Hydrophobic adhesin of *E coli* in ulcerative colitis

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SUMMARY Pathogenic *E coli* have adhesive properties which are mirrored by an increase in their surface hydrophobicity. *E coli* isolated from patients with ulcerative colitis possess a mannose resistant adhesin similar to that found in pathogenic *E coli*. In this study 42 *E coli* isolates from patients with colitis have been compared with 15 from controls to assess hydrophobicity and cellular adherence. The salting out method and the buccal epithelial cell technique were used respectively. *E coli* isolated from colitics are significantly more hydrophobic than control *E coli* ($p < 0.001$). The salting out score correlates negatively with the buccal epithelial cell adhesion index. When *E coli* are grown at 18°C both properties are temporarily reduced suggesting that they are related to each other. The salting out method clearly differentiates between *E coli* isolated from colitics and controls, and offers a simple method of detecting adhesive *E coli* in inflammatory bowel disease.

E coli which cause diarrhoea in man¹ and animals² possess an adhesive property which is a virulence factor, without it, toxin producing and enteropathogenic organisms lose their pathogenic potential. Patients with ulcerative colitis harbour *E coli* which possess an adhesive property which is mannose resistant,³⁻⁵ however, the nature of the adhesin is unknown. Serotyping alone provides little information about adhesins as these are often plasmid mediated.

Bacteria to cell interactions are complex and varied, but include lectin like, electrostatic, and hydrophobic mechanisms. When the surface hydrophobicity of a bacterial cell is increased the charge on the cell surface is reduced, this has the effect of diminishing the repulsive forces which normally exist between two negatively charged bodies and increases the chances that adhesion may occur.⁶ The fimbrial adhesins found in pathogenic *E coli* are predominantly composed of hydrophobic amino acids,^{6,7} these increase the surface hydrophobicity and reduce the cell surface charge.⁸ Bacterial cell adhesins can be ranked on the basis of their hydrophobicity with recognised pathogenic *E coli* showing a greater surface hydrophobicity⁸ than non-pathogens.

In this study we have examined the hydrophobic properties of *E coli* isolated from patients with ulcerative colitis and have compared them with *E coli* obtained from a control population.

Methods

PATIENTS

E coli were isolated from 42 patients with ulcerative colitis in relapse and 15 controls who comprised patients and members of staff with no evidence of inflammatory bowel disease. One individual colony of *E coli* was chosen at random from each subject, stored on Dorsett egg slopes, and protected from exposure to light.

The salting out method similar to that described by Lindahl *et al*⁸ was used to assess hydrophobicity. Test bacteria were grown at 37°C for 18 hours on nutrient agar slopes and suspended in 0.002 M sodium phosphate buffer (pH 6.8) at a concentration of $\approx 5 \times 10^9$ bacteria per ml. Twenty five microlitres of the bacterial suspension was mixed with an equal volume of varying concentrations of ammonium sulphate in 0.002 M sodium phosphate (pH 6.8). Concentrations of ammonium sulphate ranged from 0.2 M $\times$ 0.02 M increments and from 0.2–4 M $\times$ 0.2 M increments. The bacteria and salt solution mixture was gently rocked for two minutes at 20°C on a black

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Figure Lowest molar concentration of ammonium sulphate resulting in autoagglutination and buccal epithelial cell adhesion indices of colitic, control, and standard *E coli* isolates.

backed glass slide. The lowest molar concentration of ammonium sulphate which produced autoagglutination of the test strain was determined for each isolate.

The buccal epithelial cell adhesion assay was done as previously described.⁵ *E coli* H10407, a recognised CFA1 fimbriate enterotoxigenic *E coli*⁹, *E coli* E851/71 a mannose resistant adhesive enteropathogenic *E coli*,¹⁰ and *E coli* SC13 an Hep 2 non-adhesive *E coli* were included as standards.

STATISTICAL ANALYSIS

Wilcoxon's rank-sum; Wilcoxon's signed-rank test; and Spearman's rank correlation were used.

Results

The lowest molar dilution of ammonium sulphate resulting in autoagglutination for colitic *E coli* (median 0.6 M) was significantly lower ($p < 0.001$)

than that of control isolates (median 2 M), (Figure). The buccal epithelial cell adhesion indices of the colitic *E coli* isolates (median 43.5%), were significantly greater than those of the control group (median=2%) $p < 0.0001$ (Figure). Buccal epithelial cell adhesion indices correlate negatively with the lowest molar dilution of ammonium sulphate causing autoagglutination ($p < 0.001$), indicating that the higher the adhesion index the greater the cell surface hydrophobicity.

Ten adhesive *E coli* isolates were grown at 18°C. There was a significant decrease in their buccal epithelial cell adhesion index from a median 48% to 1.5% ($p < 0.01$) with a concomitant increase in the lowest molar dilution of ammonium sulphate to result in autoagglutination from a median of 0.7–1.8 M, indicating a decrease in surface hydrophobicity ($p < 0.01$). The buccal epithelial cell adhesive ability was regained when they were further subcultured at 37°C. The salting out score and buccal epithelial indices of the control standards were for E851/71 0.8 M, and 47%, H10407 0.6 M, and 42%, and for SC13 2.6 M, and 6% respectively.

Discussion

The adhesion of bacteria to cells may be mediated by fimbrial and afimbrial adhesins, but in either case *E coli* expressing mannose resistant adhesins have a higher relative surface hydrophobicity¹¹ suggesting that this is an important factor in the mediation of bacterial adhesion. In the present study the adhesive property was directly related to the degree of surface hydrophobicity. The fact that both buccal epithelial cell adhesive property and increased hydrophobicity is lost when organisms are grown at suboptimal temperatures suggests that the adhesion mechanism and increased surface hydrophobicity are inter-related.

The commoner type 1 mannose sensitive fimbriae expressed by many *E coli* is not pathogenic in man. Furthermore Lindahl *et al* have shown that the surface hydrophobicity of this particular adhesin is less than that expressed by any intestinal pathogenic *E coli*. This suggests that the adhesiveness of pathogenic organisms is related to the hydrophobicity conferred by their adhesins.

The data presented here show that the adhesive property of *E coli* in ulcerative colitis is similar to that found in recognised pathogenic *E coli* in its buccal epithelial cell adhesive ability and relative surface hydrophobicity.

Intestinal mucosal adhesion of *E coli* may be species specific as shown by the K88 antigen in pigs and the CFA1 antigen of man. It may be receptor dependent as shown by both the somatotrophic

localisation¹² and genotypic expression of receptors.¹³ Whatever the receptor specific attachment mechanism, surface hydrophobicity appears to be involved. The importance of this is shown by the ability of hydrophobic gels to prevent the occurrence of diarrhoea in rabbits infected with enterotoxigenic *E coli* possessing the hydrophobic fimbrial antigen CFA1.¹⁴

The demonstration of an adhesive property in *E coli* isolated from patients with ulcerative colitis which is similar to that found in human pathogenic *E coli* suggests that these organisms may have a role in the pathogenesis of the disease. This finding suggests the possibility of a new approach to treatment and the prevention of relapse in ulcerative colitis. The salting out method clearly differentiates between *E coli* isolated from colitics and controls and offers a simple reproducible method of detecting adhesive *E coli* in inflammatory bowel disease.

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