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Protection Against Acute, Ascending Pyelonephritis Caused by *Escherichia coli* in Rats, Using Isolated Capsular Antigen Conjugated to Bovine Serum Albumin

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We found that isolated *Escherichia coli* K13 antigen conjugated to bovine serum albumin, in contrast to isolated, non-conjugated K13, was highly immunogenic and induced protection against acute pyelonephritis caused by *E. coli* O6K13H1 in rats.

Most upper urinary tract infections (UTIs) in infants and children are caused by strains of *Escherichia coli*.

In spite of antibiotic therapy, UTIs often recur, especially in young children (18). In addition to the symptoms of the disease, the patients run the risk of kidney scarring. Therefore, prevention of upper UTIs is a worthwhile goal. The K acidic polysaccharide antigens have been shown to confer pathogenic properties to *E. coli* strains causing upper UTIs. Only a limited number of the many K antigens are associated with these *E. coli* strains. Antibodies to *E. coli* K antigens induced by whole bacteria are highly protective in an ascending pyelonephritis model in laboratory rats. A vaccine consisting of some relevant *E. coli* K antigens has been suggested (7). Isolated K antigens which are poor immunogens in infants and children may not be effective for immunization against infection in this age group (10, 14).

The aim of the present investigation was to compare a purified *E. coli* K13 polysaccharide, one of the common K antigens causing UTI, to a K13-protein conjugate as immunogens against acute pyelonephritis in rats, as it has been suggested that binding polysaccharide to immunogenic carrier proteins increases their immunogenicity.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats weighing 200 to 300 g were given pellets and tapwater ad libitum.

Bacterial strains. *E. coli* O6K13H1 (World Health Organization [W.H.O.] designation Su4344/41) and *E. coli* O22K13H1 (W.H.O. designation E14a) from the Collaborative Centre for Reference and Research on Escherichia (W.H.O.), Statens Seruminstitut, Copenhagen, Denmark, were used.

Infection procedures. The rats were infected intravesically via the urethra with 0.5 ml of live *E. coli* O6K13H1 (10^9 organisms per ml) as described previously (8, 11). After the animals were sacrificed, the kidneys were removed under sterile conditions and examined. The degree of kidney damage was graded as previously described (11): 0, unaffected; 1, microscopic pyelitis; 2, microscopic pyelonephritis with one or two discrete macroscopic pinpoint abscesses; 3, several macroscopic pinpoint abscesses showing coalescence; 4, confluent macroscopic lesions occupying less than half of the kidney surface; 5, confluent macroscopic lesions occupying more than half of the kidney surface.

Antigens. K13 antigens were isolated by the cetavlon (E. Merck AG, Darmstadt, Germany) precipitation technique as described by Schneerson (16) and modified by Kaijser (4) with *E. coli* O6K13H1 or O22K13H1. Conjugation of K13 from *E. coli* O6K13H1 to bovine serum albumin (BSA), designated K13/BSA, was performed as described by Schneerson et al. (16). *E. coli* O6 lipopolysaccharide antigen was prepared as described by Westphal and Jann (17).

Immunization. Five groups of animals were treated (see Table 2). Group A, 20 animals were given two doses of K13/BSA subcutaneously, each dose corresponding to 2.5 μg of K13. Group B, 10 rats were given similar doses intraperitoneally, with a 4-week interval. Group C, nine rats were given one dose of 2.5 μg of K13/BSA perorally; after 1 and 2 weeks they were again injected subcutaneously with the same dose. Group D, 20 rats were given BSA, 1.25 μg intraperitoneally, in two doses with a 4-week interval. This dose corresponded to the amount of BSA in the K13/BSA conjugate dose. Seventeen rats were immunized with two doses of 2.5 μg of isolated K13 and 1.25 μg of BSA in two different syringes subcutaneously, with a 4-week interval. Group E, 50 control rats received phosphate-buffered saline (PBS) intraperitoneally twice, with a 4-week interval. All animals were challenged 1 week after the last dose of antigen or PBS.

