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# Site-Specific Growth of *Nocardia asteroides* in the Murine Brain

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**The growth of *Nocardia asteroides* GUH-2 and two mutants (NG-49 and I-38-syn) in regions of the brains of BALB/c mice was determined by microdissection and viable counting. GUH-2 grew throughout the murine brain but at different growth rates that depended on the specific location. The rate of increase in total CFU per brain during GUH-2 infection was unaffected by the inoculum size; however, in five of eight brain regions, an alteration in the inoculum size resulted in altered nocardial growth rates. Mutant NG-49 showed a significantly slower rate of increase in total CFU per brain than did the parental strain, GUH-2, and significantly decreased growth rates in seven brain regions. Mutant I-38-syn showed a rate of increase in total CFU per brain similar to that of the parental strain; however, this mutant grew significantly faster in the cerebellum and pons-medulla. Growth appeared to be a necessary precursor to the cellular damage that resulted in the variety of neurological disorders observed in mice infected with *N. asteroides* GUH-2, because mutant NG-49 exhibited a decreased ability to grow in specific regions of the brain and did not induce signs of neurological damage. In contrast, mutant I-38-syn induced neurological signs in a larger percentage of the infected animals than did parental strain GUH-2 and grew better in certain regions of the brain than did the parental strain. Furthermore, there appeared to be a relationship between the growth of *N. asteroides* in the substantia nigra and the induction of an L-dopa-responsive head shake that was observed in some of the mice following a sublethal intravenous injection of *N. asteroides* GUH-2.**

In 1891, Eppinger (14) reported a case in which a 52-year-old man developed meningitis, a cerebral abscess, and left-sided hemiplegia during infection with the organism *Cladothrix asteroides*. This is the earliest reported case of central nervous system involvement during infection with the bacterium that is now known as *Nocardia asteroides*. The predilection of this organism for the brain as a secondary site of infection has been reported with a frequency of at least 23% for metastasis following pulmonary infection (24). Despite this frequency, *N. asteroides* has been perceived as an unusual and opportunistic pathogen of limited significance (1, 26). Thus, very little is known about the interactions of *N. asteroides* with the host brain.

Recently, a murine model for *N. asteroides*-induced neurological disorders was reported (19). Neither an inflammatory response nor brain abscess formation was observed, contrary to the disease process recognized in humans, in which a brain abscess is considered a hallmark. Although most reported human cases involve abscess formation (11), there are numerous cases in which there are none (9, 10). As a result, it is not unreasonable to suggest that the murine model reflects a similar, but unrecognized, disease process in humans. Thus, in the absence of an inflammatory response, the presence of the organism would be overlooked. As a consequence, the numerous neurological manifestations that are known to be caused by nocardial infections in humans (23) could be misdiagnosed. Therefore, a significant percentage of patients who have been diagnosed as having neurological diseases caused by unknown etiologies may be suffering from either an active or a previous nocardial infection.

The purpose of this investigation was to examine site-specific nocardial growth during infection of the murine brain. We determined the characteristics of growth of *N. asteroides* GUH-2 in eight brain regions. In addition, two mutants of GUH-2 were studied, and their growth was compared with that of the parent.

## MATERIALS AND METHODS

**Microorganisms.** *N. asteroides* GUH-2 was isolated from a kidney of a patient with a fatal infection at Georgetown University Hospital, Washington, D.C. Its pathogenesis has been studied extensively (4). Mutants of GUH-2, designated I-38-syn and NG-49, were selected. Their characteristics have been presented elsewhere (23). Mutant I-38-syn was shown to induce an L-dopa-responsive head shake in mice at a significantly higher frequency than the parental strain, GUH-2 (i.e., 58 versus 10% at comparable nonlethal doses). Furthermore, when injected with nonlethal doses, a higher percentage of the mice infected with I-38-syn (94%) than with GUH-2 (40%) developed visible neurological signs. Mutant NG-49 is a pigmentless mutant of GUH-2; it is relatively avirulent and does not induce an L-dopa-responsive head shake in mice. Previous studies showed that these two mutants exhibit a decreased level of adherence in regions of the murine brain (23).

Frozen stocks of GUH-2 and NG-49 were prepared from 5-day-old cultures in brain heart infusion (BHI) broth, and I-38-syn was grown from 5-day-old cultures in MI(200), a minimal medium containing 0.5% glutamate and supplemented with 200 μg of isoniazid per ml. The stock cultures were centrifuged at 55 × *g* for 5 min, and the supernatants were mixed 1:1 with 2% skim milk in sterile, capped polystyrene tubes and stored at −70°C until used.

