Basis for N-acetyllactosamine-mediated inhibition of enteropathogenic Escherichia coli localized adherence

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In a previous article, the authors reported that exposing wild-type enteropathogenic Escherichia coli (EPEC) to chemically synthesized N-acetyllactosamine glycosides covalently coupled to BSA (LacNAc–BSA) inhibited localized adherence (LA) by these organisms and also caused them to lose their bundle-forming pili (BFP), the filamentous surface appendages responsible for their LA phenotype. This effect has now been further investigated by screening a panel of LacNAc–BSA-related glycosides for their ability to inhibit EPEC LA, which revealed that LacNAc–BSA retained its status as the most effective inhibitor of EPEC LA. It was also shown that LacNAc–BSA did not cause the loss of BFP in an EPEC strain containing a non-polar mutation in the bfpF gene and, as a consequence, unable to retract its BFP. LacNAc–BSA also effectively inhibited LA of the bfpF mutant EPEC. Taken together, these observations suggest that, as well as triggering BfpF-mediated BFP retraction, LacNAc–BSA likely functions as a competitive inhibitor of EPEC binding to LacNAc-related receptors on host cells. Moreover, transmission electron microscopy revealed that LacNAc conjugated to gold nanoparticles bound specifically to BFP. This observation indicated that either the major BFP structural subunit (BfpA) itself or, possibly, an accessory protein co-assembled with BfpA into the BFP filaments, contains a LacNAc-specific EPEC adhesin. The results suggest a mechanism whereby the initial binding of EPEC to LacNAc-like receptors on host cells triggers BfpF-mediated BFP retraction. This could then expedite the intimate adherence phase of the multi-step EPEC colonization process by drawing the organisms closer to the host-cell plasma membrane.

INTRODUCTION

Enteropathogenic Escherichia coli (EPEC) serotypes cause neonatal diarrhoea in developing nations (Hart et al., 1993) and occasional diarrhoea in adults who travel to endemic regions of the world (Bieber et al., 1998; Hart et al., 1993). EPEC infect and colonize their hosts through a highly interactive process thought to involve three steps: initial binding, signal transduction, and intimate adherence (Campellone & Leong, 2003; Delahay et al., 2001; Donnenberg & Kaper, 1992; Donnenberg & Whittam, 2001; Kenny, 2002). In the first stage of this process, EPEC form micro-colonies on the host-cell surface through a process known as localized adherence (LA) (Donnenberg et al., 1992; Giron et al., 1993). EPEC micro-colony formation during LA is dependent on rope-like surface appendages called bundle-forming pili (BFP) (Donnenberg et al., 1992; Giron et al., 1993). While the role of BFP in the actual adherence of EPEC to the host cell is unknown, human volunteer studies have shown that BFP are required for full EPEC virulence in adults (Bieber et al., 1998). BFP have also been shown to be required for efficient adherence of EPEC to intestinal brush border cells during the early stages of infection (Cleary et al., 2004). Moreover, Tober and Sasakawa (2001) have demonstrated a clear requirement for BFP both in the initial attachment of EPEC to human intestinal tissue culture Caco-2 cell surfaces and in LA to this cell line. This group have also demonstrated that isolated BFP attach to

Abbreviations: BFP, bundle-forming pili; EPEC, enteropathogenic Escherichia coli; LA, localized adherence; LacNAc, N-acetyllactosamine; MBP, maltose binding protein; Pk, αGal(1,4)βGal(1,4)βGlc.
Caco-2 cells, and it appears that contact with the Caco-2 cell surface induces EPEC to shed their BFP, concomitant with micro-colony dispersal and intimate adherence. EPEC serotypes have been shown to bind to complex carbohydrate receptor sequences displayed on host-cell glycolipids and glycoproteins (Cravioto et al., 1991; Idota & Kawakami, 1995; Jagannatha et al., 1991; Vanmaele et al., 1999). Previous research in our laboratory has demonstrated that EPEC lose their BFP by an undetermined mechanism when the organisms are exposed to synthetic glycan sequences, as described in our previous reports on the Caco-2 cell-membrane-mediated BFP-shedding events described by Tobe and Sasakawa (2001). Clearly, the expression, regulation, and precise function of BFP in EPEC colonization and pathogenesis in the human intestine remain enigmatic. Regardless of the continuing debate surrounding the regulation and function of BFP in EPEC colonization in vivo, the studies reported herein were designed to further investigate the interaction of EPEC with complex carbohydrate sequences, as described in our previous reports on the subject.

