Human intestinal tissue tropism in *Escherichia coli* O157:H7 – initial colonization of terminal ileum and Peyer's patches and minimal colonic adhesion *ex vivo*

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Enterohaemorrhagic *Escherichia coli* (EHEC) are an important cause of diarrhoeal and renal disease in man. Studies of a single prototypic O157:H7 strain have shown tropism for follicle-associated epithelium (FAE) of distal ileal Peyer's patches without colonization of either small or large intestine. This study determined tropism in a range of Shiga toxin (Stx)-negative EHEC strains and looked for factors that might induce colonic colonization using human *in vitro* intestinal organ culture (IVOC). An FAE-restricted colonization was confirmed in two strains; four strains additionally colonized ileal villous surfaces, and adhesion to proximal small intestinal FAE was observed. All strains showed minimal adhesion to non-FAE regions of proximal small intestinal and to the transverse colon. Extensive large-bowel IVOC studies using three O157:H7 strains, an O26:H11 and an O103:H2 strain, and tissue from caecum to rectum found colonization and attaching/effacing lesion formation in only 4 of 113 (3.5%) IVOCs. Colonic adhesion was not enhanced by altering the IVOC technique or environment. Co-incubation of O157:H7-infected ileal FAE with colonic samples enhanced colonic colonization, producing a novel, non-intimate adhesive phenotype. Thus, in the initial stages of colonization Stx-negative EHEC preferentially infect FAE and villi of the terminal ileal region *ex vivo*; colonic colonization is infrequently observed as an initial event but may represent a subsequent stage of infection.

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INTRODUCTION

Enterohaemorrhagic *Escherichia coli* (EHEC) cause acute gastroenteritis, bloody diarrhoea and haemorrhagic colitis and are responsible for serious disease outbreaks associated with contaminated food; 10% of cases may develop severe complications including haemolytic uraemic syndrome with a 5% case fatality (Nataro & Kaper, 1998; Tarr, 1995). Systemic diseases associated with EHEC infections are linked to the expression of the bacteriophage-associated Shiga toxin (Stx) (Karmali *et al.*, 1983, 1985); however, an O157:H7 strain depleted of the bacteriophage-encoded Stx is as pathogenic, in terms of diarrhoeal disease, as the parent EHEC strain in animal models (Li *et al.*, 1993).

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Abbreviations: A/E, attaching/effacing; D4, fourth part of duodenum; EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; FAE, follicle-associated epithelium; IVOC, *in vitro* organ culture; LEE, locus of enterocyte effacement; Lpf, long polar fimbriae; PP, Peyer's patches; REPEC, rabbit enteropathogenic *E. coli*; SEM, scanning electron microscopy; Stx, Shiga toxin.

The most prominent EHEC serotype is O157:H7 (Slutsker *et al.*, 1997). Although attaching/effacing (A/E) lesion formation has been shown for EHEC in animal models (Dean-Nystrom *et al.*, 1997; Tzipori *et al.*, 1986) and on cell culture (Knutton *et al.*, 1989), *in vivo* examples in man have not been demonstrated. We demonstrated *in vitro* A/E lesion formation on human intestine in organ culture, and found it was restricted to the follicle-associated epithelium (FAE) from the Peyer's patch (PP) region of the distal ileum (Phillips *et al.*, 2000). This tropism has also been demonstrated for an O103:H2 EHEC strain (Fitzhenry *et al.*, 2003), for the initial stages of infection in rabbit enteropathogenic *E. coli* (REPEC) strains (Cantey & Inman, 1981; Heczko *et al.*, 2000), and for the mouse pathogen *Citrobacter rodentium* (Wiles *et al.*, 2004). Enteropathogenic *E. coli* (EPEC) strains show a variable phenotype, with the prototype strain E2348/69 exhibiting adhesion to proximal and distal small intestine, as well as to FAE (Phillips *et al.*, 2000), whereas O55 strains show an FAE-restricted adhesion (Fitzhenry *et al.*, 2002b). One bacterial factor that influences tropism is the outer-membrane protein intimin (Fitzhenry *et al.*, 2002a; Phillips & Frankel, 2000; Tzipori *et al.*, 1995),

encoded by the *eae* gene (Jerse *et al.*, 1990) and common to A/E organisms (Frankel *et al.*, 1998), including EPEC, EHEC and *C. rodentium*.