Blood samples. Blood samples were obtained from the tail veins of the rats before immunization, at 1-

TABLE 1. Antibody response in serum as measured by ELISA in rats immunized with K13/BSA at two doses subcutaneously with a 4-week interval

Rat no.	Anti-*E. coli* K13 antibody activity (ELISA value)[a]			
	IgG		IgM	
	Preimmunization	Postimmunization	Preimmunization	Postimmunization
1	69	1,529	58	291
2	248	489	0	326
3	51	777	73	187
4	20	2,180	40	516
5	62	548	80	197
6	85	1,299	197	154
7	275	1,559	161	313
8	72	535	56	441
9	0	1,798	0	376
10	0	1,475	0	537
11	17	685	128	492
12	0	535	8	147
13	0	1,538	78	226
14	80	1,111	52	133
15	51	879	41	834
16	22	2,068	5	156
17	6	1,131	0	88
18	0	1,117	0	395
19	0	279	0	520
20	67	1,145	0	374

[a] ELISA values for IgG and IgM are given for sera taken before immunization and 1 week after the last dose (immediately before infection).

week intervals during the course of immunization, and when the rats were killed.

Typing of bacteria. *E. coli* organisms cultured from the kidneys were O typed as described by Lidin-Janson et al. (12) and K typed as described by Kaijser (4).

Antibody determinations. *E. coli* O6, K13, and BSA antibody determinations were made as previously with the enzyme-linked immunosorbent assay (ELISA) (1, 3). For determination of K13 antibodies, the K13/BSA conjugate was used at a concentration of 0.1 μg/ml for coating of polystyrene plates (Heger Plastics, Stallarholmen, Sweden). For BSA or O6 lipopolysaccharide antibodies, a coating concentration of 1 μg/ml was used.

The antibody concentration was defined as the extinction value × 1,000 obtained with serum diluted 1/100 after an enzymatic reaction time of 120 min.

Statistical methods. Confidence intervals of proportions were obtained from the use of the binomial distribution.

For testing the equality of different proportions, the hypergeometric distribution was utilized (13).

For testing the correlation between anti-K13 antibodies and protective effect, the Wilcoxon rank sum test was used.

TABLE 2. Protection against ascending *E. coli* O6K13H1 pyelonephritis after immunization with K13/BSA

Group[a]	Immunization procedure	No. of rats with pyelonephritis/no. of rats in group (%)
A	K13/BSA, subcutaneously (4-week interval)	9/20 (45)
B	K13/BSA, intraperitoneally	6/10 (60)
C	K13/BSA, perorally and subcutaneously (1-week interval)	8/9 (89)
D	BSA, intraperitoneally	19/20 (95)
	BSA and K13 separately	16/17 (94)
E	Nonimmunized; PBS intraperitoneally	42/50 (84)

[a] A ≠ D, $P < 0.01$; A ≠ E, $P < 0.01$; B ≠ D, $P < 0.05$.

RESULTS

Antibody response. K13/BSA conjugate induced a serum K13 antibody response of both the immunoglobulin G (IgG) and IgM classes (Table 1). No antibody production was induced against BSA or against K13 when it was given alone.

Neither BSA antibodies nor O6 antibodies were found in the animals after immunization with the K13/BSA antigen. No K13 antibodies were detected in the animals immunized with isolated K13 or BSA.

