INFECTION AND IMMUNITY, Nov. 1996, p. 4859–4862 Vol. 64, No. 11
0019-9567/96/$04.00+0


# Differential Binding of *Nocardia asteroides* in the Murine Lung and Brain Suggests Multiple Ligands on the Nocardial Surface

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Received 6 May 1996/Returned for modification 4 June 1996/Accepted 14 August 1996

**The adherence of *Nocardia asteroides* in the murine brain and lungs was determined. Virulent strains had increased adherence in the brain and lungs, whereas less virulent strains bound in either the brain or lungs. Nocardiae that attached apically penetrated host cells. Multiple receptors on the nocardial surface may be involved in this differential attachment and penetration.**

*Nocardia asteroides* can cause a destructive invasion of the lungs, leading to a fulminant, necrotizing pneumonia (10); acute, regional pneumonitis with diffuse pulmonary parenchymal disease resulting in adult respiratory distress syndrome (12); multiple expanding abscesses and chronic pleuropulmonary disease (8); chronic pneumonia; empyema; bronchiolitis obliterans (9); lobar infiltration and cavitation; and extensive tissue invasion (1, 8–10, 12). Often, the invading nocardiae erode through blood vessels and disseminate to other body sites (1). The most frequent target organ for these blood-borne bacteria is the brain (1). In addition, the central nervous system is a primary target for blood-borne nocardiae in the absence of recognized infection elsewhere in the body, and approximately 10% of all nocardial infections in humans reported in the world literature are primary infections of the central nervous system (1). Furthermore, intravenous (i.v.) inoculation of nocardiae into experimental animals leads to preferential invasion and growth within specific regions of the body, especially the brain (6, 11). These observations suggest that pathogenic nocardiae may possess, on their surfaces, ligands specific for distinct cell surface receptors within the brain (1, 6, 7, 11).

Several studies have focused on experimental infection of mice by *Nocardia* spp. (1). Most reports note the heterogeneity of nocardial interactions in the murine model and suggest that mice are ideal experimental hosts for studying these interactions (1). The purpose of this investigation is to determine whether strains of *N. asteroides* isolated from the human brain show differential attachment to and invasiveness of the brain compared with the lung.

Approximately 200 clinical isolates of nocardiae have been studied in our laboratory, and the relative virulence values, including central nervous system interactions, for mice have been determined for more than 65 of these strains. In this study, 10 well-characterized strains of the *N. asteroides* complex, excluding *N. farcinica* and *N. nova* (Table 1), were grown to mid-log phase in brain heart infusion broth as described elsewhere (4), and suspensions of single, branching filaments were prepared by differential centrifugation (4). The cell density was adjusted to an $A_{580}$ of 0.5 (approximately $5 \times 10^7$ CFU/ml) in Hank's balanced salt solution by using a Spectronic 20 spectrophotometer (Bausch & Lomb). Mice were anesthetized with an intraperitoneal (i.p.) injection of Nembutal (50 mg/kg of body weight), and 0.05 ml of the bacterial suspension was aspirated into the lungs by administration to the anterior nares. Previous studies had demonstrated that this resulted in a reproducible delivery of approximately $2.3 \times 10^6$ CFU of strain GUH-2 into the lungs of mice (2). Also, 0.1 ml of a 1:5 dilution of each of these suspensions was injected i.v. into separate groups of mice to determine adherence within the brain ($10^6$ CFU per mouse). The number of CFU in each

TABLE 1. Human isolates of *N. asteroides* selected for comparative analysis of interactions in the brain and lungs of BALB/c mice

| Strain | Source | $LD_{50}$ (virulence)[a] | Patient compromised by: | Outcome |
|---|---|---|---|---|
| GUH-2 | Kidney | $<5.0 \times 10^5$ CFU (high) | Renal transplant | Fatal |
| UC-33 | Brain | $1.2 \times 10^6$ CFU (intermediate) | AIDS | Fatal |
| UC-34 | Brain | $>1.0 \times 10^6$ CFU (intermediate) | Nothing | Unknown |
| UC-44 | CNS (CSF)[b] | $>2.0 \times 10^6$ CFU (intermediate) | Steroids | Unknown |
| UC-59 | Brain | $>5.0 \times 10^6$ CFU (intermediate) | Nothing | Cured |
| UC-63 | Brain | $<7.0 \times 10^5$ CFU (high) | Organ transplant | Unknown |
| UC-111 | Brain | $<5.0 \times 10^5$ CFU (high) | Nothing | Cured |
| UC-129 | Brain | $>5.0 \times 10^6$ CFU (intermediate) | SLE[c] | Fatal |
| 14759 | ATCC[d] | $>5.0 \times 10^6$ CFU (intermediate) | Lesion (none) | Unknown |
| 19247 | ATCC | $>1.0 \times 10^8$ CFU (avirulent) | Soil (type strain) | Does not apply |

