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Research Article

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Use of a Solid-Phase Radioimmunoassay and Formalin-fixed Whole Bacterial Antigen in the Detection of Antigen-specific Immunoglobulin in Prostatic Fluid

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ABSTRACT The prostatic fluid of two patients with *Escherichia coli* bacterial prostatitis was analyzed for evidence of a local immune response to bacterial infection. A solid-phase radioimmunoassay was modified to measure the immunoglobulin (Ig)A and IgG antigen-specific antibody responses to infecting bacteria in serum and prostatic fluid from each patient. Formalin-fixed whole *E. coli* were used as antigen. In one patient with acute *E. coli* prostatic infection, measurements of antigen-specific antibody confirm the presence of a systemic and local immune response. However, in another patient with a chronic *E. coli* prostatitis, a primarily local immune response was demonstrated. The response measured in the prostatic fluid appears to be locally stimulated and specific for the infecting bacteria. Furthermore, IgA was the predominant immunoglobulin involved in the local prostatic immune response to infection. Although elevations of serum IgA antigen-specific antibody levels were short-lived after treatment of prostatic infection, local IgA antigen-specific antibodies were detected for as long as 1 yr after the initial infection in both patients studied.

INTRODUCTION

Although the local antibody responses to infection at human secretory surfaces such as the nasal mucosa, bronchus, and intestine have been studied, a local response to infection of the prostate has not been described. Using direct immunofluorescence techniques, Ablin (1) demonstrated the presence of immunoglobulin (Ig)A and IgG in normal human prostatic tissue. Others (2, 3) have measured and identified

the immunoglobulin classes in the prostatic fluid. In 1965, Maged and Khafaga (4) measured elevated serum and prostatic fluid agglutinins to staphylococci in patients with staphylococcal prostatitis, but their culture technique did not clearly distinguish between urethral and prostatic organisms. Moreover, they did not characterize the response nor investigate the antibodies involved. Using direct bacterial agglutination techniques, Meares (5) described elevated serum agglutinins in response to bacterial prostatitis.

The present work was undertaken to devise a reliable method for measuring an antigen-specific antibody (A-SA)¹ response in serum and prostatic fluid and to establish whether a local and systemic response to prostatic bacterial infection exists. This investigation demonstrates the usefulness of a formalin-fixed antigen preparation with a solid phase radioimmunoassay (SPRIA) for the measurement of A-SA in serum and human secretory fluids. Furthermore, it verifies a distinct local and systemic response to infection in two patients with bacterial prostatitis. The results demonstrate the improved accuracy and information obtained from a SPRIA technique over previous techniques used to study the immunologic response in prostatic fluid.

METHODS

Bacterial culture procedures

Quantitative bacterial localization procedures, which have been described elsewhere (6), were used to culture the

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¹ *Abbreviations used in this paper:* A-SA, antigen-specific antibody; BSA, bovine serum albumin; EPS, expressed prostatic secretion; PBS, phosphate-buffered saline; RAH, rabbit anti-human; RID, radial immunodiffusion; SPRIA, solid-phase radioimmunoassay.

urethra, voided urine, and prostatic secretions. The first-voided 5–10 ml of urine, the midstream aliquot, the prostatic fluid produced from prostatic massage (EPS, “expressed prostatic secretion”), and the first 5–10 ml of urine immediately following massage were refrigerated after collection. Standard bacteriologic techniques were used to identify and quantify the localization cultures (6). Serotyping of *Escherichia coli* O antigens was performed using the technique of Edwards and Ewing (7).

Specimens

EPS were obtained by digital massage of the prostate. The secretions were immediately cultured and placed in capillary tubes. The tubes were spun in a capillary centrifuge for 10 min and the sediment was discarded. The EPS supernatant fluid was stored at -70°C and thawed to room temperature just before assay. Serum specimens were stored at -70°C in aliquots and were thawed no more than twice.

Measurement of A-SA

The indirect SPRIA described by Zollinger et al. (8) for purified meningococcal antigen was modified to measure A-SA in serum and prostatic fluid. The radioimmunoassay was performed in flexible polyvinyl chloride microtiter plates (obtained from Cooke Engineering Co., Alexandria, Va.). Formalin-fixed whole bacteria prepared at concentrations of 10^8 organisms/ml were used as antigen and 25 μl was placed in each well for assay. The microtiter plate was then placed in a humidified chamber at 37°C for 1 h. The bacterial antigen was aspirated from the wells and 50 μl of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS, buffered to pH 7.2) was used to wash each well. This was removed and replaced by 100 μl of BSA and incubated at 37°C for 30–60 min. The BSA was aspirated and the plate was washed with PBS (pH 7.2) twice and then dried. 25 μl of the test solution was placed into the appropriate antigen-bound wells. The entire plate was incubated at room temperature overnight in a humidified box. The following day the wells were aspirated and washed with 50 μl of BSA. The plate was then flooded with PBS twice. The wells were each filled with PBS and incubated for 1 h at 37°C . They were then washed again with PBS twice and dried by aspiration. 25 μl of ^{125}I -labeled class-specific rabbit anti-human (RAH) immunoglobulin fraction, diluted to contain 20 ng/25 μl , were placed in the wells in a humidified box for 12–16 h at room temperature. This was aspirated and washed with 50 μl of BSA followed by five washes with PBS. The wells were then dried and separated. Each well was placed in a glass test tube and counted for 1 min in a Packard Gamma Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Serial twofold dilutions in 1% BSA from 1:50 to 1:102,400 for each serum specimen and from 1:50 to 1:819,200 for EPS were assayed. All assays were performed in duplicate. Standards for each assay included a high-titered immune specimen from a patient with bacterial prostatitis to determine maximum binding of ^{125}I -RAH immunoglobulin fraction, and a BSA specimen to determine nonspecific binding. RAH colostrum IgA and IgG fractions, specific for heavy chains (obtained from Bio-Rad Laboratories, Richmond, Calif.), were iodinated by the lactoperoxidase method (8, 9) using carrier-free [^{125}I]Na (New England Nuclear, Boston, Mass.).

