PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1198–1201 (1975)

Immunoglobulin Levels and Antibody-Coated Bacteria in Urines from Patients with Urinary Tract Infections (38716)

VIRGINIA L. THOMAS, MARVIN FORLAND, AND ALEXIS SHELOKOV

Department of Microbiology, and the Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

We have recently reported the use of direct immunofluorescence as a means of visualizing antibody complexed with bacteria in urine specimens from patients with urinary tract infections (1). Our results indicated a remarkable correlation between the presence or absence of antibody-coated bacteria and the site of the infection in the kidney or the bladder, respectively. These studies were confirmed by Jones *et al.* (2) who found that the presence of antibody-coated bacteria correlated closely with renal bacteriuria as determined by a direct localization method, the bladder washout technique.

We determined the class of antibody complexed with the bacteria in immunofluorescence-positive specimens from patients with pyelonephritis (1). All tests were positive for IgG coating the bacteria in the urines; tests for IgA and IgM were also positive in some of the specimens. These results led us to question whether or not the antibody coating the bacteria was associated with elevated urine levels of total protein or immunoglobulins. We found that total protein and immunoglobulin levels may or may not be elevated in urines from patients with pyelonephritis and cystitis. Results indicated that IgG, IgA, or IgM-coated bacteria may be present without elevated levels of the particular immunoglobulin in the urine, and that uncoated bacteria may occur in the presence of elevated levels of immunoglobulins in the urine.

Materials and Methods. Urine specimens were collected during the phase of active infection from 12 patients with chronic pyelonephritis (11 female and 1 male) and 12 patients with cystitis (all female). The urines, either catheter or midstream specimens taken after thorough cleansing, were cultured quantitatively and the bacterial isolates identified. The urines were tested for the presence of antibody-coated bacteria by direct immunofluorescence using fluorescein-conjugated anti-human globulin of horse origin (Roboz Surgical Instrument Company, Washington, DC). Fluorescein-conjugated monospecific antiserums (goat) against human IgG, IgA, and IgM (Hyland, Los Angeles, CA) were used to determine the class of antibody coating the bacteria (1). Levels of IgG, IgA, and IgM in the urines were measured by radial immunodiffusion (3). If a urine did not contain measurable amounts of immunoglobulins, a portion of the specimen was concentrated 50- to 100-fold by ultrafiltration and the measurements repeated. Total urinary protein was measured by the sulfosalicylic acid turbidity method (4). Serum antibody titers against the patients' own infecting bacteria were determined by indirect immunofluorescence (1).

Patients were selected with and without proteinuria in order to determine if results of fluorescent antibody (FA) tests for antibody-coated bacteria were related to the levels of total protein or of immunoglobulins in the urine; thus, patients with infections and underlying noninfectious renal diseases were included. The diagnosis of chronic pyelonephritis was based on the clinical course, characteristic radiographic changes, and impairment of renal function. Direct localization by ureteral catheterization or bladder-washout catheterization substantiated the renal origin of the bacteriuria in eight patients. Microscopic examination of renal tissue from one other patient confirmed the clinical diagnosis of chronic pyelonephritis.

Cystitis was diagnosed on the basis of lower urinary tract symptoms in the absence of fever and costovertebral-angle tenderness and demonstration of significant bacteriuria

($\geq$100,000 bacteria per milliliter) by at least two cultures. Intravenous pyelography in these 12 patients gave no evidence of pyelonephritic changes. Renal concentrating ability after 14 hr of water deprivation was normal in the seven patients with cystitis but without underlying renal disease, the mean ($\pm$SD) value being 936 $\pm$ 71 mOsm per kg.

Results. Specimens from 24 patients with urinary tract infections were studied to explore the possibility of a relation between positive FA tests and elevated urine levels of total protein and immunoglobulins. The classes of antibody bound to the infecting bacteria in 12 of the patients with pyelonephritis were examined in relation to the levels of unbound immunoglobulins in the urine (Table I). All specimens from these 12 patients were found to contain antibody-coated bacteria, although not all of the urine specimens had elevated levels of total protein or immunoglobulin. Of 13 urines collected from these 12 patients with pyelonephritis, the total protein levels were elevated in seven (>10 mg per dl). The IgG levels were within the normal range ($\leq$1.0 mg per dl) in three; IgG-coated bacteria were detected in each of these three specimens. IgG levels were >1.0 mg per dl in 10; IgG-coated bacteria were detected in each of these 10 specimens. IgA levels were within the normal range ($\leq$0.1 mg per dl) in three; IgA-coated bacteria were present in each of these three specimens. IgA levels were >0.1 mg per dl in 10; IgA-coated bacteria were present in seven and were absent in the remaining three specimens. IgM was not detectable in 10; IgM-coated bacteria were present in one and were absent in the remaining nine specimens.

