

Significance of urinary immunoglobulins in tests for antibody-coated bacteria

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SUMMARY The relationship between urinary immunoglobulin levels and coating of a panel of bacteria by these immunoglobulins has been investigated. The results indicate that elevated urinary immunoglobulin levels are a distinct hazard in interpreting tests for antibody-coated bacteria as indicating upper or lower urinary tract infections.

Several studies have indicated that the detection of antibody-coated bacteria in urinary sediment by immunofluorescence (ACB test) is a useful screening test to discriminate between upper and lower urinary tract infections. The test was initially evaluated in studies on limited numbers of closely followed patients (Thomas *et al.*, 1975a; Thomas *et al.*, 1974) and was subsequently applied to unselected individuals (Jones *et al.*, 1974; Fries *et al.*, 1975; 1977; Boisivon *et al.*, 1976; Forsum *et al.*, 1976; 1978; Montplaisir *et al.*, 1976; Gürtler, 1977; Rumans and Vosti, 1978). The occurrence of IgG and IgA antibodies coating bacteria in the urine in pyelonephritis, but not in cystitis, was experimentally verified by Smith *et al.* (1977), who showed that in experimental pyelonephritis in rabbits antibody-coated bacteria occurred in a time sequence that is consistent with an immune response.

However, there are now reports on false-positive results using antibody coating as a screening test for upper urinary tract infections in patients with prostatitis (Jones, 1974), urinary tract cancer (Forsum *et al.*, 1978), and elevated urinary immunoglobulin contents (Braude and Block, 1977) and in urines contaminated with bacteria from the urethral flora (Jones and Johnson, 1977). The risk of false-positive results must be considered because elevated immunoglobulin levels in the urine might coexist with contaminating bacteria that bind Forssman antibodies. Likewise urinary bacteria might bind immunoglobulins via Fc-receptors in such numbers as to make a false-positive diagnosis probable.

We have studied reactions between urinary immunoglobulin from non-infected patients with

urinary tract cancer and a panel of bacterial species in order to elucidate the type of non-specific reactions that can be expected to occur in tests for antibody-coated bacteria in the urine. The results indicate that reactions with the Fc-segment of IgG occur with urinary immunoglobulin, thereby complicating the detection of bacteria coated with specific antibodies.

Material and methods

Fourteen patients with bladder tumours were randomly selected and their tumours were classified according to the International Union against Cancer (UICC).

Eighteen urine specimens were collected. From four of the patients urine was sampled both before and after irradiation of the tumour. None of the patients had any sign of urinary tract infection, and culture on cystine-, lactose-, electrolyte-deficient agar (CLED) (Oxoid, England) and blood agar with 0.07% sodium azide with calibrated loop technique revealed no bacterial growth. Urine from 10 healthy workers served as controls. The bacteria used were taken from fresh clinical specimens sent to the laboratory, with the exception of streptococcus group A types M 12 and M 17 and streptococcus group C type 81 C, which were obtained from Dr Poul Christensen, Institute of Medical Microbiology, Lund, Sweden. *Shigella flexneri* 1a was obtained from the National Bacteriological Laboratory (SBL), Stockholm, Sweden.

All bacteria except the streptococci were sub-cultured seven times in a medium devoid of blood, sera, egg yolk, and peptone (Springer *et al.*, 1961) to avoid cross-reactions with these substances. The

streptococci that did not grow in this medium were grown in Todd Hewitt Broth for 18 hours and then washed seven times in phosphate-buffered saline (PBS), pH 7.4, before testing.

The indirect immunofluorescence test was performed as follows: one drop of the bacterial suspension was placed on a clean glass slide, allowed to dry, and gently heat-fixed. Then one drop of urine, dialysed extensively against PBS, was layered on the bacteria and allowed to react for 20 minutes at 37°C. After washing in PBS and drying, one drop of fluorescein-isothiocyanate (FITC)-labelled (F_{ab})₂-fragments of human IgG, IgA, and IgM (Kallestad, USA, lot numbers 139J011-2, 137K151-1, and 140K083-1, respectively) was layered on the slides and allowed to react for 20 minutes. Thereafter the slides were washed and examined in a Leitz Orthoplan microscope equipped with incident light, narrow blue-band activation, and HBO 200 mercury lamp. In some experiments 4% bovine serum albumin (BSA) was added to the urine before being layered on the bacteria.

The immunoglobulin content of urines was determined by radial immunodiffusion with Partigen and LG Partigen plates (Behringwerke, West Germany).