For each test specimen assayed, a plot was made of the reciprocal of the dilution on a log scale versus the counts per minute obtained from ^{125}I -RAH immunoglobulin fractions. These counts per minute were normalized so that the maximum counts obtained from the high-titered immune standard

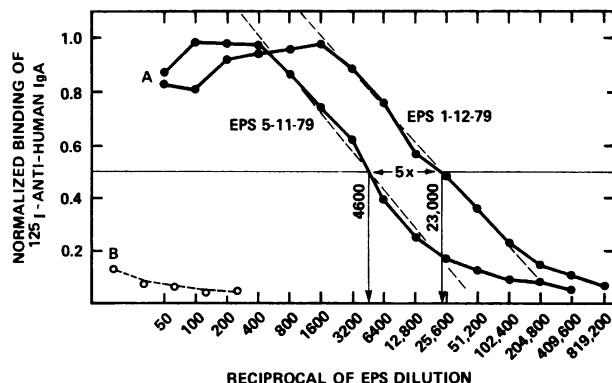


FIGURE 1 Examples of the EPS-AS-A binding curves in a patient with chronic bacterial prostatitis (A) and a healthy volunteer (B). EPS from patient 2 with *E. coli* 075 chronic bacterial prostatitis on two dates (1/12/79, 5/11/79) were assayed and labeled A on the figure. Formalin-fixed *E. coli* 075 was used as antigen and ^{125}I -RAH IgA was used to detect IgA A-SA. The standard SPRIA was performed in duplicate for all dilutions assayed. Best-fit lines were drawn through the linear portion of the binding curve. The dilution corresponding to 50% binding or 0.5 normalized binding is designated as the titer. The titer for 1-12-79 is 23,000 and for 5-11-79 is 4,600. The nonspecific binding curve (B) for an EPS from a healthy volunteer is plotted for comparison.

was equal to one. A plot of these results was then designated the normalized binding curve for the test specimen. These curves were characterized by a plateau at the region of excess specimen antibody and an asymptotic area at the region of excess ^{125}I -RAH immunoglobulin with a region of positive antibody binding between these two areas. For the purposes of this study a best-fit line was drawn in the linear region of positive antibody binding. The specimen dilution at which the normalized binding of ^{125}I -RAH immunoglobulin was 0.5 was defined as the A-SA titer. Examples of the normalized binding curves for two EPS specimens are seen in Fig. 1.

Quantitation of immunoglobulins

Total IgA and IgG were measured using commercially prepared radial immunodiffusion (RID) plates. Kallestad Endoplates (obtained from Kallestad Laboratories, Chaska, Minn.) were used for detection of serum IgA and IgG and Kallestad low-level plates were used for determination of prostatic immunoglobulins. The coefficient of variation was 10–15%. Behring low-level Partigen IgA plates (Behring Diagnostics, Somerville, N. J.) were used to repeat quantitation for specimens with values < 0.042 mg/ml. The coefficient of variation for these plates is estimated at 8.5% or less. As little as 0.012 mg/ml IgA or IgG could be determined.

In addition to RID quantitation, prostatic IgA was also measured using the SPRIA in a method used by Zollinger² in which heavy chain specific RAH IgA fraction is placed in the microtiter wells with EPS and ^{125}I -heavy chain specific RAH IgA fraction is used to assay for bound EPS IgA.

IgA determinations for 25 specimens containing secretory IgA (not presented in this study) were compared using RID and SPRIA methods of measurement; they showed a linear correlation. Because of this relationship, discussion referring

² Personal communication.

to RID values applies to the corresponding SPRIA values as well.

Preparation of bacterial antigens

Previously stored bacterial isolates were inoculated into tryptose broth and incubated for 18 h at 37°C. A second volume of tryptose was added to the inoculum and reincubated for 2 h. The organisms were centrifuged at 5,000 g for 10 min, the supernatant fluid was discarded, and the sediment was washed with PBS three times. After final resuspension the organisms were incubated for 30 min at 37°C and formaldehyde was added to make a 0.5% formalin solution. After 18 h the organisms were recentrifuged at 5,000 g for 10 min and PBS with 0.1% sodium azide was added to form a concentration $\geq 10^9$ organisms/ml. Bacterial concentration was determined by particle counting in a ZBI Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.).