TABLE I. RESULTS OF IMMUNOFLUORESCENCE TESTING FOR ANTIBODY-COATED BACTERIA COMPARED TO AMOUNTS OF IMMUNOGLOBULIN AND PROTEIN IN URINE SPECIMENS FROM 12 PATIENTS WITH CHRONIC PYELONEPHRITIS (CPN).

	FA test for antibody-coated bacteria in urine[b]				Immunoglobulin (mg/100 ml)			Total protein (mg/100 ml)	Serum antibody titer[c]
Patient identification[a]	Globulin	IgG	IgA	IgM	IgG	IgA	IgM		
GF-CPN, bilateral renal calculi	++++	++++	+	0	0.3	0.0	0.0	0.7	512
MB-CPN, bilateral renal calculi	+++	++	±	0	0.2	0.1	0.0	1.5	2048
EP-CPN, hypoplastic right kidney	+++	++	±	0	0.7	0.0	0.0	1.5	1024
MS-CPN, urethral stenosis	++	++	0	0	3.0	0.3	0.0	2.0	128
ET-CPN, bilateral renal calculi	+++	+++	+	0	4.1	2.8	0.0	5.0	2048
VM-CPN	++++	+++	++	0	3.9	0.3	0.0	5.6	256
AP-CPN, meningomyelocele with spastic neurogenic bladder	+++	+++	++	+	4.0	1.0	0.0	31.3	4096
MC-CPN, bilateral renal hypoplasia	++++	++++	±	0	7.2	0.8	0.0	33.0	64
AJ-CPN, diabetic glomerulosclerosis, papillary necrosis	++	++	0	0	9.8	0.2	0.0	37.0	1024
AV-CPN, diabetic glomerulosclerosis	+++	+++	++	0	8.0	0.2	0.0	54.0	256
ED-Asymptomatic renal bacteriuria, post-renal transplant	++++	+++	++	++	31.0	1.8	0.2	54.0	4096 (*Proteus*) 256 (*E. coli*)
AR-CPN, diabetic glomerulosclerosis									
(left ureter)	+++	+++	0	0	23.5	2.7	1.0	125.0	2048
(right ureter)	++++	++++	++	++++	34.9	15.0	41.0	190.2	

[a] The infecting bacteria were: *Pseudomonas aeruginosa* (patients GF, MB, ET, and AP); *Klebsiella pneumoniae* (patients EP, VM, MC, and AV); *E. coli* (patient MS); *Citrobacter diversus* (patient AJ); *Serratia marcescens* (patient AR); and *Proteus mirabilis* and *E. coli* (patient ED).

[b] Results of FA test recorded as negative (0) or positive with gradations of +, ++, +++, and ++++ or borderline (±).

[c] Serum antibody titers against the patients' own infecting bacteria were determined by the indirect fluorescent antibody method.

IgM was measurable in three; IgM-coated bacteria were present in two and were absent in the other specimen. Eight of the 12 patients with chronic pyelonephritis had elevated serum antibody titers ($\geq$512) against their own infecting microorganisms.

Protein and immunoglobulins present in the urines from 12 patients with cystitis did not coat the infecting bacteria, even when the levels were high, as evidenced by negative FA tests for antibody-coated bacteria in all 12. Each urine specimen from patients with cystitis had measurable IgG (10 with >1.0 mg per dl and two with $\leq$1.0 mg per dl). Five urines had >0.1 mg per dl of IgA. None of the urines contained measurable IgM. Two urines contained elevated levels of total protein; these two specimens were from patients who, besides cystitis, had renal diseases of noninfectious etiology. Each patient with cystitis had a low serum antibody titer ($\leq$128) against his own infecting microorganism.

Discussion. Urine specimens from 12 patients with chronic pyelonephritis and 12 patients with cystitis were studied to determine if results of FA tests for antibody-coated bacteria in urinary tract infection are directly associated with levels of total urinary protein or particular classes of immunoglobulins. We found that of the urine specimens with normal protein levels and small or nonmeasurable amounts of a particular class of antibody (IgG, IgA, or IgM), some contained bacteria coated with antibody of that class and some did not. Similar results were observed in studies of urine specimens with elevated total protein and large amounts of a particular class of antibody. Thus, the occurrence of antibody-coated bacteria in the urines of patients with pyelonephritis did not depend on marked elevations of total urinary protein or immunoglobulins. Further evidence of the independence of FA test results and total urinary protein and immunoglobulin levels was shown by testing urines from patients with cystitis. All patients with cystitis had negative FA tests for antibody-coated bacteria, yet the protein, IgG, and IgA levels ranged from normal values (Table II, patient CS) to markedly elevated values

TABLE II. RESULTS OF IMMUNOFLUORESCENCE TESTING FOR ANTIBODY-COATED BACTERIA COMPARED TO AMOUNTS OF IMMUNOGLOBULIN AND PROTEIN IN URINE SPECIMENS FROM 12 PATIENTS WITH RECURRENT CYSTITIS.

