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Immunoglobulin M Antibody Titers in the Diagnosis of Legionnaires Disease

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The purpose of this study was to determine whether measurement of immunoglobulin M (IgM) antibodies against *Legionella pneumophila* serogroup 1 can aid in the diagnosis of Legionnaires disease. On the basis of measurements of antibody levels in 1,942 control sera, we used an IgM titer of 1:256, observed in 2.3% of the controls, as presumptive evidence of Legionnaires disease. Measurement of IgM titers permitted us to presumptively or definitively diagnose Legionnaires disease in 13 of 34 patients earlier than we would have if only IgG titers had been measured. Of the 13 patients, 5 were diagnosed serologically only by IgM antibody determination. IgM titers were presumptively diagnostic in week 1 of clinical symptoms in 4 of the 13 patients. We conclude that conjugates used for antilegionella indirect fluorescent-antibody tests should be capable of detecting IgM antibodies so that the value of serological results in diagnosing and managing Legionnaires disease will be maximized.

Since the initial discovery of the causative agent of Legionnaires disease in 1977, laboratory diagnosis has been based primarily on isolation of *Legionella pneumophila* from patients, demonstration of organisms in clinical specimens by the direct fluorescent-antibody (DFA) test, and demonstration of a significant rise in antilegionella antibody levels. The method most commonly used for serological diagnosis is the indirect fluorescent-antibody (IFA) test (1). Patients with Legionnaires disease may produce immunoglobulin G (IgG), IgM, or IgA antibodies (or any combination of the three) against the organism (10). Laboratories performing the IFA test with material supplied by the Bureau of Biological Products, Centers for Disease Control (CDC), Atlanta, Ga., determine a combined-antibody titer with fluorescein isothiocyanate-tagged antihuman immunoglobulin from an animal immunized with IgG, IgM, and IgA. With this test, a rise in titer of fourfold or greater to a level of ≥1:128 (seroconversion) is considered diagnostic of *L. pneumophila* infection (1). A single specimen with a titer of ≥1:256 is considered presumptive evidence for infection at an unknown time in the past (1). Diagnostic titer changes usually do not occur early enough in the course of illness to be useful to clinicians in managing the disease (4). This study was undertaken to determine whether patients with Legionnaires disease develop an isolated IgM antibody response detectable by the IFA test and whether measurement of antilegionella IgM titers enables Legionnaires disease to be diagnosed earlier than it would have been had IgG levels alone been measured.

(This work was presented in part at the 81st Annual Meeting of the American Society for Microbiology, Dallas, Tex., 1981 [abstract no. C142, p. 286].)

MATERIALS AND METHODS

Patients and controls. The patients for this study were selected by reviewing laboratory reports at the Indiana University Hospitals for evidence of Legionnaires disease, including results of culturing, DFA assays, and urinary antigen assays and serology reports from April 1979 through October 1981. All patients who had any laboratory evidence for Legionnaires disease, at least one serum specimen tested for antilegionella IgG and IgM antibodies, and pneumonia clinically consistent with Legionnaires disease were included. Thirty-four patients met these criteria; the laboratory evidence for Legionnaires disease is as follows. Five patients had positive cultures for *L. pneumophila*. Of the 29 patients who had negative cultures or for whom cultures were not done, the evidence was as follows: IFA seroconversion plus positive result in DFA assay, urinary antigen assay, or both, 13 patients; single or stable IFA IgM or IgG (or both) titer of ≥1:256 plus positive result in DFA, urinary antigen assay, or both, 5 patients; positive results in DFA and urinary antigen assay and negative result in serological assay, 2 patients; seroconversion only, 4 patients; single or stable IFA, IgM or IgG (or both) titer ≥1:256 only, 3 patients; and positive result

TABLE 1. Anti-*L. pneumophila* serogroup 1 IFA titers in control populations[a]

