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Onset and Duration of Urinary Antigen Excretion in Legionnaires Disease

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The purposes of this study were to determine whether antigen is excreted by patients with Legionnaires disease early enough after the onset of symptoms to be useful for making therapeutic decisions and whether antigen excretion ends when successful treatment is concluded. Specific antigen was detected in the urine of 14 (88%) of 16 patients with Legionnaires disease during days 1 to 3 of symptoms, 33 (80%) of 41 patients during days 4 to 7, 25 (89%) of 28 patients during days 8 to 14, and 11 of 11 patients after day 14, by solid-phase immunoassays for serogroup 1 *Legionella pneumophila* antigen. Antigen excretion persisted for 42 days or longer after the onset of treatment in at least 15 patients. The longest documented duration of excretion was 326 days. We conclude that antigen can be detected approximately as often early after symptoms begin as later, allowing meaningful therapeutic decisions to be made, but that prolonged antigen excretion may negate the diagnostic value of urinary antigen detection for relapsing or recurrent *L. pneumophila* pneumonia.

Approximately 80% of patients with serogroup 1 *Legionella pneumophila* pneumonia excrete detectable levels of an antigen, presumably derived from the organism, in their urine (7). Assays developed to detect this antigen appear to be highly specific, yielding positive results only in patients with legionellosis (7, 9, 13, 14). To be optimally useful as diagnostic tools for physicians making therapeutic decisions, these assays must detect antigen early in the course of the illness. Furthermore, antigen excretion must end with, or soon after, symptomatic recovery from the illness if the assays are to be useful for detecting relapses or reinfections with legionella organisms. We therefore analyzed our experiences with urinary antigen detection over a 54-month period to determine whether antigen detection in Legionnaires disease is possible early in the illness and whether antigen excretion stops after successful treatment of the illness.

MATERIALS AND METHODS

Patient population. From 1 January 1979 through 30 June 1983, urine specimens from 124 patients with laboratory evidence for serogroup 1 *L. pneumophila* infection were tested for urinary antigen. Twenty-four of these were not included in this analysis because the dates of onset of symptoms and therapy could not be obtained. Of the remaining 100 patients, all but one had pneumonia clinically consistent with Legionnaires disease. The remaining patient had a prolonged febrile illness after renal transplantation without evidence for pneumonia; the illness responded promptly to erythromycin therapy. Serogroup 1 *L. pneumophila* was isolated in specimens from 29 patients. In specimens from 47 others, a fourfold rise in the serogroup 1 *L. pneumophila* indirect fluorescent antibody (IFA) titer, to 1:128 or greater, occurred. Eight other patients had stable indirect fluorescent antibody titers of 1:512 or greater (four patients), 1:256 (three patients), and 1:128 (one patient). Three patients had only positive direct fluorescent-antibody examinations of respiratory secretions for serogroup 1 *L. pneumophila* organisms. In the remaining 13 patients, urinary antigen was the only corroborative laboratory evidence for Legionnaires disease; urine from 6 of these patients failed to demonstrate seroconversion by indirect fluorescent-antibody examination, and the other 7 had no convalescent specimen examined.

Thirty-one of the 100 specimens were analyzed as part of a prospective evaluation of diagnostic tests during an outbreak of Legionnaires disease (6, 8). Fifty-four other specimens were submitted by physicians as part of their diagnostic evaluations of patients with pneumonias suspected to be Legionnaires disease. The remaining 15 specimens were collected for analysis from patients already known by other means to have Legionnaires disease. When possible, usually because of prolonged hospitalization, proximity to Indiana University Hospitals, frequent outpatient evaluations, or some combination of these conditions, urine specimens were collected until contact with the patient was broken through discharge or death or until antigen could no longer be detected.

Dates of onset of illness were determined by reviewing the medical records of the patients. For community-acquired infections, the date of onset of illness was determined from information recorded in the admitting patient history, and for nosocomial infections, the date of onset was determined by evaluation of daily temperature records and serial chest radiographs. For patients from other institutions information was obtained by telephone or letter from a physician involved in care of the patient.

Antigen detection. Urine specimens were examined for serogroup 1 *L. pneumophila* antigen by solid-phase radioimmunoassay or enzyme-linked immunoassay as previously described (8, 14). The two methods produced comparable results (14).

RESULTS

Dates of onset of symptoms could not be determined for 4 of the 100 patients; these 4 patients were all antigen excretors. The relationship between antigen detection and the time of urine collection after symptom onset for the remaining 96 patients is shown in Table 1. The specimens obtained during the Burlington outbreak are shown separately be-

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TABLE 1. Urinary antigen detection as a function of day after onset of symptoms: results of first specimen tested

Patient origin	Day after onset	No. positive	No. tested	% Positive[a]
Burlington outbreak	1–3	9	11	82
	4–7	8	13	62
	8–14	6	7	86
All others	1–3	5	5	100
	4–7	25	28	89
	8–14	19	21	90
	15–21	5	5	100
	21	6	6	100

[a] No differences statistically significantly different (all probabilities > 0.05) by Fisher's exact test when compared within "Burlington outbreak" or "all others" groups.

cause they were collected prospectively when the index of suspicion for Legionnaires disease was high, and they may therefore better represent the entire spectrum of disease severity than the remaining specimens. Serological follow-up on this subgroup of patients was also better than in the remaining patients, who tended to have convalescent serological evaluation only if a culture, direct fluorescent-antibody examination, or urinary antigen test was positive. Only the first specimen collected from each patient is represented in Table 1. The differences between the proportions of patients excreting antigen at the various times shown in Table 1 are small and, by Fisher's exact test, not statistically significant. Overall, antigen was detected in the urine of 14 (88%) of 16 patients examined during days 1 to 3 of symptoms, 33 (80%) of 41 patients during days 4 to 7, 25 (89%) of 28 patients during days 8 to 14, and 11 of 11 patients after day 14. The urine of one patient, included in Table 1 as negative, was negative in a specimen from day 4 after symptoms began and positive in a specimen from day 10. No other patient who was initially negative later became positive. Thus, patients with serogroup 1 *L. pneumophila* infections excreted detectable amounts of antigen in their urine approximately as often during the first 3 days after symptoms began as later.

