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### Research Article

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# The Local Immune Response to *Escherichia Coli* O and K Antigen in Experimental Pyelonephritis

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**ABSTRACT** Although the systemic and local immune response to the O antigen of *Escherichia coli* has been well characterized, little information is available on the immune response to K antigen. Experimental hematogenous pyelonephritis was produced with *Escherichia coli* 06 K13 H1 and the serum and local (intrarenal) antibody response to O and K antigens was determined with the enzyme-linked immunosorbent assay. Both local and serum antibody responses to the K antigen were significantly less than that to the O antigen. The K antigen induced low titer IgM and IgG antibody responses in fewer than one-half of the animals and did not induce a local IgA response in any animal. In contrast, the O antigen induced local antibody responses in each of the immunoglobulin classes in all animals from day 9 of infection. Similarly, the serum IgM and IgG antibody titers to the K antigen were significantly less than those evoked in response to the O component of the *Escherichia coli*. No serum IgA anti-K antibodies were detected.

These observations help clarify the roles of these two antigens in pyelonephritis. Although the K antigen of *Escherichia coli* functions as a virulence factor in upper urinary tract infections, this antigen does not elicit a significant immune response, whereas the O antigen does induce a significant antibody response which could be of protective or diagnostic benefit.

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## INTRODUCTION

The role of both O and K antigens of *Escherichia coli* (*E. coli*)<sup>1</sup> in the initiation and course of pyelonephritis has been the object of intense investigation (1-3). The O antigen (lipopolysaccharide) has been shown to be a potent immunogen, inducing both systemic and local antibody (1, 4). Clinical studies have shown that serum antibody to O antigen can be of diagnostic value in patients with pyelonephritis, whereas patients with bladder infection do not develop a serum antibody response (1, 5, 6). Furthermore, O antigen has been shown to persist in the kidney beyond the stage when viable bacteria can be cultured (7, 8); however, its significance in the pathogenesis of progressive renal destruction is unclear.

In contrast, the K antigen (polysaccharide) has been shown to be a significant virulence factor in upper urinary tract infections (9, 10).<sup>2</sup> *E. coli* isolates from the upper urinary tract more commonly have K antigen than do isolates from stool of the same individuals or from patients with bladder infection. The K antigen has been shown to elicit serum antibody less frequently in pyelonephritis than does the O antigen (11), but no studies have evaluated the local immune response to this antigen. Since antibody to both O and K antigens has been shown to be protective against systemic infections (12), it would be important to determine if a local immune response develops to K antigen. Thus, the aim of the present investigation was to examine the local immune

<sup>1</sup> Abbreviations used in this paper: *E. coli*, *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay.

<sup>2</sup> Kaijser, B., L. A. Hanson, G. Lidin-Jansson, and J. B. Robbins. Frequency of *E. coli* K antigens in urinary tract infections in children. Submitted for publication.

response to K antigen of *E. coli* in acute hematogenous pyelonephritis in rabbits. The assay utilized was the enzyme-linked immunosorbent assay (ELISA) (13) which has been shown to be a very sensitive assay for measuring serum and local antibody in the three major immunoglobulin classes to *E. coli* in animals with pyelonephritis (14).

## METHODS

**Bacterial strains.** The *E. coli* strain O6 K13 H1 was obtained from the World Health Organization Escherichia Center, Statens Seruminstutut, Copenhagen, Denmark, (WHO designation SU 4344/41).

**Experimental pyelonephritis.** Experimental pyelonephritis was produced in male New Zealand white rabbits as described previously by injecting  $10^8$  *E. coli* O6 K13 H1 bacteria intravenously while the right ureter was transiently obstructed (4). The animals were bled and sacrificed on various days, and the right kidney was examined for evidence of pyelonephritis. Tissue wedges were cultured for viable organisms and minced kidney slices were incubated for determination of protein and immunoglobulin synthesis as described previously in detail (4, 15). Immunoglobulin synthesis was determined on protein chromatographed on DEAE by precipitating radioactively-labeled immunoglobulin with goat anti-rabbit IgG, IgA, and IgM. IgG antibody to lipopolysaccharide was detected by the binding of labeled IgG to heat killed organisms (4).

Urine was obtained in some of the animals at sacrifice. After the sediment had been removed by centrifugation, the urine was held at  $-20^{\circ}\text{C}$  for antibody study.

