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Complement Fixation Antibody Test for Human Nocardiosis

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Complement-fixing antibody was detected in 13 of 16 patients with histological and/or culture evidence of infection with *Nocardia* species. The antigen used was a filtrate of soluble substances secreted into liquid growth medium by cultures of *N. asteroides*. Apparent false positive reactions were found in three of three patients with leprosy and two of five patients with tuberculosis—results similar to some previously reported methods. *N. asteroides* and mycobacteria share antigens. Only the false positives with tuberculosis are considered a diagnostic problem. No reactions were obtained with sera from 26 patients with other infections and 41 uninfected individuals. Whereas previous nocardia serodiagnostic methods have a sensitivity of approximately 50%, our overall sensitivity (81%) compares favorably and included 9 of 11 positive tests in immunocompromised nocardiosis patients (a source of false negative reactions with previous methods).

Nocardiae are gram-positive, partially acid-fast, branching, filamentous bacteria. There are approximately 750 human cases of invasive nocardiosis each year in the United States (1). Nocardiae are common pathogens of cattle and dogs (11). Because many human cases present as progressive pulmonary infections in compromised hosts, aggressive diagnostic efforts, often invasive, are necessary to institute early, appropriate chemotherapy (8, 9).

Serological diagnosis has been attempted in animals (12, 14, 15, 20) and humans (2, 5) with variable success. The methods have differed in antigen used (cellular, extracellular) and antibody sought (precipitin, slide agglutinin, complement-fixing). In this study with an extracellular antigen, we detected complement-fixing antibody in 81% of 16 humans infected with nocardia, including 82% of 11 immunosuppressed patients. This initial result is encouraging and suggests that this test antigen detects an antibody response in humans infected with *N. asteroides* and that the clinical implementation of this complement fixation test may facilitate the rapid, noninvasive diagnosis of a life-threatening disease.

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MATERIALS AND METHODS

The extracellular nocardia antigen was prepared as described previously (7, 13; Fig. 1). Initial efforts in testing for complement-fixing antibodies were performed by a standard macrodilution technique, and then the system was standardized in a micromethod (10), modified as indicated, for subsequent development and testing. An 18-h, overnight incubation at 4°C was utilized. The complement source was freshly thawed samples of lyophilized guinea pig complement, previously reconstituted and frozen at −70°C. An amount equal to 7.5 50% hemolytic complement units was employed. The hemolysin was a commercial preparation (Grand Island Biological Co.) of 50% glycerol-treated anti-sheep erythrocyte rabbit antiserum. Erythrocytes were obtained commercially in Alsever solution and used as a 3.0% suspension. Sera were heat inactivated at 56°C for 30 min. Dilutions were performed in Veronal-buffered saline without gelatin. The end point of serum titrations was the highest dilution showing at least 3+ complement fixation (corresponding to 30% hemolysis) (10). Appropriate complement, anticomplementary, hemolysin, and sheep erythrocyte controls were used in each run. An experimental rabbit antiserum of known titer against the extracellular antigen was included in each run. Results with this antiserum seldom varied from the known titer of 1:256 and never exceeded one dilution above or below 1:256.

Sera were obtained from 17 patients with culture-proven nocardia infection. One serum was anticomplementary. The ages of the patients ranged from 20 to 82 years. Of the 16 patients tested, 11 were male. Diagnosis was made by lung aspiration or biopsy in 11 of the 16 patients, by wound or skin culture in 3, and by sputum culture, brain biopsy, and kidney biopsy in 1 each. Of the 16 patients, 12 had documented pulmonary infection; two of these also had infections at other sites. Nine of the 16 *Nocardia* isolates were identified as *N. asteroides;* the remainder were not identified to species. We are indebted to Edward Stin-

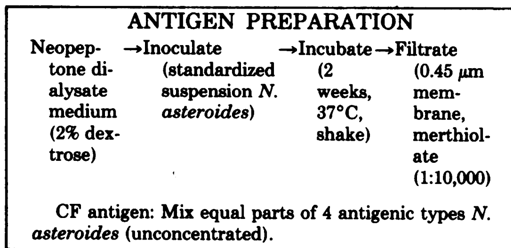

FIG. 1. *Schema for preparation of antigen (7, 13).*

son, Department of Cardiovascular Surgery, Stanford University, Stanford, Calif., and Kenneth Holmberg, National Bacteriological Laboratory, Stockholm, Sweden for many of these sera.

RESULTS

Complement-fixing antibodies at a titer of ≥1:4 were detected in 13 of 16 patients with nocardia infection. Nine of 11 immunosuppressed patients had a positive test. The distribution of positive titers (last dilution with ≥3+ fixation) was: 1:4, 5 sera; 1:8, 4 sera; 1:16, 4 sera; and ≥1:32, no sera. The analysis of the experience with immunocompromised hosts by diagnosis and by immunosuppressive medication is given in Table 1. Underlying diseases in the nonimmunocompromised patients included diabetes mellitus, chronic tuberculosis, and alveolar proteinosis (1 each). One patient developed a nocardial empyema in the absence of apparent underlying disease, and no underlying disease was recorded for the remaining patient, although clinical data were sparse. Two of the three patients with extrapulmonary disease in whom active pulmonary disease could be excluded were seropositive, as were three of four patients who died due to their nocardial infection. These results do not suggest differences in these small subgroups from the overall results.