**Animals.** Female BALB/c mice weighing 18 to 20 g were obtained from Simonsens (Gilroy, Calif.) or Bantin & Kingman (Fremont, Calif.). Following infection, the mice were kept in a climate-controlled animal room and provided food and water ad libitum. They were maintained by the Animal Resource Service, University of California, Davis.

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**Inocula.** Inocula were prepared as previously described (23). In brief, 50-ml aliquots of BHI broth were inoculated with 0.1 ml of either GUH-2 or NG-49 frozen stock cultures and incubated at 37°C with rotational agitation (150 rpm) until the cells were in the log phase (16 to 18 h for GUH-2 and 40 h for NG-49). Following incubation, the cells were centrifuged at 55 × *g* for 5 min to produce a single cell suspension; the cell concentrations were estimated by measuring the optical density at 580 nm (an optical density of 0.10 is approximately $10^7$ CFU/ml), and all dilutions were made in fresh BHI broth. When necessary, cells were concentrated by centrifugation (1,400 × *g*, 5 min) and resuspended in fresh BHI broth. All cell concentrations were quantitated by plate counting on tryptic soy agar (Difco, Detroit, Mich.).

Inocula from cells grown in a minimal medium containing 0.5% glutamate (MMG) were prepared as follows. Starter cultures of I-38-syn in MI(200) and GUH-2 in MMG were prepared from frozen aliquots. After 4 days of incubation (37°C, 150 rpm), the cells were centrifuged (55 × *g*, 5 min), and the supernatants were used to inoculate 50 ml of fresh MMG. The cells were incubated for 25 to 27 h at 37°C. On the basis of growth curves, these cells were determined to be in the mid-log phase. Log-phase cells were collected in a sterile, disposable filtration unit with a 0.45-μm-pore-size cellulose nitrate membrane. The cells from the filter were resuspended in fresh MMG to produce a 100-fold concentration relative to that of the original culture. The resultant suspensions were centrifuged at 55 × *g* for 2 min to produce suspensions of single cells in the supernatants. The $A_{580}$ of each inoculum was adjusted with fresh MMG, and dilutions were plated on tryptic soy agar for viable counting.

**Time course.** Mice were injected intravenously with 0.10 ml of inoculum. Mice infected with GUH-2 grown in BHI broth were inoculated with either a nonlethal dose ($5 \times 10^5$ CFU per mouse) or a lethal dose ($2 \times 10^6$ CFU per mouse). MMG-grown GUH-2 was used at a dose of $8 \times 10^5$ CFU per mouse. Mice infected with NG-49 and I-38-syn received nonlethal inocula that contained approximately $2 \times 10^6$ CFU per mouse. Mice were sacrificed at 3, 24, 48, 72, and 168 h postinjection for all groups, except the mice that received the lethal dose of GUH-2. These mice were sacrificed at 3, 24, and 36 h postinjection.

**Microdissection.** Brains were collected and treated as described previously (23).

**Statistical analysis.** The rates of increase were determined as the slopes of the lines defined by the CFU counts at 3 and 24 h. Since bacterial growth is geometric rather than arithmetic, the CFU counts were converted to logarithms by use of the equation $x' = \log(x + 1)$, in which $x$ is the CFU count; this transformation linearizes the data so that the rates of increase can be correctly determined and allows the inclusion of zero values in the analysis (28). Linear regression of the transformed CFU counts was performed to determine the slopes; this method was chosen because it includes the variability of the values at both 3 and 24 h rather than just that of the 24-h values, as would be the case if the number of doublings were used. The calculated slopes were compared by use of Student's *t* test for the difference between two slopes (28). A *P* value of less than 0.05 was considered significant.

## RESULTS

**Growth in the murine brain of BHI broth-grown *N. asteroides* GUH-2.** The total number of CFU per brain versus time following both nonlethal and lethal injections of GUH-2 grown in BHI broth is presented in Fig. 1A. After a nonlethal injection, the counts in the brain increased rapidly during the first 24 h and then gradually decreased (Fig. 1A). This same growth pattern was seen in each region of the microdissected brain (Fig. 2A; data for regions other than the cerebellum are not shown). In contrast, after a lethal injection, the number of CFU in the brain rose rapidly during the 36-h time course, after which the animals began to die.

