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Antibody-coating of bacteria in the urine in relation to various immunologic indexes

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Antibody-coating of bacteria in the urine in relation to various immunologic indexes. Little is known about the immunologic aspects of antibody coating, though the test for determining antibody-coated bacteria in urine has been examined for its diagnostic uses by many authors after its inauguration in 1974. In adults with chronic pyelonephritis *with* and *without* antibody-coated bacteria in the urine, we tested whether bacterial coating is correlated with the homologous O-antibody titre in the urine. We also determined Ig levels in urine and serum, as well as homologous O-antibody titres in serum. By means of indirect immunofluorescence technique, we were able to show homologous O-antibodies in the urine of all patients with and without antibody-coated bacteria. IgG and IgA levels in urine were mostly raised, as were often the O-antibody titres in the serum. There were no significant differences in the immunologic parameters within the patient groups with or without antibody-coating. The presence of homologous O-antibodies in urine does not therefore necessarily lead to coating of the bacteria.

Recouvrement des bactéries par des anticorps en relation avec divers index immunologiques. Les connaissances concernant l'aspect immunologique du recouvrement par des anticorps sont peu nombreuses bien que le test de détection dans l'urine de bactéries recouvertes d'anticorps ait été évalué pour son utilité diagnostique par de nombreux auteurs depuis sa description en 1974. Chez des adultes atteints de pyélonéphrite chronique avec et sans présence dans l'urine de bactéries recouvertes d'anticorps nous avons recherché une corrélation entre ce recouvrement et le titre d'anticorps homologue O dans l'urine. Nous avons aussi déterminé les concentrations d'Ig dans l'urine et le sérum, de même que le titre de l'anticorps homologue O dans le sérum. Au moyen de la technique d'immunofluorescence indirecte nous avons pu mettre en évidence des anticorps homologues O dans les urines de tous les malades, qu'elles contiennent ou non des bactéries recouvertes d'anticorps. Les concentrations d'IgG et d'IgA dans l'urine étaient le plus souvent élevées de même que les titres d'anticorps O dans le sérum. Il n'a pas été observé de différence significative dans les index immunologiques dans les groupes de malades avec ou sans recouvrement des bactéries par des anticorps. La présence d'anticorps homologues O dans l'urine n'implique pas nécessairement le recouvrement des bactéries.

Since the method of detecting antibody-coated bacteria in the urine was first introduced by Thomas, Shelokov, and Forland [1] and Jones, Smith, and Sanford [2], it has been examined by many other authors for its diagnostic relevance [3–11]. Not all authors have confirmed these initial findings, namely, that results are nearly always positive in pyelonephritis but negative in cystitis. The differing results obtained by various workers raises the question of the needed prerequisites for the antibody-coating of bacteria to take place.

We asked how well the presence of homologous O-antibodies in the urine of patients with chronic pyelonephritis might correlate with the antibody-coating of the organisms. At the same time, we determined quantitatively the homologous O-antibodies in serum, as well as IgG, IgA, and IgM levels in urine and serum, so as to gain insight into the behavior of these immunologic parameters in pyelonephritis patients with and without antibody-coated bacteria.

Methods

Patients. Nine patients with and six patients without antibody-coated bacteria in urine, selected arbitrarily from 206 adults with chronic pyelonephritis, were investigated. We examined these 15 adults and took midstream urine specimens (MSU) for bacteriologic examination, as well as serum, and urine collected over a 24-hour period, so as to determine the O-antibodies and levels of IgG, IgA, and IgM.

The diagnosis of chronic pyelonephritis was based on a past history of symptomatic urinary tract infections, documentation of significant bacteriuria

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(greater than 10^5 bacteria/ml) on at least three occasions, serum creatinine concentrations greater than 1.2 mg/dl, and characteristic changes on Addis counts of the urine sediment (leukocytes more than 5 Mio per 24 hours, erythrocytes more than 1.5 Mio per 24 hours). In addition, i.v. pyelography performed in each patient demonstrated one or more of the following changes: scar formation, displacements of calyx-parenchyma relation, deformation of the renal pelvis (distortion with clubbing), and clubbing of calyces with or without shrinkage. During the time of observation, only 4 of our 15 patients were treated with chemotherapeutics (Tables 1 and 3).