False positive results were obtained in three of three patients with lepromatous leprosy and in two of five patients with *Mycobacterium tuberculosis* infection. The distribution of titers in these patients was indistinguishable from that in the patients with nocardia infection. Negative results (titers < 1:4) were obtained in 10 patients with tissue-proven systemic fungal infections (7 coccidioidomycosis, 1 blastomycosis, 1 candidiasis, and 1 cryptococcosis), 9 patients with a variety of bacterial infections, 6 patients with viral infections, 1 patient with *Pneumocystis carinii* infection, 29 uninfected random hospitalized patients, and 12 healthy volunteers.

DISCUSSION

Whereas extracellular nocardia antigen has been used in a complement fixation test and in skin testing in animals (12, 15), it had not been employed in human testing. The complement fixation test used in the animal studies was a more cumbersome macrotube technique. In this study the method was modified to a microtiter technique, as is commonly employed in most diagnostic laboratories.

Previous studies of nocardia serology in humans (2, 5) have employed a precipitin test, a method generally accepted to be less sensitive, with a cellular antigen. Bojalil et al. (2), using a purified polysaccharide antigen derived from a whole-cell extract, detected antibodies in humans infected with *Nocardia brasiliensis*. Patients with *N. asteroides* infection were not studied. Although a common cause of mycetoma in skin and lung in Central and South America, *N. brasiliensis* accounts for less than 7% of nocardial infection in North America (1, 17). It has been reported only rarely in the United States as a cause of disseminated disease in immunosuppressed patients (6). Humphreys et al. (5) used a crude whole-cell sonic extract antigen to study the serological response of humans infected with *N. asteroides*. They found an overall sensitivity of 45% (9 of 20). A total of 78% (7 of 9) of otherwise healthy patients and only 22% (2 of 11) of immunosuppressed patients with nocardia infection had positive precipitin results in their test.

Nocardiosis in "normal" individuals is a chronic, indolent infection, and it is not surprising to find circulating antibodies. Response in immunosuppressed patients, however, might be less predictable. Pier and Enright (12) documented the appearance of complement-fixing antibodies 2 to 3 weeks after experimental infection of cattle. The interval between onset of illness and timing of the serum specimen was documented in only a few of our cases. In all instances the serum sample tested was obtained at the time of bacteriological diagnosis. In two immunosuppressed patients antibodies were

TABLE 1. *Nocardia serology in immunocompromised patients*

Diagnosis	No. of patients with positive sera/total	No. of patients receiving steroid therapy	No. of patients receiving other cytotoxic drugs[a]
Heart transplant	3/4	4	4
Renal transplant	1/2	2	2
Cancer	3/3	2	2
Other[b]	2/2	2	0

[a] Azathioprine, antithymocyte globulin, cyclophosphamide, vincristine, methotrexate, and 5-fluorouracil.
[b] Multiple fractures; temporal arteritis.

present at 19 days after direct wound contamination and 30 days after presumed onset of illness. In another immunosuppressed patient, no antibody was detected in a single specimen obtained at 9 days after onset of illness (onset of illness was defined in retrospect after careful review of a series of chest X rays). These findings are in agreement with the experimental data cited. The exact time relationship between infection and seroconversion needs to be determined by further study.

Negative reactions were observed in patients with a variety of infectious and noninfectious illnesses and in healthy subjects. Two of five sera from tuberculosis patients gave positive reactions. The problem of false positive tests in tuberculosis has been discussed in animal studies and probably relates to cross-reacting antigens (2, 15, 22). *Mycobacteriaceae* and *Nocardiaceae* are two families in the order *Actinomycetales,* and they share common antigens. Precipitin assays with nocardia antigen in humans (5) showed false positive reactions in 15% of tuberculosis patients and 85% of leprosy patients. We observed apparent false positive reactions in three of three sera from leprosy patients without obvious nocardial disease. Cross-reactions in leprosy are likely to be of little clinical relevance because this is rarely a problem in differential diagnosis, particularly in temperate climates. However, cross-reactions with tuberculosis are likely to be a more significant problem. If acid-fast organisms are seen in sputum or tissue smears, nocardiae and mycobacteria may be distinguished from each other by differences in the degree of the acid-fast staining property (16, 18). The fluorochrome stain for acid-fast bacilli might similarly differentiate them. However, failure to isolate *Nocardia* from sputum in pulmonary cases confirmed with invasive procedures is not uncommon (8, 9), and coexistence of pulmonary tuberculosis and nocardiosis is well known (4, 19, 21). Assessment of the clinical setting with considerations of the prior history of the patient, epidemiological circumstances, skin test results for tuberculosis, and/or the ancillary use of invasive techniques might be needed if the present test were to be used in clinical decision making before the results of cultures. A positive test would be of value to alert the clinician to the possibility of nocardiosis. Our study population is relevant to this point, because in our cases it required 4 to 19 days for the organism to grow in culture, even on appropriate media (3).

Information is lacking in this and other serological assays for nocardia on possible cross-reactivity induced by infections due to *Actinomyces* species. *Actinomycetaceae* is a third family in the *Actinomycetales* and might share common antigens.

The results with this assay are encouraging. However, anticomplementary activity in the serum sample limits the use of any complement fixation test. The hyperlipemic state of many patients on prednisone often results in anticomplementary activity of their serum. The titers in the assay system described were low, although immunosuppression in most of these patients may have contributed to this. Increased sensitivity of the assay would be a desirable feature, and a more rapid method of assay may be desirable in some cases. Although *N. asteroides* is the preponderant nocardial pathogen in North America, we are uncertain whether our results would be duplicated in infections due to other nocardia species. For these reasons, other methods of assay are currently being pursued. More sera from confirmed and suspected cases will be needed for such studies, and we encourage our colleagues to send these for testing. These studies could give us important information, presently lacking, on the correlation between the clinical course of an infection and the serological titers. Further studies of the antigens involved are also indicated to derive more specific reagents.

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