At 24 h after infection, the total CFU in the entire murine brain for both the nonlethal and the lethal infections had increased by 26-fold (approximately five doublings) above the inoculum levels at 3 h postinjection (Fig. 1A). No significant differences between the rates of growth were observed with these two doses. Since the lag phase of growth under these conditions was about 8 h (2), the doubling time for GUH-2 in the brain was approximately 3 h. Even though there were no differences in the rates of growth of GUH-2 when averaged throughout the entire brain, examination of the rates of growth in specific regions of the brain revealed that during a lethal infection growth in the substantia nigra ($P < 0.02$), cerebellum ($P < 0.001$), hypothalamus ($P < 0.001$), and pons-medulla ($P < 0.005$) was significantly more rapid than that during a nonlethal infection (Table 1). However, during a nonlethal infection, the nocardial rate of growth in the cortex was significantly increased ($P < 0.001$). Since the cortex had the highest proportion of nocardial cells in the brain, the faster rate of growth in the cortex during a nonlethal infection offset the increased rates of growth in the substantia nigra, cerebellum, hypothalamus, and pons-medulla during a lethal infection.

**Growth in the murine brain of MMG-grown *N. asteroides* GUH-2.** The effect of culture media on the growth of *N. asteroides* GUH-2 in the murine brain was evaluated by growing GUH-2 in a chemically defined mineral salts medium with glutamate as the sole source of carbon (MMG) and comparing these cells with those grown in complex BHI broth (Fig. 1). The total CFU per brain over a period of 168 h following injection of $8 \times 10^5$ CFU of MMG-grown GUH-2 differed from that following injection of $5 \times 10^5$ CFU (nonlethal dose) of BHI broth-grown GUH-2 (Fig. 1). Rather than a linear decrease in the number of CFU from 24 h to 7 days, like that seen with BHI broth-grown cells, the number of CFU of GUH-2 grown in MMG remained constant for 72 h and then gradually decreased (Fig. 1). With the exceptions of the substantia nigra and the cerebellum, the growth curves for each brain locale were identical to that for the entire brain. In the cerebellum, the cell counts remained relatively constant for 1 week after the initial growth at 24 h (Fig. 2B). In contrast, the behavior in the substantia nigra was extremely variable (data not shown). The rates of growth in the brain during the initial 24 h after infection were essentially the same for both MMG- and BHI-broth grown GUH-2 cells. However, a comparison of regional growth rates for MMG-grown GUH-2 and BHI broth-grown GUH-2 resulted in the finding that MMG-grown cells grew significantly faster ($P < 0.05$) in the hypothalamus (Table 1). No differences were observed among the rates of growth in the other regions of the brain.

**Growth of mutant NG-49 in the murine brain.** Strain NG-49 is a mutant of *N. asteroides* GUH-2. This mutant shows decreased virulence and decreased attachment in the brain and does not induce any significant neurological signs in mice (23). Following intravenous injection of $2 \times 10^6$ CFU of strain NG-49, only a few organisms were deposited in the brain by 3 h (Fig. 1A). Furthermore, this organism grew

