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Persistence of E. coli O- and K-Antigens in Experimental Pyelonephritis

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Summary. Experimental chronic pyelonephritis was produced in guinea pigs by ligation of a ureter and injection of various strains of E. coli. Using direct and indirect immunofluorescence the localisation and persistence of E. coli somatic O-antigen and capsular K-antigen was demonstrated in sterile kidneys. The possible pathogenetic role of persistent antigen as the cause of renal inflammation is discussed.

Key words: Experimental pyelonephritis, E. coli O- and K-antigens, immunofluorescence.

Introduction

Clinical and experimental observations have shown that it is often impossible to stop the progression of chronic pyelonephritis. Amongst possible causes of the chronicity, a disturbance of immunity has been postulated (11), as well as persistence of the somatic O-antigen of gram-negative bacteria (10, 12). It is not known whether chronic pyelonephritis results from persistence of residual antigens of the initial infecting organisms, or whether these antigens are responsible for the development of specific immunity (9). However, it has also been suggested (10) that combination of circulating antibodies with antigen retained in the kidney and subsequent fixation of complement might result in local damage. Little is known about the behaviour and localisation of the capsular K-antigen of E. coli strains that have infected the renal parenchyma.

The present study was designed to compare the pathogenicity and behaviour of E. coli O- and K-antigens in experimental, chronic pyelonephritis by bacteriological methods, and immunofluorescence microscopy.

Materials and Methods

Animals and mode of infection: Male and female guinea pigs weighing 280-630 gm were used. After anaesthetising them with Ketanest^{R} (Parke-Davis), 80 mg/kg, a midline lower abdominal incision was made to expose the bladder. The left ureter was ligated with 3-0 catgut. E. coli strains 02:K1; 06:K13; 06:K62; and 0131:K?, isolated from urine of patients with urinary tract infections, were used as the infecting organisms. 0.5 ml of the bacterial suspension (about 10^7 organisms) was injected into the ligated ureter. Controls included animals with one ligated ureter into which 10^9 heatkilled organisms were injected, and others with a ligated ureter into which physiological saline 0.5 ml was injected. An additional control group was normal animals without ureteric obstruction into which 10^7 organisms were injected. Bladder urine for bacteriological culture was aspirated during the operation.

Preparation of the infected kidneys: Both kidneys were removed aseptically between 2 h and 5 months after infection and were cut into three parts. One was homogenized in a high-speed homogenizer (Ultra-Turrex) and examined bacteriologically. The second part was fixed in 4% formalin for histological examination and the third part was frozen and cryostat sections cut for direct and indirect immunofluorescence microscopy.

Production of conjugated antisera: The homologous O-antibody titres of the rabbit-anti-E. coli-sera used were: 02 = 1:3 200; 06 = 1:4 800; 0 131 = 1:25 600 (microtiter Cooke). The globulin fraction was obtained from the antisera by repeated ammonium sulphate precipitation (three times at 50% and 33% saturation) and centrifugation at 10 000 g at low temperatures. The gamma-globulin-fraction was dialyzed against PBS, 0.005 M, pH 8.5; the protein concentration was adjusted to about 1-2% by ultrafiltration. Fluorescein-isothiocyanate (FITC, Fluka) was added with constant stirring at 4° C in 0.5 M carbonate-bicarbonate buffer. Free dye was removed from conjugated antisera by gel-filtration (Sephadex G-25 fine, Pharmacia). Fluorescent antibody-

stained sections were examined by ultraviolet light in a Zeiss Photomikroskop Universal.

Controls for specific immunfluorescence: No specific fluorescence was seen in a blocking test; no specific fluorescence was obtained with a non-homologous antibody; and, none after the homologous labelled antibody had been absorbed first with the specific antigen (7).

K-Antisera: 11 rabbits were infected with E. coli 0 6 : K62 and their pooled anti-0K-sera had an anti-K-titre of 1 : 800. In order to obtain specific monovalent anti-K-sera, the OK-sera were twice absorbed with 0 6-antigen.

Immunoelectrophoresis then showed no 0 6-antibody activity. The purified antiserum was labelled in the same way as described above and both fluorescein-conjugated and unlabelled sera were used to identify K-antigen in cryostat sections by direct and indirect immunofluorescence. For indirect staining a fluorescent anti-rabbit-gamma-globulin prepared in goats was used (Behring-Werke).

Controls for specific immunofluorescence were performed as described above. They showed only the specific reaction.

Results

All but four of the kidneys of animals sacrificed earlier than three weeks after infection contained relatively large numbers of E. coli (10^5 organisms per gm of kidney, wet weight Table 1).

Animals in Group I with sterile kidneys had been treated with chloramphenicol 50 mg because of their very poor condition. There were differences of virulence between certain OK-combinations, E. coli 06 : K13 and 0 6 : K 62 causing the largest number of deaths (Tables 1 and 2).

