Interactions of Yersinia enterocolitica with polarized human intestinal Caco-2 cells

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Abstract The in vitro interactions of Yersinia enterocolitica, Salmonella typhimurium and Escherichia coli with polarized human colonic carcinoma (Caco-2) cells are described. Invasion of a confluent Caco-2 cell monolayer by Yersinia and Salmonella took place within 4 h after contact, which was in marked contrast to E. coli which did not invade Caco-2 cells. Cytoplasmic extrusions developed on the apical membrane and indicated the site of entrance of bacteria into the Caco-2 cells. Intracellular Yersinia and Salmonella were surrounded by a vacuolar membrane. Single as well as multiple bacteria were enclosed within a single vacuole. At 6 h after contact some of the intracellular yersinia were found free in the cytoplasm. Furthermore, morphological signs of degeneration of Caco-2 cells such as vacuolization and autophagy were observed. Caco-2 cells infected with Salmonella also showed degenerative changes but the salmonellae resided within membrane-bound vacuoles in contrast to Yersinia. These observations are in contrast to those described for the invasion of other cell lines (not derived from intestinal epithelium) by Yersinia and may reflect more closely the interactions between Yersinia and the intestinal epithelium during gastrointestinal infection.

Key words Yersinia · Salmonella · Escherichia · Caco-2 · Invasion, ultrastructural

Introduction

An important determinant of virulence of pathogenic bacteria is the invasion of normally nonphagocytic host cells.

Original Investigation

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Yersinia enterocolitica is the invasion of normally nonphagocytic host cells. Binding to host cells can result in extracellular adhesion followed by internalization of the pathogens. Many enteropathogenic organisms such as Salmonella, Shigella and Yersinia species have the ability to adhere to and penetrate epithelial cell barriers (Moulder 1985; Finlay and Falkow 1988, 1989; Falkow et al. 1992; Conconnier et al. 1994). Oral infection with Y. enterocolitica is presumably followed by penetration of the intestinal epithelium permitting the bacteria access to the underlying lamina propria, where they may be internalized by macrophages and/or leukocytes. After invasion multiplication and translocation to other tissues where they inflict inflammatory responses may follow. Therefore, clinical symptoms may not be limited to gastroenteritis or terminal ileitis but may also include extraintestinal symptoms such as mesenteric lymphadenitis. Additionally, a variety of postinfectious sequelae such as arthritis and erythema nodosum may develop.

Initial interactions between host and pathogens have been studied in animal models and in vitro. In the intestine of orally infected mice invasion of Y. enterocolitica O:8 predominantly occurs in ileal Peyer’s patches, although some invasion into the remaining mucosa can be observed (Hanski et al. 1989). Grützkau et al. (1990) demonstrated in mice that uptake of Y. enterocolitica takes place by microfold (M) cells of the follicle-associated epithelium of the Peyer’s patches. However, oral infection with Y. enterocolitica O:8 is usually lethal for mice and conclusions made from observations made in mice may not apply to human yersiniosis. In vitro studies with Yersinia and/or Salmonella have used CHO, HEP-2, MDCK, HeLa, A431 and Henle cells and human fibroblasts (Brunius 1980; Schieman and Crane 1987; Finlay and Falkow 1988, 1989; Rosenshine et al. 1994). These studies show similar results for both organisms and, therefore, Yersinia is supposed to enter its host by invading the epithelium of the intestine through a mechanism similar to that used by Salmonella. However, although some of the studied cell lines are polarized, none of them develop a well-defined brush border and, thus, may not resemble the intestinal epithelium.

Three human adenocarcinoma cell lines (Caco-2, HT-29 and T84) have been shown to form polarized mono-
layers and well-defined brush borders mimicking human intestinal epithelium (Pinto et al. 1983; Dharmasathaphorn et al. 1984; Huet et al. 1987). Previous studies of Caco-2 cells with Salmonella sp. (Finlay and Falkow 1990) and Campylobacter jejuni (Russel and Blake 1994) have shown that bacteria interacted with apical microvilli and cause disruptions in the brush border followed by internalization. Recently Coconnier et al. (1994) have shown that Y. pseudotuberculosis is able to enter Caco-2 cells by an α5β1 integrin-dependent mechanism. However, the behavior and morphology of Yersinia inside Caco-2 cells remains unclear. Here we present a comparative study of the interactions of Y. enterocolitica and S. typhimurium with Caco-2 cells.

Materials and methods

Caco-2 cells

Human colon carcinoma-derived Caco-2 cells (Pinto et al. 1983) were grown to confluence on surfactant-free nitrocellulose membrane filters (12-mm diameter, 0.4-μm pore size, Costar, Cambridge) placed in 24-well tissue culture plates (Costar, Cambridge) in Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis) without antibiotics, supplemented with 20% heat-inactivated fetal calf-serum, and 1% non-essential amino acids. Cells were incubated at 37 °C in 5% CO2 in a critical point drying apparatus. Recently Caco-2 cells were reported to be fully differentiated which was assessed by positive immunofluorescence labeling for sucrose-isomaltase, a marker for the functional differentiation of Caco-2 cells (Semenza 1986). Cells were ready for experiments when they were in culture for 12 or more days.

The cells were used only when they satisfied two conditions. (1) They had to form a tight monolayer. The tightness of the monolayer was tested by inducing a higher level of medium in the apical chamber (Klumpperman et al. 1991). The cells were only used when this difference was maintained for at least 4 h. (2) Caco-2 cells had to be fully differentiated which was assessed by positive immunofluorescence labeling for sucrose-isomaltase, a marker for the functional differentiation of Caco-2 cells (Semenza 1986). Cells were ready for experiments when they were in culture for 12 or more days.

