INFECTION AND IMMUNITY, Apr. 1978, p. 78-81
0019-9567/78/0020-0078$02.00/0

Vol. 20, No. 1
Printed in U.S.A.

Protection Against Ascending *Escherichia coli* Pyelonephritis in Rats and Significance of Local Immunity

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Received for publication 23 September 1977

Acute pyelonephritis in rats caused by *Escherichia coli* O6K13H1 produced serum and urinary antibodies against O6 and K13 antigens. This was also seen after intravesical immunization with Formalin-killed bacteria. Both intraperitoneal and intravesical immunization with these Formalin-killed bacteria protected against ascending urinary tract infection induced by homologous bacteria. Passive transfer of urine containing both O6 and K13 antibodies also protected against infection. By absorption experiments it was shown that K13 antibodies were especially important.

Studies of immunization against experimental *Escherichia coli* infection in animals have shown that antibodies against *E. coli* can be protective (2, 4, 9, 11, 13, 15, 18, 20). So far such experiments have usually analyzed the significance of serum antibodies in intraperitoneal infections or hematogenous urinary tract infections. With the exception of some of the urinary tract infections of probable hematogenous origin in young infants and in the aged, most cases in man are considered to be ascending infections (3). Thus, the local immune response in the urinary tracts might be of some importance for protection.

This study investigates the significance of humoral immunity, and in particular locally formed antibodies, in protection against ascending urinary tract infections induced in rats with *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The following *E. coli* standard strains from the Collaborative Centre for Reference and Research on Escherichia (W.H.O.), Statens Serum Institut, Copenhagen, Denmark, were used (W.H.O. designation in parenthesis): O2K2abH1 (Su 1242), O6K2a2cH1 (Bi 7458/41), O6K13H1 (Su 4344/41), and O22K13H1 (E14a).

Animals. Female Sprague-Dawley rats weighing 200 to 300 g (Anticimex, Stockholm, Sweden) were used. They were given pellets and tap water ad libitum.

Immunization procedures. (i) Active immunization. Formalin-killed (0.5%) broth cultures of the standard strains in 0.25 ml (10^9 organisms per ml) were given intraperitoneally three times with 7-day intervals. For intravesical immunization, the same dose of bacteria was introduced into the bladder via the urethra by using a blunt needle.

(ii) Passive immunization. Urine (0.3 ml) was injected via the urethra into the bladder just before infection as described below. The following types of urine samples were used. (a) Urines pooled from 10 selected rats infected twice as described below, with a 1-week interval. These urine samples were chosen because of presence of O6 and K13 antibodies of the immunoglobulin G (IgG) and IgA classes but no IgM, as tested by the enzyme-linked immunosorbent assay (ELISA) technique. (b) The urine pool described in (a), but absorbed twice with K13 antigen. The absorption was done by adding 10 mg of isolated (see below) K13 antigen per ml of urine and incubating the mixture for 1 h at 37°C and then for 12 h at 4°C. No K13 antibodies were detected after absorption by the ELISA technique. The level of O6 antibodies was unaffected. (c) Urines, containing O6 antibodies of IgG and IgA but not of IgM class and without detectable K13 antibodies as found with ELISA, were absorbed with isolated (see below) O6 lipopolysaccharide. This was performed as described for K13 antigen [see (b) above], using 2 mg of O6 lipopolysaccharide per ml of urine. No O6 antibodies were detectable after absorption, when checked with ELISA. (d) Urines from healthy rats.

Infection procedures. The rats were infected intravesically via the urethra with 0.5 ml of live *E. coli* O6K13H1 (10^9 bacteria per ml). In the case of passive immunization, 0.3 ml of sterile urine was injected into the bladder just before infection with 0.5×10^9 bacteria in 0.2 ml of buffered saline.

Obtaining samples. Serum samples were taken before the experiments started, 1 week after the third antigen dose, and at sacrifice. Urine samples were obtained by voiding the rats into test tubes kept over the orificium of the urethra while the animal was firmly held. Since the volumes were small, the samples from 4 consecutive days for each animal were pooled.

