Increased incidence of faecal coliforms with \textit{in vitro} adhesive and invasive properties in patients with ulcerative colitis

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SUMMARY Faecal samples were collected from 23 patients with active ulcerative colitis, 15 patients with established ulcerative colitis in remission, 20 patients with active colitis of cause other than ulcerative colitis, and 20 normal control subjects. Ten coliform colonies were randomly selected from the faecal sample cultures and serotyped before the testing of each different serotype from each sample for adhesive or invasive properties on HeLa cell monolayers. In the patients with both active ulcerative colitis and ulcerative colitis in remission and those with other types of colitis one serotype tended to dominate the faecal coliform flora. In normal controls more variety was encountered. Thirty-five per cent of the patients with active ulcerative colitis and 27% of the patients with ulcerative colitis in remission had at least one adhesive or invasive faecal coliform as compared with 5% of the patients with other types of colitis and 5% of the normal controls. These findings are significant (p < 0.05) and may have aetiological and therapeutic significance.
Other active colitis
Twenty patients with active colitis thought to be due to conditions other than ulcerative colitis. In all cases the diagnosis was based on the clinical history and findings, taken in conjunction with the results of routine bacteriological, radiological, and histological investigations. Five had Crohn's disease of the colon, six antibiotic related colitis or pseudomembranous colitis and three infective colitis with recognised pathogens. The other six all had a short history of diarrhoea of sudden onset, and, although no intestinal pathogens were identified, an infective cause for the colitis was considered most probable on clinical and investigational grounds. No patient in this group had the rectal findings of ulcerative colitis.

Normal controls
Twenty adults who had neither been in hospital nor received antibiotics for at least one year.

Procedure
Faecal samples were obtained as soon as possible after the presentation of the patient to hospital. The samples were inoculated on to blood agar and incubated overnight at 37°C.

A typical coliform colony was then randomly selected and identified as *E. coli* or *Klebsiella* species as previously. This colony and nine others similarly selected were then stored on Dorset Egg medium at room temperature in the dark until use.

The *E. coli* and *klebsiellas* studied were from the first faecal sample obtained, except in the case of a patient with bacteriological evidence of staphylococcal enterocolitis who had no faecal coliforms until five days after admission.

Serotyping
*E. coli* were 0-serotyped and *klebsiellas* capsular typed as previously, using 156 0 antisera and 77 capsular antisera respectively. Every colony from each sample was serotyped and then at least one representative of each different serotype from each faecal sample was tested for its ability to adhere to or invade HeLa cells in culture.

HeLa cell studies
Adhesiveness and invasiveness were investigated on HeLa cell monolayers after the method of La Brec et al. (1964).

HeLa Ohio cells were maintained in tissue culture in Minimum Essential Medium (Glasgow's modification of Eagle's medium). Immediately before use each 150 ml of medium were enriched with 20 ml tryptose phosphate broth, 20 ml calf serum, 2 ml glutamine solution (containing 29.2 mg glutamine/ml), and 10 ml 3% sodium bicarbonate. The stripping medium comprised 100 ml phosphate buffered saline (PBS—pH 7.2) containing 2 ml 1% versene, 0.5 ml trypsin, and 3.5 ml 3% sodium bicarbonate.

The test HeLa cells (approximately 10⁶) were transferred to plastic petri dishes containing glass coverslips and maintained in an atmosphere of 95% air 5% CO₂ at 37°C until nearly confluent. The cells were then infected with approximately 3.5 × 10⁹ washed bacteria obtained from overnight incubation in nutrient broth. The petri dishes were reincubated at 37°C in an atmosphere of 95% air, 5% CO₂, and coverslip samples were taken at three, five, and seven hours, and sometimes 10, 12, and 18 hours after infection. Before taking coverslip samples the petri dishes were emptied of culture medium and washed twice with Hank's balanced salt solution. Fresh medium was then added and the petri dishes reincubated. The coverslip samples were washed in PBS, fixed in three parts methanol: one part acetic acid, and stained with dilute Giemsa at pH 7 before examination under the light microscope.