TABLE 2. Duration of antigen excretion after onset of therapy: patients followed until negative

Last day antigen detected[a]	First day antigen not detected[a]	Associated factors
326	327	Renal transplant
274	294	Renal transplant
207	214	Corticosteroids
123	129	None
109	123	Renal transplant
80	130	Renal transplant
75	116	Renal transplant
72	86	Renal transplant
44	159	Corticosteroids
42	116	None
39	50	COPD[b]
38	41	Alcoholism; acute renal failure
15	30	None
10	21	None
10	14	No information
4	201	Acute renal failure
4	53	No information
4	9	No information
3	35	None
2	5	Renal transplant[c]
1	4	No information
1	4	Acute renal failure
−1	18	No information

[a] Number of days after erythromycin therapy was begun. −1, One day before therapy was begun.
[b] COPD, Chronic obstructive pulmonary disease.
[c] This patient had a prolonged febrile illness which responded to erythromycin therapy. There was no clinical or radiological evidence of pneumonia.

TABLE 3. Duration of antigen excretion after onset of therapy: patients still positive when last tested

Days after onset of therapy	No. of patients
172	1
160	1
128	1
79	1
47	1
38	1
29–35	3
22–28	4
15–21	5
8–14	11
1–7	26
Pretherapy	4

Altogether, antigen was detected in 88 patients. In six of these patients, no analysis of duration of antigen excretion after onset of therapy could be performed because either no treatment was given or the date of onset of treatment could not be obtained. Multiple urine specimens were obtained from 23 patients initially excreting antigen until antigen could no longer be detected. Urine specimens were not collected at regular intervals, so a variable period of time intervened between the last positive and first negative specimens. Nonetheless, it was possible to document that antigen excretion was prolonged in many patients (Table 2). The longest duration of antigen excretion was 326 days. Ten of the 23 patients excreted antigen for 42 days or longer. All these patients recovered from their Legionnaires disease and felt well despite continued antigen excretion; thus, persistent antigen excretion did not reflect persistent clinical infection. Fifty-nine other patients continued to excrete antigen when last tested, in five cases for longer than 40 days (Table 3). Although most of the patients who excreted antigen for prolonged periods were receiving immunosuppressant medication, five who excreted antigen for more than 40 days (two from Table 2, three from Table 3) had no apparent immunological abnormalities. Thus, antigen excretion may end soon after treatment is begun in some patients but may persist for months in others.

DISCUSSION

To be optimally useful to physicians making therapeutic decisions, the immunoassays for *L. pneumophila* antigen must detect antigen early in the course of the illness. The data presented in Table 1 indicate that, if antigen is going to be detected at all, it can be detected as often during the first 3 days after symptoms begin as at later times. Thus, we conclude that the immunoassays should enable physicians to determine, early in the course of the illness and in time to initiate appropriate antibiotic therapy, that serogroup 1 Legionnaires disease is present.

Once present, however, antigen may continue to be excreted in detectable quantities in urine for prolonged periods of time despite appropriate treatment and apparent recovery from infection. Our initial experience suggested that antigen excretion is often short-lived (9). Since then, however, the

assays have been improved by the use of higher-titered antiserum (10). As a result, those patients who apparently stopped excreting antigen after 3 to 14 days of erythromycin therapy (9) have subsequently been shown to be excreting antigen in all urine specimens collected. That infected humans may excrete microbial antigens for prolonged periods is not a new observation. Using a relatively insensitive precipitation technique, Dochez and Avery reported in 1917 that three patients who had recovered from pneumococcal pneumonia still excreted pneumococcal polysaccharide at 40, 42, and 58 days after onset of their illnesses (3). Their observations were confirmed by others (2, 12). More recently, O'Reilly et al. detected circulating capsular polysaccharide, complexed with immunoglobulin, 145 days after hospitalization in the serum of a patient who had recovered from type b *Haemophilus influenzae* meningitis (11). Prolonged urinary excretion of polysaccharides after infections with *Cryptococcus neoformans* (4) and *Salmonella typhi* (1) has also been documented. After injection into mice, pneumococcal polysaccharide is present in many tissues 8 to 12 months later (5, 15). Thus, some microbial polysaccharides appear to be degraded very slowly, or not at all, by mammalian tissues, probably accounting for their prolonged appearance in urine. Although the exact nature of the *L. pneumophila* urinary antigen is not known, it withstands boiling for 30 min and treatment with pepsin, trypsin, pronase, and proteinase K (9; Kohler, unpublished observations) suggesting that it is a polysaccharide.

The biological implications of the prolonged urinary excretion of *L. pneumophila* antigen are unclear. The involved patients were not carefully studied but appeared to have recovered from their illnesses with no ill effects. However, the observation that antigen excretion may be prolonged means that urinary antigen detection cannot be relied upon to provide useful diagnostic information in patients with recurrent pneumonias in the months following recovery from *L. pneumophila* pneumonia.

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