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Nocardia Species as an Etiologic Agent in Parkinson's Disease: Serological Testing in a Case-Control Study

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To test the hypothesis that *Nocardia* spp. may be an etiologic factor in Parkinson's disease (PD), we used a serodiagnostic panel to determine if PD patients had antibodies specific for *Nocardia* spp. To validate the serological test panel, sera from healthy volunteers and from patients with culture-proven nocardiosis (n = 307) were compared in part 1 of the study. The sensitivity of the panel was 88% for detection of culture-proven nocardial infections, and specificity was 85% (excluding cross-reactive leprosy cases). In part 2, no difference in seropositivity was found when PD patients were compared with their age- and gender-matched controls (n = 140). We found a high exposure rate of humans to nocardial antigens, especially among men and older individuals. Our results offer no support to the hypothesis that *Nocardia* spp. are causative in PD; however, it is possible that serological testing may not be optimal for detection of nocardial central nervous system infection.

The cause of Parkinson's disease (PD) is uncertain. Current etiological hypotheses center on environmental toxins (9, 22, 25) and genetic factors (6, 12). It is conceivable that multiple factors singly or in combination result in PD. Postencephalitic parkinsonism is an illness resembling PD, but it bears distinguishing features, both clinically and pathologically (26). Postencephalitic parkinsonism is considered to be a sequela of encephalitis and occurred in association with viral pandemics in the first part of this century (2); thus, it is rarely diagnosed at this time, as survivors of the exposed cohort are few in number. In more recent years, rare reports of parkinsonian syndromes following acute infectious illnesses have been made (7, 8, 11, 18); however, no clear association between acute or chronic infection and PD has been demonstrated (3–5, 16, 17, 21).

Kohbata and Beaman, in 1991, reported that a sublethal injection of *Nocardia asteroides* produces a syndrome in mice that resembles PD both clinically and pathologically (14). These findings prompted us to hypothesize that *Nocardia* spp. may be an etiological factor in PD in humans. To test this hypothesis, we used a serological test panel for nocardiosis which had recently been developed in the murine model (13). All serological testing was performed at the Department of Medical Microbiology and Immunology, University of California (Davis), by methodology previously described by Kjelstrom and Beaman (13). On the basis of test panel results, samples were considered to be positive, negative, or indeterminate. Data were analyzed by the chi-square test (Statistical Analysis Software).

Because the panel had not previously been used to test human sera, we first sought to determine its validity in healthy human subjects and individuals with nocardiosis and related infections. Of 121 healthy young subjects recruited from among staff and students at the University of California (Davis), 26 were seropositive, 72 were seronegative, and 23 were indeterminate. Sera from individuals with culture-proven nocardial infections (n = 24) were also tested. Serological testing was positive in 19 of 24 cases. Testing was negative in 2 of 16 cases of *N. asteroides* infection; in both instances, patients were immunocompromised. In three of six cases of primary brain nocardial infection, test results were negative. The sensitivity of the serological test panel was 88% for the detection of *N. asteroides* infections and 79% for nocardial infections as a whole. To test specificity, the serology panel was performed for 22 cases of nonnocardial opportunistic infection; *Mycobacterium leprae* was the etiological organism in five of seven seropositive cases. Specificity of the nocardial serology panel was 68% overall and 85% when cases of leprosy were excluded.

Sera were obtained from a study cohort of PD patients and age-matched controls as previously described (9). PD subjects did not differ from controls with regard to *Nocardia* serological status. Overall seropositivity in this study group was higher than that for the young healthy subjects initially studied (49 and 21% positivity, respectively). In an effort to account for this unexpectedly high rate of seropositivity, we examined several variables as possible predictors for *Nocardia* seropositivity in this group of subjects. A variable was selected because it either had been reported as a risk factor for PD or could theoretically influence the likelihood of *Nocardia* exposure. These variables were previously described (9) and included gender, rural living, agricultural and outdoor occupations, exposure to pesticides, smoking, alcohol use, history of encephalitis, and head trauma. Significantly more males were seropositive compared with females (Figure 1). All other variables examined were not predictive of serological status. Serological cross-reactivity of *Nocardia* and *Mycobacterium* species was postulated; therefore, information was sought from all subjects regarding tuberculosis skin test (purified protein derivative tine test) results and tuberculosis *Mycobacterium bovis* BCG vaccination exposure. No relationship between tuberculosis status and nocardial serological test results was found.

In summary, we offer evidence that serological testing may

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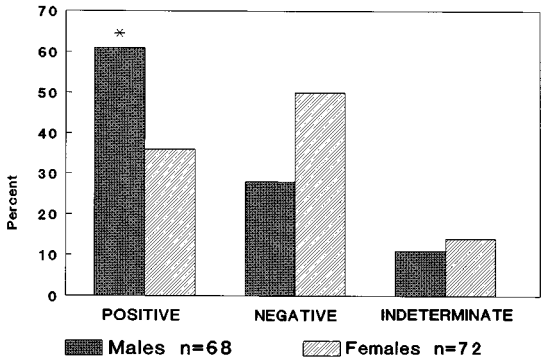

FIG. 1. Results of serological tests for *Nocardia* spp., broken down by gender. The asterisk indicates a probability of <0.05 by chi-square analysis.

provide reasonably sensitive and specific screening for *N. asteroides* exposure. The test panel identifies *N. asteroides* infections in nearly 90% of culture-proven cases. About 20% of young healthy subjects were seropositive in this study; this may represent antigenic cross-reactivity (false positivity). However, some of these subjects were strongly positive in all aspects of serological testing, suggesting occult *N. asteroides* infection. An even greater degree of seropositivity was found in the PD patients and their age-matched controls (49% of the serum samples were positive). These two study populations differed in that part 1 healthy subjects were young (>75% were less than 50 years old) and lived in California while the PD and control subjects were much older (4% were less than 50 years old) and lived in Kansas. The relatively high levels of seropositivity in the older subjects from Kansas may suggest that *Nocardia* exposure is a concomitant of increasing age or rural residency. The latter explanation is particularly intriguing since *Nocardia* spp. are soil-contained pathogens and rural living is a reported risk factor for PD (15). We specifically examined the Kansas data set for an association between seropositivity and rural residence, outdoor occupation, or other rural lifestyle features; no association was found. In addition to rural living, other variables which may have a relationship to PD or parkinsonism include age, family history, ethnicity, cigarette smoking, alcohol consumption, encephalitis, head trauma, depression, and gender (1, 9, 10, 19, 20, 24). We found no link between *Nocardia* serological status and these variables, except for gender. Significantly more males than females were seropositive. The reason for this gender difference is not apparent. It is possible that males are more likely to come in contact with *Nocardia*-containing soil or water because of occupational exposures. Interestingly, a male preponderance in PD is also reported by some authors (23), although a plausible explanation for this gender disparity in PD is lacking. A larger sample size matched for potentially confounding variables may be needed to pursue this issue further. It is also possible that serological testing does not offer the best method for examining the role of *Nocardia* spp. in a neurodegenerative disorder such as PD. The serological test panel appears to be quite useful in confirming nocardial infections in most instances; however, false negatives may occur when the immune response is lacking or when the brain is the primary infection site. A means of reliably detecting *Nocardia* spp. in postmortem brain tissue may ultimately be required to confirm or refute the proposed link between this bacterium and PD.

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