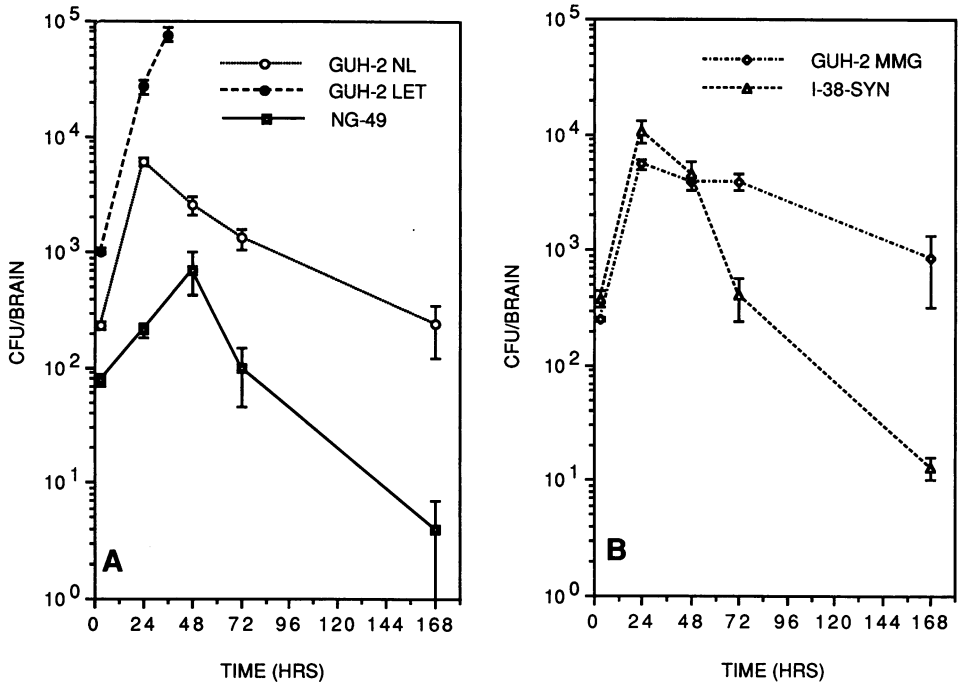


FIG. 1. Total colonies per brain over time during infection with a nonlethal inoculum of GUH-2 (NL), a lethal inoculum of GUH-2 (LET), or NG-49 (A) and during infection with MMG-grown GUH-2 or I-38-syn (B). Values are presented as the average CFU per brain ± the standard error of the mean. (Each point represents an average for 10 mice, with the exception of those for MMG-grown GUH-2, which represent an average for 5 mice). The inoculum used for GUH-2 LET, NG-49, and I-38-syn was $2 \times 10^6$ CFU per mouse, and the inocula used for GUH-2 NL and MMG-grown GUH-2 were $5 \times 10^5$ and $8 \times 10^5$ CFU per mouse, respectively.

slowly for the first 48 h and then was rapidly removed from the brain (Fig. 1A). Neither the initial increase nor the final decrease in CFU in the brain paralleled those seen with the parental strain. The regional patterns of growth of NG-49 seen during infection were similar to the patterns of growth seen for the total brain (Fig. 1A and 2A). The most dramatic difference in the regional growth patterns was the total absence of CFU in the substantia nigra at 24 h of infection.

In sharp contrast to the results for parental strain GUH-2, the total counts of NG-49 in the brain during the first 24 h of infection increased only threefold. There were significant differences between the rates of increase in CFU at 24 h after infection for NG-49 and both nonlethal ($P < 0.001$) and lethal ($P < 0.001$) doses of GUH-2. A comparison of the regional growth rates revealed that only in the hypothalamus did a nonlethal dose of GUH-2 show a growth rate similar to that for NG-49 (Table 1). The rates of growth of NG-49 in all other regions of the brain were significantly lower ($P < 0.05$) than those found for parental strain GUH-2 (Table 1).

**Growth of mutant I-38-syn in the murine brain.** Strain I-38-syn is a mutant of *N. asteroides* GUH-2 that shows decreased virulence (based on 50% lethal doses) but induces significantly more neurological signs when injected into mice than does the parental strain. Following an intravenous injection of $2 \times 10^6$ CFU of I-38-syn, about $4 \times 10^2$ CFU bound in the brain. During the first 48 h after infection, the total number of CFU per brain increased and then began to decrease at a rate similar to those for nonlethal doses of both GUH-2 grown in BHI broth and GUH-2 grown in MMG (Fig. 1). After 48 h, I-38-syn was removed from the brain more rapidly than the parent. The growth curves for I-38-syn in different regions of the brain were similar to those for I-38-syn in the entire brain (Fig. 1B and 2B). The rate of growth of I-38-syn during the first 24 h after infection in the entire brain was similar to those of BHI broth-grown and MMG-grown GUH-2. Thus, the counts of the mutant increased 28-fold in the brain during this time period (Fig. 1). However, comparisons of different regions of the brain demonstrated that localized rates of growth of mutant I-38-syn were significantly faster than those of the nonlethal dose of GUH-2 in the cerebellum ($P < 0.005$) and the pons-medulla ($P < 0.05$) (Table 1), while comparisons with the rates of growth of GUH-2 in MMG showed that I-38-syn grew faster only in the cerebellum ($P < 0.05$).

**Comparison of growth in the substantia nigra.** The results of Kohbata and Beaman (19) suggested a probable relationship between damage in the substantia nigra and the induction of the L-dopa-responsive movement disorder following sublethal infection with *N. asteroides*. For this reason, we compared the patterns of growth of I-38-syn, NG-49, and BHI broth-grown GUH-2 (nonlethal dose) in this region. Since the substantia nigra regions of fewer than 100% of the mice were infected at 3 h after injection of each inoculum, the zero values were not averaged into the mean counts of CFU that were used for this comparison, unless all counts at a given time point were zero. By manipulating the data in this manner, it was possible to examine the variability of growth that does occur without the influence of values from uninfected substantia nigra regions. Growth curves produced from these non-zero values are presented in Fig. 3. During infection with I-38-syn, the numbers of CFU in the substantia nigra at 24 h were higher and significantly less variable than those obtained during infection with a nonlethal dose of GUH-2 grown in BHI broth. Since the standard deviation of growth in the substantia nigra was large for GUH-2 (Fig. 3B), the much smaller variation of growth seen