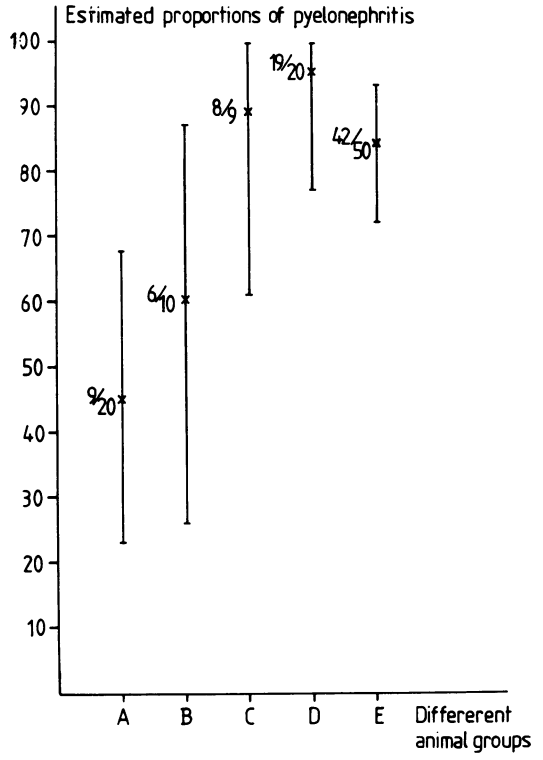

FIG. 1. Estimated proportions of pyelonephritis as percentages, and the corresponding intervals (95% confidence level) in the different animal groups (Table 2). A, Two doses of K13/BSA subcutaneously, 4-week interval; B, K13/BSA intraperitoneally; C, K13/BSA perorally and subcutaneously (two doses, 1-week interval); D, BSA intraperitoneally; E, nonimmunized.

Protection against experimental pyelonephritis. A protective effect against ascending *E. coli* O6K13H1 pyelonephritis after immunization with K13/BSA conjugate was obtained with two subcutaneous injections given with a 4-week interval (Table 2, Fig. 1). No protection was observed in rats immunized with BSA, K13, or PBS.

A correlation between preinfection anti-K13 antibody activity and protection against acute, experimental pyelonephritis was shown in the rats subcutaneously immunized with two doses of K13/BSA (Table 2, group A), ($P \leq 0.05$, Table 3; Fig. 2 and 3).

DISCUSSION

In earlier investigations it has been shown that antibodies can protect against experimental pyelonephritis in animals (2, 6, 8, 9, 15). We have shown that O and K antibodies can protect against acute, hematogenous pyelonephritis in rabbits and ascending pyelonephritis in rats (8, 9). It has also been shown that anti-*E. coli* K antibodies are more protective than anti-*E. coli* O antibodies are against intraperitoneal challenge with living organisms (6).

In the present investigation, *E. coli* K13 antigen conjugated to BSA was an excellent immunogen (Table 1). A protective effect against experimental pyelonephritis was noted after two injections given subcutaneously with an interval of 4 weeks (group A, Table 2). Immunization with corresponding amounts of isolated K13 or

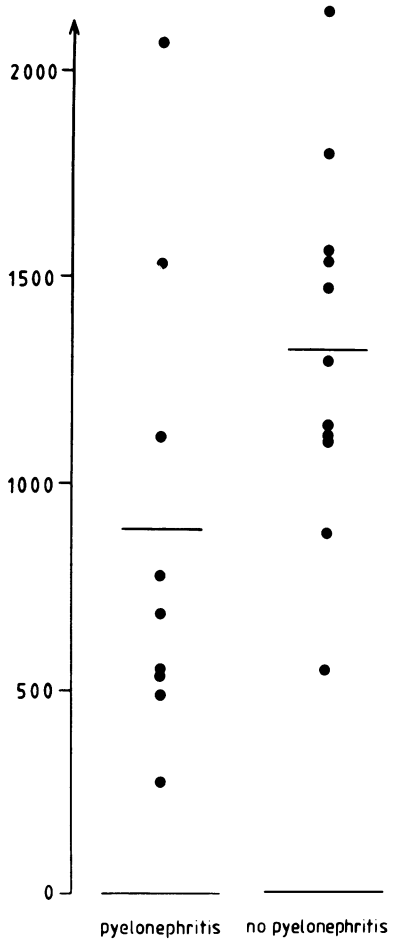

FIG. 2. Correlation between anti-K13 antibodies of the IgG class in postimmunization serum samples taken immediately before infection and protection against experimental pyelonephritis in rats immunized with two subcutaneous doses of K13/BSA antigen with a 4-week interval (group A, Table 2 and 3). The left-hand scale indicates the ELISA value for anti-K13 antibodies after vaccination. First column, pyelonephritis present; second column, no pyelonephritis present. Horizontal lines are mean values. Each dot represents one animal.