Absorption with sheep red blood cells was performed by washing the blood cells three times in PBS and then mixing equal volumes of urine and packed blood cells. The mixture was kept for 1 hour at 37°C before centrifugation. The sediment was discarded.

Serum IgA and secretory IgA were kindly provided by Dr Ingemar Björk, Institute of Veterinary Biochemistry, Biomedical Centre, Uppsala, Sweden.

Pepsin cleavage of IgA was performed as described by Stanworth and Turner (1973); IgG was cleaved by the method of Turner *et al.* (1970).

Finally, IgG from the urine of one patient with bladder cancer was purified as follows: 2 litres of urine was collected, dialysed extensively against PBS, concentrated 100 times with polyethylene glycol, and passed through a column of Protein A Sepharose (Pharmacia Fine Chemicals, Sweden). The retained material was eluted with 3M MgCl₂ and, after dialysis against PBS, shown to be pure IgG by immunoelectrophoresis.

Results

To see if a reaction with urinary immunoglobulin could be found among strains with known immunological relations to human tissue, urine specimens from the bladder cancer patients were tested against a panel of bacterial strains. *Escherichia coli*, proteus, salmonella, and pseudomonas all contain blood

group-like substances in their cell walls (Springer *et al.*, 1961). *Sh. flexneri*, however, has not been found to contain any such substance (Springer, 1971). Certain *E. coli* strains have also been shown to cross-react immunologically with renal tissue (Holmgren *et al.*, 1975), as has the streptococcus group A (Lyampert and Danilova, 1975). Streptococcus group C carbohydrate has been found to have antigen determinants in common with Forssman antigen (Coligan *et al.*, 1977). The cell walls of pseudomonas have been shown to be antigenically related to the Rh factors of human erythrocytes (Lyampert and Danilova, 1975).

The result of the immunofluorescence test can be seen in the Figure. Most of the urines reacted with streptococcus group C when anti-IgG was used. About one-third of the urines reacted with streptococcus group C and half the urines reacted with a *Staphylococcus albus* strain when anti-IgA was used. Reactions were not recorded when testing with anti-IgM. One of the urines from a healthy laboratory worker reacted with a streptococcus group C alone when anti-IgG was used. Reactions were not seen with anti-IgA or anti-IgM. Addition of 4% BSA to urines did not interfere with the reactions recorded. Most of the urines that reacted with anti-IgG or anti-IgA had elevated urinary contents of the corresponding immunoglobulin (Table). Reactions were not recorded when using the following bacterial strains: *Streptococcus faecalis*, *Staph. albus*

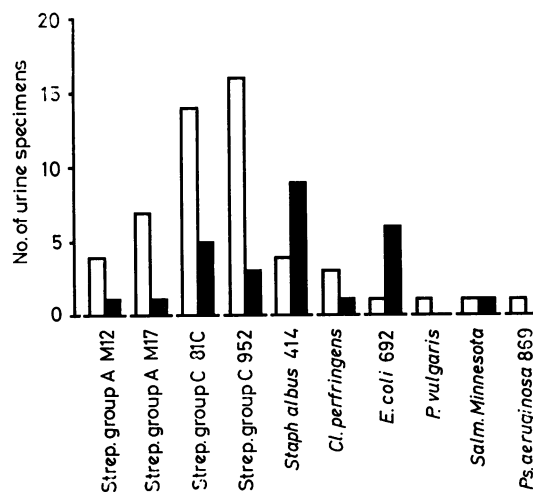


Figure Bacterial strains reacting with uninfected urine specimens from patients with bladder tumours. Open columns: number of urines reacting with FITC anti-IgG; hatched columns: number of urines reacting with FITC anti-IgA.

Table Immunoglobulin content of urine from patients with bladder tumours at different stages of development in relation to immunoglobulin coating with a panel of bacteria

Clinical stage of tumour	No. of urines	Mean levels of immunoglobulin (mg/100 ml)			No. of urines reacting with panel of bacteria by		
		IgG	IgA	IgM	IgG	IgA	IgM
II TI	4	47.6	11.0	0	3	3	1
II TII	1	8.0	0	0	1	0	0
IIb TI	1	0	0	0	1	1	0
III TI	4	0	0	0	3	3	0
III TIIa	3	18.6	1.5	0	3	2	0
III TIVb	5	23.1	2.9	0	5	3	0
Normal	10	2.1	0	0	1	0	0

308, *E. coli* 699, *Proteus mirabilis*, *Sh. flexneri*, and *Pseudomonas aeruginosa* 690.

