Haemagglutination and Tissue Culture Adhesion of *Gardnerella vaginalis*

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Six strains of *Gardnerella vaginalis* were studied to examine the adhesin-receptor mechanism involved in their attachment to human red blood cells and an epithelial tissue culture cell line (McCoy). The adhesins involved in the attachment of the bacteria to each of these cells were proteinaceous but showed marked differences after various chemical or physical treatments, indicating that separate adhesins were present. Haemagglutinating strains were more hydrophobic than tissue-culture-adherent strains. Haemagglutination of human red blood cells by strains of *G. vaginalis* was inhibited by galactose, lactose, N-acetylneuraminic acid and phosphatidylserine. In contrast, the tissue-culture adherence of strains was not inhibited by these substances.

**INTRODUCTION**

The claim that *Gardnerella vaginalis* is the sole aetiological agent in bacterial vaginosis (Gardner & Dukes, 1955) has been a matter of controversy (Amsel et al., 1983; Easmon & Ison, 1986). A number of investigators have demonstrated an association between anaerobes, *G. vaginalis* and bacterial vaginosis (Easmon & Ison, 1986). A common feature of many studies has been the presence of clue cells (epithelial cells coated with Gram-variable bacilli) in the vaginal discharges of patients with bacterial vaginosis (Amsel et al., 1983).

There has been relatively little investigation of the adhesiveness of *G. vaginalis*. Edmunds (1962) first showed that *G. vaginalis* possessed haemagglutinating properties. The adherence of *G. vaginalis* to vaginal epithelial cells has also been demonstrated (Mårdh & Westrom, 1976; Sobel et al., 1981). These investigators showed that strains of *G. vaginalis* demonstrated a higher degree of adhesion to vaginal epithelial cells than did bacteria of the indigenous vaginal flora, and that *Gardnerella* strains attached equally well to exfoliated vaginal epithelial cells as to vaginal epithelial tissue culture cells. Recently it was shown that this haemagglutination was mannose-insensitive and was inhibited by D-galactose (Ison & Easmon, 1985). The mechanism of adhesion of *G. vaginalis* to exfoliated vaginal epithelial cells has been investigated (Peeters & Piot, 1985). The adhesin appeared to be heat-labile and was affected by UV-irradiation. Adherence could be inhibited by pre-treatment of epithelial cells with sodium periodate. While adherence was mannose-resistant, none of the other carbohydrates tested in adhesion inhibition experiments, including D-galactose, markedly reduced adherence.

In a recent study the association between the presence of clue cells in and adhesiveness of isolates of *G. vaginalis* from respective vaginal discharges was investigated (Scott et al., 1987). An apparent association between the degree of adhesiveness of isolates, as determined by a tissue-culture adhesion test using an epithelial cell line (McCoy), and the presence of clue cells in respective vaginal discharges was found. A similar association between the level of bacterial haemagglutinating activity and clue cell presence was not observed. In addition, the tissue culture adhesion indices and the haemagglutination titres of strains did not correlate. Taken together, the adhesion mechanism studies and the clinical association data suggested that
separate mechanisms might be involved in in vitro epithelial cell adhesion and haemagglutination.

The purpose of the present study was to examine the mechanism of adherence to human red blood cells and tissue culture cells (McCoy), to determine whether or not separate adherence systems were involved.

**METHODS**

**Chemicals.** All reagents used for treatment of bacterial cells, tissue culture cells and red blood cells, and for inhibition studies, were from Sigma except for lactose, glucose, sucrose and hexadecane, which were from BDH.

**Bacterial strains and growth conditions.** The six strains of G. vaginalis used were identified according to the criteria of Taylor & Phillips (1983). Five of these were selected from a collection acquired during a study relating adhesiveness of clinical isolates of G. vaginalis to the presence of clue cells in the vaginal exudates of respective patients (Scott et al., 1987). The sixth, strain 624, was isolated from a urinary tract infection in a male attending a sexually transmitted diseases clinic. The strains selected (Table 1) showed either haemagglutinating properties, tissue culture adhesion properties or a combination of both. The six subcultures utilized were derived from single colonies from primary cultures of stock strains and were stored at -70 °C in thioglycollate broth containing 10% (v/v) glycerol and 10% (v/v) defibrinated horse blood. Strains were subcultured on Casman blood agar (Difco) for 48 h at 37 °C in approx. 5% CO₂ (GasPak, Baltimore Biological Laboratories).