METHODS

Reagents and bacterial strains. The wild-type EPEC strain E2348/69 (serotype O127:H6), initially isolated from an infant with diarrhoea, was provided by Dr M. Finlayson (Provincial Laboratory of Alberta, Edmonton, AB). JPN15, a spontaneous EAF plasmid-containing pMSD217) EPEC strains (Anantha et al., 1998) were provided by Dr M. Donnenberg (University of Maryland School of Medicine, Baltimore, MD). These bacteria were routinely cultivated on conventional tryptic soy agar (TSA) plates at 37 °C to obtain individual colonies. Rabbit BFP-specific antiserum was provided by Dr J. A. Giron (University of Arizona, Tucson, AZ). The rabbit maltose binding protein (MBP) antiserum was purchased from New England Biolabs. Goat anti-rabbit IgG conjugated to 6 nm gold particles was purchased from Cedarlane.

All the glycoconjugates listed in Table 1 consisted of chemically synthesized 8-methoxycarbonyloctyl (MCO) glycosides (Lemieux et al., 1977) covalently coupled to BSA. The conjugation ratios (N, mole MCO glycoside sequence per mole BSA) were determined by mass spectroscopy analysis. The glycoconjugates were dissolved in distilled water to a final concentration of 5 mg ml⁻¹.

Preparation of gold glyconanoparticles. The LacNα–disaccharide and αGal(1,4)βGal(1,4)βGlc(Pk)–trisaccharide glycosides containing decamethylene extensions terminating in a disulfide functionality were conjugated to gold nanoparticles (nominal size 5 nm) purchased from Sigma (G1402), essentially as described elsewhere (Lin et al., 2002). In a typical procedure, a solution of gold colloid (5 ml) was slowly added to a near-boiling solution of either glycoside derivative (~10 mg) in 30 ml water. The mixtures were maintained for 2 h at 80 °C, then concentrated to 10 ml under reduced pressure. Next, the preparations were centrifuged to remove large particulates. The resulting supernatant solutions were dialysed (12–14 kDa molecular weight cut-off) exhaustively against ultra-pure water and then lyophilized to dryness.

Preparation of tissue culture cell monolayers and LacNα–BSA LA binding inhibition assays. The HEp-2 (CCL-23), Caco-2 (HTB-37) and T84 (CCL-248) cell lines were obtained from and propagated according to the American Type Culture Collection recommendations. Cell monolayers were prepared in 96-well plates containing polystyrene discs, as described in our previous article (Vanmaele et al., 1999).

Prior to each experiment, BFP expression was induced by inoculating 10 μl of a tryptic soy broth (TSB) bacterial culture, grown statically overnight at 37 °C from a single colony picked from a TSA plate, into 1 ml Dulbecco modified Eagle medium (DMEM, Gibco) which had been pre-equilibrated overnight in a CO₂ incubator and supplemented with 44 mM NaHCO₃, 40 μM phenol red and 25 mM glucose (Vanmaele et al., 1995, 1999). After 30 min at 37 °C in the CO₂ incubator, these cultures were added to 96-well plates containing the BSA glycoconjugates and incubated for an additional 30 min at 37 °C in a CO₂ incubator. The contents of each of these wells were then transferred to the corresponding wells of a 96-well microtitre plate containing the subconfluent HEp-2, Caco-2 or T84 cell monolayers on polystyrene discs. After 30 min at 37 °C in the CO₂ incubator, the tissue culture cells were washed three times with physiological PBS, pH 7.2. HEp-2 cells were fixed with methanol for 10 min and then stained with Giemsa stain for 20 min. The polystyrene discs were removed from the microtitre plate wells, and EPEC LA was quantified microscopically as described previously (Vanmaele et al., 1999).