The antibody response was determined in animals injected with either purified K antigen or heat-killed organisms. Animals were injected every 5 days for five occasions with either 1 mg of purified K antigen or with 0.5–4.0 ml of heat-killed organisms ( $10^9/\text{ml}$ ). Animals were bled 1 wk after the last injection and the serum was analyzed for antibody to O6 or K13 antigen.

**Antigen preparation.** The *E. coli* O6 antigen was produced by the phenol-water method (16). Isolation of *E. coli* K13 antigen was achieved using a modification of a previously described method (17). The bacteria were cultivated in 3 l trypticase soy broth containing 0.5% glucose, after adjusting pH to 7.4. After overnight growth, the supernate was saved and 1 g of Cetavlon (cetylmethylammonium bromide) was added to 3 l of solution. After another centrifugation, the sediment was dissolved in 500 ml of water. This suspension was stored at  $+4^{\circ}\text{C}$  overnight and thereafter  $\text{CaCl}_2$  was added to a final concentration of 0.5 M. After stirring for 15 min, sufficient ethanol was added to achieve a final concentration of 20%. After centrifugation, ethanol was added to the supernate to a given final concentration of 70%. 500 ml of 10% saturated potassium acetate was added to the sediment. Phenol was added, the mixture was stirred for 20 min, and it was centrifuged for 1 h at 15,000 rpm. The supernate contained K antigen which was shown to be free of O and protein antigens by double diffusion in agar with known antiserum and by absence of sialic acid.

**Antibody determination.** Serum and kidney antibodies were quantified using the ELISA technique as described previously (13, 14). Polystyrene plastic tubes were coated with 1 ml of optimal concentration (20  $\mu\text{g}/\text{ml}$ ) of the purified *E. coli* O and K antigens\* (Fig. 1). Sufficient O and K antigen attached to the tubes to be able to detect

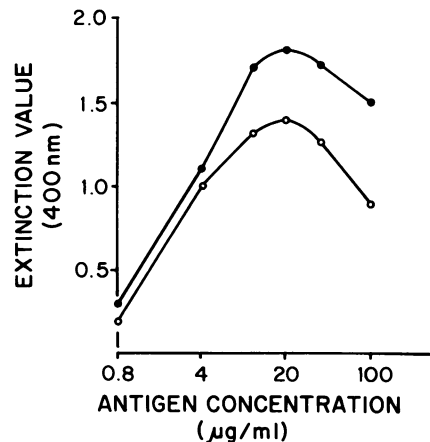


FIGURE 1 Spectrophotometric determinations of varying concentrations of K (●—●) and O (○—○) antigens reacted with a  $10^{-2}$  dilution of serum from infected animal followed by alkaline phosphatase conjugate of goat anti-rabbit IgM.

antibody in presence of high concentration ( $10^{-1}$  dilution) and low concentration ( $10^{-3}$  dilution) antiserum from infected animals. After thorough washing to remove unattached antigen from the tubes, dilutions of serum and kidney fractions which had been chromatographed on DEAE (4) were added and mixed in a shaking water bath at  $22^{\circ}\text{C}$  for 6 h. Unattached protein was removed by repeated washing with a Tris buffer (pH8) containing Tween 20. The antigen-antibody reaction was detected by adding alkaline phosphatase conjugates of goat anti-rabbit IgG, IgM, or IgA. The enzyme activity of the bound conjugates was measured spectrophotometrically after adding paranitrophenylphosphate. In the ELISA assay, the calculated titer was the  $\log_{10}$  of the reciprocal of the serum dilution which had an extinction 0.15 above the base-line level.

Studies of antibody present in kidney fractions could represent either antibody synthesized during incubation or tissue bound or serum antibody. In this and the previous study (14), evidence was consistent with the thesis that the ELISA technique measured newly synthesized antibody. Firstly, a significant correlation was noted in this study and the previous study (14) between IgG antibody detected in kidney by ELISA and by the binding of  $^{14}\text{C}$  antibody to heat-killed suspensions of organisms, whereas less correlation was noted with serum antibody, secondly, there was little correlation between the avidity of serum and local antibody (14), and thirdly, antibody titers in the present studies were 1.5–2.0  $\log_{10}$  higher on material studied after incubation with  $^{14}\text{C}$ -amino acids than in preincubation material, indicating that greater than 90% of the antibody was newly synthesized.

