Kidney International, Vol. 8, (1975), p. 316–319

Immunogenicity of *Escherichia coli* O antigen in upper urinary tract infection

JUDITH A. WHITWORTH, KENNETH F. FAIRLEY, CYNTHIA M. O'KEEFE and THOMAS E. MILLER

Department of Medicine, The Royal Melbourne Hospital, Victoria, Australia and Auckland Hospital, Auckland, New Zealand

Immunogenicity of *Escherichia coli* O antigen in upper urinary tract infection. The role of immunogenicity of the infecting organism (*Escherichia coli*) in the antibody response to O antigen in upper urinary tract infection was investigated. Heat-killed vaccines were prepared from "immunogenic" organisms which had produced upper urinary tract infection associated with high titers of hemagglutinating antibody to O antigen and "nonimmunogenic" organisms which had produced upper urinary tract infection without a rise in antibody titer. "Immunogenic" 06 vaccine produced high titers of antibody in patients regarded as possibly "poor producers" of antibody, but "nonimmunogenic" 011 vaccine was not associated with a rise in titer in patients previously regarded as "good producers". These vaccines were significantly different in immunogenicity ($P < 0.05$). Five vaccines were tested in 50 rats. The difference in hemagglutinating titers to O antigen between 06 and 011 was highly significant ($P < 0.001$). Immunogenicity of the infecting organism appears to be a significant factor in determining antibody response to O antigen in upper urinary tract infection.

Immunogénicité de l'antigène O d'*Escherichia coli* dans les infections du haut appareil urinaire. Le rôle de l'immunogénicité de l'organisme infectant (*Escherichia coli*) dans la réponse immune à l'antigène O au cours des infections du haut appareil urinaire a été étudié. Des vaccins tués par la chaleur ont été préparés à partir d'organismes "immunogéniques" qui ont été responsables d'infection du haut appareil urinaire associées à des titres élevés d'anticorps hémagglutinants contre l'antigène O et d'organismes "non immunogéniques" qui ont produit une infection du haut appareil sans augmentation du titre d'anticorps. Le vaccin "immunogénique" 06 produit des titres élevés d'anticorps chez des malades considérés comme de faibles producteurs d'anticorps et le vaccin "non immunogénique" 011 ne détermine pas d'augmentation du titre chez des malades antérieurement considérés comme de "bons producteurs". Ces vaccins diffèrent significativement en "immunogénicité" ($P < 0,05$). Cinq vaccins ont été essayés chez 50 rats. La différence dans les titres d'hémmaglutination vis à vis de l'antigène O est très significative entre 06 et 011 ($P < 0,001$). L'immunogénicité de l'organisme infectant paraît être un facteur important dans la détermination de la réponse immune à l'antigène O au cours des infections du haut appareil urinaire.

A rise in the serum antibody titer following urinary infection with *Escherichia coli* is well documented and this rise was linked to the site of the infection in the upper urinary tract by Brumffit and Percival [1]. The finding that many infections localized to the upper tract bacteriologically did not produce a rise in hemagglutinating antibody to the somatic O antigen of the infecting strain of the *E. coli* [2] was an unexpected finding. In an attempt to find an explanation, three possible factors have been considered: namely, a defect in the patient's immune mechanisms; the possibility that the site of infection in the upper tract could be confined to the renal pelvis (pyelitis); and lastly (thirdly) the immunogenicity of the strain of *E. coli*.

The immunological capacity of the patients producing high and low antibodies to upper urinary tract infections was tested by challenge with flagellin [3] and no significant difference in immunological response was found.

The possibility that pyelitis may occur in human infections was investigated using a technique of lavage of the renal pelvis and determination of subsequent bacterial excretion rates in urine collected through ureteric catheters [4]. This provided little if any evidence that urinary infections are limited to the renal pelvis in man.

This paper reports an attempt to test the third possibility, namely that the immunogenicity of different strains of *E. coli* differs. The role of an antibody response to O antigens in upper urinary tract infection has been studied.

As the deliberate induction of upper urinary tract infection in patients is ethically unacceptable, heat-killed vaccines, the vaccine from the 011, were made from three *E. coli* organisms which had produced a high O antibody response in patients and two *E. coli* organisms which had failed to do so, inspite of proven upper urinary tract infection.