[a] Virulence was determined on the basis of acute lethality (death within 2 weeks) by a modified Reed-Muench method (3, 4) following i.v. injection of log-phase cells into BALB/c mice (in most instances, mice that would not survive were euthanized prior to actual death in accordance with animal care guidelines). The degree of nocardial virulence is defined for normal animals as follows: a 50% lethal dose ($LD_{50}$) of $<10^5$ CFU, very high virulence; an $LD_{50}$ of $10^5$ to $10^6$ CFU, high virulence; an $LD_{50}$ of $10^6$ to $10^7$ CFU, intermediate virulence; an $LD_{50}$ of $10^7$ to $10^8$ CFU, low virulence; and an $LD_{50}$ of $>10^8$ CFU, avirulent (3–5).
[b] CNS, central nervous system; CSF, cerebrospinal fluid.
[c] SLE, systemic lupus erythematosus.
[d] ATCC, American Type Culture Collection.

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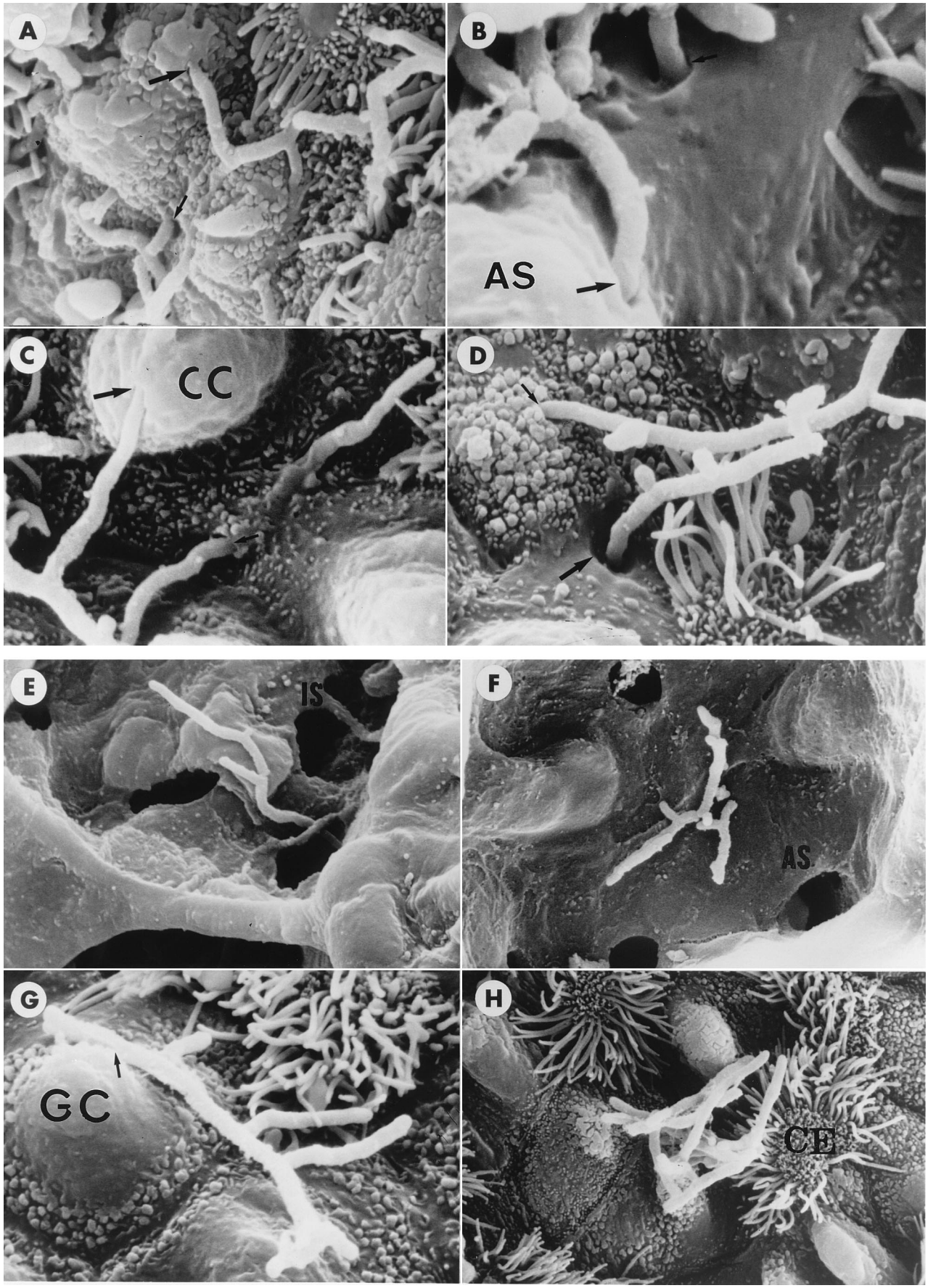
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FIG. 1. Scanning electron micrographs of log-phase cells of eight strains of *N. asteroides* (Table 1) in the lungs of BALB/c mice 3 h after intranasal administration. (A) UC-111; (B) UC-44; (C) GUH-2; (D) UC-59; (E) UC-129; (F) UC-34; (G) UC-63; (H) ATCC 19247. All of the nocardial cells shown in panels A to H have approximately the same filament diameter of 0.4 to 0.6 μm. The arrows in panels A to D point to areas of penetration of the host cell by the nocardial filament. The arrow in panel G indicates longitudinal adherence of a nocardial filament to a goblet cell. Abbreviations: AS, alveolar surface; CC, Clara cell; CE, ciliated epithelial cell; GC, goblet cell; IS, interalveolar septum.