Table 1. Synthetic BSA glycoconjugates used in the EPEC receptor specificity study

<table>
<thead>
<tr>
<th>Ligand name</th>
<th>BSA conjugation ratio (N)</th>
<th>Glycan sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis B</td>
<td>30</td>
<td>αFuc(1,2)βGal(1,3)αFuc(1,4)βGlcNAc</td>
</tr>
<tr>
<td>Diglucoside</td>
<td>30</td>
<td>αGlu(1,3)αGlu</td>
</tr>
<tr>
<td>Linear B (IV)</td>
<td>35</td>
<td>αGal(1-3)βGal(1-4)βGlc</td>
</tr>
<tr>
<td>Sialyl-Lewis A</td>
<td>28</td>
<td>NeuNAc(2,6)βGal(1,3)αFuc(1,4)βGlcNAc</td>
</tr>
<tr>
<td>Lewis A</td>
<td>42</td>
<td>βGal(1,3)αFuc(1,4)βGlcNAc</td>
</tr>
<tr>
<td>Sulfο-Lewis A</td>
<td>38</td>
<td>3'-sulfo-βGal(1,3)αFuc(1,4)βGlcNAc</td>
</tr>
<tr>
<td>Sulfο-Lewis X</td>
<td>42</td>
<td>3'-sulfo-βGal(1,4)αFuc(1,3)βGlcNAc</td>
</tr>
<tr>
<td>Sialyl-Lewis X</td>
<td>36</td>
<td>NeuNAc(2,6)βGal(1,4)αFuc(1,3)βGlcNAc</td>
</tr>
<tr>
<td>Lewis X</td>
<td>30</td>
<td>βGal(1,4)αFuc(1,3)βGlcNAc</td>
</tr>
<tr>
<td>LacNAc</td>
<td>35</td>
<td>βGal(1,4)βGlcNAc</td>
</tr>
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Since individual cells could not be distinguished, it was not possible to enumerate EPEC LA to Caco-2 and T84 cell monolayers by the same microscopic method used to determine EPEC LA to Hep-2 cells. Therefore, EPEC attachment to these cell lines was determined by solubilizing the washed monolayers at 37 °C for 30 min in 100 μl PBS containing 0.01% (v/v) Triton X-100. The solubilized cells were then serially diluted in sterile PBS and spread on TSA plates. After incubation overnight at 37 °C, c.f.u. were enumerated to determine the number of adherent EPEC per individual monolayer. In addition, one disc from each Caco-2 and T84 LA inhibition assay was fixed, stained and observed microscopically as described above to confirm the LA phenotype of the adherent organisms (Vanmaele et al., 1999).

LacNAc–BSA-mediated reduction of BfpA, as assessed by immunoblotting. Overnight TSB cultures of all the strains used in this study were incubated in DMEM to induce BFP expression (Vanmaele et al., 1999). These cultures were then mixed with BSA, LacNAc–BSA, Pk–Au or LacNAc–Au at a concentration of 0.4 mg ml⁻¹ in DMEM and incubated at 37 °C for an additional 30 min in the CO₂ incubator. After centrifugation at 14,000 g for 10 min, the supernatant solutions were removed and the resulting bacterial cell pellets were dissolved in SDS sample buffer containing 50 mM DTT and subjected to conventional one-dimensional SDS-PAGE using 12.5% (v/v) separating gels and 5% (v/v) stacking gels. The separated proteins were then electrophoretically transferred to Immobilon-P (Millipore) membranes, and the immunoreactive BfpA and MBP (an invariant E. coli housekeeping protein used to control for sample-loading inconsistencies) and housekeeping protein bands were subsequently detected as per the procedure described in our previous article (Vanmaele et al., 1999). The resulting immunoblots were subjected to densitometry analysis using the Kodak Image Station 2000MM and associated Kodak ID image analysis software (version 3.6.5). Sample-loading inconsistencies were then corrected for by normalizing the densitometry intensities of each of the BfpA bands in each gel lane to the intensity of the accompanying invariant E. coli MBP band.

Immunogold and gold nanoparticle labelling procedures. BFP were isolated for these studies as described elsewhere (Giron et al., 1991). The bacteria for these studies were grown at 37 °C overnight on TSA plates supplemented with 5% (v/v) defibrinated sheep blood to induce BFP expression (Giron et al., 1991). Single bacterial colonies were then picked for analysis. These were gently suspended in 200 μl monoethanolamine (Sigma-Aldrich) buffer (pH 10.0). Twenty microlitres of each of the suspensions were subsequently applied onto Formvar and carbon-coated copper grids (Cedarlane). These were incubated for 10 min at 4 °C, after which time excess fluid was gently wicked from the grids. All remaining steps in the procedure were performed at 4 °C, a condition chosen to prevent BFP retraction. Immunogold labelling was performed by floating the grids, sample side down, for 20 min on a 50 μl drop of PBS containing 5% (w/v) BSA. The grids were then exposed for 1 h to rabbit anti-BFP serum diluted 100-fold in PBS. After a thorough washing with PBS, the grids were incubated for an additional 30 min in a solution containing goat anti-rabbit IgG conjugated to 6 nm gold particles, diluted 30-fold in PBS. The grids were again washed thoroughly with PBS, stained with 1:0% (w/v) phosphotungstic acid (PTA) for 5 min and gently wicked dry. The glycoside–gold nanoparticle labelling procedure was performed by floating the grids, sample side down, for 45 min on a 50 μl drop of either LacNAc–Au or Pk–Au suspended at a concentration of 10 mg ml⁻¹ in PBS. The grids were then thoroughly washed with PBS and stained using PTA, as described above. Finally, all the grids were examined using a Hitachi S-7000 transmission electron microscope.