**Determination of tissue culture adhesiveness and haemagglutination properties.** Coverslip preparations of McCoy cells were prepared as described before (Scott et al., 1987). The density of bacterial suspensions was adjusted by an opacity method (Paik, 1980) to 1 x 10⁸ organisms ml⁻¹ in Dulbecco A phosphate-buffered saline (PBS), pH 7.3 (Oxoid). The bacterial suspension (1-0 ml) was added to each coverslip and incubated for 1 h at 37 °C. The preparations were subsequently washed in Dulbecco A PBS and stained with Giemsa stain. The stained coverslips were examined by light microscopy. An adhesion index was calculated as the percentage of 200 cells examined which had four or more organisms attached.

Haemagglutination tests were performed by a rocked tile test on crushed ice. Bacterial suspensions of approximately 5 x 10¹⁰ organisms ml⁻¹ were prepared in PBS (0.15 m-NaCl with 0.02 m-sodium phosphate buffer) pH 6.8 from 48 h cultures on Casman blood agar. A 3% (w/v) packed red blood cell suspension of human group O cells was used. Drops (approx. 50 μl) from Pasteur pipettes were used. The tests were performed at 0 °C and were graded semi-quantitatively (Duguid et al., 1979).

**Treatment of bacterial cells prior to adhesion studies.** Bacterial strains were treated with sodium periodate (10 mg ml⁻¹) or 0.4% (w/v) formalin in Dulbecco A PBS at room temperature for 30 min. Strains were also treated with Pronase E, trypsin and wheat germ lipase (2 mg ml⁻¹) in Dulbecco A PBS at 37 °C for 2 h. After treatment, strains were washed three times and resuspended at 1 x 10⁸ organisms ml⁻¹ in Dulbecco A PBS for tissue culture studies and at 5 x 10¹⁰ organisms ml⁻¹ in PBS pH 6.8 for haemagglutination tests. Bacterial suspensions of 5 x 10¹⁰ organisms ml⁻¹ were heated at 60 °C for 30 min or were added in 0.2 ml amounts to open Trac tubes (Sterlin) and UV-irradiated at 1240 W cm⁻² for 1 h. Suspensions of 1 x 10⁸ organisms ml⁻¹ were prepared from these treated organisms for tissue culture tests. Control suspensions were tested in parallel.

**Treatment of red blood cells and McCoy cells prior to adhesion studies.** Coverslip preparations of McCoy cells and 20% (v/v) packed red blood cell suspensions were treated with sodium periodate (10 mg ml⁻¹) and 0.4% (w/v) formalin in PBS (pH 7.2) for 30 min at room temperature. Tissue culture preparations and red blood cell suspensions were also treated with Pronase E (10 μg ml⁻¹) and neuraminidase (Sigma type X, 1 unit ml⁻¹) in 0.05 m-sodium acetate buffer (pH 5.5) at 37 °C for 1 h. Cells incubated with sodium acetate buffer in an identical manner were used as control. Following all treatments, red blood cells were washed three times in PBS (pH 6.8) and suspended to a 3% (v/v) packed red blood cell suspension; tissue culture cells were washed six times in Dulbecco A PBS.

**Haemagglutination of human red blood cells of different blood group systems.** Strains 102, 138, 184 and 624 were tested against 3% (v/v) packed red blood cell suspensions of A, B, O, P, p⁺, MM, MN, NN, S, s⁺, Fy⁺, Fy⁰, Jk⁺ and Jk⁰ blood group types, kindly provided by the National Blood Transfusion Board, Dublin. Haemagglutination tests were performed as described above.