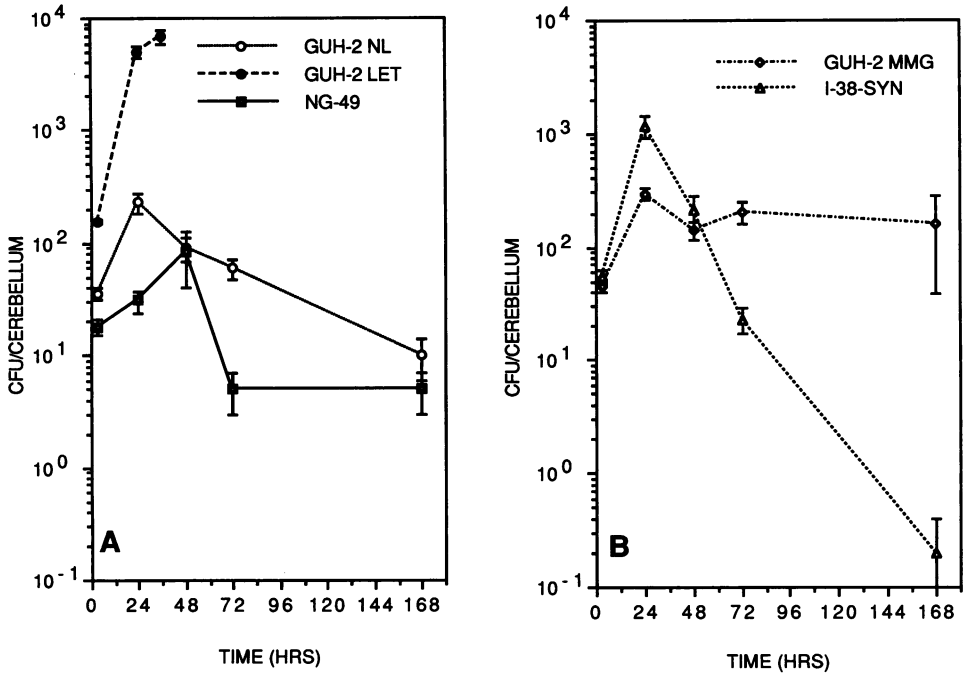


FIG. 2. Growth in the cerebellum for a nonlethal inoculum of GUH-2 (NL), a lethal inoculum of GUH-2 (LET), and NG-49 (A) and for GUH-2 grown in MMG and I-38-syn (B). Zero counts were set at 0.1. Values are presented as the average CFU per cerebellum ± the standard error of the mean. (Points for MMG-grown GUH-2 represent the average values for 5 mice; all other points represent the average values for 10 mice). Doses were as described in the legend to Fig. 1.

with mutant I-38-syn may be critical in interpreting the different frequencies of induction of the L-dopa-responsive head shake in mice (i.e., I-38-syn induced 58% head shake, whereas GUH-2 induced only 10% head shake, at comparable nonlethal doses). In contrast, at 24 h, NG-49, which does not induce a head shake, could not be isolated from the substantia nigra of eight mice.

## DISCUSSION

In this study, the characteristics of growth of *N. asteroides* GUH-2 and two mutants, NG-49 and I-38-syn, in the murine brain were examined. Comparisons of both the total amount of growth and the rates of growth of GUH-2 in specific regions of the brain during nonlethal and lethal infections were made. The data demonstrated that different inocula resulted in the same rates of increase in CFU in the entire brain; however, there were significant differences within specific localized regions of the brain. The variability of the growth rates in response to the altered inocula may have been a reflection of regional differences in the immune defenses in the brain. Astrocytes have been proposed to promote immune reactivity in the brain through their ability to serve as antigen-presenting cells (27). In addition, it has been reported that subpopulations of human astrocytes possess different antigen-presenting capabilities and that, for rats, numbers of type 1 and type 2 astrocytes vary from one brain region to another (27). Thus, an increased inoculum may have a significant effect on the nocardial growth in one region of the murine brain and little effect in another.

The comparison of GUH-2 with NG-49 and I-38-syn provided insights into the significance of regional growth in the brain to the induction of specific neurological signs. As noted previously, NG-49 produced a low frequency of neurological disorders in infected mice (23). This mutant grew significantly more slowly and to lower levels than the parental strain in all regions of the brain. These observations suggest that nocardial growth was a necessary precursor of