Bacterial agglutination and immunofluorescence techniques for determining antibody titers

Antibody-coated bacteria and serum antibody against infecting bacteria were detected by the techniques described by Thomas et al. (10). Specimens were judged to show positive fluorescent antibody coating if at least 25% of the organisms fluoresced. The method of Percival et al. (11) was used to determine antibody titers by bacterial agglutination. Each test was run in duplicate.

Absorption of antibodies from serum and secretory fluids

Specific antibodies were removed from serum or secretory fluid using the absorption techniques described by Fireman

et al. (12). Heavy-chain RAH class-specific immunoglobulin fractions were used to absorb antibodies; absorbed specimens were then assayed for A-SA activity. In a similar manner, formalin-fixed bacteria were used to absorb specimen A-SA. After removal of these bacteria, the specimen was assayed for A-SA activity and the bacteria were examined for fluorescent antibody coating.

Patients

Patient 1. A 50-yr-old male with an 11 yr history of recurrent urinary tract infections came to this institution in December 1978 with 3 d of chills, dysuria, and 102°F fever. A few days before his visit he had been given nitrofurantoin and then tetracycline. His past history revealed that he was treated for a well-documented acute *E. coli* 04 prostatitis in 1973. On examination his right prostatic lobe was rock hard, markedly elevated, and irregular; microscopic examination of the EPS revealed oval fat macrophages and leukocytes too numerous to count. Cultures revealed no growth in the bladder urine, but 1,000 colonies/ml of *E. coli* 018 grew from the prostatic fluid (Table I). The patient was then switched to treatment with trimethoprim-sulfamethoxazole (160 mg/800 mg) twice daily and became asymptomatic. He continued this treatment for 90 d. During that interval and the subsequent 6 mo his cultures remained sterile for any gram-negative organisms; on rectal examination the prostate gradually returned to normal.

This patient demonstrates an episode of acute bacterial prostatitis characterized by systemic symptoms of fever and chills during infection and a grossly distorted, inflamed prostate.

Patient 2. A 52-yr-old male was seen in December 1978 in the urology clinic and found to have an asymptomatic *E. coli* bacteriuria. In 1975 he had been seen for a chronic *E. coli* 01 prostatitis which was cured after removal of a prostatic calculus containing *E. coli* 01. Transurethral prostatic biopsies obtained at that time showed chronic prostatitis and nodular hyperplasia. He was then lost to further followup.

After his visit in December 1978, he was treated with nitrofurantoin to sterilize the urine but to allow prostatic localization. Quantitative bacteriologic localization cultures on the day 10 of nitrofurantoin therapy showed the *E. coli* 075 to be in the prostate (Table II). Cystoscopy revealed a smooth bladder without evidence of prostatic or bladder calculi. He was then placed on trimethoprim-sulfamethoxazole for the next 60 d, after which he remained asymptomatic for urinary tract complaints even though an *E. coli* 016 colonized his prostate (Table II). Subsequent cultures showed *E. coli* 016 persisted in his lower tract.

In contrast to patient 1, this man presented with an asymptomatic bacteriuria secondary to chronic bacterial prostatitis; he had no systemic symptoms and his prostate felt normal on rectal examination.

RESULTS

Reliability of the SPRIA using formalin-fixed whole bacteria. To investigate the reproducibility of the SPRIA results, serial twofold dilutions of a high-titered immune serum specimen from a male with bacterial prostatitis and that of a low-titered specimen from an uninfected male volunteer were assayed 18 times on a single day. The coefficient of variation of A-SA titers for a given serum dilution was 3–8%. In a second series of tests these same two serum specimens were

TABLE I
Bacterial Localization Cultures in Patient 1 with
Recurrent Acute Bacterial Prostatitis

Date	Drug	Days on or off	VB1*	VB2*	EPS	VB3*
colonies/ml						
12/18/78	nitrofu- rantoin	–2†	0	0	1,000	5,000
12/18/78	tetra- cycline	+2†				
12/29/78	tmp-smx§	+10	0	0	0	0
01/26/79	tmp-smx	+38	0	0	0	0
03/06/79	tmp-smx	+77	0	0	0	0
04/03/79	tmp-smx	–15	0	0	0	0
05/08/79	tmp-smx	–38	0	0	0	0
07/31/79	tmp-smx	–122	0	0	0	0
11/06/79	tmp-smx	–220	0	0	0	0
01/22/80	tmp-smx	–297	0	0	0	0

Patient infected with *E. coli* 018.

* VB1, first voided 5–10 ml urine; VB2, midstream urine; VB3, first voided 5–10 ml urine after prostatic massage.

† +, on; –, off.

tmp-smx, trimethoprim-sulfamethoxazole.

