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Evaluation of Antibody Coating of Yeasts in Urine as an Indicator of the Site of Urinary Tract Infection

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Antibody coating of yeasts (*Candida* sp. and *Torulopsis* sp.) found in urine specimens was investigated to ascertain whether the presence of such coating might identify the site of urinary tract infection. Washed yeast cells obtained by centrifugation of fresh urine specimens were reacted with fluorescein-conjugated goat antihuman immunoglobulins (Ig) G, A, and M and examined by fluorescent microscopy. IgG was found on the surface of all species of yeasts encountered in all urine specimens evaluated, whereas there was variability of IgA and IgM coating. Antibody coating with IgG, IgA, and IgM was also demonstrated on yeasts from other body sites (sputum, gastrostomy, oral, etc.). Control experiments confirmed the specificity of the reactions. Thus, it appears that yeasts from any body site are coated with antibodies. These results are in contrast to recent work with bacteria which showed that the presence of antibody-coated (IgG) bacteria indicates upper urinary tract infection (pyelonephritis) while bacteria are not coated with antibodies in lower urinary tract infection. Since all yeasts from all body sites tested were found to be coated with antibody regardless of the clinical situation, the presence of surface antibody has no diagnostic value in identifying the site of urinary tract infection with yeasts.

Yeasts found in a urine specimen by direct examination or culture may result from either lower urinary tract infection or simple colonization. Similar laboratory results may be encountered in renal infection or in disseminated infection with renal involvement. The evaluation of the significance of these mycology laboratory findings is fundamental to appropriate clinical and therapeutic decisions. Candiduria, when appropriate culture techniques are used, is considered significant by Schonebeck (6). However, clinical management and therapeutic measures will be selected according to the extent to which the urinary tract is involved.

In a review by Lehner (4) of autopsied cases of disseminated candidiasis, the kidneys were the most frequently involved organs. Forty of the 45 cases had renal involvement; candiduria was present in the majority of these cases. Schonebeck (6) reviewed invasive candidiasis in the lower and upper urinary tracts. He emphasized that fatal renal candidiasis occurred in the absence of pyuria (8). Earlier studies (5) attempted to use quantitation of organisms per milliliter to determine threshold of significance, but studies by Schonebeck et al. (7, 9) demonstrated that quantitation may be misleading and will not identify all serious yeast infections.

A laboratory technique that could indicate the type of urinary tract involvement by species of *Candida* and related yeasts would be useful. A direct immunofluorescent technique for identifying antibody coating of bacteria in urine was described by Thomas et al. (12). This noninvasive, rapid assay permitted excellent correlation between the presence of antibody-coated bacteria and renal involvement. Antibody coating of bacteria was generally not found if the infection was limited to the bladder. Jones et al. (2), using a bladder washout technique, also found the direct immunofluorescent technique to be a sensitive, reliable method for identifying the site of bacterial infection or colonization even in patients with asymptomatic bacteriuria and negative radiological findings.

The purpose of this study was to evaluate the correlation of antibody coating of yeasts with the site of urinary tract infection with the direct immunofluorescent technique.

MATERIALS AND METHODS

Twenty-five patients (15 females and 10 males) were selected for this study because they had freshly collected urine specimens that contained yeasts or yeasts and hyphal elements on direct examination of centrifuged specimens sent for culture to the Mycology or Bacteriology Laboratories of The Johns Hopkins Hospital. The urine specimens were cultures on routine mycological media, and fungal isolates were identified by standard procedures.

Three aliquots (2 to 5 ml) of each urine sample were centrifuged at 2,000 × *g* for 10 min and the supernatants were discarded. The sediments were suspended in 5 ml of phosphate-buffered saline (PBS), pH 7.4, mixed on a Vortex mixer for 1 min and centrifuged at 2,000 × *g* for 10 min. This washing procedure was done three times. After the third wash, 0.25 ml of a 1:5 dilution in PBS of fluorescein-conjugated monospecific anti-immunoglobulins (Ig) G, A, or M of goat origin (Meloy Laboratories) was added to the separate aliquots of sediment. The samples were then incubated for 30 min at 37 C and again washed three times with PBS. A drop of the sediment was air-dried on a microscope slide, mounted with PBS-buffered glycerol and examined microscopically for fluorescent coating of the fungal elements. An A/O series U20 Fluorestar microscope with an FITC exciter filter and Schott OG-1 and GG-9 barrier filters was used. Two observers independently read and graded the fluorescence as strongly positive, weakly positive, or negative.

Controls included cultural isolates recovered from five patients in order to show the conjugated antiserum to be free of anti-*Candida* activity. A second control, incubation with fluorescein-conjugated anti-Group A Streptococcal antiserum, was performed to demonstrate there was no nonspecific absorption of fluorescent material by the cultured yeasts. To further rule out nonspecific absorption of IgG, a control was included with cultured yeasts that had been incubated previously in human serum in which anti-*Candida* antibodies had not been detected. As a fourth control, three isolates of the cultured yeasts were incubated for 2 h at 37 C with urine that had been filtered through a 0.45-μm filter (Falcon Corp.) to rule out nonspecific coating of the yeasts by substances in urine to which the conjugated antiserum may have had activity.