Group	No. tested	No. (%) with ≥1:256 titer	
		IgM	IgG
Indianapolis venereal disease clinic	400	9 (2.2)	1 (0.2)
Other venereal disease clinics	250	0	0
Indiana University Medical Center employees	73	4 (5.5)	2 (2.8)
Indianapolis industrial employees	1,020	28 (2.7)	28 (2.7)
Other industrial employees	199	3 (1.5)	2 (1.0)

[a] A total of 2.3% of the control population had ≥1:256 IgM titers, and 1.7% had ≥1:256 IgG titers ($P = 0.249$; Fisher exact test).

in urinary antigen assay only, 2 patients. Multiple serum specimens were available from 29 patients, and single specimens were available from the remaining 5. Control sera were obtained from 1,219 industrial employees and from 73 employees of the Indiana University Medical Center. An additional 400 specimens were obtained from an Indianapolis venereal disease clinic, and 250 specimens were obtained from venereal disease clinics in other Indiana cities.

IFA test. A modification of the IFA test developed by CDC was used (9). *L. pneumophila* strain Philadelphia 1 serogroup 1 was obtained from CDC. Ten-well slides were coated with the antigen suspension, dried by the CDC procedure, and stored at −20°C until use. Starting with a 1:64 dilution, 20-μl portions of diluted test sera were placed on the slides. Slides for IgG determination were incubated at 37° for 30 min, and slides for IgM determination were incubated at 37° for 60 min. Slides were washed for 10 min in phosphate-buffered saline (pH 7.2), rinsed in distilled water, and dried. Fluorescein isothiocyanate-labeled sheep anti-human IgG and antihuman IgM were obtained from Burroughs Wellcome Co. (Research Triangle Park,

TABLE 2. IFA IgM and IgG titers

Category	Patient no.	Titer at indicated week after onset of symptoms						
		IgM						
		Preonset	1	2	3	4	5	6
A. Seroconversion, IgM only	1		<64		512	2,048	512	
	2		<64			128		256
	3			<64				1,024
B. Seroconversion, IgM before IgG	1				<64	256		2,048
	2	<64	1,024	4,096				
	3	<64	512		512	512		64
	4	<64		128	256	2,048	512	1,024
C. Significant IgM titer preceding significant IgG titer or IgG seroconversion	1		1,024	512		512		512
	2		2,048			4,096	2,048	
	3			512	1,024			
	4			256		4,096		
	5			4,096		8,192	4,096	
	6			256				
D. Seroconversion, IgG only	1		<64			<64	<64	
E. Simultaneous IgM and IgG seroconversion	1		<64		256	512		
	2		<64		4,096			
	3		<64		1,024			512
	4			64	256			
	5		<64			8,192		
	6		<64				128	
	7		64					256
	8	64	1,024					1,024
	9	<64		16,384	16,384			
F. Simultaneous significant IgG and IgM titers[a]	1		32,768					
	2			16,384		4,096		
	3				512			
G. No seroconversion or significant titer[b]	1		<64				<64	
	2		128					
	3		64	<64				<64
	4		<64					

N.C.). These antisera were purified for class specificity by absorption and were demonstrated to be class specific by gel diffusion and DFA testing. Optimal dilutions for anti-IgG and anti-IgM antisera were 1:20 and 1:10, respectively. We added 20 μl of the appropriate conjugate to each well of the slides. The slides were incubated for 30 min at 37°C, rinsed for 10 min in phosphate-buffered saline, rinsed in distilled water, and dried. A glycerol-based mounting solution (pH 9.0) was applied, and cover slips placed on the slides. Slides were read with a Zeiss epifluorescence microscope equipped with a 50-W mercury vapor lamp incident-light source and appropriate filters.

Sera were initially diluted 1:16 in normal yolk sac suspension obtained from CDC. Twofold dilutions were prepared in phosphate-buffered saline. *L. pneumophila* serogroup 1-positive human serum was obtained from CDC and used as a positive control. In one case, one lot of this control serum was positive for IgG but not IgM, and serum from an Indiana University Medical Center patient previously shown to have specific anti-*L. pneumophila* IgM was used. A single normal serum was used each time as a negative control.