TABLE 1. Comparisons of regional rates of increase determined from 3- and 24-h CFU counts[a]

| Inoculum | Rate of increase ± SE in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CERE | SN | HIPPO | STR | HYPO | COR | P-M | M-B |
| GUH-2 (in BHI broth, nonlethal) | 3.3 ± 0.6 | 1.8 ± 0.8 | 7.5 ± 0.5 | 5.2 ± 0.7 | 2.6 ± 1.3 | 7.3 ± 0.2 | 5.2 ± 0.3 | 6.8 ± 0.3 |
| GUH-2 (in BHI broth, lethal) | 7.0 ± 0.3[b] | 4.9 ± 0.8[b] | 6.6 ± 0.3 | 4.6 ± 0.4 | 7.4 ± 0.4[b] | 6.1 ± 0.3[b] | 6.4 ± 0.2[b] | 7.0 ± 0.2 |
| GUH-2 (in MMG) | 3.8 ± 0.3 | 2.5 ± 1.1 | 6.2 ± 0.5 | 6.2 ± 0.4 | 6.0 ± 1.1[b] | 6.7 ± 0.3 | 6.0 ± 0.5 | 6.6 ± 0.4 |
| NG-49 | 1.0 ± 0.5[b] | −0.3 ± 0.2[b] | 2.1 ± 0.3[b] | 0.02 ± 1.0[b] | 1.0 ± 0.8 | 2.4 ± 0.5[b] | 1.8 ± 0.6[b] | 1.9 ± 0.6[b] |
| I-38-syn | 5.9 ± 0.6[b] | 3.0 ± 1.1 | 6.0 ± 1.2 | 5.1 ± 1.1 | 4.9 ± 1.3 | 6.8 ± 0.5 | 6.6 ± 0.5[b] | 6.3 ± 0.7 |

[a] Regional rates of increase (change in log CFU over time) for GUH-2 (in BHI broth, lethal), GUH-2 (in MMG), NG-49, and I-38-syn were compared with those for GUH-2 (in BHI broth, nonlethal). Abbreviations for regions: CERE, cerebellum; SN, substantia nigra; HIPPO, hippocampus; STR, striatum; HYPO, hypothalamus; COR, cortex; P-M, pons-medulla; M-B, midbrain.

[b] $P < 0.05$.

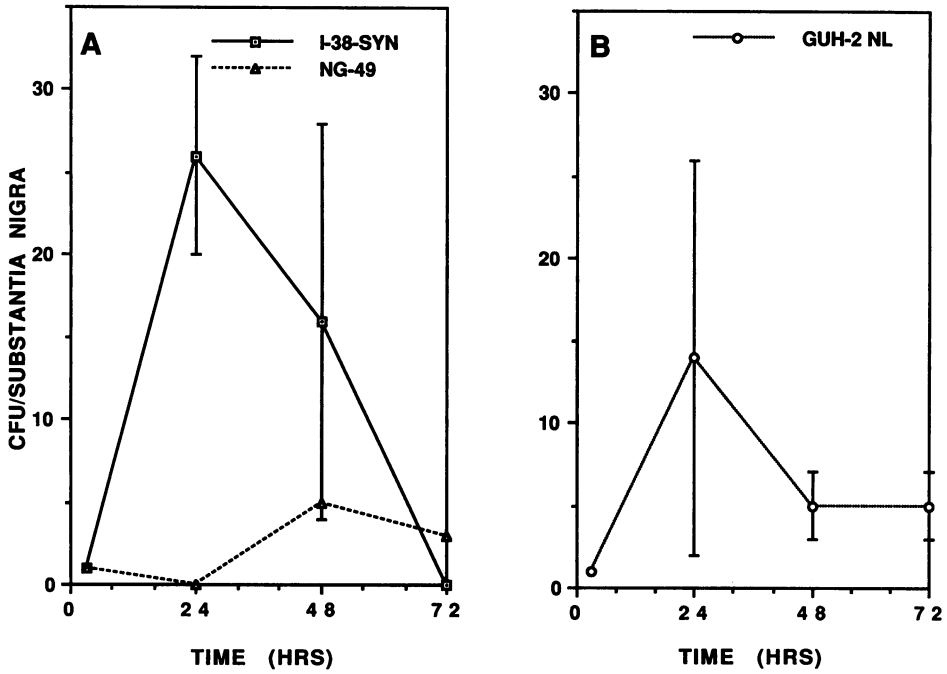


FIG. 3. Growth in the substantia nigra for I-38-syn and NG-49 (A) and for a nonlethal inoculum of GUH-2 (NL). Values are presented as the average CFU per substantia nigra ± the standard error of the mean. Values were determined from non-zero values (zero values were used only when there were no non-zero values for a time point). (The number of mice that each point represents is variable because of the use of non-zero values). Doses were as described in the legend to Fig. 1.

the induction of sufficient cellular damage to result in the movement disorders apparent in the infected mice. A comparison of the regional rates of growth of I-38-syn with those of a nonlethal dose of GUH-2 demonstrated that this mutant grew faster than the parent in both the cerebellum and the pons-medulla. It is known that a variety of motor dysfunctions can occur as a result of cerebellar damage (16). These include dysmetria, dysdiadochokinesia, ataxia, and tremors. In addition, cerebellar function is influenced by projections from the pons, such as those from the locus coeruleus (13, 22). Therefore, the enhanced growth of I-38-syn in the cerebellum and pons-medulla suggests that these regions were significant in the higher frequency of neurological signs induced by this mutant in the murine model used here.