Bacteria

Y. enterocolitica serotype O:8 strain WA (ATCC 9610), serotype O:3 (NCTC 11176) and serotype O:9 (NCTC 11174) all harboring the virulence plasmid were grown overnight at 26 °C in brain heart infusion (BHI) broth followed by 4 h growth at 37 °C. S. typhimurium (ATCC 13311) and Escherichia coli (ATCC 25922) were grown at 37 °C in BHI.

Monolayer penetration assay

After washing with PBS, bacterial suspensions of 2 × 108 CFU/ml in DMEM without supplements were made and 0.5 ml of this suspension was added to the apical side (brush border) of Caco-2 cells. Cells with bacteria were incubated at 37 °C for 4 and 6 h and prepared for electron microscopy. Additionally, 6 h after incubation Giemsa-stained preparations were examined by light microscopy for determination of the percentage of infected cells (100 cells/filter were counted). All experiments for electron microscopy were performed in triplicate.

Scanning electron microscopy

Filters containing bacteria and cells were washed thoroughly with DMEM followed by overnight fixation in 2% glutaraldehyde in 0.1 M phosphate buffer (PB) at 4 °C. Dehydration was performed in an ascending series of alcohols followed by critical point drying with CO2 in a critical point drying apparatus. After mounting and coating with gold, samples were examined in a JEOL 6310 scanning electron microscope.

Transmission electron microscopy

Monolayers were fixed as described above and after washing with PB, cells were post-fixed in 1% OsO4 in PB for 1 h and dehydrated in an ascending series of alcohols and embedded in Epon 812. Ultrathin sections were cut with a Diatome diamond knife on a Reichert-Jung microtome. Sections were stained with 3% uranyl acetate (10 min) and lead citrate (10 min) at ambient temperature. Sections were examined in a JEOL 1010 transmission electron microscope.

Results

Determination of number of infected Caco-2 cells

Light microscopic examination of Caco-2 cells after 6 h of incubation revealed that more than 90% of the cells contained one or more Yersinia bacteria. This was independent on the serotype. In the experiments with Salmonella 87% of the Caco-2 cells were infected. No bacteria were found in Caco-2 cells incubated with E. coli.

Scanning electron microscopy

Yersinia enterocolitica

After 4 h of incubation Y. enterocolitica O:8WA can be seen in close contact with the brush border of the Caco-2 cells, and at the interaction site of some of the bacteria with the brush border the formation of cytoplasmic extrusions (blebs) can be observed (Fig. 1 A). The formation of blebs may indicate entrance of bacteria into Caco-2 cells. Apart from these extrusions the brush border remains largely intact without any indication of cell degeneration.

At 6 h after incubation, extracellular bacteria are mainly located at the region of contact between adjacent cells (Fig. 1 B). Protoplasmic extrusions are rarely observed. Furthermore, the surface of the Caco-2 cell monolayer (brush border) appears to be undamaged, similar to that seen at 4 h.

No difference is observed between serotypes O:3, O:8 and O:9. All three strains show a comparable interaction with the apical surface of the Caco-2 cells.

Salmonella typhimurium

At 4 and 6 h after incubation, numerous bacteria can be seen interacting with the apical surface of the Caco-2 cells. Degenerative changes of the brush border have become apparent and comprise elongation, thinning and broadening of the villi. Cytoplasmic blebs are a prominent feature (Fig. 2 A).
Inoculation of the Caco-2 cells (Fig. 1 C). At 4 h after inoculation, intracellular bacteria can be demonstrated 4 and 6 h after inoculation (Fig. 2 C).

The E. coli strain used in our experiments does not induce any changes in the brush border at either 4 or 6 h after inoculation. Escherichia coli.
Disscussion

No intracellular bacteria are found inside the Caco-2 cells. In addition, the presence of the Caco-2 cell in the experimental system makes for the expression of the presence of the intracellular bacteria. In contrast, the bacteria are not found inside the Caco-2 cells. The expression of the presence of the intracellular bacteria is not reported in this study.
single as well as multiple bacteria inside one vacuole suggests coalescence of vacuoles or inclusion of several bacteria by a single vacuole during uptake, which is contradictory to what has been described for Yersinia in non-intestinal cell lines (Brunius 1980; Finlay and Falkow 1988). The differences in the way the vacuolar membrane surrounds Yersinia compared to Salmonella may arise from different internalization strategies as hypothesized by Isberg and Tran Van Nhieu (1994). They suggest that Yersinia uptake occurs through an "exclusive" or "zippering" mechanism, whereas uptake of Salmonella is "inclusive" or "triggering".

Our observation that after 6 h of incubation yersiniae are found intra- and extracellularly without indications of invasion or blebs suggests that at this timepoint further invasion or endocytosis of bacteria has stopped. The majority of the Caco-2 cells already harbor several bacteria per cell and one can imagine that if invasion indeed depends on the microfilament rearrangement of the Caco-2 cells, as described by Buckholm (1984) for Yersinia infection in HEp-2 cells, internalization of additional bacteria may be limited.

No indications have been found that Yersinia might be able to disrupt the tight junctions between adjacent Caco-2 cells within 6 h of contact, suggesting that in vivo bacteria might be able to transmigrate through the cells of the epithelial layer of the intestine rather than between the cells. However, no evidence for transcytosis was found within 6 h after incubation of Yersinia with Caco-2 cells. It remains to be established with the help of non-lethal in vivo experiments whether uptake occurs through an "exclusive" or "zippering" mechanism, whereas uptake of Salmonella is "inclusive" or "triggering".

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