## RESULTS

**Pyelonephritis and immunoglobulin synthesis.** All animals studied had diffuse severe pyelonephritis as shown previously with another *E. coli* strain (4). Organisms were not present in kidney after day 19, but persistent

\*Kajiser, B. *E. coli* K-antibody determination and K antigen quantitation using ELISA. In preparation.

TABLE I  
Microbiologic Data and Intrarenal Synthesis of Immunoglobulin  
in Rabbits with Experimental Pyelonephritis

Day of infection	Isolation of <i>E. coli</i> 06 K13 H1		Total protein synthesis <i>cpm/g</i>	Immunoglobulin* synthesis		
	Kidney	Urine		IgG	IgA	IgM
5	+	0	4,520	10	2	1
9	0	+	24,160	22	4	1
11	0	+	17,380	44	<1	<1
11	0	+	39,680	70	5	2
13	0	+	77,150	68	1	<1
13	0	+	103,400	95	1	<1
14	+	+	9,040	87	16	5
16	+	+	13,950	100	4	6
19	+	+	21,725	71	5	2
19	0	+	38,990	63	5	2
20	0	+	14,050	72	4	2
26	0	+	29,900	60	4	4
26	0	+	47,710	57	5	8
27	NS	+	50,190	69	5	4
34	0	+	37,690	66	5	5
34	0	+	24,380	66	1	<1
46	0	0	15,290	62	3	2
Normal	0	0	2,650	3	4	2

\* Percent of TCA-precipitable 14-C protein which was precipitated by goat anti-rabbit IgG, IgA, and IgM, respectively.

bacteriuria was detected (Table I). All bacteria recovered in urine or kidney had O and K antigens.

Significant synthesis of immunoglobulin, particularly IgG, was noted by day 9 after infection and persisted through day 46 although considerable variation occurred between individual animals. As shown previously with *E. coli* 075 (4), synthesis of IgA and IgM was also increased but not to the same level as was IgG (Table I).

*Serum antibody in experimental pyelonephritis.* All rabbits developed serum antibody in IgM and IgG classes to the O antigen (Table II). The antibody titer showed little variation from day 9 through day 46. Only

TABLE II  
Serum Antibody Response to O and K Antigen  
in Experimental Pyelonephritis

Day of infection	O Antigen			K Antigen		
	IgM	IgG	IgA	IgM	IgG	IgA
5	2.6	2.6	0	2.6	0	0
9	4.4	3.7	2.5	3.0	2.6	0
11	3.6	3.8	0	1.7	0	0
11	4.6	4.5	4.0	3.4	2.5	0
13	4.6	4.5	0	2.9	0	0
13	4.7	5.1	0	3.8	0	0
14	4.3	5.6	3.5	1.9	2.8	0
16	4.7	5.6	3.9	2.8	0	0
19	4.8	4.4	3.2	1.7	0	0
19	4.9	5.5	0	3.5	0	0
20	4.6	4.7	0	2.3	0	0
26	3.6	5.0	0	0	0	0
26	4.6	5.2	3.5	3.1	3.3	0
27	4.4	5.4	0	2.8	4.0	0
34	4.5	5.0	0	3.7	0	0
34	3.7	4.1	0	1.4	0	0
46	3.8	4.9	3.7	3.0	0	0
No. positive (17)	17	17	7	16	5	0
Mean (positive titers)	4.3*	4.7*	3.5*	2.7	3.0	0
±SE	±0.2	±0.2	±0.2	±0.2	±0.3	0

Antibody titer is expressed as log<sub>10</sub> of reciprocal of the dilution of serum which has an extinction 0.15 above baseline.

\* Antibody response to O antigen was significantly greater in each immunoglobulin class than to K antigen at  $P < 0.001$  by Student's *t* test.

7 of 17 animals studied developed serum antibody in the IgA class, and titers were slightly lower than in other classes.

All rabbits save one developed serum antibody (day 5-46) to the K antigen in the IgM class. Only 5 of 17 had antibodies in the IgG class, and none had antibody in IgA class. The mean serum antibody response was significantly greater to the O antigen than to the K antigen in all three classes ( $P < 0.001$  by Student's *t* test).