of the inocula was determined by performing viable plate counts. At 3 h after intranasal administration, the mice were sacrificed by Nembutal overdose. Their lungs were then lavaged three times with 1.5 ml of Hank's balanced salt solution to remove nonadherent bacteria and perfused with 2.5% glutaraldehyde in cacodylate buffer, after which sections of the mid-left lobe were cut, dehydrated, critical point dried with $CO_2$, mounted on stubs, coated with gold, and photographed by scanning electron microscopy.

The relative abundance of binding, degree of either apical or longitudinal binding, types of host cells binding nocardiae, and evidence of cellular penetration were evaluated by scanning electron microscopy for each strain (Fig. 1). *N. asteroides* UC-111, UC-44, GUH-2, and UC-59 bound to and penetrated goblet cells, Clara cells in terminal bronchioles, the alveolar surface, and the interalveolar septum (Fig. 1A to D). These bacteria neither adhered to nor penetrated into ciliated epithelia (Fig. 1). In contrast, strains UC-129, UC-34, and UC-63 (Fig. 1E to G) adhered to alveolar surfaces primarily, but these bacteria were not observed to penetrate into any cell type within the lung. Strains UC-33 and ATCC 14759 appeared the same as shown in Fig. 1F; however, cells of strain ATCC 14759 showed greater adherence within the lungs than strains UC-33, UC-34, UC-63, UC-129, UC-59, and ATCC 19247 (Fig. 2). The adherence of each of these strains was significantly lower than that of GUH-2 and UC-111 (Fig. 2). Cells of the avirulent reference strain ATCC 19247 were occasionally observed to be associated with ciliated epithelial cells, but penetration was not observed (Fig. 1H) and relatively few bacteria were recovered from the lungs, which indicated a general lack of adherence (Fig. 2). Stationary-phase cells (coccobacillary cells) of these strains of nocardia did not show a binding pattern similar to that of log-phase cells, and none of them were observed to penetrate into pulmonary cells (data not shown).