The earliest histological changes were seen after 24 h, when infiltrates of leucocytes were found in the wall of the pelvis and in the renal parenchyma. From the second day onwards characteristic pyelonephritic lesions were seen, namely casts of leucocytes, destruction of the tubules and small abscesses. Granulation tissue and fibrosis were observed after one week.

After four weeks atrophy of the kidney with loss of parenchyma, dilatation of the remaining tubules and gross scars had developed.

Bacteria were demonstrated by immunofluorescence minutes after infection in the urine of

Table 1. Relation between survival, results of bacteriological culture and degree of pyelonephritis

Survival in days	Number of animals	Organism g/kidney 10^3	sterile	Pyelonephritis +	++	Atrophy	Normal
1 - 4	53	51	2+	27	0	0	26
5 - 20	20	18	2	8	12	0	0
21 - 150	27	9	18	0	7	20	0
Total	100	79	21	35	19	20	26

+ animals receiving chloramphenicol

Table 2. Survival of animals correlated with serotype of E. coli injected

Group	E. coli Serotype	Survival in days 1 - 4	5 - 20	21 - 150	Total
I	02 : K1	13 (30%)	12 (28%)	18 (42%)	43 (100%)
II	06 : K13	17 (81%)	2 (9.5%)	2 (9.5%)	21 (100%)
III	06 : K62	7 (70%)	2 (20%)	1 (10%)	10
IV	0131	16 (62%)	4 (15%)	6 (23%)	26 (100%)
Total		53	20	27	100

$p > 0.05$; $p < 0.1$

the obstructed ureter and on the urothelium of the ureter. Twelve hours later they appeared in the urothelium of the pelvis, in the tubules and pathily in the interstitial parenchyma of the medulla. In the early stages deposits of O-antigen was clearly shaped like single bacteria. After the second week "amorphous" bacterial antigen was seen in the interstitium, and it was apparent in stripe-like formations in connective tissue after the seventh week. Only in two cases was fluorescent antigenic material detected in scarred glomeruli.

The fluorescence of K-antigen was not as bright as O-antigen due to the lower antibody titre, and single fluorescent bacteria were rarely seen. K-antigen was found diffusely in the renal parenchyma around collecting tubules from which it appeared to have originated. Phagocytosis of K-antigen was seen after 24 h, which was much earlier than of the O-antigen. After four weeks most of the antigenic material had been absorbed by macrophages, although in some areas it was seen in the connective tissue between atrophic tubules. In all cases the persistent connective tissue antigen was accompanied by an inflammatory infiltration, primarily of immunocompetent cells. The cell types most often associated with antigen were the plasma cell and, to a considerable extent, the lymphocyte. The persistence of O- and K-antigens in sterile atrophic kidneys was also proved in some cases by immunoelectrophoresis against homologous antisera. No antigen could be detected in control animals injected with a suspension of heat-killed E. coli, even after 12 hours, neither by immunfluorescence nor by immunoelectrophoresis.

Discussion

In the pathogenesis of chronic pyelonephritis the possible role of persisting bacterial antigenic

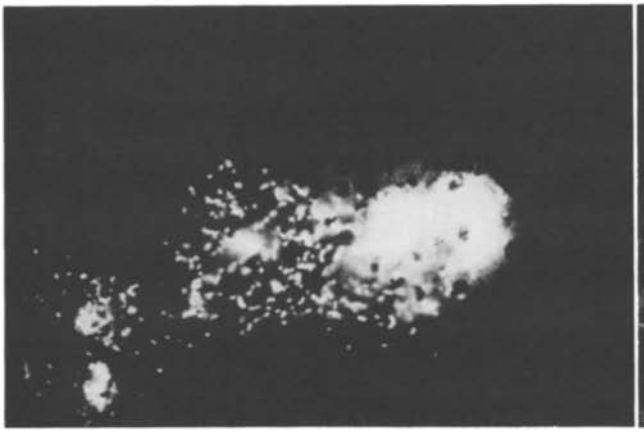

Fig. 1. Isolated foci of E. coli and O-antigen 2 days after infection

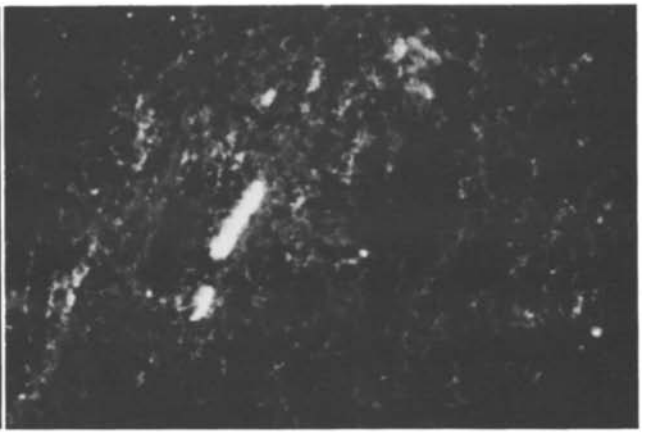

Fig. 2. Persistent O-antigen 4 months after infection Kidney sterile on culture.