Non-adhesive and non-invasive strains were usually not evident on any of the coverslip preparations. Scattered bacteria or bacterial clumps unrelated to the cell membranes were also regarded as negative. Adhesive strains had attached to the cells within three hours, usually in a patchy distribution. Adhesion increased variably with further incubation but invasion of the monolayer and its consequent disruption did not occur, even if incubation was prolonged up to 12 hours after infection (Fig. 1). Invasive strains usually affected the entire monolayer in a pattern initially similar to that of the adhesive strains. After five hours' incubation bacteria could usually be seen within the cells and, by seven hours, disruption of the monolayer had invariably occurred (Fig. 2).

All strains deemed adhesive or invasive were tested at least twice and categorisation was based on the agreement of two observers one of whom was unaware of the clinical diagnosis.

Results
The results of this study are summarised in Tables 1, 2, and 3. In all but one patient, the 10 coliform colonies were identified as *E. coli*. In the exceptional case all the colonies were *Klebsiella* and these comprised three different serotypes.

It will be seen that the faecal samples from all the patients with active colitis of whatever cause and from those with quiescent ulcerative colitis yielded generally fewer serotypes than those from the normal controls. Furthermore, differences in serotype among the 10 colonies from the patients with
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Active colitis and quiescent ulcerative colitis were often from typable to rough or non-typable and possibly therefore variants of the same strain. This occurred less frequently in the normal controls who yielded a wide variety of serotypes.

Adhesive or invasive serotypes were encountered at an increased frequency in the patients with active ulcerative colitis and quiescent ulcerative colitis when compared with the patients with other colitis or normal controls ($p < 0.05$ in both instances by Fisher's test). The adhesive or invasive strains were usually representative of the dominant serotype, although one patient with active ulcerative colitis had two adhesive strains one of which, *E. coli* 0153, was represented by a single colony.

**Discussion**

In any study of the faecal flora there are inevitable questions concerning the validity of results obtained at bacteriology. The bacterial population of the flora is so large that the sampling methods used must be reliable before any deductions can be made relating laboratory findings to the pathogenesis of

**Table 1** Details of diagnostic groups, number of serotypes isolated, and frequency of adhesive or invasive strains

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>No. of patients</th>
<th>No. of serotypes tested</th>
<th>No. of adhesive serotypes</th>
<th>No. of invasive serotypes</th>
<th>Mean no. serotypes/sample</th>
<th>% patients with adhesive or invasive serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active UC</td>
<td>23</td>
<td>33</td>
<td>6*</td>
<td>3</td>
<td>1.4</td>
<td>35*</td>
</tr>
<tr>
<td>Quiescent UC</td>
<td>15</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>1.3</td>
<td>27</td>
</tr>
<tr>
<td>Other colitis</td>
<td>20</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>1.3</td>
<td>5</td>
</tr>
<tr>
<td>Normal control</td>
<td>20</td>
<td>37</td>
<td>0</td>
<td>1</td>
<td>1.9</td>
<td>5</td>
</tr>
</tbody>
</table>

*One patient had two adhesive serotypes in one sample.
Table 2  
**Detail of O serotype of E.coli and capsular type of species Klebsiella in each group of patients studied (dominant serotype given first)**