*Antibody response to purified antigens.* The antibody response to purified K antigen and heat-killed organisms

TABLE III  
Serum Antibody Response to Various Preparations of *E. coli* Antigens\*

Antigen injected	Animal	Antibody response					
		O Antigen			K Antigen		
		IgM	IgG	IgA	IgM	IgG	IgA
K Antigen	1	0	0	0	2.2	0	0
K Antigen	2	0	0	0	2.6	0	0
Heat-killed 06K 13 H1 in saline	1	4.6	5.4	0	0	0	0
	2	3.5	3.0	0	0	0	0
Heat-killed 06K 13 H1 in 0.1N NaOH	1	1.7	0	0	0	0	0
	2	3.2	0	0	0	0	0

\* Animals injected on five occasions with either K antigen (1 mg) or heat-killed organisms (10<sup>9</sup>/ml) every 5 days and bled 1 wk after last injection.

Antibody titer is expressed as log<sub>10</sub> of reciprocal of the dilution of serum which has an extinction 0.15 above baseline.

was determined (Table III). Injection of purified K antigen induced only a low titer IgM response to this antigen, while repeated injections of heat-killed organisms induced a high titer IgM and IgG response to O antigen but no antibody to K antigen. Organisms heat-killed in 0.1 N NaOH to cleave lipid bonds induced only low titer IgM response. None of these antigens induced an IgA response.

**Local antibody production.** All infected kidneys from day 9 on had antibody to O antigen in IgM and IgG class, and most had antibody in IgA class (Table IV). Little variation was noted in antibody levels in O antigen after day 11 of infection in all three classes. Local antibody response to K antigen was not detected until day 14. Of 11 animals followed for longer than 14 days, IgM antibody was detected in 5, IgG in 4, and IgA antibody in none. The mean local kidney antibody response to O antigen was significantly greater than the response to K antigen ( $P < 0.01$  by Student's *t* test).

**Urinary antibody.** Urine obtained from animals sacrificed before day 14 contained no antibody to O or K antigen, but all six animals studied from day 14 on had IgM and IgG antibody to O antigen in their urine (Table V). None had antibody to K antigen.

## DISCUSSION

These studies demonstrate that K antigen is less immunogenic in experiment pyelonephritis than the O

TABLE IV  
Local Antibody Response to O and K Antigen  
in Experimental Pyelonephritis

Day of infection	O Antigen			K Antigen		
	IgM	IgG	IgA	IgM	IgG	IgA
5	0	0	0	0	0	0
9	3.1	3.1	2.4	0	0	0
11	2.8	4.1	0	0	0	0
11	4.1	3.5	3.3	0	0	0
13	3.7	4.3	0	0	0	0
13	2.8	4.5	2.3	0	0	0
14	3.8	4.0	2.7	2.0	2.3	0
16	3.8	4.1	3.5	2.5	0	0
19	2.8	5.0	0	0	0	0
19	4.0	4.3	3.2	0	0	0
20	4.0	3.8	3.0	0	0	0
26	3.0	5.5	2.8	0	0	0
26	3.8	4.2	3.4	0.9	1.8	0
27	4.0	5.6	4.0	1.1	3.2	0
34	4.0	3.6	3.5	2.0	0	0
34	3.7	4.4	3.5	0	0	0
46	3.1	3.7	3.4	0	2.6	0
No. positive (17)	16	16	13	5	4	0
Mean (positive titers)	3.5	4.2	3.1	1.7	2.5	
±SE	±0.1	±0.2	±0.1	±0.3	±0.3	

Antibody titer is expressed as  $\log_{10}$  of reciprocal of the dilution of kidney fraction which had an extinction 0.15 about baseline.

Antibody response to O antigen was significantly greater than response to K antigen at  $P < 0.001$  for IgM and  $P < 0.01$  for IgG by Student's *t* test.