Distinct intimin types (α , β , δ , γ , ε , etc.) have been identified (Adu-Bobie *et al.*, 1998; Jenkins *et al.*, 2003; Oswald *et al.*, 2000) and results suggesting that intimin type plays a role in the pattern of colonization include intimin exchange studies in piglets (Tzipori *et al.*, 1995) and our human *ex vivo* investigations. The latter showed that while EPEC expressing intimin α colonized PP as well as proximal and distal small intestine (Phillips *et al.*, 2000), a restricted tropism towards PP followed expression of intimin γ from EHEC in the EPEC background (Phillips & Frankel, 2000). These results were based on one prototype strain, 85/170, and, although we have shown that an EHEC intimin ε -expressing O103:H2 strain also shows PP-restricted tropism (Fitzhenry *et al.*, 2003), it is unclear if other intimin γ -expressing O157:H7 strains are similarly restricted. In particular, although O157:H7 is associated with colonic pathology (Griffin *et al.*, 1990; Kelly *et al.*, 1987) and is considered a colonic pathogen (Nataro & Kaper, 1998), strain 85/170 does not show adhesion to large bowel (Phillips *et al.*, 2000). We have shown colonic colonization by both enteroaggregative *E. coli* (EAEC) (Hicks *et al.*, 1996) and EPEC (Phillips *et al.*, 2000), indicating that the lack of colonic adhesion is not a general result of the organ culture conditions or host related. The aim of this study was to determine if FAE-restricted tropism was typical for EHEC strains and to see if modulation of the *in vitro* organ culture (IVOC) system could change this phenotype.

METHODS

Bacterial strains. The bacterial strains used in this study are shown in Table 1. We included an O26:H11 strain for comparison and used O103:H2 strain PMK5 (Fitzhenry *et al.*, 2003) for further colonic studies. In consideration of health and safety (Nataro &

Kaper, 1998), only Stx-negative strains were used. EAEC strain O42, which colonizes colonic samples in IVOC (Hicks *et al.*, 1996), was used as a positive control.

IVOC. Human tissue was obtained, with fully informed parental consent and local ethical committee approval, during routine investigation of potential intestinal disorders. Mucosal biopsies of the fourth part of the duodenum (D4), terminal ileum, terminal ileal PP region and areas of the colon from caecum to rectum, were taken during video-endoscopy (Fujinon EG/EC-41 paediatric endoscope) using a grasp biopsy forceps. All biopsies were from areas without obvious pathology, and histology was subsequently reported to be normal. IVOC was performed as described previously (Hicks *et al.*, 1998). Briefly, 25 μ l (2.5×10^7 c.f.u.) of an overnight bacterial culture was inoculated onto the biopsy samples. IVOC medium was changed every 2 h and the assay terminated at 8 h. Each bacterial strain was examined on at least three occasions using tissue from different children. An uninoculated specimen was included with each experiment to exclude *in vivo* bacterial colonization. After incubation with bacteria or appropriate control solutions, IVOC specimens were washed thoroughly three times to remove non-adherent bacteria and prepared for scanning electron microscopy (SEM). Samples were fixed with glutaraldehyde and post-fixed in osmium tetroxide. Specimens were critical-point dried using liquid carbon dioxide in an Emitech K850 apparatus, sputter coated with gold-palladium using a Polaron E5100 sputter coater, and viewed in a JEOL 5300 SEM at an accelerating voltage of 25 kV.