Bacteriologic urine diagnosis. The number of organisms was determined from the MSU and the different types biochemically differentiated. For *E. coli,* we also undertook serotypic identification with 44 anti-E. coli O-sera [12].

Determination of antibody-coated bacteria in urine. We centrifuged 2 ml of MSU at ×3,500 rpm and incubated this twice with PBS-washed sediment (pH, 7.3), together with FITC-labeled anti-human globulin, for 45 min at 37° C. We then washed again the sediment-conjugate mixture with PBS and prepared it for a slide. This we assessed under a fluorescence microscope at ×1,000 magnification. A positive urine sediment was marked by the presence of numerous bacteria with marginal fluorescence in most of the visual fields. We have described the details of the technique and its evaluation in earlier papers [4, 16].

Preparation of urine samples. The 24-hour urine was collected by the patients in glass flasks without adding any antiseptic. A 200-ml aliquot was taken from the total volume and dialyzed for 2 to 3 days against twice-distilled water. The urine was then lyophilized and the lyophilisate dissolved, so that we were left with urine in a ×100 concentration. This was stored at −18° C until further processing.

Determination of IgG, IgA, and IgM in urine and serum. Quantitative determination of immunoglobulins was carried out with the Mancini technique as described by Rogos and Wagner [17], slightly modified. Reference sera from Behringwerke (Federal Republic of Germany) were used as standard, and antisera from Sevac (Prague, CSSR) were used for the production of the immune diffusion plates. Patients' sera were diluted as follows: IgG determination, 1:20; IgA, 1:5; and IgM, 1:3. The urine was used in a 1:100 concentration. Details of the Mancini technique have been described by Budde [16].

Quantitative antibody determination in urine and serum. For this, we used the O-antigen of the patient strain, taking a suspension of the organism previously treated in a steam sterilizer and subsequently washed; this suspension was fixed at a concentration of about 10^7 organisms/ml [16]. For the indirect immunofluorescence technique, we fixed the antigen onto slides and incubated it with the diluted serum or urine at 37° C for 45 min in the humid chamber. We then fixed FITC-labeled anti-human globulin onto the slides, and after renewed incubation removed this by rinsing. The titres, which were shown by the stage of dilution still exhibiting a definite fluorescent outline, were determined under the fluorescence microscope. We have described previously the details of the immunofluorescent technique [14, 16].

Results

Out of a large number of patients with chronic pyelonephritis and significant bacteriuria, we tested 15 patients for the presence of antibody-coated bacteria in urine. Antibody-coating was found in 9 patients, and none in 6. The two groups tested do not reflect the real ratio of antibody-coating in our patients. We also determined the homologous O-antibodies and IgG, A, and M levels in their urine and serum. Bacteriologic-immunologic results in *the 9 patients with evidence of antibody-coating* are shown in Tables 1 and 2.

All 9 patients had O-antibodies against the urine strain in the 24-hour urine samples. The O-antibody titres differed considerably; the highest was 1:256 in patient 8. Serum O-titres concurrently determined lay between 1:320 and 1:2560 (patients 5 and 9, and 4 and 6, respectively). Table 1 shows no evidence of

Table 1. Bacteriologic and immunologic results in the urine and serum of patients with antibody-coated bacteria

Patient	Urinary strain	Reciprocal immunofluorescence titre	
		Urine[a]	Serum
1	*E. coli* 024	1	1280
2[b]	*E. coli* nt[c]	4	1280
3	*E. coli* nt	4	640
4	*E. coli* 06	8	2560
5	*E. coli* nt	1	320
6	*Klebsiella pneumoniae*	128	2560
7[d]	*Proteus inconstans*	1	640
8	*E. coli* 07	256	640
9	*E. coli* nt	16	320

[a] Urine concentrated 100-fold

[b] Ampicillin therapy (4 g/day) started two days before examination

[c] Not able to be typed with the antisera used

[d] Gentamicin therapy (120 mg/day) started 6 days before examination

Table 2. Immunoglobulins in the urine and serum of patients with antibody-coated bacteria