DFA test. The DFA test was performed as previously described (2). The reagents were supplied by CDC.

Radioimmunoassay. The radioimmunoassay for detecting urinary legionella antigen was performed as previously described (5), except that a higher-titer anti-*L. pneumophila* IgG obtained from a more extensively immunized rabbit was used to coat the tubes. This made the test more sensitive and enabled detection of antigen for longer periods of time after the initiation of therapy.

RESULTS

Of the 1,942 control specimens, 44 (2.3%) had an antilegionella IgM titer of ≥1:256 (Table 1), and 33 (1.7%) had an antilegionella IgG titer of ≥1:256. These differences were not statistically significant ($P = 0.249$; Fisher exact test). On the basis of these results, we considered an antile-

TABLE 2—*Continued*

Titer at indicated week after onset of symptoms						
IgG						
Preonset	1	2	3	4	5	6
	<64		<64	64	<64	
	<64			<64		<64
		<64				64
			<64	<64		1,024
<64	64	512				
<64	<64		<64	128		<64
<64		<64	64	256	512	256
	<64	<64		<64		512
	64			256	2,048	
		<64	256			
		64		1,024		
		<64		<64	<64	
		128				
	<64			256	128	
	<64		256	256		
	64		1,024			
	<64		512			256
		64	256			
	<64			2,048		
	<64				128	
	<64					128
<64	1,024					2,048
<64		8,192	8,192			
	16,384					
		8,192		8,192		
			1,024			
	<64				<64	
	<64					
	64	<64				<64
	<64					

[a] For three patients, the dates of infection onset are unknown.
[b] For one patient, the date of infection onset is unknown.

gionella IgG or IgM titer of ≥1:256 as presumptive evidence for Legionnaires disease.

The serological test results for the 34 patients, arranged according to the week of illness during which the specimen was obtained, are shown in Table 2. Of the 34 patients, 5 had sera in which neither the IgM nor the IgG antilegionella titer was helpful (group G). For 15 other patients, IgG and IgM titers simultaneously allowed definitive or presumptive diagnoses (groups E and F). For one patient, only the IgG titers were helpful (group D). In the remaining 13 patients (38%), determination of antilegionella IgM titers enabled presumptive or definitive serological diagnoses to be made earlier than would have been possible if an assay capable of detecting only IgG antibodies had been used (groups A, B, and C). Of the 13 patients who demonstrated IgM seroconversion, 2 did not demonstrate IgG seroconversion, by ≥6 weeks after the onset of symptoms, and 1 did not demonstrate IgG seroconversion by 5 weeks after the onset of symptoms. Of the other 10 patients (groups B and C), 4 gave sera in which a presumptively diagnostic IgM titer of ≥1:256 or IgM seroconversion occurred during week 1, five gave sera in which one of the phenomena occurred during week 2, and one gave sera in which one of the phenomena occurred during week 4. The IgG level enabled presumptive or definite diagnosis in none of the 10 patients during week 1, in 1 during week 2, in 1 during week 3, in 4 during week 4, and in 2 at ≥6 weeks; 2 patients (group C, no. 5 and no. 6) were never diagnosed by IgG levels. Thus, IgM titers provided the only serological evidence of infection in 5 of the 34 patients tested and enabled earlier presumptive or definitive diagnosis in 5 others.