Technical factors and colonic adhesion. Routine IVOC incorporates a medium change every 2 h (Hicks *et al.*, 1998). This could remove secreted bacterial factors that may influence colonization of target host epithelium via quorum-sensing-mediated regulation of virulence mechanisms, such as locus of enterocyte effacement (LEE) gene expression and flagella production (Sperandio *et al.*, 1999). The impact of reducing the frequency of medium change to one change at 4 h and to no change at all was tested on adhesion of strains Sakai 813 and EAEC O42 to transverse colon.

A modified version of the IVOC system (Cleary *et al.*, 2004) used DMEM-activated bacterial cultures to inoculate explants immersed in organ culture medium for 1.5 h on a rotary mixer at 37 °C. Using this protocol, the authors demonstrated adhesion of the Δeae mutant of strain E2348/69 (CVD206) to duodenal explants whereas CVD206 is non-adherent in routine IVOC (Hicks *et al.*, 1998). This immersion

Table 1. *E. coli* strains used in the study

Strain	Serotype	Reference/source
85/170	O157:H7	Tzipori <i>et al.</i> (1987)
O42	O44:H18	Nataro <i>et al.</i> (1995)
AGT300	O157:H7	Torres <i>et al.</i> (2002)
TT12B	O157:H7	Feng <i>et al.</i> (2001)
TUV 93-0	O157:H7	Derived from strain EDL933 and supplied by A. Donohue-Rolfe, Division of Infectious Diseases, Tufts University School of Veterinary Medicine, Massachusetts, USA
Sakai 813	O157:H7	Supplied by S. Chihiro, University of Tokyo, Tokyo, Japan; derived from Sakai outbreak strain (Hayashi <i>et al.</i> , 2001)
12900	O157:H7	Supplied by H. Smith, Division of Enteric Pathogens, Health Protection Agency, Colindale, London, UK
3801	O26:H11	Supplied by J. Kaper, Center for Vaccine Development, University of Maryland, Baltimore, USA
PMK5	O103:H2	Fitzhenry <i>et al.</i> (2003); Mariani-Kurkdjian <i>et al.</i> (2001)

system was tested to see if it influenced colonic adhesion. Explants from the transverse colon were fully immersed in organ culture medium at 37 °C on a rotator with Sakai 813 or O42 either for 1.5 h followed by 6.5 h of the standard IVOC system, or for 8 h. Samples were then processed for SEM as above.

Environmental factors and colonic adhesion. Various compounds were either added to (40 mM sodium bicarbonate, 30 mM sodium acetate and 20 mg taurocholic acid ml⁻¹ as a representative bile acid), or removed from (D-mannose and newborn calf serum), the organ culture medium to determine if they influenced the adhesion of O157:H7 to colonic mucosa.

HEp-2:HEp-2 and HEp-2:IVOC relay experiments. HEp-2 cell monolayers were grown on 13 mm glass coverslips in 24-well tissue culture plates. Cells were seeded at 5 × 10⁵ cells (ml culture medium)⁻¹ and incubated at 37 °C in 5% CO₂ for at least 24 h to achieve 70–80% confluency. For infection assays, the wells were inoculated with 10 µl of an overnight culture of bacteria (1 × 10⁷ c.f.u.) grown in BHI broth and incubated for 3 h (strain E2348/69) or 6 h (strain TUV 93-0) at 37 °C in 5% CO₂. After incubation, the HEp-2 cell monolayers were washed with sterile PBS to remove non-adherent bacteria and lysed in 1 ml of 0.1% Triton X-100. The lysed contents were transferred to a 1.5 ml Eppendorf tube and washed twice with sterile PBS to remove traces of Triton X-100. The cell debris and bacterial pellet were resuspended in 100 µl LB [(1–5) × 10⁸ c.f.u. ml⁻¹] and 10 µl [(1–5) × 10⁶ c.f.u.] was added to fresh uninfected HEp-2 cell monolayers for the relay assay. The relay assay continued for 1 h (strain E2348/69) or 3 h (strain TUV 93-0) when the cells were washed with sterile PBS to remove non-adherent bacteria and processed for fluorescent actin staining (FAS) (Knutton *et al.*, 1989). The appearances at these times were compared to those at the same incubation times in initial assays. In addition, 25 µl of the inoculum recovered from the initial assay was added onto intestinal explants for the HEp-2:IVOC relay assay, which was performed for 8 h (Hicks *et al.*, 1998).