Six other specimens (two sputa, one gastrostomy drainage, and three scrapings of oral mucosa) containing yeasts or yeasts and hyphal elements on direct (KOH) examination also were tested by this direct immunofluorescent method. Samples were suspended in 5 ml of PBS and processed as described above for urine samples.

Serological tests for anti-*Candida* antibodies were performed on sera from 16 of the 25 patients. A whole-cell agglutination test was performed using patient's serum diluted in saline from 1:20 to 1:1,280 in tubes 12 by 75 mm. To each 0.25 ml of diluted serum, 0.25 ml of a stock suspension of heat-killed *Candida albicans* containing 1.5×10^6 organisms/ml was added. The tubes were incubated at 37 C for 3 h and then refrigerated overnight. They were read for microscopic agglutination with indirect light and the titer was the last tube with visible agglutination.

The agar gel diffusion test was performed using barbital buffered agar plates prepared by Schering Corp. Wells measuring 4.5 mm in outer diameter were filled with 50 μl of patients' sera. These wells were spaced 3 mm from antigen wells measuring 3 mm in outer diameter that were filled with a cell-free extract antigen provided by Schering Corp. (lot #118-17-15). The plates were incubated for 24 h at 25 C and were examined for precipitin bands with indirect light.

The counterimmunoelectrophoresis test was performed with the Austigen II counterelectrophoresis system (Hyland Laboratories). The wells on the anodal side were filled with undiluted patients' sera. The wells on the cathodal side were filled with a cell-free extract of *C. albicans* provided by Schering Corp. (lot #120G-17-1 V1). The antigen was employed undiluted and diluted 1:5 and 1:10 with the 0.01 M barbital buffer, pH 8.6. Each serum was tested against the above three antigen concentrations. Electrophoresis was run at 30 mA for 120 min. The plates were flooded with 0.85% saline for 30 min and read for precipitin bands with indirect light.

Clinical data were obtained by chart review with a prospectively designed protocol that included the following data: general information (age, sex, and clinical diagnosis); predisposing factors (immunosuppression, antibiotic therapy, neoplastic disease, surgical procedures, intravenous or urinary catherization, hyperalimentation, renal disease, and diabetes); laboratory data (serum urea nitrogen, creatinine, leukocytes, urinalysis, and immunoglobulin levels); and clinical findings suggesting possible fungal infection. The clinical information and microbiological culture results were evaluated only after the fluorescent testing and serological testing was completed.

RESULTS

Urine specimens from 25 patients and 6 specimens from other body sites were tested by direct immunofluorescence for antibody coating of yeasts. Yeasts in all urine specimens regardless of the type of clinical disease showed antibody coating. The clinical diagnoses included systemic candidiasis, pyelonephritis, cystitis with and without an indwelling urinary catheter, and asymptomatic funguria with and without an indwelling urinary catheter (Table 1). Antibody coating of yeasts occurred in all specimens examined from other superficially infected or colonized sites such as oral mucosa, gastrostomy drainage, and the respiratory tract.

TABLE 1. *Correlation of clinical diagnosis with antibody-coated yeasts in urine*

Clinical diagnosis	No. of patients	No. positive[a]
Disseminated candidiasis	4	4
Pyelonephritis	4	4
Cystitis		
With indwelling catheter	5	5
Without indwelling catheter	2	2
Asymptomatic funguria		
With indwelling catheter	7	7
Without indwelling catheter	3	3

[a] Brilliant fluorescence of yeasts (strongly positive) with conjugated anti-IgG antiserum.

The reaction with anti-IgG was strongly positive in all specimens as demonstrated by a brilliant fluorescence outlining the cell wall. Hyphal elements, when present, also showed strongly positive fluorescent reaction with anti-IgG (Fig. 1). Anti-IgA antiserum demonstrated reactivity varying from strongly positive in 18 cases and weakly positive in 6 cases to negative in one case. Reactivity of the yeasts with the anti-IgM antiserum also varied from strongly positive in 11 cases and weakly positive in 8 cases to negative in 6 cases. The patterns of fluorescence, with the three antisera, encountered in urines and specimens from other sites are outlined in Table 2. The one patient who showed strongly positive fluorescence with anti-IgG and negative reactions with anti-IgA and anti-IgM was a 3-day-old infant.

The types of yeasts recovered from specimens included in this study were: *C. albicans*, 23; *C. tropicalis*, 4; *Torulopsis glabrata*, 2; *C. krusei*, 1; and *C. guilliermondii*, 1. All species of yeasts examined showed antibody coating.

All five controls with cultural isolates showed negative reactions for coating with IgG, IgA, and IgM. Incubation of the cultured yeasts with filtered urine did not result in antibody coating; reactions with anti-IgG, anti-IgA, and anti-IgM were uniformly negative. There was no nonspecific absorption of fluorescein-conjugated anti-Group A *Streptococcus* antiserum. The yeasts, preincubated in serum known to lack anti-*Candida* antibodies, also were negative, indicating no adsorption of IgG.

