Urological Research 8, 163-165 (1980)

Urological Research

E. Coli Adherence to Bladder Epithelial Cells of Mice

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Accepted: October 30, 1979

Summary. Adherence of E. coli to bladder cells was studied by mixing E. coli with cells scraped from the surface of the normal mouse bladder. E. coli adherence to bladder epithelium did not correlate with renal infection, the ability of E. coli to resist phagocytosis, the growth of the strains, the presence of K-antigen or dulcitol fermentation. There was also no correlation with proportion of deaths, motility, or rough mutation. Pili were observed in three of the 22 strains of E. coli and their presence was not associated with increased virulence. In this model of renal infection neither adherence of E. coli to bladder epithelial cells nor the presence of pili were significant virulence factors.

Key words: Urinary Tract Infection - E. coli Adherence - Pili.

INTRODUCTION

A number of studies have suggested a possible correlation between the virulence of an organism and the ability of the organism to adhere to the surface of an organ or cell (6). This ability to adhere to surfaces has been correlated in some instances with the presence of pili or fimbriae or other surface factors on the surface of the organism or cell. We have examined the possibility that the ability of strains of E. coli to adhere to bladder epithelial cells may be a virulence factor in the pathogenesis of urinary tract infection and pyelonephritis in the mouse. In addition, we have examined the strains of E. coli for the presence of pili.

MATERIAL AND METHODS

E. coli

The 22 strains of E. coli and their virulence in producing renal infection has been previously reported (5). The E. coli strains were stored frozen at -70°C. Before use in the experiment, the E. coli were cultured on blood agar and a colony inoculated into trypticase soy broth (TSB) and incubated at 37°C for 48 hours. Each strain was transferred twice in TSB for 24 hours before use in the experiments.

Adherence to Bladder Epithelial Cells

Epithelial cells were scraped from the surface of 12 normal mouse bladders with a metal spatula, pooled and washed once by centrifugation in phosphate buffered saline pH 7.2 (PBS). The number of cells was counted in a haemocytometer and adjusted to 2.0×10^6 ml and 0.05 ml of cells mixed on a slide with the same volume of each strain of E. coli. A 24 hour culture of each strain of E. coli was concentrated by centrifugation and resuspended in PBS at approximately 1×10^{10} bacteria/ml. The mixture of bladder cells and E. coli was shaken by hand for one minute and left at room temperature for 15 minutes. The cells were examined by phase contrast microscope and the presence of three or more E. coli bacteria attached to the surface of the epithelial cell was regarded as positive. Duplicate samples were examined for each strain of E. coli. The attachment of E. coli to 50 epithelial cells in each specimen was expressed as the percentage of epithelial cells with three or more attached bacteria. Each strain was examined on two separate days.

The precision of the adherence technique was examined by calculating the relative standard de-

0300-5623/80/0008/0163/$ 01.00

viation. The standard deviation (SD) was calculated using the formula

$$SD = \sqrt{\frac{\Sigma (x_1 - x_2)^2}{2n}}$$

where x_1 and x_2 were the values of percent adherence obtained for each strain of E. coli and n was the number of samples (8). The relative SD was calculated as (SD ÷ M) x 100 where M was the mean of all values determined. The relative SD was 8.0% for duplicate samples obtained on the same day and 15.9% for the same strains measured on different days.

Electron Microscopy

A drop of E. coli culture was placed on a Formvar coated grid for two minutes. The excess fluid was then withdrawn and the surface washed with a drop of distilled water. A drop of uranyl acetate (0.5%) was added and the excess taken off after 30 seconds. The grid was allowed to dry and examined.

Analysis of Results

In a previous study (5) we examined a number of potential virulence factors for E. coli. Two parameters were used to examine virulence. (A) The ability of the E. coli to produce pyelonephritis was measured by bacterial infection of the kidneys. A rank order of virulence was obtained by giving equal weight to the renal bacterial population and the proportion of kidneys infected. In that study the number of E. coli ranged from log 1.74 to 7.53 per gram of kidney with two to 98% of kidneys infected. (B) Virulence was also measured by the lethality of the infection produced by the E. coli. Deaths following infection with E. coli occurred in the first few days; the majority within 24 hours. The percent mortality ranged from 0 to 79.6%.