<table>
<thead>
<tr>
<th>Active UC (n=23)</th>
<th>Other colitis (n=20)</th>
<th>Quiescent UC (n=15)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR 019</td>
<td>ONT 053</td>
<td>OR 01</td>
<td>015, 016, OR</td>
</tr>
<tr>
<td>021</td>
<td>06</td>
<td>02</td>
<td>06, 011</td>
</tr>
<tr>
<td>OR 018ab</td>
<td>075, ONT</td>
<td>OR 086, ONT 04</td>
<td>016, 02, 09</td>
</tr>
<tr>
<td>019, 08, ONT</td>
<td>OR, ONT</td>
<td>04</td>
<td>091, 028</td>
</tr>
<tr>
<td>02, 077</td>
<td>083</td>
<td>OR 01</td>
<td>08, 051</td>
</tr>
<tr>
<td>08, 0153</td>
<td>0153</td>
<td>01</td>
<td>022</td>
</tr>
<tr>
<td>0102</td>
<td>07</td>
<td>01</td>
<td>075, 065</td>
</tr>
<tr>
<td>063, 0153</td>
<td>ONT 018ab</td>
<td>01, OR</td>
<td>059</td>
</tr>
<tr>
<td>OR 02</td>
<td>02</td>
<td>02, ONT</td>
<td>0155</td>
</tr>
<tr>
<td>087</td>
<td>0111, 06</td>
<td>ONT 06</td>
<td>048</td>
</tr>
<tr>
<td>0153, OR</td>
<td>075</td>
<td>0146, 018ab, ONT</td>
<td>018ab, 021</td>
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<tr>
<td>OR, ONT</td>
<td>075</td>
<td>0153</td>
<td>ONT</td>
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<tr>
<td>OR 018ab</td>
<td>075, 018ab</td>
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<td>018ab, 09</td>
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<tr>
<td>02</td>
<td>05</td>
<td>081, 021</td>
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</tr>
<tr>
<td>020, OR 01</td>
<td>018ab, 04, 08</td>
<td>088, 0126</td>
<td>021, 062</td>
</tr>
<tr>
<td>023, ONT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K45, K2, K58*</td>
<td>ONT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: Rough strain.  
ONT: Non-typable strain.  
* Klebsiella capsular type.

Table 3  
**Details of adhesive and invasive strains in each diagnostic group**

<table>
<thead>
<tr>
<th>Active UC (n=8)</th>
<th>Quiescent UC (n=24)</th>
<th>Other colitis (n=7)</th>
<th>Controls (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli 021</td>
<td>invasive</td>
<td>E.coli OR adhesive</td>
<td>E.coli 0153 adhesive</td>
</tr>
<tr>
<td>E.coli OR</td>
<td>adhesive</td>
<td>E.coli 01 adhesive</td>
<td>E.coli 018ab adhesive</td>
</tr>
<tr>
<td>E.coli 02</td>
<td>invasive</td>
<td>E.coli 0146 adhesive</td>
<td></td>
</tr>
<tr>
<td>E.coli 063</td>
<td>adhesive</td>
<td>E.coli 0153 adhesive</td>
<td></td>
</tr>
<tr>
<td>E.coli 0153</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli 01</td>
<td>adhesive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli 087</td>
<td>invasive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli 02</td>
<td>adhesive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella K45</td>
<td>adhesive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is recognised that the faecal coliform flora in health may comprise a variety of serotypes, some of which are represented in small measure, and that the greater number of coliform colonies tested in each faecal sample, the greater the theoretical yield of serotypes. In order to identify the dominant serotype in each sample, we have chosen to select 10 colonies, as there is good evidence from a number of previous studies that this method will reveal the dominant serotype of that sample. There is also evidence from a colonscopic study that serotypes that dominate the faecal sample also dominate the entire colonic flora and these observations suggest that our sampling methods are valid. In this study we have usually restricted ourselves to studying one faecal sample per patient. While it could be argued that the results we have obtained might be due to chance shedding of faecal pathogens, this would seem unlikely, especially as second samples obtained from five patients soon after admission to hospital either showed no change in O-serotype of the colonies tested, or, if the original coliforms were non-typable, no change in antibiotic sensitivity in similarly non-typable strains.

In order to facilitate testing for other properties, we have assumed that each coliform colony with the same O-serotype from the same patient had similar properties with regard to adhesiveness or invasiveness. This assumption might be invalid if adhesiveness and invasiveness are both plasmid-mediated, or if different bacterial strains with the same serotype can coexist in the same faecal sample. As all samples were treated similarly, however, it is unlikely that the results we have obtained can be accounted for thus and, in any event, while there is evidence to suggest that there is heterogeneity of properties among E.coli colonies with the same O-type in the faeces of normal persons, the coliforms in disease conditions are
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probably more homogeneous (Varian and Cooke, unpublished observations), as is shown by our finding that one serotype tended to dominate the flora in patients with active and quiescent colitis, but not in normal persons.