To test the possibility that a reaction with Forssman-like antigens on the bacterial surface had been detected, absorption of four of the urines with sheep red blood cells was performed, and the urines were tested by immunofluorescence. No difference was found between absorbed and unabsorbed urines applied to either Gram-positive or Gram-negative bacteria.

The IgG purified from the urine of one patient and its (F_{ab}')₂-fragment were tested against the bacterial panel by immunofluorescence using anti-IgG. Positive reactions could be seen with only the two streptococcus group C strains and one *Staph. albus* strain. Reactions were not seen with (F_{ab}')₂-fragments.

We also tested pure serum IgA and pure secretory IgA and their (F_{ab}')₂-fragments. A positive reaction could be seen between serum IgA and streptococcus group C and between secretory IgA and *E. coli* 692. Reactions were not recorded with (F_{ab}')₂-fragment of secretory IgA, but the reaction between serum IgA and *E. coli* 692 remained after pepsin cleavage.

Discussion

Since Thomas *et al.* (1974) described the ACB test for determination of the level of urinary tract infection there have been many reports that confirm the usefulness of the method (Jones *et al.*, 1974; Fries *et al.*, 1975; 1977; Boisivon *et al.*, 1976; Montplaisir *et al.*, 1976).

However, there have also been a few reports that point out some difficulties in interpreting the results. Jones (1974) has made it clear that many patients with prostatitis have antibody-coated bacteria in their urine. Braude and Block (1977) and Rumans and Vosti (1978) have reported false-positive reactions in urine from patients with proteinuria. Indwelling catheters have resulted in the introduc-

tion of bacteria into the urine that are antibody-coated (Gürtler, 1977). We have in a previous report shown that infants should not be investigated by this method (Forsum *et al.*, 1976) since infants do not mount a vigorous antibody response in the urine. We have also shown that false-positive results occur in patients with cancer of the urinary tract (Forsum *et al.*, 1978).

By studying experimental pyelonephritis in rabbits, Smith *et al.* (1977) found that antibody-coated bacteria did not occur in the urine before the 11th day of infection. That leads to the conclusion that urine specimens taken early in the infection will probably be negative in the ACB test.

In the present investigation we used urines from patients with cancer of the urinary tract not complicated with a concurrent urinary tract infection. All the urines reacted with at least one of 16 bacteria when tested by indirect immunofluorescence. Most reacted with beta-haemolytic streptococci group C when anti-IgG was used (Figure). We have excluded the possibility that this was due to reactions with contaminating blood group-like substances from the medium. Bacteria were grown in a medium devoid of blood, sera, egg yolk, and peptone. When such media could not be used the bacteria were carefully washed. Most Gram-negative bacteria have blood group-like substances on their surface (Springer *et al.*, 1961). However, such bacteria were rarely coated by urinary immunoglobulins.

Forssman-like antigens have been found in beta-haemolytic streptococci group C (Coligan *et al.*, 1977), but when we absorbed the possible Forssman antibodies on sheep red blood cells we found no difference in coating before and after absorption.

There are reasons to believe that urinary immunoglobulins can bind to bacteria via F_C-receptors. Evidence to support this proposition was obtained in the current study by the fact that IgG but not (F_{ab}')₂-fragments could bind to several of the bacteria used in the panel.

When testing IgA we found a reaction that was probably a reaction between the F_{ab}-part of the IgA molecule and streptococcus group C. Most of the cancer patients had elevated urinary immunoglobulins. One of the control urines contained an elevated level of IgG (15.2 mg/100 ml) that was the control urine that reacted with the bacterial panel.

Thomas *et al.* (1975b) concluded that the immunoglobulin level of the urine was of no importance in the outcome of the ACB test. However, uninfected urines were not included in their study and they did not test the urines against many different bacteria. Therefore they might not have seen false-positive results. Further discussions relating to false-positive results have stated that protein levels of the urine do affect the ACB test, but it has been claimed to be of minor importance (Thomas *et al.*, 1977). That may be true in uncomplicated cases but when one is dealing with cancer of the urinary tract or other diseases that are urologically complicated the risk that the patients are infected with bacteria is increased. Since it is a well-known fact that some bacteria, especially *Staphylococcus aureus* and certain streptococci, are able to bind to the F_c-part of the IgG molecule (Forsgren and Sjöquist, 1966; Kronvall, 1973), the risk that contaminating bacteria are coated with immunoglobulin is also increased when the immunoglobulin content is high. We conclude that the immunoglobulin content of the urine has to be considered when the result of the ACB test is being interpreted.

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