**Inhibition tests.** The following solutions were prepared in PBS (pH 6.8) and Dulbecco A PBS for haemagglutination tests and tissue culture tests, respectively: α-glycoprotein and mucin (2 mg ml⁻¹); serine (20 mg ml⁻¹); D-galactose, D-galactosamine, N-acetylglactosamine, lactose, sucrose, glucose, L-fucose, lactulose, N-acetylmuramic acid and N-acetylneuraminic-lactose (all 40 mM). Dispersions of sphingomyelin, phosphatidylcholine, phosphatidyoserine and phosphatidic acid were prepared by dissolving the phospholipids in ethanol (10 mg ml⁻¹) and diluting them (1 mg ml⁻¹) in the appropriate buffer. Human blood group O cells (3%, v/v) were used for haemagglutination inhibition tests. Bacterial suspensions were incubated in the presence of the inhibitor for 5 min at 0 °C for haemagglutination and for 30 min at 37 °C for tissue culture adhesion prior to testing. Control
suspensions were incubated with diluent in parallel. In the case of the phospholipids, control suspensions were incubated with 10% (v/v) ethanol in PBS (pH 6-8) or Dulbecco A PBS (pH 7-3). Inhibition was judged by a reduction in the intensity of the haemagglutination reaction or a reduction in the adhesion index of the tissue culture adhesion assay.

Hydrophobicity studies. The cell-surface hydrophobicity of strains 26, 53, 102, 138 and 624 was tested using adhesion to the hydrocarbon hexadecane (Rosenberg et al., 1980; Rosenberg & Kjelleberg, 1986). Bacterial suspensions from 48 h cultures on Casman blood agar were prepared in phosphate/urea/magnesium (PUM) buffer pH 7.1 (97 mM-K2HPO4, 53 mM-KH2PO4, 30 mM-urea, 0.8 mM-MgSO4; Rosenberg et al., 1980). The suspensions were adjusted to OD100 = 1.00. To 1.2 ml bacterial suspension 0.2 ml hexadecane was added and the mixture was incubated at 30 °C for 15 min. This mixture was blended on a vortex mixer (Gallenkamp Spinmix with the setting at 7.5) for 30 s, 1 min, 1.5 min and 2.0 min. It was allowed to stand for 30 min before the aqueous layer was removed and its OD100 read in a spectrophotometer. The OD100 value of the aqueous layer of the test mixture was expressed as a percentage of the OD100 value of the original suspension. To determine the effects of proteases on hydrophobicity, strains were treated with Pronase E (2 mg ml⁻¹) or trypsin (2 mg ml⁻¹) in Dulbecco PBS and washed as previously described prior to resuspension in PUM buffer. Hydrophobicity values of enzyme-treated and untreated strains were determined after mixing with hexadecane for 2 min.

**RESULTS**

Effect of pre-treatment of bacterial strains, red blood cells and tissue culture cells on tissue culture adhesion and haemagglutination

The haemagglutinating activity of the six *G. vaginalis* strains (Table 1) was not affected by formalin, trypsin, or lipase treatment except in the case of strain 624, which showed some reduction of haemagglutination after trypsin treatment (relative haemagglutination reduced from + ++ to +). The pre-treatment of strains with sodium periodate, heat or pronase completely inactivated haemagglutinating activity (data not shown). UV-irradiation markedly reduced haemagglutination (relative haemagglutination of strains 26 and 53 abolished; for strains 138, 184, 624 and 102 relative haemagglutination reduced to +).

Tissue culture adhesion was markedly reduced by the pronase and heat treatments of bacteria (Table 2). In contrast to the haemagglutination results, periodate treatment and UV-irradiation produced no reduction in tissue culture adhesion, while trypsin treatment markedly reduced it (Table 2).

Pre-treatment of red cells with neuraminidase markedly reduced haemagglutination by the *G. vaginalis* strains but pre-treatment with formalin had no effect. In contrast, neuraminidase treatment of McCoy tissue culture cells had no effect on adhesion of *G. vaginalis* strains while formalin treatment of McCoy cells markedly reduced adhesion (Table 3).