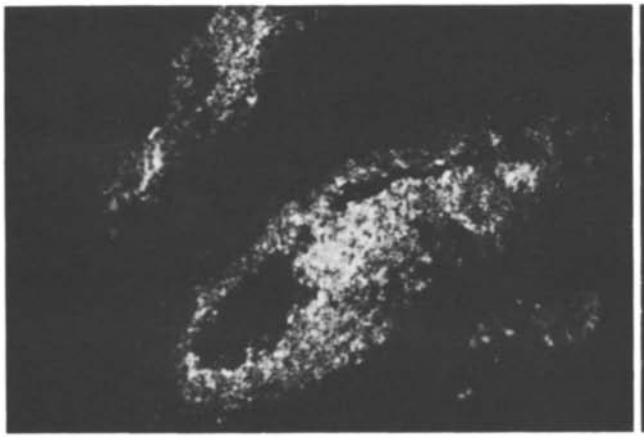

Fig. 3. K-antigen one week after infection (direct immunfluorescence)

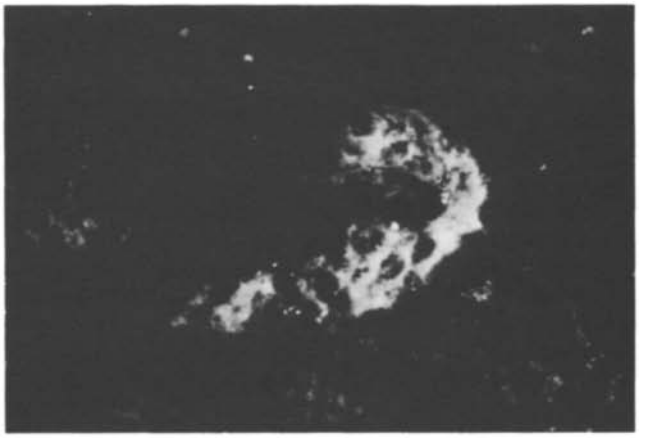

Fig. 4. K-antigen 4 weeks after infection (Kidney sterile on culture.) indirect immunfluorescence

products has been the subject of speculation. Sanford et al. (8) found persisting E. coli somatic antigen in renal connective tissue in rats when the kidneys were sterile.

He concluded that the antigen was not reponsible for the progression and chronicity of the pyelonephritis. Cotran (4) localized gamma globulin and antibody production to the plasma cells in the renal inflammatory infiltrates. Schafer (10) demonstrated complement fixation in loci of persistent antigen. Buckingham et al. (3) found that E. coli antigen applied to fibroblasts caused a marked increase in mucopolysaccharide synthesis and that endotoxin= somatic O-antigen was bound to the fibroblasts. Bacterial antigen was detected by Aoki (2) in six of seven patients with "abacterial pyelonephritis". The report by Angell et al. (1) "active chronic pyelonephritis without evidence of bacterial infection" cannot exclude the possibility of a prior asymptomatic bacterial infection of the urinary tract. It is still uncertain whether persistence of antigen is associated with progressive scarring, but in most studies it has been pointed out that scars containing antigenic materials are a "locus minoris resistentiae" that could lead to continued creeping inflammation.

The present investigations have confirmed previous demonstrations (2, 8, 9, 10, 12) of the persistence of E. coli antigens. The differences between the lethality of strains 06:K13 and 06:K62 indicates the importance of the K-antigen as a factor in the pathogenicity of E. coli. The special significance of K-antigen in pyelonephritis is also shown by its particular morphological behaviour in the immunofluorescent sections, i.e. diffuse spread in the renal parenchyma, a process which resembles the easy dissociation of K-antigen from the cellular complex. The ease of dissociation at 37°C has already been shown in vitro by immunological techniques (13). Wolberg (14) found special pathogenicity of the K-antigen (L-type) in vivo experiment and Glynn et al. (5) postulated that the ability of certain E. coli strains to infect the renal parenchyma was related to their K-antigen, as it prevented bacterial agglutination by O-antibodies. The proposed mechanism of renal invasion is that part of the K-antigen resists the lytic and opsonic activities of antibody and complement. (Myerowitz et al. 6). The present results show that the immunofluorescent demonstration of K-antigen is a good way to obtain detailed knowledge about its role in cases of pyelonephritis.

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