Distal ileal IVOC:colonic IVOC relay experiments. In order to establish the procedure, initial experiments were performed with duodenal explants in duplicate. Paired duodenal samples were taken from a single patient and underwent 8 h IVOC, one being inoculated with strain E2348/69. After 8 h, the infected sample was placed next to the uninfected sample and IVOC was continued for a further 12 h. An uninfected D4 sample was incubated for 20 h as a negative control.

For O157:H7 strains, distal ileal (PP containing) and transverse colon biopsy samples were obtained from a single patient. The colonic sample was placed in organ culture without bacterial inoculation, while the ileal sample was incubated with bacterial strain 12900 (*n*=6) or TUV 93-0 (*n*=5) for 8 h routine IVOC. At 8 h the sample was washed in PBS, placed in close proximity to the colonic sample in fresh organ culture medium and IVOC was continued for a further 12 h. The medium was changed every 4 h. Each of the 11 experiments was performed with different patients providing paired ileal and colonic biopsy samples. Control IVOC included uninfected colonic samples incubated for 20 h and colonic samples incubated with O157:H7 strains 12900 and TUV 93-0 for 20 h.

RESULTS

We previously described a single O157:H7 strain 85/170 (O157:H7), which adhered to FAE and not to colon (Phillips *et al.*, 2000). For this study, five further O157:H7 strains and an O26:H11 Stx-negative strain (Table 1) were tested for tropism to duodenum, distal ileum, PP and transverse colon using IVOC.

Table 2 shows the results. Prototype strain 85/170 again showed FAE-restricted tropism, as did AGT300 (Fig. 1a). Other strains showed FAE adherence (Table 2); however, four strains (12900, Sakai 813, TUV 93-0 and 3801) also adhered to ileal villi around PP, sometimes in large colonies (Fig. 1b, c), and strains 12900, TT12B, Sakai 813 and TUV 93-0 showed some adhesion to proximal small intestine (Fig. 1d). On two occasions isolated follicles were present in D4 biopsies, to which both TT12B (Fig. 1e) and 85/170 (Table 2) adhered. In contrast to the accepted dogma that EHEC strains adhere to colon, only 1 of 40 transverse colon IVOC showed A/E lesion formation (strain TT12B, Fig. 1f).

In view of this finding, we performed extensive studies along the large bowel for five selected strains, three O157:H7 (intimin γ), one O26:H11 (intimin β) and one O103:H2 (intimin ε). We performed 113 colonic IVOCs to determine if there was selective adhesion to a particular colonic region (Table 3), but only 4 (3.5%) IVOCs from distal colonic sites

Table 2. Tissue tropism of Stx-negative EHEC strains

Values correspond to A/E lesion formation as a proportion of biopsies inoculated; D4, fourth part of the duodenum; FAE, follicular associated epithelium; PP, Peyer’s patch; NA, not available.

O157:H7 strain	D4	FAE (D4)	Terminal ileum	FAE-PP terminal ileum	Transverse colon
O157:H7 strains					
85/170 (prototype)	0/8	1/1	0/9	11/11	0/20
AGT300	0/11	NA	0/6	2/4	0/5
TT12B	2/10	1/1	0/6	7/8	1/5
TUV 93-0	1/3	NA	8/12	3/5	0/4
Sakai 813	1/4	NA	10/16	5/9	0/3
12900	2/6	NA	6/8	2/3	0/3
O26:H11 strain					
3801	0/4	NA	1/4	1/3	0/3

Fig. 1. SEM of representative IVOCs. (a) AGT300 A/E lesion on distal ileal FAE (bar, 5 μ m), (b) TUV 93-0 on ileal villus, low power (bar, 10 μ m), (c) Sakai 813 A/E lesion on the villous surface of terminal ileum (bar, 5 μ m), (d) Sakai 813 A/E lesion on D4 (bar, 5 μ m), (e) TT12B A/E lesion on FAE of D4 follicle (bar, 1 μ m), (f) TT12B A/E lesion on transverse colon (bar, 1 μ m).