Patient	Sex	Immunoglobulin in urine *mg per 24 hr*			Immunoglobulin in serum *mg/dl*		
		IgG	IgA	IgM[a]	IgG	IgA	IgM
1	F	21.7	5.9	ND	810	140	65
2	F	11.0	3.9	0.5	1680	330	123
3	F	20.2	7.0	1.6	1350	120	165
4	F	4.9	1.6	ND	1250	205	168
5	M	8.7	4.0	ND	1050	390	105
6	F	19.6	3.2	ND	1385	175	234
7	F	40.5	9.0	ND	1050	390	220
8	F	7.2	1.3	ND	960	105	195
9	F	27.0	10.5	ND	1250	160	187

[a] ND is not detectable.

a connection between heights of urine and serum titres. Thus, patient 1 had homologous antibodies only in the concentrated urine, but the serum titre was 1:1280. Patient 8's urine titre was 1:256 and serum 1:640. Table 2 shows IgG values in serum and urine for these patients. Urine IgG levels varied between 4.9 and 40.5 mg per 24 hours in the 9 patients. Urine IgM was found only in 2 patients. Urine IgA levels varied from 1.3 to 10.5 mg per 24 hours (Table 2). IgG serum levels were between 810 and 1680 mg/dl, serum IgA between 105 and 390 mg/dl, and IgM between 65 and 234 mg/dl. All were female except for patient 5.

Bacteriologic-immunologic results obtained from *the 6 patients without evidence of antibody coating* of bacteria are summarized in Tables 3 and 4.

These 6 pyelonephritis patients had homologous O-antibodies in their urine. Antibody titres fluctuated between 1:4 and 1:32. Serum titres (also determined by the indirect immunofluorescence technique) varied from 1:20 to 1:2560 (patients 4 and 6, and also patient 1 in Table 3). Ig levels also fluctuated greatly from one patient to another (Table 4). Urine IgG levels varied between 2.4 and 107.3 mg per 24 hours, and IgA from 0.2 to 20.9. IgM was found only in the urine of patient 2. As with the patients with antibody-coated bacteria, we were unable to find a connection between serum and urine Ig levels. Also we could not find any relationship between the height of the homologous O-titres in urine and serum and the urine Ig. Thus, patients 3, 4, and 5 (Tables 3 and 4) had a urine titre of 1:32, but the urine Ig levels lay under 8 for IgG and under 3 for IgA per 24 hours.

Only 4 patients had received treatment with antibiotics during the observation time. Details of the chemotherapy are given in Tables 1 and 3.

Table 3. Bacteriologic and immunologic results in the urine and serum of patients without antibody-coating of bacteria

Patient	Urinary strain	Reciprocal immunofluorescence titre	
		Urine[a]	Serum
1[b]	*Proteus mirabilis*	8	2560
2[c]	*Proteus mirabilis*	4	1280
3	*E. coli* nt	32	320
4	*E. coli* nt	32	20
5	*E. coli* nt	32	640
6	*E. coli* nt	4	20

[a] Urine concentrated 100-fold
[b] Chloramphenicol therapy (1.4 g/day) started 12 days before examination
[c] Gentamicin therapy (120 mg/day) started 7 days before examination

Table 4. Immunoglobulins in the urine and serum of patients without antibody-coating of bacteria

Patient	Sex	Immunoglobulin in urine *mg per 24 hr*			Immunoglobulin in serum *mg/dl*		
		IgG	IgA	IgM[a]	IgG	IgA	IgM
1	F	6.2	3.4	ND	750	98	135
2	M	107.3	20.9	15.5	2040	250	360
3	F	7.4	2.0	ND	905	120	216
4	F	2.6	0.3	ND	1980	90	168
5	F	2.4	0.2	ND	4350	455	384
6	F	3.0	0.6	ND	1260	255	234

[a] ND is not detectable.

Discussion

Since the test for determining antibody-coated bacteria in urine sediment of patients with urinary tract infection [1, 2] was introduced, its diagnostic relevance has been examined by several workers. Though special immunologic aspects connected with it have also been examined, the prerequisites responsible for the sequence of the antigen-antibody reaction, and leading to bacterial coating, are largely unknown. Also, no investigations in man correlating quantitative behavior of urine O-antibodies with the coating of bacteria have been published.