Investigation of receptors by use of human blood group antigens and inhibitor substances

Human red blood cells of all the blood groups tested were haemagglutinated by the four *G. vaginalis* strains tested (102, 138, 184 and 624). Complete inhibition of haemagglutination of human blood group O red cells was achieved in each case with phosphatidylserine and partial inhibition by phosphatidic acid. Serine, x-glycoprotein, sphingomyelin and phosphatidylcholine, however, produced no inhibition. Of the carbohydrates tested, galactose, galactosamine, N-acetylgalactosamine, lactose and lactulose inhibited haemagglutination. The effects of lactose and lactulose were greater than those of galactose, galactosamine or N-acetylgalactosamine.

N-Acetylmuraminic acid also inhibited haemagglutination and a combination of lactose and N-acetylmuraminic acid produced marked inhibition at low molarity (Table 4). N-Acetylneuramin-lactose enhanced haemagglutination. None of the test inhibitor substances produced more than a marginal reduction in the adhesion of strains 26, 53, 138 and 184 to tissue culture cells.

Hydrophobicity studies

Of the five strains chosen for hydrophobicity studies, strains 26 and 53 showed a marked tissue culture adherence index but weak haemagglutinating properties, while strains 102 and 624 showed marked haemagglutinating properties but a very weak tissue culture adhesion index. Strain 138 was moderately haemagglutinating and markedly tissue-culture adherent (Table 1).
Table 1. Strains of G. vaginalis used in haemagglutination and tissue culture adhesion studies

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Biotype*</th>
<th>Source</th>
<th>Relative haemagglutination+</th>
<th>Tissue culture adhesion index‡ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>1</td>
<td>Vagina</td>
<td>+</td>
<td>95.2 ± 1.9</td>
</tr>
<tr>
<td>53</td>
<td>2</td>
<td>Vagina</td>
<td>+</td>
<td>57.8 ± 2.2</td>
</tr>
<tr>
<td>138</td>
<td>1</td>
<td>Vagina</td>
<td>++</td>
<td>98.8 ± 0.3</td>
</tr>
<tr>
<td>184</td>
<td>1</td>
<td>Vagina</td>
<td>++</td>
<td>45.8 ± 2.2</td>
</tr>
<tr>
<td>102</td>
<td>1</td>
<td>Vagina</td>
<td>+++</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>624</td>
<td>1</td>
<td>Urine (male)</td>
<td>++</td>
<td>5.6 ± 1.3</td>
</tr>
</tbody>
</table>

* According to Piot et al. (1984).
† + ++. Strongly positive, coarse clumping within about 20 s; ++, moderately strong, coarse clumping in about 2 min; +, weak positive, fine clumping in 10 min (Duguid et al., 1979).
‡ Mean of five experiments ± SEM: percentage of 200 cells examined showing four or more adherent bacteria.

Table 2. Effects of pretreatments of G. vaginalis strains on adhesion to McCoy tissue culture cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Untreated</th>
<th>Periodate</th>
<th>Formalin</th>
<th>Pronase</th>
<th>Lipase</th>
<th>Heat</th>
<th>UV</th>
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<tbody>
<tr>
<td>26</td>
<td>94 ± 2</td>
<td>98 ± 2</td>
<td>98 ± 2</td>
<td>6 ± 2</td>
<td>18 ± 3</td>
<td>93 ± 5</td>
<td>17.5 ± 3.5</td>
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<tr>
<td>53</td>
<td>61 ± 3.5</td>
<td>67 ± 2</td>
<td>68 ± 3</td>
<td>7.5 ± 1.5</td>
<td>7 ± 2</td>
<td>62 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>138</td>
<td>98 ± 2</td>
<td>100</td>
<td>100</td>
<td>12 ± 4</td>
<td>15 ± 4</td>
<td>95 ± 5 ± 2.5</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>184</td>
<td>47.5 ± 3.5</td>
<td>50 ± 2</td>
<td>52 ± 3</td>
<td>8.3 ± 1.5</td>
<td>11 ± 2</td>
<td>50 ± 5 ± 3.5</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>102</td>
<td>8 ± 3</td>
<td>10 ± 2</td>
<td>6.5 ± 1.5</td>
<td>0</td>
<td>0</td>
<td>8 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>624</td>
<td>6 ± 2</td>
<td>3.5 ± 1.5</td>
<td>4 ± 1</td>
<td>0</td>
<td>0</td>
<td>4 ± 2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean of two experiments ± SEM: percentage of 200 cells examined showing four or more adherent bacteria.