Patient identification[a]	FA test for antibody-coated bacteria in urine	Immunoglobulin (mg/100 ml)			Total protein (mg/100 ml)	Serum antibody titer[b]
		IgG	IgA	IgM		
CS	0[c]	0.4	0.0	0.0	0.2	64
CD	0	1.3	0.0	0.0	0.6	128
SS	0	2.1	1.0	0.0	0.9	128
MW	0	1.7	0.0	0.0	2.7	32
MA	0	0.2	0.0	0.0	3.9	128
BH	0	1.1	0.0	0.0	4.0	64
LM	0	2.8	0.3	0.0	5.0	64
PF	0	4.0	1.2	0.0	5.8	128
DM	0	3.3	0.0	0.0	7.2	128
MS	0	2.2	0.0	0.0	8.3	128
RG	0	13.6	1.3	0.0	42.4	64
JR	0	25.4	6.2	0.0	250.0	64

[a] The infecting bacteria were: *E. coli* (patients CS, CD, SS, MW, MA, BH, DM, and JR); *Proteus mirabilis* (patients LM, PF, and MS); and *Streptococcus sp.* (patient RG). Patients with noninfectious renal disease: PF—lupus erythematosus; RG—recurrent cystitis and arteriolar nephrosclerosis; and JR—recurrent cystitis and diabetic glomerulosclerosis.

[b] Serum antibody titers against the patients' own infecting bacteria were determined by the indirect fluorescent antibody method.

[c] 0—negative FA test.

(patient JR); thus, the antibody present in these 12 urine specimens could be assumed to be nonreactive with the infecting bacteria.

The site of production of unbound antibody found in urines from patients with cystitis is probably different from the source of antibody that complexes with the infecting bacteria in urines of patients with pyelonephritis. The antibody coating the bacteria in urines from patients with pyelonephritis is most likely antibody synthesized in response to the infection. This antibody may be locally synthesized in the kidney as suggested by others (5, 6). The low levels of IgG and IgA in specimens from patients with cystitis (Table II, CS through MS) may represent antibody normally present in urine (7) or antibody released directly into the bladder as a result of the local inflammatory response due to bladder infection. Two patients (RG and JR) with cystitis had marked proteinuria due to renal disease unrelated to their bladder infection. The elevated IgG and IgA levels in these two patients were probably due to altered glomerular permeability rather than local synthesis caused by infection.

In conclusion, we observed a lack of correlation between the presence of antibody-coated bacteria and elevated levels of total protein and immunoglobulins in the urine. The results indicate the specificity of the FA test in that a positive test appears to be due to antibody produced as a result of kidney infection. This further strengthens the use of the FA test for localizing the site of urinary tract infection.

Summary. The presence of antibody-coated bacteria in urines from patients with urinary tract infections has previously been reported to correlate with renal infection as opposed to bladder infection. Urine specimens from 12 patients with pyelonephritis and 12 patients with cystitis were studied to determine whether the antibody coating the bacteria is associated with elevated urine levels of total protein or of particular classes of immunoglobulins. The classes of antibody bound to the infecting bacteria in urines from the patients with pyelonephritis were compared to the levels of unbound antibody in the urine. Each specimen was found to contain antibody-coated bacteria, but not all of the specimens had elevated levels of total protein or immunoglobulins. Thus, the occurrence of antibody-coated bacteria in pyelonephritis did not depend on marked elevations of total urinary protein or immunoglobulins. Studies of patients with cystitis showed that immunoglobulins and protein present in the urines, even in elevated quantities, did not react with the infecting bacteria in patients with bladder infections, as each of these patients had negative FA tests for antibody-coated bacteria.

1. Thomas, V., Shelokov, A., and Forland, M., N. Engl. J. Med. **290,** 588 (1974).
2. Jones, S. R., Smith, J. W., and Sanford, J. P., N. Engl. J. Med. **290,** 591 (1974).
3. Mancini, G., Carbonara, A. O., and Heremans, J. F., Immunochemistry **2,** 235 (1965).
4. Todd, J. C., and Sanford, A. H., *in* "Clinical Diagnosis by Laboratory Methods" (I. Davidsohn and J. B. Henry, eds.), p. 48. Saunders, Philadelphia (1969).
5. Cotran, R. S., J. Exp. Med. **117,** 813 (1963).
6. Lehmann, J. D., Smith, J. W., Miller, T. E., Barnett, J. A., and Sanford, J. P., J. Clin. Invest. **47,** 2541 (1968).
7. Uehling, D. T., and Steihm, E. R., Pediatrics **47,** 40 (1971).

Received Nov. 4, 1974. P.S.E.B.M., 1975, Vol. 148.