The results from earlier studies of GUH-2 may be significant to our current findings. For example, the poor growth of NG-49 in the brain and the rapid removal of I-38-syn from the brain may have been the results of altered resistance to destruction by phagocytic cells such as microglia (17). Although it is not known how refractory *N. asteroides* GUH-2 is to destruction by these phagocytes, various cellular components (3, 5) and capabilities of the organism (8, 12) have been reported to be significant to its interactions with other phagocytic cells. These factors probably play a role in nocardial interactions with microglia as well; therefore, NG-49 and I-38-syn may have lost one or more of these protective properties. Additionally, studies of nocardial interactions with phagocytic cells have led to the discovery that GUH-2 can utilize acid phosphatase, a lysosomal enzyme, as a sole carbon source and that the addition of acid phosphatase to glutamate-containing media enhances nocardial growth (6). Histochemical studies have shown that acid phosphatase is present in the brain and that its distribution is variable at both the structural and the cellular levels (15, 21, 25). This variability of the levels of acid phosphatase may be related to the regional variability of nocardial growth.

The temporary stoppage of the head shake signs in mice following injections of L-dopa suggested that this movement disorder was the manifestation of a dysfunction in the dopaminergic neurons of the basal ganglia. Kohbata and Beaman (19) demonstrated histopathological alterations in the substantia nigra of mice manifesting this abnormality. I-38-syn induces a higher frequency of the L-dopa-responsive movement disorder and appears to attach to the substantia nigra more efficiently than does parental strain GUH-2 (23). Examination of the counts in the substantia nigra at 24 h postinjection indicated that the level of growth of I-38-syn was higher and significantly less variable than that of the parental strain. In contrast, NG-49, which does not induce the L-dopa-responsive movement disorder, was not isolated from the substantia nigra of infected mice at 24 h postinjection. Therefore, the ability to grow in the substantia nigra and the ability to induce the movement disorder appear to be related, and it is possible that in mice infected with I-38-syn, the greater extent of growth in this region led to the higher frequency of induction of the L-dopa-responsive head shake that occurred as a result of infection with I-38-syn.

The significant variability noted when the CFU at 24 h during a nonlethal infection with GUH-2 were compared with those during a comparable I-38-syn infection was due to the presence of a single increased value in one mouse in the GUH-2 group. When this value (85 CFU) was removed, the average CFU per substantia nigra for GUH-2 decreased from 14 to 2 and the standard deviation decreased from 31 to 1. These values indicated a significant difference in growth between GUH-2 and I-38-syn ($p < 0.002$). The significant variability that was created by the inclusion of this value

indicated that during a nonlethal infection with GUH-2 the neurological disorders due to damage in the substantia nigra should occur at a low frequency. These data were consistent with the reported low frequency of induction (10%) of the L-dopa responsive head shake during a nonlethal infection with GUH-2. Furthermore, these observations support the hypothesis that the substantia nigra is the major region of the brain involved in the production of the head shake signs (18). Therefore, the increased frequency of the L-dopa-responsive head shake induced in the mice infected with I-38-syn is related to the increased predilection for the substantia nigra and the greater consistency of growth of the mutant in this region. The involvement of the substantia nigra in this murine model suggests that the origin of the L-dopa-responsive movement disorder may be analogous to that of the tremors observed in humans with Parkinson's disease (i.e., damage in the substantia nigra [7, 20]).

The results of this study have shown that *N. asteroides* GUH-2 has the ability to grow in all regions of the brain, thus explaining the wide variety of *N. asteroides*-induced movement and psychological disorders in humans (23) that are reported in the literature. In addition, the selective growth of GUH-2 and its mutants, NG-49 and I-38-syn, in regions of the murine brain, resulting in a variety of visible neurological deficits, provides a model for analyzing the mechanisms of regional damage in these movement disorders.