A comparative analysis of the relative binding in the murine brain of log-phase cells from the same strains of *N. asteroides* as described above was performed (Table 1). Most clinical isolates selected for this study were from the human brain, and at the log phase of growth, all of them had similar morphologies (size and shape). Therefore, only if there were binding sites for the brain on the surfaces of some strains of *N. asteroides* but not on others would the numbers of CFU per brain following administration of a standardized inoculum be different. *N. asteroides* cells were grown to mid-log phase and prepared as described above, and groups of mice (four mice per group) were injected i.v. with approximately $10^6$ CFU per mouse. At 3 h after injection, the mice were anesthetized and perfused through the heart with 20 ml of sterile phosphate-buffered saline to remove blood and nonadherent bacteria from the brain (11); the brains were then transferred to preweighed tubes containing sterile water, weighed, homogenized, and plated on Trypticase soy agar. The CFU from each brain were enumerated, and the numbers were normalized to an inoculum size of $10^6$ CFU per mouse.

Figure 3 shows that some strains of *N. asteroides* differed in their adherence properties within the murine brain. For example, the numbers of CFU of strains GUH-2, UC-63, and UC-111 that bound within the brain were not different from each other ($P > 0.05$); however, they were significantly different from those of strains UC-33, UC-59, ATCC-14759, and ATCC-19247 ($P < 0.01$).

Among the strains studied, *N. asteroides* UC-63 demonstrated the highest degree of adherence in the brain (Fig. 3). In contrast, strain UC-63 neither bound apically to nor penetrated into pulmonary cells; however, some of these bacteria

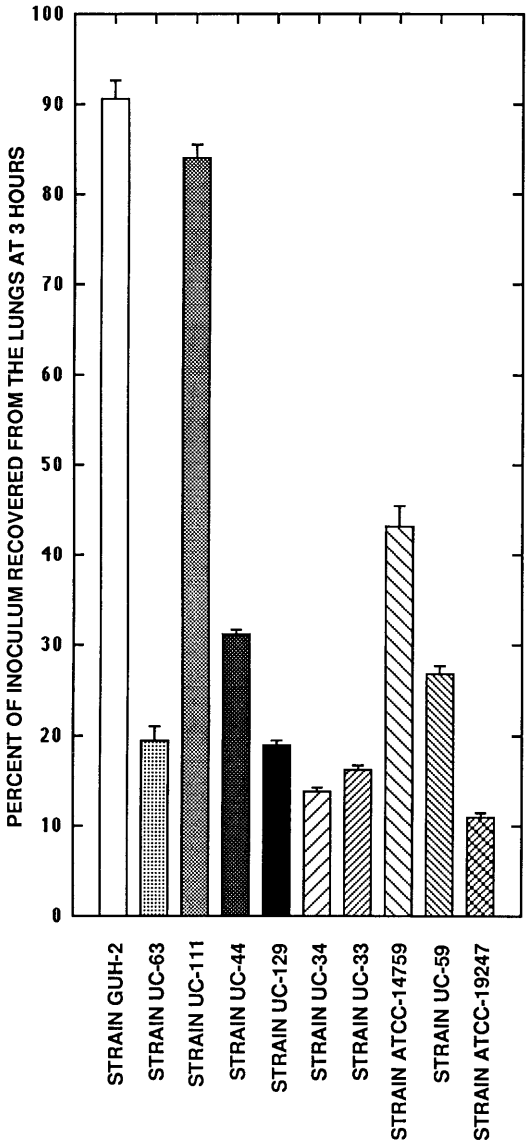


FIG. 2. Binding of 10 strains of *N. asteroides* (Table 1) in the murine lung 3 h after intranasal administration of approximately $1 \times 10^6$ to $2.5 \times 10^6$ CFU of log-phase cells. The lungs were lavaged three times with 1.5 ml of minimal essential medium containing 10% fetal calf serum to wash out nonadherent bacteria, removed from the thorax, placed in 3.0 ml of sterile distilled water, and homogenized, and dilutions were plated on brain heart infusion agar. The percentage of inoculum was calculated by the equation (CFU recovered from lung) ÷ (CFU in inoculum) × 100. The bars represent the standard error ($n = 6$).