TABLE II
Bacterial Localization Cultures in Patient 2 with Recurrent Chronic Bacterial Prostatitis

Date	Drug	Days on or off	VB1*	VB2*	EPS	VB3*	Organism
<i>colonies/ml</i>							
12/18/78	none		>100,000	>100,000	>100,000	>100,000	<i>E. coli</i> 075
01/02/79	none		NS	>100,000	NS	NS	<i>E. coli</i> 075
01/12/79	nitrofurantoin	+ 10	40	0	5,000	2,000	<i>E. coli</i> 075
02/09/79	tmp-smx§	+28	0	0	0	0	
03/09/79	tmp-smx	-25	100	0	200	50	<i>E. coli</i> 016
05/11/79	tmp-smx	-88	3,000	380	10,000	NS	<i>E. coli</i> 016
06/29/79	tmp-smx	-137	1,000	0	10,000	NS	<i>E. coli</i> 016

* VB1, first 5–10 ml voided urine; VB2, midstream urine; VB3, first 5–10 ml voided urine after prostatic massage.

† NS, no specimen.

§ tmp-smx, trimethoprim-sulfamethoxazole.

repeatedly assayed eight times over a 20-d period. Except for the ^{125}I -RAH immunoglobulin fraction, all dilutions and reagents were freshly prepared for each test. These eight binding curves demonstrated a coefficient of variation of 13–18% without considering variation related to radioactive decay of the RAH ^{125}I -immunoglobulin fraction. In both series of tests, maximum variability appeared to occur at dilutions with low binding and minimum variability at dilutions with high binding.

Antigen concentration and fixation. The optimal bacterial antigen concentration for the SPRIA was studied by assaying, at different concentrations of the infecting bacteria, serial twofold dilutions of a high-titered immune serum specimen from a patient with bacterial prostatitis. These studies showed that there was a decrease in maximal counts if the bacterial concentration was $<0.29 \times 10^9$ organisms/ml. Therefore $\geq 10^9$ bacteria/ml were used to determine A-SA titers.

Multiple preparations of *E. coli* 04 antigen were made using varying solutions of formalin for fixation (0.1, 0.5, 1.0, 3.0, 6.0, and 10.0%). These bacterial suspensions were cultured at 1.5, 7, and 16–18 h. In 0.1% formalin solution, bacterial growth persisted at all cultured time intervals. In 0.5% formalin solution, bacterial growth was present at 1.5 h but absent at 7 and 16 h. For convenience it was decided to study only those bacterial suspensions in which bacterial growth was absent at 16 h. Serial twofold dilutions of a high-titered immune serum specimen were assayed against the various fixed bacterial suspensions. Each suspension was adjusted to a concentration of $\geq 10^9$ organisms/ml by Coulter Counter just before assay; optimal binding occurred at a 0.5% formalin solution and appeared to decrease with increasing concentrations of formalin. Furthermore, reincubation of the final suspension for 30 min before formalin fixation, as opposed

to immediate fixation, increased the maximal counts per minute.

Glutaraldehyde was also investigated as a potential antigen fixative, but clumping of the bacterial particles made quantitation by the Coulter Counter difficult and therefore unsatisfactory.

To test the acceptability of formalin fixation of bacterial antigen in the SPRIA, two further tests were performed. First, two bacterial preparations were made using live refrigerated bacteria and formalin-fixed bacteria. The SPRIA results for the two sets of bacterial antigen were compared using the previously described high- and low-titered immune sera. The differences between the two preparations were negligible. Second, both of these bacterial preparations were used for indirect fluorescence studies to measure serum antibody titers against the same high- and low-titered immune sera. On multiple trials the fluorescent antibody titers using the formalin-fixed bacteria were within one dilution of those for the live bacteria.

Absorption of antigen-specific antibodies. Samples of secretory fluid and sera were absorbed by bacteria to study the specificity of the assay. The stored sera from a patient with chronic bacterial prostatitis and a healthy volunteer were absorbed with formalin-fixed preparations of the infecting organism three times. Serial twofold dilutions of both the absorbed and unabsorbed sera were then assayed against the infecting organism; bacterial absorption effectively removed A-SA and successive absorption attempts resulted in a binding curve that reflected only base-line nonspecific binding (Fig. 2). Furthermore, indirect fluorescent antibody studies performed on these specimens using infecting organism were positive for the unabsorbed serum of the infected patient, trace positive for the same serum absorbed once, and negative for the sera absorbed two or three times. Similar studies on the uninfected

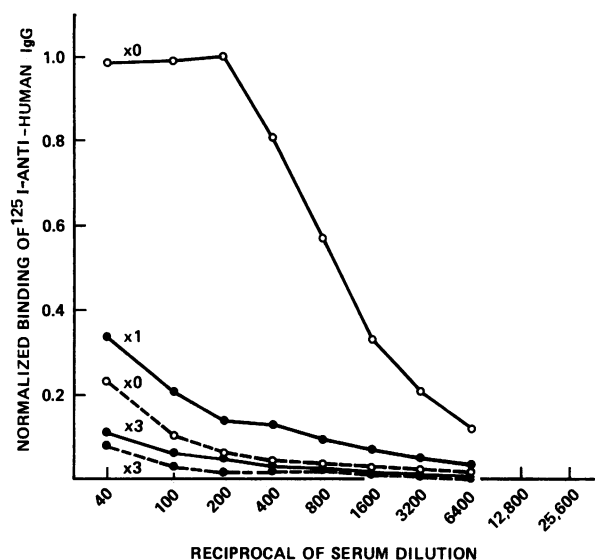


FIGURE 2 Bacterial absorption of A-SA from a serum specimen. The sera of a patient with *E. coli* 04 prostatitis (infected) and that of a control volunteer were absorbed with *E. coli* 04. These absorbed and unabsorbed sera were assayed with antigen *E. coli* 04 and ^{125}I -heavy-chain specific RAH IgG fraction was used to detect antigen-specific IgG. Both sera were absorbed with *E. coli* 04 three times ($\times 3$). The binding curves of the specimens after absorption show that bacterial absorption by the infecting organism effectively removes the positive antigen-antibody binding activity and only non-specific binding results. \circ , before absorption; \bullet , after absorption; —, infected; ---, uninfected.

volunteer serum, for the same organism, were negative before and after bacterial absorption.