Methods

Nine women and one man, ranging in age from 22 to 62 yr, with frequent recurring urinary tract infections, were studied. All had a documented upper tract infection determined bacteriologically by the bladder

Received for publication June 20, 1974;
and in revised form May 12, 1975.

washout technique [5], five had failed to show a rise in hemagglutinating antibodies to the infecting organisms and five had shown a significant rise to a titer of 1 in 640 or more. (Although the importance of any previous infection with the serotype used in the vaccine is realized, it is not possible to document the serotypes of all previous infections with *E. coli* going back to infancy in any patient.) Serotyping had been available in the laboratory for three years prior to the study and the previous urinary infections known to have occurred in these patients over this period had been diagnosed by this laboratory, and serotyping of all *E. coli* infections was carried out over this period. No patient had had a known infection with a coli of the same serotype as the vaccine given.

Heat-killed vaccines were prepared by Dr. Gulasekharam, Commonwealth Serum Laboratories, Melbourne, in strengths of 50×10^6 and 1000×10^6 organisms/ml. The organisms used were *E. coli* 06, 075 and 05, which had produced infections associated with a high hemagglutinating titer and were thus theoretically immunogenic, and *E. coli* 011 and a nontypable *E. coli*, which had produced infection associated with a very poor antibody response and were thus possibly nonimmunogenic. All of these infections had been localized to the kidney by the simple bladder washout test [5]. O serotypes had been determined by the method of Needell et al [6] and hemagglutinating antibodies by the technique of Neter et al [7].

The vaccines were made by the standard method of the above Laboratories. *E. coli* were grown up in veal infusion agar in Roux bottles incubated at 37°C for 24 hr. The bacteria were harvested in saline solution and heated to 60°C for 30 min, and 0.5% phenol was added. Cell density was determined by opacity measurement against standard opacity tubes and the vaccine diluted to give the cell a density of 50×10^6 and 1000×10^6 organisms/ml. Vaccines were tested routinely for sterility and abnormal toxicity. The 06 and 011 vaccines were administered subcutaneously to patients in an initial dose of 0.1 ml of 50×10^6 organisms/ml (5×10^6 organisms). A total of nine injections was given at three-day intervals, the total dose being 2575×10^6 coli. The dose of *E. coli* per injection in millions was 5, 10, 20, 40, 100, 200, 400 and 800 with a final dose of 1000 million *E. coli*. Blood for serum antibodies was collected at monthly intervals (see Table 1).

06 vaccine was given to five women who had persistently low antibodies to the O antigen of their homologous organism during an episode of symptomatic urinary infection prior to this study, theoretically "poor producers" of antibody. 011 vaccine was given to four women and one man in whom a significant antibody rise had previously been demonstrated to an infecting organism of another *E. coli* serotype, theoretically "good producers" of antibody.

The vaccines were tested in laboratory rats as follows: Blood was taken for titer estimation before treatment in all rats. Fifty adult rats (ten for each vaccine) were injected i.m. with 0.2 ml of 1000×10^6 organisms/ml of vaccine (200×10^6 organisms) on day 0 and were bled for a titer on day 5. On day 10 and day 20, a further 0.2 ml of vaccine was injected and blood taken for titer estimation. A final blood sample was taken on day 30. Antibody titers were determined by the same method used in the human studies.

Results

Results of vaccination in patients with recurrent urinary tract infection are shown in Table 1. Five patients had had documented renal infection with persistently low antibodies to their infecting organisms, "poor producers." These patients received 06 vaccine. Four showed a subsequent titer rise of at least two tube dilutions and three had titers higher than 1:640. In the remaining patient, no such rise was demonstrated.

Table 1. Hemagglutinating antibody response to 06 vaccine in "poor producers" and to 011 vaccine in "good producers" of antibody to O antigen expressed as reciprocal titer

Patient	Before treatment	1 month	2 months	3 months
		Response to 06 vaccine in "poor producers"		
1	80	640	1280	640
2	160	320	640	640
3	80	640	320	320
4	80	320	160	80
5	20	40	20	20
		Response to 011 vaccine in "good producers"		
6	40	80	80	80
7	160	80	40	40
8	40	80	80	80
9	40	80	80	40
10	20	160	80	20

Five patients regarded as "good producers" had had high titers of antibody to their homologous infecting organism. These patients received 011 vaccine. The maximum titer obtained was only 1:160.

Thus, following vaccination a titer above 1:640 occurred in three of five patients receiving 06 vaccine, in whom no such response to a urinary tract infection with other coliform organisms had previously been demonstrated. These patients were thus not "poor producers" of antibody as they showed a response to 06 vaccine.