Results and Discussion

Screening of potential EPEC LA inhibitors

In the present study, we extended our screening of potential EPEC glycan receptors reported in our previous article (Vanmaele et al., 1999) to include the synthetic glycoconjugates listed in Table 1. These all consisted of LacNAc, or modified LacNAc, sequences containing various fucosyl, sialyl or sulfo substitutions and additions. The outcome of this screening assay confirmed our previous finding (Vanmaele et al., 1999) that LacNAc–BSA is the most significant (P<0.001, Student’s t test) glycan-based inhibitor of LA (data not shown). At a concentration of 0.8 mg ml⁻¹, LacNAc–BSA caused a 98.5% (±1.72%) reduction in the number of HEp-2 cells with LA EPEC compared to the BSA control. LacNAc–BSA was followed in relative inhibitory potency by linear B (IV) (54.3±3.25% reduction of LA EPEC) and sialyl-Lewis A (49.1±11.0% reduction of LA EPEC) glycoconjugates, both of which significantly inhibited EPEC LA (P<0.005) at a concentration of 0.8 mg ml⁻¹. The remaining glycoconjugates listed in Table 1 inhibited LA weakly or not at all. We therefore focused the remainder of the present study on further investigating the interaction of EPEC with LacNAc.

EPEC binding inhibition experiments were also performed using LacNAc glycosides coupled to 5 nm gold nanoparticles. LacNAc–Au, at a concentration of 0.8 mg ml⁻¹, inhibited LA to HEp-2 cells by 73.2±7.89% (Fig. 1). In contrast, gold nanoparticles containing the Pk glycan sequence had no inhibitory effect on LA relative to BSA alone (Fig. 1). Therefore, LacNAc–BSA and LacNAc–Au both specifically inhibit EPEC LA to HEp-2 cells. These observations confirm LacNAc as the active inhibitory

![Fig. 1. LacNAc–Au nanoparticle-mediated inhibition of EPEC E2348/69 LA to HEp-2 cells. EPEC were pre-incubated at 37 °C in DMEM to induce BFP expression and subsequently with 0.8 mg ml⁻¹ LacNAc–BSA, BSA, LacNAc–Au or Pk–Au. LA was determined as described previously (Vanmaele et al., 1999). The data represent the means and standard errors of three trials per inhibitor. The difference between the inhibitory capacity of LacNAc–BSA versus BSA was significant, as was that between the inhibitory capacity of LacNAc–Au versus Pk–Au or BSA (P<0.001, Student’s t test).](http://jmm.sgmjournals.org)
component in the two different glycoconjugate preparations.

**LacNac–BSA-mediated inhibition of EPEC LA to human intestinal cell lines**

The EPEC binding inhibition experiments were also conducted using Caco-2 and T84 cells, both human intestinal cell lines. At a concentration of 0.8 mg ml\(^{-1}\), LacNac–BSA inhibited LA to Caco-2 cells by 87.1 ± 5.6% compared to the BSA control (Fig. 2). At the same concentration, LacNac–BSA inhibited LA to T84 cells by 74.2 ± 21.7% (Fig. 2). We also examined the effect of LacNac–BSA on the attachment of the EPEC JPN15 strain to Caco-2 and T84 cells. JPN15 is a derivative of the wild-type EPEC E2348/69 strain lacking the EAF plasmid and, as a consequence, the ability to express BFP as well as the capacity for LA in tissue-culture cell-binding assays (Levine et al., 1985). In both Caco-2 and T84 cells, LacNac–BSA failed to inhibit the residual non-LA BFP-independent adhesion of JPN15 to Caco-2 or T84 cells (data not shown), thereby confirming that LacNac–BSA was effectively inhibiting BFP-mediated EPEC LA in these experiments.