Absorption of class-specific immunoglobulins. RAH IgG, IgA, and IgA secretory piece fractions were used to absorb IgG, IgA, and secretory IgA from specimens. A high-titered immune serum from a patient with chronic bacterial prostatitis and serum from a healthy volunteer were absorbed with RAH IgG and IgA. The sera were absorbed seven times. Serial dilutions of the absorbed and unabsorbed sera were assayed for IgG and IgA A-SA. In the assay for IgG A-SA, after absorption with RAH IgG fraction, no IgG A-SA could be detected in the sample, whereas after absorption with RAH IgA fraction, IgG-A-SA levels remained the same as preabsorption. Conversely, when assaying for IgA A-SA, no IgA A-SA could be detected after absorption by RAH IgA fraction, whereas no difference was found in detection of IgA A-SA after absorption by RAH IgG fraction.

IgA in serum and secretory fluid before and after selective absorption with RAH IgA secretory piece and RAH IgA antisera. To study the specificity of the ^{125}I -heavy chain-specific RAH IgA (colostrum) fraction for the detection of both IgA and secretory IgA, the SPRIA binding curves for IgA quantitation were

studied for both human serum and secretory fluid. Both specimens were absorbed separately with RAH IgA and IgA secretory-piece antisera until no further precipitation was observed and no further changes were seen in the SPRIA binding curves for IgA quantitation. The curves for serum-IgA quantitation before and after absorption with IgA secretory-piece antiserum do not show a significant change (Fig. 3); however, similar absorption of the secretory fluid with antiserum caused decreased binding (Fig. 4). After absorption by RAH IgA-antibody fraction, the IgA quantitation binding curves for absorbed serum and secretory fluid show no region of the positive antigen-antibody binding. This indicates that the ^{125}I -heavy chain-specific RAH IgA (colostrum) fraction used in these studies to demonstrate IgA A-SA did not discriminate between secretory and nonsecretory IgA.

A-SA measurements in serum and EPS for two patients with bacterial prostatitis. Total nonspecific IgG and IgA and specific IgA and IgG antibodies directed against infecting organisms were measured using the previously described SPRIA for serum and EPS. Longitudinal measurements of total IgA and IgG showed substantial variation between specimens in both serum and EPS, although the total immunoglobulin concentration in prostatic fluid was less than that in serum in all cases (Tables III–VI).

The patient with acute bacterial prostatitis (Patient 1) showed a parallel rise and fall in IgG A-SA per milligram of IgG in both EPS and serum that suggested

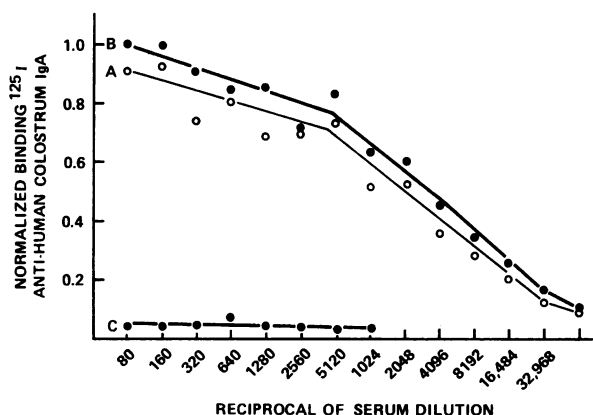


FIGURE 3 Absorption of a serum specimen with heavy-chain specific RAH IgA and secretory piece fractions. Binding curves to determine IgA content were performed in duplicate on serum specimens using heavy-chain specific RAH IgA fraction as the antigen and ^{125}I -RAH IgA fraction to detect bound IgA A-SA. Binding curve A shows the serum specimen without absorption; curve B shows the same serum after absorption with RAH IgA secretory piece fraction; curve C shows the serum after absorption three times with RAH IgA fraction.