No titer above 1:160 occurred after 011 vaccination in patients known to have produced antibody in high titer to a previous urinary tract infection. These patients were therefore not invariably "good producers" of antibody. Using one-factor variance analysis, peak titers after immunization with 06 vaccine were significantly different from those against 011 using *t* test analysis of variance.

Results of vaccination in the rats, expressed as geometric means of titer, are shown in Table 2 and Fig. 1. Peak titers were usually recorded at 5 days and generally showed a slow decline over the 30-day interval. In rats, of the three organisms associated with high antibody titers in human urinary infection, 06 was the most and 011 the least immunogenic in the rat. The response to 011 and the nontypable strain followed the pattern seen in human infection, being associated with lower antibody titers. Using one-factor variance analysis, peak titers following administration of 06 vaccine were significantly higher than those produced by any other vaccine ($P < 0.01$) and the difference between 06 and 011 was highly significant ($P < 0.001$).

Discussion

Escherichia coli is by far the commonest urinary pathogen in man [8], and although there is considerable geographical variation in O-type distribution, a limited number of serotypes are responsible for most urinary infections.

Various factors have been suggested to explain the prevalence of certain strains of *E. coli* in patients with urinary tract infection. Glynn, Brumfitt and Howard [9] showed a greater proportion of *E. coli* strains rich in K antigen in patients with urinary infection than in controls and suggested that the excess of K-rich strains was due to patients with renal infection. They suggested that strains of *E. coli* reached the bladder in proportion to their frequency in fecal flora but that strains rich in K antigen subsequently invaded the kidney because of their relative resistance to phagocytosis and killing by complement, and these would be more likely to evoke an O antibody response.

The correlation of K-rich strains with titers of antibody to O antigen could reflect the immunogenicity of these organisms, rather than their pathogenicity. Mabeck, Ørskov and Ørskov [10] found no correlation between the individual O, K or H antigens of the infecting strain and renal involvement, although some serotypes—02:K1:H4, 04:K12:H5 and 06:K2ac:H1 were associated with a disproportionately high frequency of symptomatic acute pyelonephritis. They speculated that the antigen combination determining serotype may be genetically linked to other characteristics, which together constitute pathogenetic strains.

High titers of hemagglutinating antibody to the O antigen of the homologous infection *E. coli* are found more commonly in patients with upper than lower urinary tract infection. Nonetheless, upper tract infection frequently fails to evoke an antibody response [2].

In the present study, vaccination in both human subjects and rats revealed significant differences in antibody response to the O antigen of different strains of *E. coli* studied. All these organisms had produced renal infection in patients and were thus pathogenic. The vaccines from the particular 06, 05 and 075 *E. coli* used in this study produced higher titers in rats than 011 and nontypable vaccines, as they had in the original urinary infections in the patients from whom they were isolated. Similarly, in patients, although the

Table 2. Range and geometric mean of reciprocal hemagglutinating antibody titers following *E. coli* vaccination in adult rats

Vaccine	Before treatment	Day 5	Day 10	Day 20	Day 30
06 geometric mean	4	830	194	174	194
Range	0–8	64–4096	64–1024	32–1024	128–512
05 geometric mean	9	304	147	84	60
Range	0–16	64–1024	64–512	16–512	8–256
075 geometric mean	5	36	41	46	42
Range	0–16	8–256	16–128	16–128	16–128
NT[a] geometric mean	4	16	9	9	11
Range	0–8	8–32	8–16	8–16	8–16
011 geometric mean	5	7	6	11	15
Range	0–16	0–32	0–16	8–32	0–64

[a]NT = nontypable.

numbers were small, this 06 vaccine produced significantly higher titers than the 011 vaccine. No direct comparison could be drawn between titers obtained in differing species using different methods of immunization, but the 06 vaccine was significantly more immunogenic in both patients and rats than the 011 vaccine. The same was true in the episodes of urinary tract infection in the two patients from whom the 06 and 011 organism had been isolated. It is possible that vaccine from other 06 and 011 strains (with differing K and H antigens) could produce widely differing antibody responses from those obtained with the vaccines used in this study. In our experience with human renal infection (C. M. O'Keefe, unpublished data), *E. coli* 06 produced HA titers 1:640 in only four of 14 patients with kidney infection due to this serotype. For 011, 05 and 075 the numbers were 0/1, 0/1 and 3/7, respectively. Thus, the O antigen is not the only determinant of the specific antibody response.