To avoid the risk of hematogenous infection, different volumes (0.25, 0.50, 0.60, 0.75, and 1.0 ml) for the infection dose were tested in 51 animals. At 20 to 30 min after infection, blood samples (0.5 to 1.0 ml) were taken by cardiac puncture and cultivated for bacterial growth. In animals infected intravesically with 1.0 ml,

90% showed positive blood cultures; of those infected with 0.75 ml, 50% were positive for the infecting *E. coli* strain. All other animals showed negative blood cultures after infection.

Confirmation of pyelonephritis. After sacrifice, the kidneys were removed under sterile conditions and examined. As a control of the infection, cut surfaces were inoculated on Drigalski agar plates for growth of *E. coli* (14). O grouping (16) and K typing (8) were performed on positive cultures. The kidneys were fixed in 10% Formalin for light microscopy. The microscopic examinations were done blindly. Definition of pyelonephritis was done according to Heptinstall (6).

Isolation of antigen and antibody determination. O6 and K13 antibody determination was performed by ELISA (5), using rabbit anti-rat IgA, IgG, and IgM from Nordic Laboratories, Tilburg, The Netherlands. O6 lipopolysaccharide was purified from *E. coli* O6K2a2cH1 by the phenol-water procedure described by Ørskov et al. (17), and K13 antigen was isolated from *E. coli* O22K13H1 by using the modification of cetavlon precipitation described by Kaijser (8).

For coating of tubes, an optimal concentration of 0.01 g/liter was used both for O6 and K13 antigens.

In control experiments, absorption of sera with the K13 antigen did not affect O6 titers, nor did absorption with the O6 antigen affect the K13 antibody values. Furthermore, the purity of the K13 antigen preparations was checked with immunodiffusion using rabbit hyperimmune serum, indirect hemagglutination technique, immunization of rabbits, and limulus assay. Presence of endotoxin was indicated in none of these tests.

The definition of antibody titer using the ELISA was the extinction value at 405 nm of serum diluted 1:100 or undiluted urine after an enzymatic reaction time of 100 min. For each animal tested, serum and urine samples were taken before immunization or infection. In these samples no or very low values were seen. An increase of the antibody value ≥20% was considered an antibody response.

Statistical evaluations were performed using the chi-square test.

RESULTS

Serum and urine antibody levels in rats with acute pyelonephritis. All rats produced serum antibodies against O6 antigen of IgA, IgG, and IgM classes (Table 1). Only around half the number of rats gave measurable serum antibodies against K13 antigen.

In urine, most animals (16/20) showed O6 antibodies of IgG and IgA classes, whereas development of K13 antibodies was more irregular (6/20). All samples obtained on days 7 to 10 were tested and compared with samples taken before infection.

Serum and urine antibody levels in rats intravesically immunized with Formalin-killed bacteria. The antibody response in urine was about the same in animals immunized intravesically with Formalin-killed *E. coli* O6K13H1 and in rats with acute pyelonephritis (Table 1). In some of the immunized animals, serum antibodies were detectable. All samples obtained on days 7 to 10 were tested and compared with samples taken before immunization.

The concentration of antibodies detected in urine was less than 1/1,000 per volume of that in serum. There was no correlation between presence of antibodies in urine and serum.

Protective effect of active and passive immunization against acute pyelonephritis. A protective effect could be demonstrated against the ascending infection when the rats had been immunized with Formalin-killed bacteria of the homologous O6K13H1 strain, as seen in Table 2. This effect was found both after intraperitoneal and after intravesical administration of the vaccine. Immunization with the other strains gave no statistically significant protection against pyelonephritis.

The protective effect of passive transfer of

TABLE 1. *Number of rats with a detectable antibody response*[a]

Determination	Antigen	No. of rats with detectable serum levels of:			No. of rats with detectable urine levels of:		
		IgG	IgM	IgA	IgG	IgM	IgA
Infected rats[b]	O6	20[c]	20[c]	9	16	0	16
	K13	11[d]	8[e]	1	6	0	6
Immunized rats[f]	O6	8[c]	8[c]	5	15	0	15
	K13	2[d]	2[e]	2	6	0	6

[a] Serum and urine samples on day 7 to 10 (sample II) were compared with samples taken before infection or immunization (sample I), as measured with the ELISA. Antibody values were always ≤0.15 in preinfection serum samples and not detectable in urine. An antibody response was regarded as significant if the antibody value increased ≥20% from sample I to II.