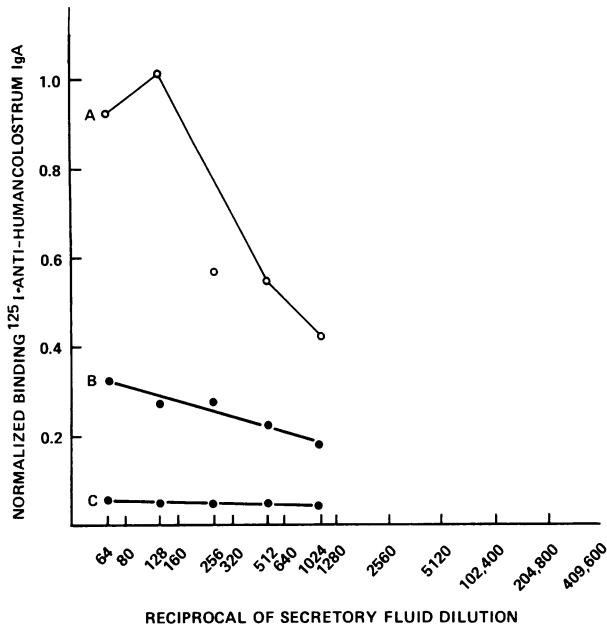


FIGURE 4 Absorption of a secretory fluid specimen with heavy-chain specific RAH IgA and antiseretory piece fractions. Binding curves to determine IgA content were determined using the SPRIA. Heavy-chain specific RAH IgA was used as antigen. Heavy-chain specific ^{125}I -RAH IgA was used to detect bound IgA A-SA. A shows the SPRIA binding curve for a secretory specimen before absorption; B shows the curve after the specimen was absorbed three times with RAH-secretory piece immunoglobulin; C shows the curve after it was absorbed three times with RAH IgA.

serum transudation of specific antibody into the prostate during acute inflammation (Table III). During this same interval the total IgG in the EPS and serum remained relatively constant, reflecting little of the change seen in the A-SA. IgA A-SA per milligram IgA for the same patient showed an increase in both serum and EPS (Table IV); however, the EPS elevation was higher than that in serum and persisted for at least 1 yr, whereas the serum elevation lasted only 4 mo. Furthermore total IgA in both EPS and serum was elevated following the infection with an *E. coli* 018.

Patient 2, who had a chronic bacterial prostatitis unaccompanied by systemic signs of inflammation or infection, showed disproportionate elevation of his EPS IgG when compared with his serum values. Antibiotic treatment of his infection was followed by a drop in EPS-IgG A-SA that was minimally reflected in the serum (Table V). Neither serum nor EPS total IgG reflected this fall in specific antibody. Likewise EPS-IgA A-SA decreased after treatment and there was no significant change in serum IgA A-SA. Total EPS IgA varied, but elevations in serum and EPS total IgA correlated with periods of bacterial infection.

The antigen specificity of the antibody response is illustrated in patient 2 who demonstrated infection by two separate organisms. His EPS IgA A-SA showed a rising titer to the newly acquired *E. coli* 016 at a time when titers to the previously infecting *E. coli* 075 were falling. This was months after the initiation of antimicrobial treatment for the *E. coli* 075 (Table VI).

TABLE III
Measurement of Total IgG, IgG A-SA, and Serum Bacterial Agglutination Titers in Patient 1 with Recurrent Acute Bacterial Prostatitis

Date	Total serum IgG	Serum A-SA		Total EPS IgG	EPS A-SA 018	Bacterial agglutination	
		04	018			04	018
	mg/ml RID	titer/mg IgG		mg/ml RID	titer/mg		
07/05/73*	12.8	78	<10†	0.20	<10	ND§	ND
12/18/79¶	NS	NS	NS	NS	NS	NS	NS
12/29/79	14.3	<10	182	0.45	133	1:160	1:320
01/26/79	11.5	ND	313	0.20	400	1:320	1:320
03/06/79	13.0	ND	246	0.20	200	1:160	1:320
04/03/79	12.2	ND	352	0.15	267	1:320	1:160
05/08/79	13.5	ND	156	0.17	112	1:320	1:160
07/31/79	13.8	ND	146	0.24	58	ND	ND
11/06/79	14.5	ND	116	0.16	81	ND	ND
01/22/80	13.3	ND	126	0.24	54	ND	ND

* *E. coli* 04 cultured from the prostate 3 mo before this date.

† <10 indicates that antigen specific antibody was detected at a titer which was felt to be below the level of accurate quantitation.

§ ND, assay not done.

¶ *E. coli* 018 cultured in at least one of the specimens from Table I on that date.

^{||} NS, no specimen.

TABLE IV
Measurements of Total IgA and IgA-A-SA in Patient 1 with Recurrent
Acute Bacterial Prostatitis

Date	Total serum IgA	Serum A-SA		EPS	
		04	018	IgA	A-SA*
	mg/ml RID	titer/mg IgA		mg/ml RID	titer/mg IgA-RID
07/05/73‡	1.48	74	<10§	0.230	217
12/18/79¶	NS	NS	NS	NS	NS
12/29/79	1.45	<10	896	0.073	4,109
01/26/79	2.65	ND**	204	0.152	2,829
03/06/79	2.73	ND	88	0.051	3,725
04/03/79	2.53	ND	61	0.035	4,857
05/08/79	2.40	ND	64	0.027	4,815
07/31/79	1.98	ND	<10	0.010	450
11/06/79	1.64	ND	<10	0.010	590
01/22/80	1.52	ND	<10	0.010	500

* *E. coli* A-SA.

‡ *E. coli* 04 cultured from the prostate 3 mo before this date.

§ <10 indicates that A-SA was detected at a titer which was felt to be below the level of accurate quantitation.