Table 3. Effects of pretreatments of human red blood cells and McCoy tissue culture cells on haemagglutination and tissue culture adhesion index of G. vaginalis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Untreated</th>
<th>Pronase</th>
<th>Neuraminidase</th>
<th>Periodate</th>
<th>Formalin</th>
</tr>
</thead>
<tbody>
<tr>
<td>138</td>
<td>++</td>
<td>+ +</td>
<td>+</td>
<td>–</td>
<td>+ +</td>
</tr>
<tr>
<td>184</td>
<td>+ +</td>
<td>+ +</td>
<td>+</td>
<td>–</td>
<td>+ +</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Untreated</th>
<th>Pronase</th>
<th>Neuraminidase</th>
<th>Periodate</th>
<th>Formalin</th>
</tr>
</thead>
<tbody>
<tr>
<td>138</td>
<td>97 ± 2</td>
<td>98 ± 2</td>
<td>92 ± 1</td>
<td>10.5 ± 2.5</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>184</td>
<td>52.5 ± 2.5</td>
<td>51 ± 3</td>
<td>48.5 ± 3.5</td>
<td>4 ± 1</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

* See Table 1.
† Mean of two experiments ± SEM: percentage of 200 cells examined showing four or more adherent bacteria.

Table 4. Effects of lactose, galactose and N-acetyl neuraminic acid on inhibition of haemagglutination by G. vaginalis strain 102

<table>
<thead>
<tr>
<th>Test saccharide(s)*</th>
<th>20 mM</th>
<th>10 mM</th>
<th>5 mM</th>
<th>2.5 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-acetyl neuraminic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose + N-acetyl neuraminic acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose + N-acetyl neuraminic acid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Each saccharide present at indicated concentration.
† See Table 1.
Adhesion of Gardnerella vaginalis

Each of the strains showed some cell-surface hydrophobicity but strains 102 and 624, which showed marked haemagglutination properties, had the highest level of cell-surface hydrophobicity (Fig. 1). The treatment of strains with pronase completely abolished their hydrophobicity. Trypsin treatment abolished the hydrophobicity of strains 26 and 53 (percentage of bacteria in the aqueous phase after blending with hexadecane increased from 33% to 92% for strain 26 and from 43% to 95% for strain 53), but only reduced that of strains 102 and 624 (percentage of bacteria in the aqueous phase after blending with hexadecane increased from 22% to 72% for strain 102 and from 19% to 67% for strain 624).

DISCUSSION

It is generally agreed that the adhesiveness of bacteria is likely to be an important factor influencing the colonization of mucous membrane surfaces. Thus while adhesiveness would be useful in the maintenance of commensal states, for many bacteria it is an important prerequisite for the establishment of infective states. In the evaluation of the role of G. vaginalis in bacterial vaginosis the adhesive properties of this organism are generating increasing interest (Ison & Easmon, 1985; Peeters & Piot, 1985; Scott et al., 1987).

Ison & Easmon (1985) and Peeters & Piot (1985) used red blood cells and exfoliated vaginal epithelial cells, respectively, to study mechanisms of adhesion in G. vaginalis. In model adhesion systems such as haemagglutination tests and epithelial cell tests the same adhesive activity of a culture may be measured with both. However, some bacteria produce more than one adhesin and the adhesion of bacteria to different surfaces may be mediated by distinct mechanisms (Jones, 1980; Smyth, 1986). The finding that D-galactose and D-galactosamine markedly reduced haemagglutination (Ison & Easmon, 1985) but failed to produce a similar effect on adhesion to exfoliated vaginal epithelial cells (Peeters & Piot, 1985) suggested that the receptor complexes on these cells may be different. The finding that haemagglutination and adhesion to tissue cells did not correlate (Scott et al., 1987), again suggested that G. vaginalis might possess at least two adhesive systems.