Of the 16 patients in the study who had serological testing done, four had no detectable anti-*Candida* antibodies in their sera by all assay techniques used. Two of the 16 had only low titers (<1:160) in the agglutination assay. Ten of the 16 patients had agglutination titers of 1:160 or greater. This titer has been suggested in other studies as an indicator of significant disease (11). Of these 10 patients, five had a positive reaction with the agar gel diffusion and the counterimmunoelectrophoresis assays. The other five with an agglutinin titer of 1:160 or greater did not demonstrate precipitating anti-*Candida* antibodies.

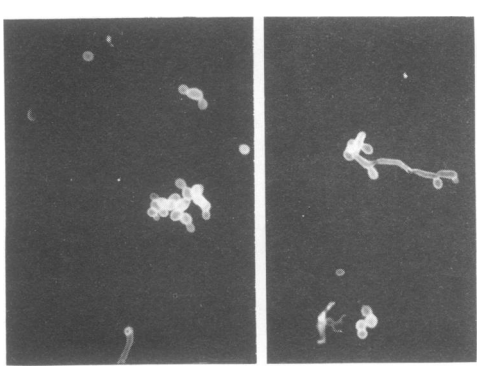

FIG. 1. *Urinary yeasts and hyphal elements showing strongly positive fluorescence with fluorescein-conjugated anti-IgG.*

TABLE 2. *Patterns of fluorescence of yeasts with antiserum to IgG, IgA, and IgM*

Fluorescein-conjugated monospecific antiserum	Test results	No. of urines	Other[a] sites
IgG, IgA, IgM	Strongly positive	11	2
IgG, IgA IgM	Strongly positive Weakly positive	2	2
IgG IgA, IgM	Strongly positive Weakly positive	6	2
IgG, IgA IgM	Strongly positive Negative	5	
IgG IgA, IgM	Strongly positive Negative	1	

[a] Sputum, oral, mucosa, and gastrostomy drainage.

DISCUSSION

The direct immunofluorescent technique applied to yeasts is an exquisitely sensitive as well as specific technique for demonstrating antibody coating of yeasts by IgG, IgA, and IgM. All yeasts from urine as well as the other body sites tested (sputum, oral mucosa, and gastrostomy drainage) were found to be coated with IgG. This study has shown that a humoral antibody response, while often present, is not a prerequisite for antibody coating of yeasts. The circulating anti-*Candida* antibodies may be absent or may be present at such low concentrations that they are not detected by our serological assays.

It is possible that the antibody found coating the yeasts in the urine was due to local production, at least in part. This can be supported by studies that demonstrated local production of antibody by the urinary tract in an experimentally induced, unilateral acute bacterial pyelonephritis in rabbits by Lehman et al. (3) and in a chronic bacterial pyelonephritis rat model by Cotran (1). Thomas et al. (12) suggested the possibility of local production of antibody in some of their patients with pyelonephritis who had antibody-coated bacteria in their urine without elevation of serum antibody titers against the infecting bacteria.

On the other hand, one patient in our study was a 3-day-old infant receiving broad spec-

trum antibiotics who had urinary tract colonization. His pattern of urinary yeast fluorescence was IgG, strongly positive, and IgA and IgM, negative. This pattern of reactivity coincides with expected levels of the immunoglobulin classes in the newborn. The IgG present reflected passive transplacental acquisition of antibody from the mother. Presumably, this IgG anti-*Candida* antibody reached the newborn's urinary tract mucosa through the circulation.

Decreased fluorescence with anti-IgA and diminished or absent fluorescence with anti-IgM did not correlate with the extent of infection or colonization. For example, strongly positive reactions for IgG, IgA, and IgM were found in all types of clinical disease as well as in apparent colonization. However, assuming that the intensity of fluorescence varies with the amount of antibody present in a given patient, the pattern of fluorescence may correlate with the duration of host-organism interaction. The yeast infection, at the time of fluorescent examination, may not have been present long enough for an IgM response or for the IgA or IgM to be present in amounts equivalent to IgG. Smith et al. (10) have shown IgG to be the class of immunoglobulins first demonstrated (11 days) in a local antibody response by rabbits with experimental bacterial pyelonephritis. IgA was found next in temporal sequence (14 days), and IgM was not demonstrated until after infection had been present for 19 days.

Thomas et al. (12) also demonstrated that bacteria in urine from patients with upper urinary tract infections were consistently coated with IgG. IgA was present in 70% of cases, whereas IgM was present in less than 50% of cases.

Our results indicate that, unlike the results of studies on bacterial infection, antibody coating of yeasts could not be used to differentiate systemic seeding or kidney infection from lower urinary tract infection or colonization. All types of yeasts from all body sites were coated with antibody including urinary yeasts from 17 patients with asymptomatic funguria or cystitis. The potential usefulness of this technique for identifying the site of infection of the urinary tract is prevented by the fact that host-organism interaction is a stimulus for antibody production even in asymptomatic urinary tract involvement.

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