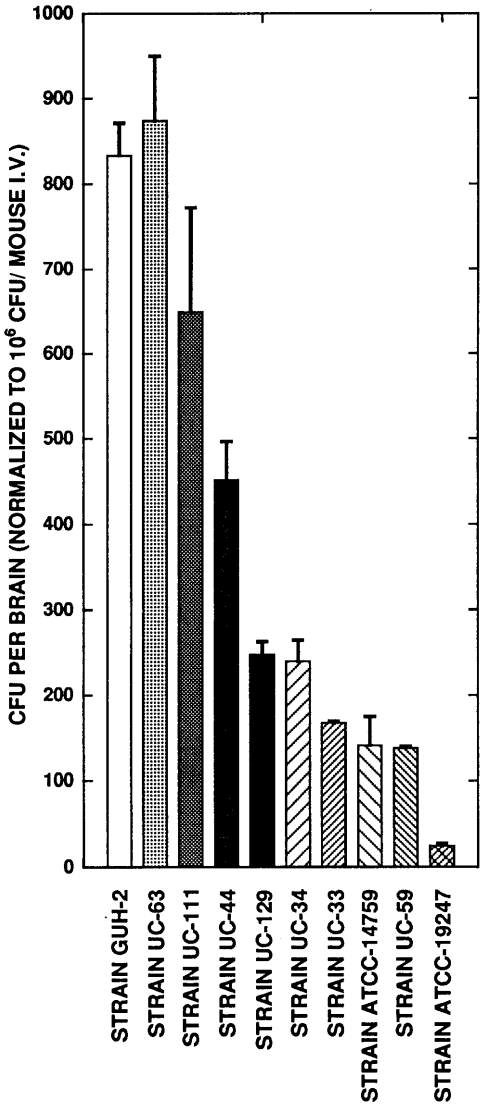


FIG. 3. Binding of 10 strains of *N. asteroides* (Table 1) in the murine brain 3 h after i.v. injection of approximately $10^6$ CFU of log-phase cells. The data were normalized to $10^6$ CFU per mouse. The bars represent the standard error ($n$ = 4).

did adhere longitudinally to goblet cells (compare Fig. 1G, 2, and 3). Even though strain UC-129 bound moderately well in the brain (Fig. 3) and grew significantly more rapidly ($P < 0.01$) than any of the other isolates tested (data not shown), this strain bound poorly in the lungs and did not grow there. Strain UC-129 was not observed to penetrate into pulmonary cells (compare Fig. 1E, 2, and 3). On the other hand, strain UC-59 adhered poorly in the brain (Fig. 3) yet had the same penetration properties as GUH-2 in the lungs (compare Fig. 1C and D). Strains GUH-2 and UC-111 bound readily in both the brain and lungs and showed prominent adherence to and penetration of cells in the brain (5) as well as the lungs (compare Fig. 1A and C, 2, and 3).

*Editor:* D. H. Howard

On the basis of these results in combination with data from the analysis of 65 additional strains, nocardial isolates could be divided into at least four groups. Group 1 was composed of nocardiae that adhered to and invaded both the brain and lungs (43.1%; 28 of 65 strains tested). These isolates were usually virulent for mice following i.v. injection. Group 2 consisted of nocardiae that adhered to and invaded the brain but not the lungs (8 of 65 strains tested), and they were usually of intermediate virulence for mice. Group 3 nocardiae were those that invaded the lungs but not the brain (14 of 65 strains tested). These isolates generally exhibit significantly decreased virulence for mice. Finally, group 4 nocardiae were those that did not adhere to or invade either the brain or lungs (15 of 65 strains tested). Isolates in this group were either avirulent or had low virulence following i.v. injection into mice. These data indicate that there are probably different ligands on the nocardial surface for adherence to and penetration of host cells in the brain and in the lungs. Furthermore, the differential ability to invade either the lungs or the brains of mice usually corresponded with the relative virulence for mice, but the source of the original clinical isolate appeared not to be as important. Nevertheless, neuroinvasive strains appear to be more acutely virulent for animals than other nocardiae (1). Since binding of nocardiae to the brain requires interactions with endothelial cells, whereas the interactions in the lungs involve epithelial cells, it is reasonable to postulate that different surface receptors in these two regions of the body may be exploited by the nocardiae for attachment. The recognition and identification of these host cell surface receptors should permit a better understanding of the basic mechanisms by which facultatively intracellular pathogens, such as mycobacteria and nocardiae, target specific regions of the body.

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

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