(transverse colon to rectum) showed A/E lesion formation in a similar appearance to that shown in Fig. 1(f); there was no adhesion in proximal colonic samples (caecum and ascending colon). EAEC strain O42 demonstrated colonic adhesion on all occasions. This suggested that either IVOC conditions prevented Stx-negative EHEC colonic adhesion, or that initial adhesion to FAE is followed at an undetermined time by colonization of other intestinal regions. This prompted an investigation of environmental factors.

The alterations to the IVOC protocol (newborn calf serum removal, D-mannose removal, limiting changes in medium, sample immersion, inclusion of bile acids or acetate, and increased bicarbonate), all failed to produce O157:H7 colonic adhesion, although EAEC strain O42 adhered to the colon on all occasions (data not shown). Most O157:H7 strains do not produce type 1 fimbriae and so no effect would be expected following mannose removal from the medium; strain 85/170 has been shown to produce such fimbriae (Fitzhenry *et al.*, 2006) but its tropism did not

Table 3. Colonic adhesion of selected Stx-negative EHEC strains

Values correspond to A/E lesion formation as a proportion of biopsies inoculated; positive results are highlighted in bold.

Serotype and strain	Caecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum
O157:H7, 85/170	0/4	0/3	0/6	0/3	1/3	0/3
O157:H7, AGT300	0/3	0/4	0/5	0/5	0/4	0/3
O157:H7, TT12B	0/3	0/4	1/5	0/5	0/4	0/3
O26:H11, 3801	0/5	0/3	0/3	0/3	0/3	1/3
O103:H2, PMK5	0/3	0/4	0/5	1/5	0/3	0/3
Total	0/18	0/18	1/24	1/21	1/17	1/15

change in mannose-free medium. Due to health and safety considerations we were not able to use Stx-positive strains and it is possible that Stx itself may influence tropism through the modulation of receptor expression (Robinson *et al.*, 2006).

HEp-2 cell:HEp-2 cell relay infection assay of EPEC and O157:H7

HEp-2 cells incubated for 1 h with strain E2348/69 showed scanty adherence (Fig. 2a), whereas the 1 h relay infection assay following Triton extraction from a standard 3 h HEp-2

cell assay produced large bacterial colonies on the majority of the cells (Fig. 2b). Strain TUV 93-0 adhered extremely poorly at both 2 h and 3 h infection of HEp-2 cells (Fig. 2c), whereas both 2 and 3 h relay assays showed large colonies (Fig. 2d). Hence, adherence in the HEp-2 relay infection assay occurred more rapidly and was enhanced in comparison to the standard assay for strains E2348/69 and TUV 93-0.

HEp-2:IVOC relay assay

Cell-membrane-recovered strain TUV 93-0 taken from 6 h HEp-2 cell incubations was used to infect explants from the

Fig. 2. (a) Routine E2348/69 HEp-2 cell assay at 1 h, with a small colony of adhering bacteria (arrow). (b) HEp2:HEp2 E2348/69 relay assay at 1 h. Note increased presence of bacteria compared to (a) (arrows). (c) Routine 12900 HEp-2 cell assay at 3 h with few adhering bacteria (arrows), (d) HEp2:HEp2 12900 relay assay at 3 h with many adhering bacteria.

terminal ileum and transverse colon under standard IVOC conditions. A/E colonies were identified on ileal samples (on villi and FAE), demonstrating no detrimental effect of Triton extraction, but none were seen on colonic IVOC, indicating that the enhanced adherence on the HEP-2 cell relay assay is not translated to the colonic situation.