DISCUSSION

This study demonstrates that determination of antilegionella IgM titers by IFA testing enables serogroup 1 Legionnaires disease in a significant proportion of patients to be detected earlier than would have been possible by testing for IgG antibodies only. Furthermore, for 15% of our study population, only IgM titers enabled presumptive or definitive diagnosis of Legionnaires disease. These findings corroborate and expand the similar observations of Nagington et al. (6), who also found that many patients produce only specific IgM antilegionella antibodies early in the illness and that some of these patients never produce detectable IgG antibodies. Similarly, Wilkinson et al. (10) demonstrated that a few patients with Legionnaires disease may have only demonstrable IgG or IgA antibodies. None of their 29 patients had only IgM antibodies, although the existence of such patients was noted parenthetically. Our study suggests that the presence of only IgM antibodies in patients is reasonably common. Whether the non-class-specific antiimmunoglobulin conjugate used by Wilkinson et al. to select patients with Legionnaires disease was capable of detecting IgM antibodies with high sensitivity was not stated. If the conjugate was not capable, the incapability might account for the absence of patients who seroconverted only for IgM antibodies. Laboratories using fluorescein isothiocyanate antihuman immunoglobulin conjugates which are not class specific should use conjugates which can detect antilegionella IgM antibodies if optimum serological diagnostic capabilities are to be achieved. These conjugates should be as sensitive as class-specific conjugates. Sera rich in specific IgM but lacking specific IgG, such as the sera of patients in group A, allow this determination to be made. We tested one antiimmunoglobulin conjugate supplied by CDC (lot no. PR 845, courtesy of Hazel Wilkinson) with four such serum specimens; the conjugate detected IgM antibodies approximately as well as did our IgM-specific conjugate.

Of our control population, only 2.3% of the individuals had an IgM titer of ≥1:256. This level appeared at some point after the onset of Legionnaires disease symptoms in 79% of the 34 patients studied. Two additional patients achieved IgM titers of 1:128, but this titer was also seen in 14.3% of the control specimens. Therefore a titer of ≥1:256 seems reasonable to use as presumptive evidence of recent or past infection if only a single serum specimen has been tested.

Rheumatoid factor in high titers could conceivably block detection of IgG antibodies by combining with specific IgG antibodies, thus resulting in fluorescent staining of IgM but not IgG. We did not screen our sera for rheumatoid factor. However, we mixed the serum of one patient with a serum containing rheumatoid factor. This did not affect the IgM or IgG titer. Subsequent removal of rheumatoid factor by absorption also produced no effect on the IgM or IgG titer. In a more extensive analysis of this potential problem, Wilkinson et al. (8) also noted no influence of rheumatoid factor on legionella IFA titers. Thus, rheumatoid factor does not appear to pose problems in performing the class-specific IFA test.

In addition to the IFA test, methods currently available to diagnose Legionnaires disease include antigen detection by radioimmunoassay or enzyme-linked immunosorbent assay, examination of specimens for bacilli by DFA testing, and growth of organisms from clinical specimens. Radioimmunoassay and enzyme-linked immunosorbent assay can diagnose the illness ear-

ly in its course and have sensitivities of approximately 80% (5, 7). However, these methods are not likely to become generally available in the near future. The DFA test is simple and permits early diagnosis. However, suitable test specimens may not be obtainable or may require invasive procedures. Furthermore, the sensitivity is only 50 to 60% (3). Growth of legionella spp. from clinical specimens requires the use of special media, may require invasive procedures to obtain appropriate specimens, and involves a 3- to 5-day delay until growth is recognizable (3). The sensitivity of culturing is not yet clear, and it is not yet known with certainty whether asymptomatic carriage of *L. pneumophila* can occur. The IFA test is easy to perform and requires no complicated procedures. It may give positive results for some patients who cannot be diagnosed by any of the other diagnostic tests. Its major drawback has been its inability to enable the diagnosis of early stages of Legionnaires disease in most patients. Our study suggests that measurement of antilegionnella IgM titer enables diagnosis of Legionnaires disease to be made earlier than would have been possible if measurement of IgG titer alone had been used. It should be a useful supplemental procedure for diagnosing and, for some patients, managing Legionnaires disease, particularly if specimens for DFA examination are negative or unobtainable. Separate assays for IgM antibodies may not be necessary as long as appropriately standardized conjugates known to be capable of detecting IgM antibodies with a sensitivity comparable to that of anti-IgM-specific conjugates are used.

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