The wide range of titers seen in rats immunized with a particular vaccine (Table 2) and the poor response of patient 5 (Table 1) imply that, as would be expected, the genetically controlled ability of the host to respond to a specific immunogenic stimulus influences the immune response in individual cases. However, it has been shown that a group defect of humoral immune capacity cannot explain the failure of many patients with proven upper urinary tract infection to produce hemagglutinating antibody in high titer to the homologous infecting organism [3]. These results further suggest that the poor response is not due simply to a failure of production of antibody to polysaccharide O antigen. Patients with low titers to the homologous organism produced high titers following immunization with 06 vaccine.

A failure of antibody response would be expected if the upper tract infection diagnosed bacteriologically on "ureteric urine" were limited to the renal pelvis. When this was investigated [4] using a technique of renal pelvic lavage followed by the measurement of the bacterial excretion rate during a profound diuresis, it was concluded that pyelitis, if it occurred, was rare. As upper urinary tract infection in the human is almost always due to pyelonephritis, a failure of antibody response cannot be attributed to lack of parenchymal invasion.

This study indicates that immunogenicity of the infecting organism appears to be a significant factor in determining antibody response to O antigen in upper urinary tract infection.

Acknowledgments

Heat-killed coli vaccines were prepared by Dr. Gulasekharam, Commonwealth Serum Laboratories, Melbourne. Prof. Raymond Ardaillou helped with the statistics.

Reprint requests to Dr. K.F. Fairley, Department of Medicine, The Royal Melbourne Hospital, Victoria 3050, Australia.

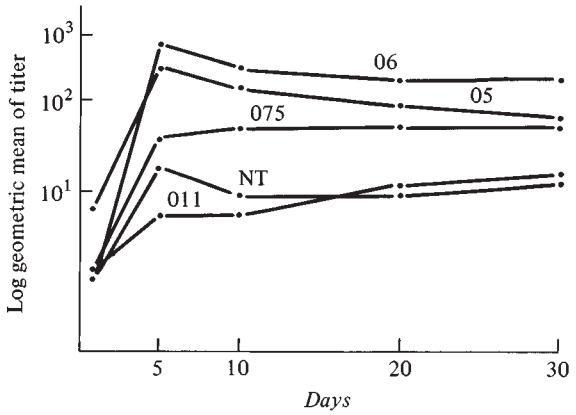

Fig. 1. Geometric mean of reciprocal hemagglutinating antibody titers in five groups of ten adult rats following vaccination. NT = nontypable.

References

1. Brumfitt W, Percival A: Serum antibody response as an indication of renal involvement in patients with significant bacteriuria, in *Progress in Pyelonephritis*, edited by Kass EH, Philadelphia, Davis, 1965, p. 118.
2. Fairley KF: The routine determination of the site of infection in the investigation of patients with urinary tract infection, in *Renal Infection and Renal Scarring*, edited by Kincaid-Smith P, Fairley KF, Melbourne, Mercedes, 1971, pp. 107–116
3. Whitworth JA, Fairley KF, MacKay IR: Humoral immune capacity in recurrent *E. coli* upper urinary tract infection. *Kidney Int* 2:287–290, 1972
4. Whitworth JA, Fairley KF, O'Keefe CM, Johnson W: The site of renal infection: Pyelitis or pyelonephritis? *Clin Nephrol* 2:9–12, 1974
5. Fairley KF, Bond AG, Brown RB, Habersberger P: Simple test to determine the site of urinary tract infection. *Lancet* 2:427–428, 1967
6. Needell MH, Neter E, Staubitz WJ, Bingham WE: The antibody (haemagglutinin) response of patients with infections of the urinary tract. *J Urol* 74:674–689, 1955
7. Neter E, Bertram LF, Zak DA, Murdock MR, Arbesman CE: Studies on haemagglutination and haemolysis by *Escherichia coli* anti-sera. *J Exp Med* 96:1–15, 1952
8. Gruneberg RN, Leigh DA, Brumfitt W: *Escherichia coli* serotype in urinary tract infection: Studies in domiciliary, antenatal and hospital practice, in *Urinary Tract Infection* edited by O'Grady F, Brumfitt W, London, Oxford University Press, 1968, pp. 68–79
9. Glynn AA, Brumfitt W, Howard CJ: K antigens of *E. coli* and renal involvement in urinary tract infection. *Lancet* 1:514–516, 1971
10. Mabeck CE, Ørskov F, Ørskov I: *Escherichia coli* serotypes and renal involvement in urinary tract infection. *Lancet* 1:1312–1314, 1971