IVOC:IVOC relay infection

These experiments involved generating O157:H7-mediated A/E lesions on FAE, which were then incubated with

transverse colonic explants for a further 12 h, to allow time for the development of colonic adhesion via bacterial spread from FAE. The ability of bacteria to spread from explant to explant was confirmed in D4:D4 relay infections carried out using strain E2348/69 (Fig. 3a). No adhesion was noted on uninfected D4 controls after 20 h incubation (data not shown).

After 20 h incubation, O157:H7 TUV 93-0- and 12900-infected distal ileal explants showed increased cellular debris on the surface. Extensive bacterial colonization occurred on

Fig. 3. (a) D4:D4 20 h relay IVOC showing E2348/69 adhesion to D4 (bar, 5 µm). (b, c) 20 h distal ileal FAE incubated with (b) TUV 93-0 (bar, 5 µm) and (c) 12900 (bar, 1 µm). (d) 20 h transverse colon IVOC showing non-intimate adhesion of 12900 (bar, 1 µm). (e, f) 20 h FAE:transverse colon relay IVOC incubated with (e) 12900 (bar, 1 µm) and (f) TUV 93-0 (bar, 1 µm) showing foci of colonization.

F AE (Fig. 3b) and bacterial-like imprints were seen on the epithelial surface of 12900-infected FAE among A/E adhering bacteria (Fig. 3c). These probably represent sites of A/E lesion formation where bacteria have become detached.

IVOC incubations of strains TUV 93-0 and 12900 with transverse colon explants for a period of 20 h generated adhesion on 0/3 and 1/3 occasions respectively without A/E lesion formation (Fig. 3d), indicating that prolonged incubation per se did not promote O157:H7 colonic adhesion. The FAE:transverse colon relay assay with TUV 93-0 and 12900 produced small foci of adhering bacteria on 2/5 and 2/6 transverse colon explants respectively, again without A/E lesion formation (Fig. 3e, f). No bacterial adhesion occurred on uninfected IVOC incubated for 20 h (data not shown).

DISCUSSION

Extensive IVOC studies were performed to investigate Stx-negative EHEC tropism. The restricted tropism of some strains to distal ileal FAE was confirmed, and it was found that other strains additionally adhered to ileal villi around the PP regions with a limited adhesion to proximal small intestinal villi. These latter results were similar to the tropism shown by a single intimin β O26:H11 strain and by an intimin ϵ -expressing O103:H2 (Fitzhenry *et al.*, 2003) strain. More strains should be tested before this can be assumed to be representative for these serotypes. However, we have now shown that Stx-negative EHEC adhesion is not limited to FAE, but includes normal absorptive epithelium, albeit in a region where lymphoid follicles are commonly found.

We were unable to demonstrate reproducible colonic adhesion of any strain using direct inoculation of IVOC samples, although four strains did adhere on single occasions. The positive control strain, an EAEC, adhered on each occasion, and EPEC strain E2348/69 shows colonic adhesion (Hicks *et al.*, 1998), indicating that this is not an absolute problem of IVOC. It is possible that technical reasons precluded colonic adhesion but simple environmental factors were investigated and no positive results were seen.

Does EHEC colonize the colon in man? Although EPEC colonic colonization has been reported *in vivo* (Lewis *et al.*, 1987; Rothbaum *et al.*, 1982), there are no such reports from studies of *in vivo* EHEC infection in man, despite colonic pathology being clearly described (Griffin *et al.*, 1990). Stx, as toxin levels can reach high levels in the intestinal lumen (Gamage *et al.*, 2003), may induce marked mucosal damage via endothelial (Jacewicz *et al.*, 1999) or epithelial (Schuller *et al.*, 2004) interaction, without bacteria being present at that site. One study specifically looked for adhering organisms in acute O157:H7 infection and concluded, as no bacteria were seen, that the pathology resulted from toxin-mediated ischaemia (Kelly *et al.*, 1987). Other reports

have described a mixture of acute infective and ischaemic changes (Griffin *et al.*, 1990), leaving the debate open. If human tissue samples are taken at late stages in the illness or post-mortem, then bacterial adhesion may have diminished and/or be difficult to identify.