TABLE V  
Urinary Antibody to O and K Antigen in  
Experimental Pyelonephritis

Day of infection	O Antigen			K Antigen		
	IgM	IgG	IgA	IgM	IgG	IgA
14	1.0	1.6	0	0	0	0
19	2.2	2.7	0	0	0	0
20	1.9	2.4	2.3	0	0	0
26	0.5	1.5	0.5	0	0	0
27	0.6	1.6	0	0	0	0
34	0.9	1.4	1.4	0	0	0
No. positive (6)	6	6	3	0	0	0

Antibody titer is expressed as  $\log_{10}$  of reciprocal of the dilution of urine which has an extinction 0.15 above baseline.

antigen. Although K antigen could elicit an IgM response uniformly in serum, K antigen was a poor immunogen locally as shown by the delay in synthesis of IgM and IgG antibody until day 14, its failure to induce high titer antibody after that date, and the failure to demonstrate antibody to K antigen in urine. The ELISA has been shown to be useful in measuring serum and intrarenal antibody (13, 14) and has been sufficiently sensitive to detect quantities of antibody at concentrations of less than 1 ng/ml (13). Our observations indicated that intrarenal antibody was newly synthesized antibody since preincubation media had significantly less antibody than did incubation material. In these studies, K antigen was shown to attach to plastic tubes as well as did O antigen. Hence, this assay would be expected to measure antibody if present.

The poor antibody response to K antigen might occur because smaller quantities of this antigen were available than of the O antigen. It has been shown previously that a smaller quantity of antigen is required to induce an IgM response than needed to induce an IgG response to both protein and lipopolysaccharide antigens (18, 19). However, the injection of large quantities of purified antigen (1 mg) failed to elicit very high titers of IgM or an IgG response at all. Hence, the failure of the K antigen to induce antibody does not appear limited because of lack of availability of this antigen.

Factors which affect the immunogenicity of K antigen of *E. coli* could relate to the particular polysaccharide structure of these antigens or to the acidic nature of these polysaccharides. It has been postulated that pneumococcal polysaccharide may have a greater affinity for IgM than IgG because it has a high density of identical epitopes (20). Furthermore, K antigen does not attach to activated lymphocytes at the infected site (21) and consequently would not stimulate helper cells which appear necessary to initiate IgG and IgA responses (22). The characteristics of K antigen which make it a poor immunogen in pyelonephritis has further clinical

relevance since K antigen is a determinant of extra-intestinal infections (23) and is closely related to other antigens, such as the group B meningococcus and *Hemophilus* strains which cause significant human infections (24).

The importance of the lipid moiety of the lipopolysaccharide in inducing an antibody response has been confirmed in these studies since removal of lipid by alkaline hydrolysis of heat-killed organisms ablated the IgG and IgA response. The lipid moiety of the lipopolysaccharide is responsible for toxicity as well as for mediating the mitogenicity of lipopolysaccharide (25-28). Unlike K antigen, the lipid moiety stimulates circulating and kidney lymphocytes in animals with pyelonephritis (29). Induction of an IgA response requires more than lipopolysaccharide alone, since IgA response develops only with significant local infection, in which extensive tissue invasion has occurred (30). Hence, the class expression of the immune response varies not only with the antigen but with the manner in which the antigens are presented to the host.

The biological significance of the local antibody in the kidney may be manifested in a number of ways: firstly local antibody might play a role in the pathogenesis of pyelonephritis by mediation of cytotoxicity by sensitized lymphocytes (31). These studies indicate that insufficient local antibody to K antigen is present to elicit cytotoxicity. Secondly, local antibody could protect against reinfection (1). Although both antibody to O and K antigen have been shown to be protective in systemic infections (12), these studies suggest that insufficient local antibody to K antigen is present to be protective. This is indeed unfortunate since recent studies indicate that antibody to K antigen is more protective than O antibodies.<sup>4</sup> Thirdly, antibody to K antigen could not be used as a diagnostic test for localization as serum and urinary antibody to O antigen has been successfully employed (1, 5, 6). In these studies, antibody in urine likely reflects local antibody since it was directed against O antigen but not K antigen. In other studies,<sup>5</sup> we have also shown that the antibody which coats bacteria in urine sediment is to the O antigen and not to K antigen.

Thus, K antigen would appear to play its most significant role in selecting out organisms which infect the upper urinary tract (9, 10). These studies indicate that after infection develops, K antigen induces little host immune response. In contrast, O antigen of the infecting organisms would account for toxicity (25), and induce

a vigorous host immune response once infection is established (1, 4). It has not been established whether K antigen persists as O antigen does (7, 8). Hence, it is not known if K antigen contributes to the persistence of organisms in the kidney as has been postulated for O antigen (1).

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