. L. Beaman, R. M. Donovan, and E. Goldstein. 1983. Effect of virulent and less virulent strains of *Nocardia asteroides* on acid-phosphatase activity in alveolar and peritoneal macrophages maintained *in vitro*. *J. Infect. Dis.* **148**:117–124.
- Black, C. M., B. L. Beaman, R. M. Donovan, and E. Goldstein. 1985. Intracellular acid phosphatase content and ability of different macrophage populations to kill *Nocardia asteroides*. *Infect. Immun.* **47**:375–383.
- Black, C. M., M. Paliescheskey, B. L. Beaman, R. M. Donovan, and E. Goldstein. 1986. Modulation of lysosomal protease-esterase and lysozyme in Kupffer cells and peritoneal macrophages infected with *Nocardia asteroides*. *Infect. Immun.* **54**:917–919.
- Davis-Scibienski, C., and B. L. Beaman. 1980. Interaction of *Nocardia asteroides* with rabbit alveolar macrophages: association of virulence, viability, ultrastructural damage, and phagosome-lysosome fusion. *Infect. Immun.* **28**:610–619.
- Filice, G. A., B. L. Beaman, J. A. Krick, and J. S. Remington. 1980. Effects of human neutrophils and monocytes on *Nocardia asteroides*: failure of killing despite occurrence of the oxidative metabolic burst. *J. Infect. Dis.* **142**:432–438.
- Filice, G. A., B. L. Beaman, and J. S. Remington. 1980. Effects of activated macrophages on *Nocardia asteroides*. *Infect. Immun.* **27**:643–649.
- Griffin, F. M., J. A. Griffin, J. E. Leider, and S. C. Silverstein. 1975. Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane. *J. Exp. Med.* **142**:1263–1282.
- Hill, H. R., N. H. Augustine, P. A. Williams, E. J. Brown, and J. F. Bohnsack. 1993. Mechanism of fibronectin enhancement of group B streptococcal phagocytosis by human neutrophils and culture-derived macrophages. *Infect. Immun.* **61**:2334–2339.
- Isberg, R. R. 1991. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. *Science* **252**:934–938.
- Kluftinger, J. L., N. M. Kelly, B. H. Jost, and R. E. W. Hancock. 1989. Fibronectin as an enhancer of nonopsonic phagocytosis of *Pseudomonas aeruginosa* by macrophages. *Infect. Immun.* **57**:2782–2785.
- Lachman, L. B., D. C. Brown, and C. A. Dinarello. 1987. Growth-promoting effect of recombinant interleukin 1 and tumor necrosis factor for a human astrocytoma cell line. *J. Immunol.* **138**:2913–2916.
- Malawista, S. E., and P. T. Bodel. 1967. The dissociation by colchicine of phagocytosis from increased oxygen consumption in human leukocytes. *J. Clin. Invest.* **46**:786–796.
- Malawista, S. E., J. B. L. Gee, and K. G. Bensch. 1971. Cytochalasin B reversibly inhibits phagocytosis: functional, metabolic and ultrastructural effects in human blood leukocytes and rabbit alveolar macrophages. *Yale J. Biol. Med.* **44**:286–300.
- Noske, W., H. Lentzen, K. Lange, and K. Keller. 1982. Phagocytic activity of glial cells in culture. *Exp. Cell Res.* **142**:437–445.
- Snyderman, R., M. C. Pike, D. G. Fischer, and H. S. Koren. 1977. Biologic and biochemical activities of continuous macrophage cell lines P388D1 and J774A.1. *J. Immunol.* **119**:2060–2066.
- Tweardy, D. J., P. L. Mott, and E. W. Glazer. 1990. Monokine modulation of human astroglial cell production of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. I. Effects of IL-1 α and IL-1 β . *J. Immunol.* **144**:2233–2341.
- Walker, T. S. 1984. Rickettsial interactions with human endothelial cells in vitro: adherence and entry. *Infect. Immun.* **44**:205–210.
- Wiker, H. G., and M. Harboe. 1992. The antigen 85 complex: a major secretion product of *Mycobacterium tuberculosis*. *Microbiol. Rev.* **56**:648–661.