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## REFERENCES

1. **Adair, J. C., I. J. Amber, and J. M. Johnston.** 1987. Peritonsillar abscess caused by *Nocardia asteroides*. J. Clin. Microbiol. **25:**2214–2215.
2. **Beaman, B. L.** Unpublished data.
3. **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.
4. **Beaman, B. L., and S. E. Moring.** 1988. The relationship among cell wall composition, stage of growth, and virulence of *Nocardia asteroides* GUH-2. Infect. Immun. **56:**557–563.
5. **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.
6. **Beaman, L., M. Palieschekey, 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.
7. **Bergman, H., T. Wickmann, and M. R. DeLong.** 1990. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science **249:**1436–1438.
8. **Black, C. M., M. Palieschekey, B. L. Beaman, R. M. Donovan, and E. Goldstein.** 1986. Acidification of phagosomes in murine macrophages: blockage by *Nocardia asteroides*. J. Infect. Dis. **154:**952–957.
9. **Buggy, B. P.** 1987. *Nocardia asteroides* meningitis without brain abscess. Rev. Infect. Dis. **9:**228–231.
10. **Carlile, W. K., K. E. Holley, and G. B. Logan.** 1963. Fatal acute disseminated nocardiosis in a child. J. Am. Med. Assoc. **184:** 477–480.
11. **Curry, W. A.** 1980. Human nocardiosis. A clinical review with selected case reports. Arch. Intern. Med. **140:**818–826.
12. **Davis-Scibienski, C., and B. L. Beaman.** 1980. The interaction of *Nocardia asteroides* with rabbit alveolar macrophages: association of virulence, viability, ultrastructural damage, and phagosome-lysosome fusion. Infect. Immun. **18:**610–619.
13. **Dietrichs, C.** 1988. Cerebellar cortical and nuclear afferents from the feline locus coeruleus complex. Neuroscience **27:**77–91.
14. **Eppinger, H.** 1891. Ueber eine neue pathogene *Cladothrix* und eine durch sie hervorgerufene Pseudotuberculosis. Beitr. Pathol. Anat. Allg. Pathol. **9:**287–328.
15. **Friede, R. L., and M. Knoller.** 1965. A quantitative mapping of acid phosphatase in the brain of the rhesus monkey. J. Neurochem. **12:**441–450.
16. **Gilman, S.** 1986. Cerebellum and motor dysfunction, p. 401–422. *In* A. K. Asbury, G. M. McKhann, and W. I. McDonald (ed.), Diseases of the nervous system, vol. I. William Heinemann Medical Books, Ltd., London.
17. **Giulian, D.** 1987. Ameboid microglia as effectors of inflammation in the central nervous system. J. Neurosci. Res. **18:**155–171.
18. **Higashide, E., M. Asai, K. Ootsu, S. Tanida, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino, and M. Yoneda.** 1977. Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from *Nocardia*. Nature (London) **270:**721–722.
19. **Kohbata, S. K., and B. L. Beaman.** 1991. L-Dopa-responsive movement disorder caused by *Nocardia asteroides* localized in the brains of mice. Infect. Immun. **59:**181–191.
20. **Langston, J. W.** 1987. MPTP: the promise of a new neurotoxin, p. 73–90. *In* C. D. Marsden and S. Fahn (ed.), Movement disorders 2. Butterworths & Co. Ltd., London.
21. **Manocha, S. L.** 1970. Histochemical distribution of alkaline and acid phosphatase and adenosine triphosphatase in the brain of squirrel monkey (*Saimiri sciureus*). Histochemie **21:**441–450.
22. **Moises, H. C., and D. J. Woodward.** 1980. Potentiation of GABA inhibitory action in cerebellum by locus coeruleus stimulation. Brain Res. **182:**327–344.
23. **Ogata, S. A., and B. L. Beaman.** 1992. Adherence of *Nocardia asteroides* within the murine brain. Infect. Immun. **60:**1800–1805.
24. **Palmer, D. L., R. L. Harvey, and J. K. Wheeler.** 1974. Diagnostic and therapeutic considerations in *Nocardia asteroides* infection. Medicine (Baltimore) **53:**391–401.
25. **Sethi, J. S., and R. K. Janwar.** 1989. Comparative distribution of acid phosphatase and simple esterase in the mouse and hippocampal formation. Acta Anat. **135:**323–329.
26. **Tsuboi, R., T. Yamaguchi, K. Matsuda, and H. Ogawa.** 1989. Extracellular proteinase production and the pathogenicity of *Nocardiae*. Arch. Dermatol. Res. **281:**78–80.
27. **Yong, V. W., F. P. Yong, A. Oliver, Y. Robitaille, and J. P. Antel.** 1990. Morphologic heterogeneity of human adult astrocytes in culture: correlation with HLA-Dr expression. J. Neurosci. Res. **27:**678–688.
28. **Zar, J. H.** 1974. Biostatistical analysis, p. 228–229. Prentice-Hall, Inc., Englewood Cliffs, N.J.