It seems likely that the PP-rich distal ileum represents the initial site of EHEC adhesion, and colonization spreads from there to other regions of the gut. The extent of the spread and the regions that are targeted are unknown. This pattern of colonization is shown by other bacteria, including REPEC (Cantey & Inman, 1981; Heczko *et al.*, 2000) and *C. rodentium* (Wiles *et al.*, 2004), but is not a universal phenotype as EPEC strain E2348/69 appears able to colonize the small intestine directly (Hicks *et al.*, 1998; Knutton *et al.*, 1987). The infective dose for EHEC is low (10^2 c.f.u. ml⁻¹) whereas that for EPEC is much higher (Nataro & Kaper, 1998). It is possible that FAE colonization affords a 'toehold' without inducing a strong host response, allowing multiplication and spread. This could be termed a 'stealth' approach. Down-regulation of antibacterial peptides of the innate immune response has been shown in the initial stages of *Shigella* infection in man (Islam *et al.*, 2001), where the infective dose is also low (DuPont *et al.*, 1989), and such interaction may be a factor that allows colonization to spread from follicular sites of infection. In comparison, direct infection of mucosal surfaces, as a 'frontal assault' approach may require higher numbers of organisms to overcome the innate responses.

In mice, *C. rodentium* spreads from the caecal follicular region to the large intestine within 4 days of infection (Wiles *et al.*, 2004). *E. coli* strain RDEC-1 takes a similar time in the rabbit (Cantey & Inman, 1981). This time is outside the possibilities of the IVOC system as tissue viability is limited to 24–48 h. However, a recent study demonstrated rapid colonization when *C. rodentium* from infected animals was used to infect mice within 24 h of excretion. They showed a temporary hyperinfective state that facilitated colonic colonization without transient infection of the caecal patch area (Wiles *et al.*, 2005). Here we tested if colonization of the FAE surface could mediate subsequent colonic colonization and found enhanced colonic adhesion but no A/E lesion formation in the time permitted. A similar enhancement, but with A/E lesion formation, was found for strain E2348/69 when transferring infection from duodenum to duodenum. There may be phase variation within the EHEC population so the initial colonization of FAE may act as a selection process for adherent bacteria, increasing the chances of colonic colonization. Alternatively, the process of FAE colonization may induce activation of genes which mediate colonization of other gut regions.

Mediators of FAE colonization have been identified in some species, i.e. long polar fimbriae (Lpf) in *Salmonella* (Baumler *et al.*, 1996) and AF/R1 fimbriae in REPEC (Von Moll & Cantey, 1997). Homologous genes to Lpf have been identified in EHEC strains, and the operons show a high degree of similarity (Fitzhenry *et al.*, 2006). However,

deletion of one or both of the Lpf operons in O157:H7 did not prevent FAE adhesion but resulted in additional colonization of the small intestine (Fitzhenry *et al.*, 2006), questioning their role in targeting PP in O157:H7.

It appears possible that O157:H7 can colonize FAE beyond the distal ileum, as we demonstrated adhesion to FAE from the duodenum. Mucosal lymphoid follicles are found along the entire length of the small and large intestine (Cornes, 1965), giving a widespread potential for sites of adhesion. Whether host factors come into play *in vivo* to limit this remains to be determined. Lymphoid follicles in the rectal region have been implicated in O157:H7 carriage in cattle (Gally *et al.*, 2003), indicating that FAE adhesion may be important in continuing colonization as well as in the initial stages of infection.

In summary, we have shown an initial *ex vivo* tropism of the distal ileal region and FAE for Stx-negative EHEC strains, and that adherent O157:H7 from distal ileal FAE can colonize the colon in a novel, non-A/E manner. Direct colonization of the colon is not seen *ex vivo*, despite the finding of extensive colonic pathology *in vivo*, and the relative contributions of direct bacterial infection and Stx to colonic pathology in EHEC infections remain to be established in man.

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