We have used the HeLa cells model of Labrec et al. (1964) to assess adhesiveness and invasiveness. This method has been criticised because of the difficulties that may be encountered in differentiating between adhesion and invasion. We did not find this distinction difficult, as strains were deemed invasive only if they produced monolayer disruption within seven hours of infection. Adhesive strains did not cause disruption even if incubation was prolonged to 12 hours but overnight incubation was misleading, as monolayer disruption, secondary to the effects of bacterial multiplication on the culture medium, usually led to non-specific bacterial adhesion and an appearance similar to that of the late effects of an invasive strain. Thus we consider the technique useful but only if the monolayers are inspected frequently and assessment of adhesion or invasion is based on a study of evolving change.

Adhesion by bacterial pathogens to host cells is well recognised and appears to be an essential step in the pathogenesis of some diseases. Adhesion to the intestine may facilitate colonisation by toxin-producing bacteria or even be a primary pathogenic property. The precise significance of the adhesion we have found is uncertain, as only one of the strains, E. coli 063, is a known enterotoxigenic serotype, but, while the mechanisms of E. coli diarrhoea remain incompletely understood, tests for adhesiveness are useful in determining potential pathogenicity. The adhesion by enteric pathogens to host cells has been best described in animals where a high degree of host-parasite specificity obtains. Adhesion to HeLa cells clearly lacks this specificity but, in studies of urinary tract E. coli, the ability to adhere to HeLa cells was shown to correlate with the more specific ability to adhere to uroepithelial cells, although more strains adhered to the latter, and it might be that the use of cells more closely related to colonic epithelium would reveal an even greater incidence of adhesive strains in a similar patient population. The fetal colon assay of McNeish et al. would be of value in this respect, as might the recently described use of intestine 407 cells. The adhesive klebsiella is of interest. Although klebsiellas are not widely recognised as enteric pathogens, they have been described as such. Additional research is indicated into the role of klebsiellas in the pathogenesis of enteric disease.

The invasion of HeLa cells by enteric pathogens is recognised as being indicative of in vivo pathogenity. Hitherto, the invasive property has been attributed to a small number of E. coli 0-serotypes (028, 0112, 0124, 0136, 0143, 0144), although studies to date have been largely confined to patients with infectious diarrhoea. Only one recognised invasive serotype, 028, was encountered in this study; the other invasive strains being 021, 02, and 087 respectively. Recent work on the pathogenicity of E. coli has, however, demonstrated that the relationship between pathogenic properties and 0-serotype is inconstant and the lack of correlation between 0-serotype and pathogenicity makes it probable that additional investigation will reveal a greater variety of 0-serotypes with which the invasive property can be associated.

In this study we have made two findings. Firstly, we have found that one E. coli-serotype tends to dominate the faecal flora of patients with both active colitis, of whatever cause, and quiescent ulcerative colitis. In some instances the dominant serotype may have been selected by a change of diet consequent upon admission to hospital or by prior treatment with sulphasalazine or other antimicrobials. However, these possibilities are unlikely to account for the results we have obtained, as the majority of the patients studied had faecal samples taken within 48 hours of admission to hospital and, while the majority of the patients with quiescent ulcerative colitis (13 out of 15) were taking antimicrobials at the time of study, only 12 out of 43 of the patients with active colitis were doing so. Secondly, we have found an increased incidence of adhesive or invasive serotypes in the patients with active and quiescent ulcerative colitis when compared with the other groups studied. This finding might be explicable on the basis of enhanced survival of such coliforms in patients with colonic disease, although their relative infrequency in the patients with other colitis would be unexpected if this were the case. Alternatively, it may provide additional evidence for an association between enteric infection and ulcerative colitis. The significance of positive faecal cultures in patients with ulcerative colitis is unclear and findings for salmonella and shigella organisms, similar to those we have made for coliforms, have been interpreted both as evidence for an increased tendency to pathogen carriage and for a primary aetiological relationship respectively. Additional work is indicated, especially as a report of raised serum antibody levels to the traditional enteroinvasive E. coli serotypes in patients with ulcerative colitis supports the possibility that pathogenic coliforms are involved in the pathogenesis of this disease. If this is so, then a rational basis would be provided for attempts to treat the disease by alteration of the aerobic faecal flora.
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