Immediately after inaugurating the test, Thomas et al [18, 19] answered questions relating to bacteriologic-immunologic matters connected with it, evaluating immunologic data of patients with pyelonephritis and cystitis in more detail. In three women with pyelonephritis, negative when tested for antibody coating, they also found low serum antibody titres, whereas patients with a positive result mainly showed raised serum titres. These were also normal in all cystitis patients [19]. In pyelonephritis pa-

tients with antibody-coated organisms, coating was mainly due to IgG, and more rarely by IgA antibodies. They noted in a later paper that there was no correlation between the existence of antibody-coated bacteria and raised Ig levels in the urine. They interpreted these results as pointing to the specificity of the test [18]. We must ask ourselves what caused such findings as our group found a positive antibody-coating with only 73.1% of adult pyelonephritis patients [4] and often none at all in children [7, 10, 20, 21].

Though we know that the urine of patients with urinary tract infections may contain antibodies against the homologous causative organism [14, 22, 23], to our knowledge no quantitative antibody determinations have been made in human urine in connection with this test. It was to be expected that O-antibodies specific to a particular organism in the urine were related to the presence or absence of antibody-coating of bacteria. The findings of Smith, Jones, and Kaijser in animal experiments [24] reflect with great sensitivity the local immune response to the organisms's O-antigen, and show that all animals infected had a positive test for antibody-coated bacteria after day 15 of infection. But only 86% and 93% of the animals had urinary antibodies of the IgG and IgM classes, respectively. These results reflect an acute form of renal infection in contrast to our patients with chronic pyelonephritis.

In all 9 patients with pyelonephritis and antibody-coated bacteria, we found evidence of homologous antibodies in the 24-hour urine samples (Table 1). The O-titres varied considerably from normal levels up to 1:256. In healthy subjects, we found urinary O-antibodies against 11 frequently occurring *E. coli* O-antigens with an upper limit of 1:2 [13]. Urine Ig levels were mostly raised (Table 2) [16]; serum Ig levels, in contrast, were often within the normal range. Here we must bear in mind the great individual fluctuation in urine and serum Ig ranges, which we have discussed elsewhere [16].

Additionally, all those pyelonephritis patients without evidence of antibody-coating possessed O-antibodies against the urine strain in collected urine obtained at the same time. Urine titres here, determined by the indirect immunofluorescence technique, were mostly within the pathologic range [14]. The other immunologic parameters did not differ significantly from those in patients with antibody-coating. Only urine IgG and IgA values were lower, except for patients 2 and 3 (Table 4). We would not attach too much importance to this, as the literature does not confirm any correlation between urine Ig levels and antibody-coating [18, 19, 25]. Still, 4 of our 6 patients without antibody-coating had urine IgG and IgA levels above the mean value found in healthy subjects [16]. Both groups studied (9 and 6 patients, respectively) do not reflect the results estimated in all patients with pyelonephritis when the test for detecting antibody-coated bacteria in urine is used. We found a positive test in 64.3% and 73.1% [16, 4], respectively. It cannot be excluded that urine samples without antibody-coating are to be found in additional investigations without homologous O-antibodies.

The above results argue against the existence of a correlation between O-antibody titres in the urine specific to a particular organism, and an antibody-coating of the organism. Our results indicate that the presence of homologous O-antibodies in the urine need not necessarily cause a coating, and this must be due to a factor present in the urine or on the bacteriologic surface. It is possible that such an influence is brought about by chemotherapeutics, or the organism's K antigen, or both.

Because in both groups only 2 patients were treated with antibiotics during the observation time (Tables 1 and 3), no exact conclusion can be drawn concerning this problem.

The results of Smith et al [24] also underscore the many facets of the problem. They found in animal experiments that urine O-antibodies, but not K-antibodies, were responsible for the coating. In contrast to these authors, who found bacterial coating in urine containing antibodies during its in vitro incubation, Kohnle et al [25] were unable to achieve antibody-coating of subculture organisms in urine that had been rendered sterile. Thomas et al [26] demonstrated that free urinary antibodies in vitro in patients with renal infections were not only reactive with the patient's own strain but also with heterologous bacteria.

These results stress the need for increased attention to special immunologic aspects of the test for determining antibody-coated bacteria in the urine.

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