[b] Acute pyelonephritis caused by live *E. coli* O6K13H1 ($n = 23$).

[c] $20 \neq 8$ ($P < 0.001$).

[d] $11 \neq 2$ ($P < 0.001$).

[e] $8 \neq 2$ ($P < 0.05$).

[f] Intravesically immunized with Formalin-killed *E. coli* O6K13H1 ($n = 23$).

TABLE 2. *Protection against ascending E. coli O6K13H1 pyelonephritis in rats after immunization with Formalinized bacteria*

E. coli strains	Intraperitoneal immunization		Intravesical immunization	
	No. of rats	Rats with pyelonephritis (%)	No. of rats	Rats with pyelonephritis (%)
O6K13H1	15	33[a]	18	28[b]
O6K2a2cH1	17	71	15	66
O22K13H1	18	45	15	60
O2K2abH1	13	69	10	70
Nonimmunized	16	75[a]	17	70[b]

[a] $33 \neq 75$ ($P < 0.05$).
[b] $28 \neq 70$ ($P < 0.05$).

sterile urine before infection of the urinary tract is seen in Table 3. Urine obtained from rats infected with the O6K13H1 strain, containing both O6 and K13 antibodies, protected against infection. This effect was not seen after absorption with K13 antigen or when using urine without detectable O6 or K13 antibodies.

TABLE 3. *Protection against ascending E. coli O6K13H1 pyelonephritis in rats after immunization with urines*[a]

Passive immunization with:	No. of rats	Rats with pyelonephritis (%)
Urines containing O6 and K13 antibodies	34	24[b,c,d]
Urines originally containing O6 and K13 antibodies, after absorption with K13 antigen	22	59[c]
Urines originally containing O6 antibodies but no detectable K13 antibodies after absorption with O6 antigen	18	61[d]
Urines from healthy rats containing no detectable O6 or K13 antibodies	22	77[b]

[a] Passive intravesical immunization with urines from infected or healthy animals.
[b] $24 \neq 77$ ($P < 0.001$).
[c] $24 \neq 59$ ($P < 0.01$).
[d] 24 ± 61 ($P < 0.01$).

DISCUSSION

Urinary tract infection, in most cases caused by *E. coli,* is a considerable problem in children as well as in adults. Many of the children have single and innocent infections, but some have repeated recurrences, sometimes with parenchymal scarring in spite of treatment.

Earlier investigations in animals have mainly been devoted to hematogenous experimental infections and the significance of serum antibodies. It has been shown that O and K antibodies can protect against *E. coli* infections and that K antibodies are more effective than O antibodies (9, 11, 13). This is concordant with findings that pyelonephritis has been observed in patients with high specific O-antibody titers in serum (1, 20, 21), but other factors in the host-parasite relationship, such as local immunity in the urinary tract, might also be relevant.

The present study shows that introduction of bacteria into the bladder can stimulate an immune response that is probably locally produced, since in many of these animals no antibodies were seen in serum. The local antibody response to *E. coli* O and K antigens in urine is somewhat irregular, especially for K antigen (19), and only very low levels can be found as determined with the ELISA. To show the protective effect of urinary antibodies we found it necessary to select urines with the highest levels of O and K antibodies. Both IgA and IgG antibodies may be of significance in rats. Absorption experiments also make it likely that the protection is mediated especially by K antibodies. Antibodies other than against *E. coli* O and K antigens, however, might also be of some importance.

Apparently serum antibodies, as well as locally produced antibodies, may contribute to protection against urinary tract infections in animals. If this is relevant also in humans, vaccination might be beneficial for problem patients with recurrent and/or intractable infections. A program for preventive immunization should aim at an optimal serum as well as local immune response. In agreement with earlier studies, the present investigation indicates that K antibodies are especially important and that a vaccine should include K antigens. The drawback with purified K antigens, however, is their lack of immunogenicity and their potential tolerogenicity (12). Therefore, studies concerning increase of K antigen immunogenicity, possibly by conjugation to carriers, are most relevant.

ACKNOWLEDGMENTS

The skillful technical assistance of Ingela Delgado, Kerstin Larsson, and Kerstin Lundquist was very much appreciated.

Grants were provided by the Swedish Medical Research Council (project no. 215) and the Faculty of Medicine, University of Göteborg.

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