**LacNac–BSA-mediated loss of BfpA**

We have previously demonstrated (Vanmaele et al., 1999) that pre-incubating wild-type EPEC E2348/69 with LacNac–BSA causes them to lose their BFP, as demonstrated by a reduction in BfpA reactivity in immunoblots. BfpA (bundlin) is the major structural subunit of BFP (Giron et al., 1991). These results led us to query whether LacNac inhibited LA by acting as a eukaryotic cell EPEC receptor analogue, thereby competitively antagonizing LA, or by promoting the disappearance of BFP. To differentiate between these two possibilities, we repeated our previous experiments (Vanmaele et al., 1999) with a non-polar bfpF insertional mutant strain (UMD916) of E2348/69. EPEC harbouring a mutation in the bfpF gene are hyper-piliated because they are unable to retract their BFP (Anantha et al., 1998; Bieber et al., 1998). Incubation of E2348/69 with 0.4 mg LacNac–BSA ml\(^{-1}\) caused an 82.1% (range 63.5%) reduction in BfpA immunoreactivity and an 81.4% (±4.7%) reduction in LA compared to the BSA control (Fig. 3a). Similarly, incubation of E2348/69 with 0.4 mg LacNac–Au ml\(^{-1}\) caused a 68.8% (±7.1%) reduction in BfpA immunoreactivity and a 76.3% (±1.2%) reduction in LA relative to the Pk–Au control (Fig. 3b). Although incubating UMD916 with either 0.8 mg LacNac–BSA ml\(^{-1}\) or 0.4 mg LacNac–Au ml\(^{-1}\) inhibited LA by 88.24 ± 0.55% and 76.35 ± 1.03%, respectively, we detected no reduction in BfpA immunoreactivity relative to the BSA or Pk–Au controls in this strain (Fig. 3a–c). In contrast, the BfpA immunoreactivity was reduced by 82.54% (range 3.9%) and by 84.6% (±10.1%), respectively, when the bfpF-complemented UMD916 strain was exposed to LacNac–BSA or LacNac–Au, confirming that BfpF-mediated retraction was involved in this phenomenon. In these experiments, there was no statistically significant difference between the effect of LacNac–BSA and the effect of LacNac–Au on LA inhibition (P=0.251) or on the reduction of BfpA immunoreactivity (P=0.536) as assessed by the Mann–Whitney rank sum test, again confirming LacNac as the active component in these two glycoconjugate preparations.

The data presented in Fig. 3 also imply that LacNac–BSA was functioning directly as a soluble competitive inhibitor of EPEC binding to HEp-2 cell glycan receptors. The data also provide, for the first time, compelling evidence that suggests that the mechanism whereby EPEC lose their BfpA and, by association, BFP, upon contact with LacNac–BSA may involve a BfpF-mediated pilus-retraction mechanism. LacNac–BSA may, therefore, represent a functional analogue of the microsomal membrane fraction from Caco-2 cells described by Tobe and Sasakawa (2001). In their report, however, these authors demonstrated that incubating a wild-type EPEC strain with Caco-2 cell membranes causes BFP to be shed from the surface of the bacteria. They also found the shedding process to be BfpF dependent. Although LacNac–BSA also caused the loss of BFP from wild-type EPEC, we have been unable to detect by immunoblotting any shed BfpA in the supernatant solutions from bacteria pre-incubated with this glycoconjugate (data not shown). In our experiments at least, the LacNac–BSA-mediated loss of BFP from wild-type EPEC appears to happen by means of a BfpF-dependent retraction and subsequent BfpA degradation, rather than by a BFP-shedding mechanism. The opposing mechanisms whereby Caco-2 plasma membranes and LacNac–BSA promote the loss of BFP from

![Fig. 2. LacNac–BSA-mediated inhibition of E2348/69 LA to HEp-2, Caco-2 and T84 cells. The data represent the means and standard errors of three trials per inhibitor. The difference between the inhibitory capacity of LacNac–BSA versus BSA was significant in all three cell lines tested (P<0.001, Student’s t test). Black bars, HEp-2 cells; white bars, Caco-2 cells; grey bars, T84 cells.](image-url)
wild-type EPEC might be explained by the physical state of the two different sources of receptors. In this regard, the bacteria may not be able to retract their BFP if these are bound to insoluble Caco-2 membranes. Rather, the membrane-bound BFP filaments might be physically separated from the bacterial surface when the BfpF-mediated retraction system is activated. These BFP would therefore not be available to the degradation mechanisms present within the bacterial outer membrane or periplasmic compartments, and would remain antigenically intact in the Caco-2 membrane fraction. By contrast, the soluble LacNAC–BSA preparation might not present such a physical impediment to BFP retraction and subsequent BfpA degradation, perhaps by the EPEC Cpx-DegP envelope stress-response system (Raivio, 2005), within the bacterial cell.