In that study various factors which may influence virulence were studied. These include the resistance of the strains to phagocytosis, the rate of growth of the E. coli, resistance to serum bactericidal activity, presence of K-antigen, carbohydrate fermentation, motility and roughness.

In the present study the relation of attachment of E. coli to some factors (renal infection, proportion of deaths, resistance of phagocytosis and serum bactericidal activity, bacterial growth) was compared by rank order correlation. To determine if attachment correlated with the presence of K-antigen, carbohydrate fermentation, motility or roughness the incidence of each of these factors was compared in the 11 strains with greatest attachment and the 11 strains with least attachment.

Table 1

Strain[a]	% Adherence
214	96
946	78
P92	26
Yale	91
P98	93
P67	40
CF1	87
113	90
CL-135	92
MP	88
P33	88
Lowell	84
0127:B8	8
J5	63
W1895	52
CL-136	97
OB248	12
OB51	27
CL-185	95
CL-143	100
H	9
CL-1B	7

[a] In descending order of virulence as measured by renal infection

RESULTS

The percentage of cells with attached E. coli ranged from 7 to 100% (Table 1). The strains of E. coli have been arranged in descending order of virulence, as measured by renal infection. Bacterial attachment to the bladder epithelial cells did not correlate with renal infection ($r = 0.225$). The attachment of E. coli to the bladder epithelial cells also failed to correlate with the proportion of deaths of the mice ($r = 0.060$), the rate of growth of the E. coli ($r = 0.174$), resistance to phagocytosis ($r = 0.055$), and resistance of serum bactericidal activity ($r = 0.148$). The attachment of E. coli to bladder cells also failed to correlate with the presence of K-antigen, carbohydrate fermentation, motility or roughness of the E. coli strains.

Pili were found in only three of the 22 strains of E. coli (CF1, Lowell, and CL-185) and the presence of pili was not associated with the more virulent strains.

DISCUSSION

Bacterial adherence has been considered to be closely related to colonisation and virulence of a number of organisms. The development of E. coli infection of the intestine has been correlated with the ability of this bacteria to attach to the in-

testine (10). Others have suggested adherence is important for bacteria in the oral cavity (4) and gonococci (7). Eden et al. (3) found that the ability to become attached to epithelial cells from the urinary tract was much greater in E. coli isolated from the urine of patients with acute pyelonephritis or cystitis than in those isolated from the urine of patients with asymptomatic bacteriuria. In previous studies we found that the virulence of E. coli in ascending pyelonephritis in mice correlated with the ability of the E. coli to grow in minimal media or urine, the presence of K-antigen in the E. coli, and the ability of E. coli to ferment dulcitol (5). In the present studies we were unable to correlate attachment of E. coli to the epithelial cells with virulence. These studies do not rule out the possibility that attachment may still be an important factor in their virulence. It is possible that cells scraped from the surface of the bladder and washed may not be the equivalent of the in vivo bladder surface. In addition, in the human genitourinary tract other uroepithelial cells may be of more importance with regard to bacterial adherence.

Other studies have suggested that pili or fimbriae (1, 2) may be important in the virulence of E. coli in urinary tract infections. Silverblatt described a pathogenic role of pili in Proteus mirabilis infection of the kidney in the rat (9). We found a low incidence of pili in the strains that we studied. This may have resulted from the fact that these were laboratory strains which had been maintained in different laboratories over a number of years. E. coli with plasmid associated pili have been shown to lose pili on repeated transfer. This, however, may not be true of the usual type of pili (type 1) associated with E. coli (1). The number of strains with the pili was so low that it was not possible from this study to rule out the possibility that pili may be a virulence factor. It should be noted, however, that these strains of E. coli did produce severe renal infection despite the lack of pili.

Finally, the lack of correlation between virulence on the production of pyelonephritis and adherence to bladder epithelium may suggest two independent phenomena. Silverblatt and Ofek found that piliated Proteus mirabilis which presumably adhere well to epithelium were more virulent than non-piliated bacteria when injected into the lower urinary tract, but survived less well than non-piliated bacteria when injected directly into the kidney (11). The effect of adherence on the host parasite interaction may be complex.

Acknowledgement. We wish to acknowledge the excellent technical assistance of Ms. Rachel Miller.

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