In the present study several distinct dissimilarities have been demonstrated between haemagglutination and tissue-culture adhesion by G. vaginalis with respect to sensitivity to
sodium periodate and trypsin, inhibition by lipids and sugars, and in the degree of hydrophobicity conferred on the bacterial cell surface. These findings strongly suggest that two adhesins are present, both of which appear to be proteinaceous judging from the responses of bacteria to treatments with pronase and with mild heat. However, the marked inhibitory effect of trypsin on tissue-culture adhesion and its failure to abolish haemagglutination implies that separate protein structures are involved. The finding that sodium periodate abolishes haemagglutination activity does not necessarily indicate the involvement of a carbohydrate moiety on this adhesin as periodate can inactivate some proteins and can cause the oxidative conversion of NH2-terminal serine, threonine and cysteine (Geoghagan et al., 1980).

The effect of sodium periodate on both red blood cells and tissue-culture cells suggests that the receptors for the adhesins discussed above may be carbohydrate moieties, although oxidation of proteins is possible. However, pronase had no effect on either cell type, which supports the view that the receptor may not be a protein. Neuraminidase had no effect on tissue-culture adhesion but reduced haemagglutinating activity, indicating a role for N-acetyleneuraminic acid in the red blood cell receptor complex. The finding that N-acetyleneuraminic acid inhibited haemagglutination confirms this. In addition D-galactose, D-galactosamine and lactose inhibited haemagglutination, indicating the presence of galactose in the red blood cell receptor. Lactose showed a greater level of inhibition than galactose and when lactose was combined with N-acetyleneuraminic acid inhibition was enhanced, indicating that galactose may be present in a particular conformation or linkage with N-acetyleneuraminic acid as part of the receptor complex.

The partial inhibition of haemagglutination by phosphatidic acid and its complete inhibition by phosphatidylserine is interesting, indicating a possible role for serine in the receptor complex on the red cell. As serine residues are found in glycoporphin A\(^\text{AM}\) (Jokinen et al., 1985) it would suggest a possible involvement of the MN blood group substance. However, the agglutination of red blood cells of a wide range of human blood group types by the strains of \textit{G. vaginalis} examined indicates that the receptor may not be a recognized blood group antigen. Furthermore, strains of \textit{G. vaginalis} have previously been reported to react with fowl, sheep and horse red blood cells (Scott et al., 1987).

The failure of test saccharides, phospholipids and serine to inhibit tissue culture adhesion would indicate the presence of a separate receptor on the tissue culture cell. This would be in agreement with the findings of Peeters & Piot (1985) that D-galactose and N-acetylgalactosamine did not markedly inhibit adherence to exfoliated vaginal epithelial cells. However, an ancillary role for the haemagglutinin in binding to epithelial cells cannot be excluded.

The hydrophobicity studies showed that although the strains had a high level of hydrophobicity, the haemagglutinating strains seemed to have the highest. The hydrophobic properties would appear to be due to surface-associated protein structures, as hydrophobicity was completely removed by pronase. The effect of trypsin treatment on hydrophobicity parallels the earlier finding that in the case of strains which are highly haemagglutinating, the haemagglutinin was not affected by trypsin.

The findings obtained in this study indicate that there are at least two distinct protein adhesin systems in \textit{G. vaginalis}. Such a finding is not unusual among bacteria and similar observations have been made in \textit{Salmonella typhimurium} (Tavendale et al., 1983) and \textit{Escherichia coli} (Smyth, 1986). While it is not clear from the present study if either or both systems are mediated by fimbriae, the effect of UV-irradiation on haemagglutination would suggest fimbrial involvement (Trust et al., 1980). Fimbriae have been found on \textit{G. vaginalis} strains (Johnson & Davies, 1984), but so far no evidence for their role in haemagglutination or epithelial cell adhesion has been reported. Preliminary findings from electron microscopic examination of bacteria attached to red blood cells and tissue culture cells indicate that haemagglutination may be mediated by fimbriae but that tissue culture adhesion may be mediated by an outer fibrillar coat (T. G. Scott, unpublished observations).

The reported association between clue cells and bacterial vaginosis indicates a possible role for adhesive strains of \textit{Gardnerella} in this condition. In the light of the findings of this present study it would be important in all future investigation attempting to assess the clinical
significance of adhesiveness of *G. vaginalis* to recognize the existence of two distinct adhesin receptor-systems.

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