Leverton and Kaper (2005) have recently reported that transcription of the bfpA gene increases in EPEC after the organisms have been in contact with HEp-2 cells for 3 h. Moreover, BfpA is also detected in Western immunoblots of EPEC-infected HEp-2 cells at both 3 and 5 h after bacterial attachment. In our experiments, the presence of BfpA was determined 30 min after the bacteria were exposed to LacNAC–BSA. It is possible, therefore, that the initial binding of BFP to host-cell glycan receptors may induce their rapid retraction and degradation, without affecting transcription of the bfpA gene. BFP retraction might serve to bring the bacteria into close apposition with the host-cell membrane, thereby expediting the microvillus effacement and intimate adherence phase of the EPEC colonization process. Following these early binding events, BFP expression may be upregulated, as reported by Leverton and Kaper (2005), as the EPEC prepare to perpetuate the colonization process in the infected host.

Identification of the EPEC LacNAC-specific adhesin

To identify the LacNAC-specific adhesin on the surface of EPEC, E2348/69 and UMD916 cells were exposed to LacNAC or Pk–Au nanoparticles at 4°C (Fig. 4). In these experiments, LacNAC–Au nanoparticles were only observed in association with BFP (Fig. 4a). No gold nanoparticles were observed in association with the bacterial cell surface (Fig. 4c). Additionally, LacNAC–Au nanoparticles were observed in association with BFP purified from the UMD916 EPEC strain (Fig. 4b). Whereas the Pk–Au nanoparticle preparation neutralized Shiga toxin 1 in the Vero-cytotoxicity assay (data not shown), thereby confirming the presence and functional integrity of the Pk glycan sequences in the preparation (Marcato et al., 2001), no Pk–Au nanoparticles were observed on either the BFP or the cell surfaces of E2348/69 (Fig. 4d) and UMD916 (Fig. 4f). The identity of BFP in these micrographs was confirmed by immunogold labelling using BFP-specific rabbit antiserum (Fig. 4e). Further, BFP, LacNAC–Au and Pk–Au nanoparticles were not detected on BFP-negative JPN15 cells (data not shown).
Collectively, these data serve as evidence that the BFP filament is responsible for the LacNAc-specific binding phenotype of *E. coli* E2348/69. Therefore, the major BFP structural subunit BfpA itself, or an accessory protein co-assembled into the BFP filament with BfpA, likely represents the LacNAc-specific adhesin. In this regard, Winther-Larsen et al. (2005) have demonstrated that pilin-like proteins expressed by *Neisseria gonnorhoeae* impart the epithelial cell adhesiveness properties of the type IV pili of this bacterium. While pilin-like proteins have been identified in the BFP operon of EPEC, these have not yet been shown to be associated with the BFP filament itself (Ramer et al., 2002), but they are required for BFP assembly (Anantha et al., 2000). Failure, so far, to detect any of these components in BFP may be due to their minor incorporation into the pilus filament as well as their similarity in molecular size to BfpA. It is possible that the bundlin-like proteins represent adhesins responsible for the LacNAc-specific gold labelling observed in Fig. 4.

In sum, the data presented herein are consistent with a model in which EPEC employ their BFP to initially attach themselves to LacNAc-like glycan receptors on host-cell surfaces. Although the intimate stages of the EPEC colonization process can occur in the absence of BFP in vitro (Hicks et al., 1998), a BfpF-mediated retraction system which can pull the BFP-tethered bacteria closer to the host-cell plasma membrane may be needed to expedite the intimate adherence phase of the EPEC colonization process in vivo. If so, innovative intervention strategies devised to either competitively inhibit BFP-mediated binding to host-cell glycan receptors or antagonize the BfpF-mediated retraction mechanism could be of therapeutic benefit in patients suffering from EPEC-mediated diarrhoea.

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