¶ *E. coli* cultured from at least one of the specimens in Table I on that date.

^{||} NS, no specimen obtained.

** ND, assay not done.

The serum bacterial agglutination titers that reflect primarily IgM do not show these subtle changes. In patient 1 during the 1973 *E. coli* 04 prostatitis and the later *E. coli* 018 acute prostatitis only a one-tube dilution decline was seen (Table III). This lack of clinically significant serum bacterial agglutination titers is further substantiated by the observation that by 1978 and 1979 the titers to the *E. coli* 04 cultured in 1973 were equivalent to those to *E. coli* 018, thereby making no distinction between the old and new in-

fections. Furthermore, in patient 2 (Table V), titers of only 1:160 or less were seen of *E. coli* 075 and *E. coli* 016; it has been observed that uninfected normal men have serum titers of 1:160 or less against the strains of *E. coli* found within their own fecal flora (5).

DISCUSSION

There has been little effort to investigate the immune response in bacterial prostatitis. In previous studies,

TABLE V
Measurements of Total IgG, IgG A-SA, and Serum Bacterial Agglutination Titers in Patient 2
with *E. coli* 075 and 016 Recurrent Chronic Bacterial Prostatitis

Date	Organism		Total serum IgG	Serum A-SA		Total EPS IgG	EPS A-SA		Bacterial agglutination	
	075	016		075	016		075	016	075	016
			mg/ml RID	titer/mg RID		mg/ml RID	titer/mg RID			
01/02/79	*		9.25	140	46	0.30	533	<10‡	1:10	1:10
01/09/79	*		9.50	158	45	0.30	967	IS§	1:10	1:10
01/12/79	*		NS¶	NS	NS	1.00	950	IS	NS	NS
02/09/79			13.90	108	31	0.15	1,266	<10	1:10	1:10
03/09/79		*	12.60	119	34	0.20	625	<10	1:10	1:10
05/11/79		*	11.75	68	68	0.80	225	68	1:160	1:80
06/29/79		*	17.60	67	54	0.99	175	194	1:80	1:80

* *E. coli* cultured from at least one of the specimens in Table II on that date.

‡ <10 indicates that A-SA was detected at a titer which was felt to be below the level of accurate quantitation.

§ IS, insufficient specimen to perform test.

¶ NS, no specimen.

TABLE VI
Measurements of Total IgA and IgA A-SA in Patient 2 with *E. coli* 075 and 016
Recurrent Chronic Bacterial Prostatitis

Date	Organism		Total serum IgA	Serum A-SA		Total EPS IgA	EPS A-SA	
	075	016		075	016		075	016
			mg/ml RID	titer/mg IgA-RID		mg/ml RID	titer/mg IgA-RID	
01/02/79	*		2.40	37	<10†	0.51	10,784	725
01/09/79	*		2.00	55	<10	0.75	17,123	18§
01/12/79	*		NS¶	NS	NS	0.50	46,000	840
02/09/79			1.80	<10	<10	0.28	19,643	1,607
03/09/79		*	1.53	<10	<10	0.23	13,043	1,043
05/11/79		*	1.40	<10	<10	0.70	6,521	2,000
06/29/79		*	2.90	<10	<10	0.66	6,358	4,624

* *E. coli* cultured from at least one of the specimens in Table II on that date.

† Indicates that antigen-specific antibody was detected at a titer which was felt to be below the level of accurate quantitation.

§ IS, insufficient specimen to perform test.

¶ NS, no specimen.

investigators have looked to immunoglobulins as markers of prostatic infection. Some have described serum agglutination titers (4, 5, 13), and others have only examined total immunoglobulin in prostatic secretion (2, 3, 14). The problems with some of these studies were reviewed by Meares (15). In addition it has been emphasized by multiple investigators that levels of total immunoglobulins in secretory fluids are often variable and changes in total immunoglobulin may not adequately reflect immunologic activity. Indeed, it has been the experience of this laboratory that measurements of vaginal fluid IgA and IgG show significant day-to-day variation in a given individual.³ It is difficult to ascertain if this observation represents variation in antibody production, alterations in the secretory rate, or variation caused by the collection technique. Similarly, in the collection of prostatic fluid, variable quantities of admixed urine will significantly change the concentration of specimens and, correspondingly, the measurement of immunoglobulin. Therefore, measurement of total nonspecific immunoglobulin levels alone may not reveal an immunologic prostatic response to bacterial infection.

For these reasons it was decided to examine the A-SA response to bacterial infection in addition to total nonspecific immunoglobulin production. Since the volume of EPS specimen was small, as was the concentration of immunoglobulin, a highly sensitive method for detecting A-SA and one that required small quantities of fluid, was needed. Bacterial agglutination and hemagglutination techniques were inappropriate for this study because they reflect the activity of serum IgM preferentially and require too large a specimen

volume. For this reason, the indirect SPRIA described by Zollinger et al. (8) was selected. This assay was modified for use with a formalin-fixed whole bacterial antigen and was found to be extremely reproducible and specific for measurements of both IgA and IgG A-SA. Absorption studies showed that bacteria incubated in the serum specimens became coated with fluorescent antibody and absorbed the reacting A-SA such that the SPRIA no longer measured a significant A-SA titer in the serum. Absorption with class-specific immunoglobulins showed that both serum and secretory IgA, or IgG antigen-specific antibodies, could be measured by the assay. Furthermore, because the pH of EPS, unlike serum, may vary during periods of infection (16), the effect of specimen pH on the antigen-antibody binding as measured by the SPRIA was studied. Because variation in specimen pH from 5.0 to 8.5 consistently decreased binding by 20–25%, all specimens were buffered to pH 7.2 before assay.