TABLE 3. Correlation between anti-K13 antibodies of the IgG class in postimmunization serum samples taken immediately before infection and protection against experimental pyelonephritis in rats immunized with two subcutaneous doses of K13/BSA antigen with a 4-week interval (group A, Table 2)

Rat no.	Degree of kidney damage[a]		Pyelonephritis present	Anti-K13 IgG antibody activity (ELISA value)
	Right	Left		
1	0	1	No	1,529
2	4	0	Yes	489
3	4	4	Yes	777
4	1	0	No	2,180
5	1	1	No	548
6	1	0	No	1,299
7	0	0	No	1,559
8	5	5	Yes	535
9	1	1	No	1,798
10	0	0	No	1,475
11	3	2	Yes	685
12	2	1	Yes	535
13	3	2	Yes	1,538
14	1	0	No	1,111
15	0	1	No	879
16	2	4	Yes	2,068
17	0	1	No	1,131
18	2	2	Yes	1,117
19	0	3	Yes	279
20	1	1	No	1,145

[a] 0 to 1, No pyelonephritis or pyelitis; 2 to 5, minor pyelonephritis to severe pyelonephritis; 0 to 1 $\neq$ 2 to 5, $P \leq 0.05$.

BSA alone induced no detectable K13 antibodies or protection against experimental pyelonephritis. This suggests that the protective effect was related to the induction of anti-K13 antibodies. This assumption was validated by the correlation found between the level of anti-K13 antibodies and the protective effect against infection in the group vaccinated with K13/BSA, two doses subcutaneously ($P < 0.05$).

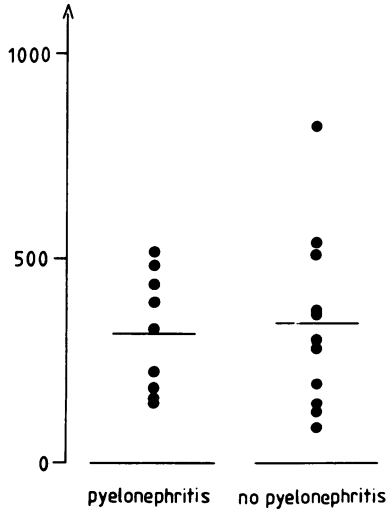

FIG. 3. Correlation between anti-K13 antibodies of the IgM class in postimmunization serum samples taken immediately before infection and protection against experimental pyelonephritis in rats immunized with two subcutaneous doses of K13/BSA antigen with a 4-week interval (group A, Table 2 and 3). Refer to the legend to Fig. 2 for explanation of figure.

In group C, no protection was seen after one oral dose and two subcutaneous injections. However, the subcutaneous injections were given with only 1 instead of 4 weeks intervening. It may be preferable to increase the time interval between injections of the K13/BSA conjugate.

In the intraperitoneally immunized rats a protective effect was also indicated. This immunization route however is less interesting, because in humans intraperitoneal injections will never be considered.

So far, only one K antigen has been tested. Future experiments will be performed with an antigen pool consisting of the most common *E. coli* K antigens in bacteria causing UTI. As a protective effect was noted with one antigen, it is reasonable to believe that protection can also be demonstrated with a mixture of antigens, as earlier studies have shown that the immunogenicity of individual *E. coli* K antigens, given as whole bacteria, is not influenced if different polysaccharide capsules are given simultaneously (5).

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