Although it has been shown that there are sites for local immunoglobulin production in the prostate (1), there have been few studies dealing with the nature of this process. The two patients studied in this report demonstrated a local response which was specific for the infecting serotype. This confirms an earlier observation by Jones (17) who showed, using an indirect fluorescent antibody technique, that the EPS of two patients with a well-localized bacterial prostatitis contained immunoglobulin specific for the infecting *E. coli*.

Of greater significance is the detection in both patients of an IgA-A-SA response in the prostate which is of greater magnitude than that measured in the serum. These elevated IgA-A-SA levels in EPS followed an independent pattern of elevation from those of serum.

³ Unpublished observations.

In patient 2 with chronic prostatitis, there was a decline in prostatic IgG A-SA disproportionate to that seen in serum values. These data provide evidence of a locally stimulated immunologic response in IgA, and possibly IgG as well.

The local antibody response seen in these two patients appears to be similar to that seen at other secretory surfaces. In patient 1, ~5 mo after the acute infection, the peak elevation in prostatic IgA A-SA is seen just after the elevation in IgG A-SA is beginning to subside. On the other hand, in patient 2 after eradication of the bacterial stimulus by antibiotic treatment, EPS-IgA-A-SA levels are seen to decline. The prolonged elevation of the EPS-IgA A-SA (presumably secretory IgA) in both these patients is characteristic of the natural history of locally stimulated responses at other secretory surfaces. Although no other data of this sort exist for the prostate, and little information on the natural history of the secretory response at other human secretory surfaces can be found, Waldman and Ganguly (18) showed that secretory antibodies stimulated by live attenuated polio vaccines produced detectable antibody in nasal secretions for up to 34 mo. These findings are in contrast to the secretory IgA-antibody response to *Neisseria gonorrhoeae* in the genital secretions of females (19, 20), in which the local response was of short duration (weeks to months) following therapy in the majority of cases.

It should be emphasized that a serum-IgA reference, rather than a pure secretory IgA reference, was used in both the RID and SPRIA-IgA quantitations and this factor may be reflected in systematically lower EPS-IgA values. Since the SPRIA-IgA quantitation method used here was shown to cross-react between secretory and serum IgA, measurements may reflect the presence of both types of IgA. Although this does not create a problem in this study (since only relative differences in IgA in either EPS or serum are of interest), investigation into the two components may prove to be relevant for future work. Furthermore, the IgA- and IgG-A-SA levels in EPS represented in Tables III-VI may be lower than actual levels available in the prostatic fluid during the time of infection for two other reasons. First, the infecting bacteria, which may have absorbed some available A-SA, were present in the specimen only at the time of infection. Since bacteria and cellular debris were removed from EPS before storage, adherent antibody may also have been removed, thereby lowering the A-SA level of the remaining specimen. Second, A-SA was measured as the titer per milligram of immunoglobulin. Since total IgA in EPS was higher at the time of infection than at other times, the resulting A-SA level per milligram of immunoglobulin may not represent completely the absolute quantity of immunoglobulin available at the secretory surface.

Although bacterial agglutination studies were performed on sera and not prostatic specimens, it can be seen that bacterial agglutination is an inadequate reflection of the patient's clinical status or cultures. Serum bacterial agglutination did not show a significant response to the infecting organism in either patient, whereas simultaneous IgA- and IgG-A-SA titers in prostate were elevated.

Although only two patients were studied, both clearly demonstrated a locally stimulated immune response in the prostate which was reflected in the EPS. The response appeared to be primarily IgA in nature and specific for the infecting *E. coli*. The prostatic IgA A-SA could be detected as long as 12 mo after infection, whereas the serum IgA antibodies were undetectable after 3 mo in the acute case and not detected at all in the chronic case. Of note is the elevation in serum IgA which is seen after prostatic infection in each case and has not been discussed by other investigators. The elevation is substantial in the case of the chronic prostatitis and may be the result of infection at a secretory surface. Further studies involving other cases of acute and chronic bacterial prostatitis, as well as non-bacterial prostatitis, are underway.

From these studies it can be concluded that measurement of A-SA by SPRIA using a simple formalin-fixed whole bacterial antigen gives reliable results and is a valuable technique, appropriate for wide application to a variety of fluids and antigens. The indirect SPRIA was a particularly efficient method with which to study the immunology of the prostate because of the small quantities of specimen needed for the assay and its high sensitivity. In addition, the prostate, in view of its susceptibility to both chronic and recurrent acute enterobacteriaceal infections, may provide an ideal environment for studying problems in secretory immunity. Easy access to relatively pure secretions that are usually free of infection increases its appeal for such studies.

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