CHANGES IN THE ACTIN CYTOSKELETON AND ACTIN-ASSOCIATED PROTEINS IN HEŁA CELLS AS A RESULT OF *E. coli* O157:H7 INFECTION

T. TOPOUZOV A-HRISTOV A, S. STOITSOV A, T. PAUNOVA-KRASTEVA A, J. DOUMANOV 3 and E. STEPHANOVA 1
1 Sofia University "St. Kliment Ohridski", Department of Cytology, Histology and Embryology, Faculty of Biology, BG – 1164 Sofia, Bulgaria
2 Bulgarian Academy of Sciences, Institute of Microbiology, BG – 1113 Sofia, Bulgaria
3 Sofia University "St. Kliment Ohridski", Department of Biochemistry, Faculty of Biology, BG – 1164 Sofia, Bulgaria

Abstract


*Escherichia coli* strains with the O157 LPS serotype may cause health disorders with variable severity. These bacteria are extracellular pathogens, which have developed a specific mode to manipulate host cell cytoskeleton by means of a Type III secretion system. On the background of the very well analyzed F-actin rearrangements, little is known about the possible interference of *E. coli* O157 adherence with other cytoskeleton-related proteins and events in non-polarized host cells. Here we describe data indicating alterations of the actin-associated proteins ZO-1 and villin during co-incubation of HeLa cells with a strain of *E. coli* O157:H7. Using laser scanning confocal microscopy our data demonstrate unusual cytoskeletal actin rearrangement in HeLa cell line comparing to polarized epithelial cells. Additionally, behavior of actin-associated proteins ZO-1 and villin, as well as lack of F-actin patches, suggested a different manner of bacterial pathogenesis in non-polarized epithelial cells.

**Key words:** EHEC, A/E lesions, villin, ZO-1

**Abbreviations:** CM-PBS – phosphate buffered saline with Ca and Mg; FAS – fluorescent-actin staining; LA – localized adhesion; LAL – localized adhesion like; EHEC – enterohaemorrhagic *E. coli*; FITC – fluorescein isothiocyanate; BSA – bovine serum albumin, NCIPD – National Center of Infectious and Parasitic Diseases; A/E attaching-effacing

**Introduction**

*Escherichia coli* strains with the O157 LPS serotype may cause health disorders with variable severity due to their specific adaptations to interact with host epithelial cells by Type III secretion system. The bacteria of some well studied model EHEC strains inject a translocated intimin receptor (Tir) into the host cells and thus initiate a signaling cascade in polarized epithelial cells, that results in host cell-actin rearrangements and formation of compact patches of F-actin in loci of altered microvilli, known as pedestals (Kaper et al., 2004). These events are considered as attaching-effacing lesions (A/E lesions). Pedestals, via the interaction of Tir with bacterial intimin ensure firm attachment of the bacteria to host membranes, without penetrating into the host cell. Despite of predominant model of A/E lesions as main type of the EHEC bacterial-epithelial cells interaction, recently a variety of adhesion patterns are described (Mora et al., 2009)

On the background of the very well analyzed F-actin rearrangements in polarized epithelial cells, little is known
about the possible interference of the *E. coli* O157 adherence with other cytoskeleton-related structures and events in non-polarized host cells. The aim of our study was to analyze changes of the actin cytoskeleton and actin-associated proteins ZO-1 and villin during co-incubation of HeLa cells with a strain of *E. coli* O157:H7.

**Materials and Methods**

*E. coli* O157:H- A2CK SS, NCIPD, a Stx-negative strain, and *E. coli* O157:H7 CCUG 44875, a strain with blocked capacity to form Stx (Schmidt et al., 1999) and HeLa cells were grown and co-cultivated as previously was described (Topouzova-Hristova et al., 2012). After incubation, the samples were washed three times with CM-PBS, fixed for 1 h in ice-cold fixative containing 2% paraformaldehyde and 2% glutaraldehyde in CM-PBS, washed in the buffered saline and blocked overnight at 4°C in 5% BSA in CM-PBS. The samples were permeabilised with 0.5% Triton X-100 and single- or double- labeled at 37°C by fluorescent probes TRITC phaloidin (Sigma); polyclonal rabbit anti-*E. coli* O157 and mouse monoclonal anti-villin (1:100) or mouse anti-ZO1 (1:100) sera (Antibodies Online) followed by 1 h in anti-mouse IgG-TRITC (1:100) and anti-rabbit IgG-FITC (NCIPD). The observations were made on Nikon TiU confocal laser scanning microscope and the images were acquired and processed using EZC1 software.

**Results**

Using FAS test, our results did not show formation of typical FAS+ A/E lesions during different periods of co-cultivation of HeLa cells with both bacterial strains (Figure 1). The actin filaments in control cells displayed a relatively uniform distribution, forming netlike structure in confluent monolayer cells (Figure 1A, B). The effect of co-cultivation with *E. coli* O157: H- is expressed in lost of the filaments and cells’ rounding, probably caused by disturbances of cell-adhesion contacts. The duration of co-cultivation has no effect on these events (Figure 1C, D). We observed similar alterations after co-cultivation with *E. coli* O157: H7, but with more extensive loss of HeLa cells after incubation with this strain (Figure 1E, F).

HeLa are epithelial cells that form confluent monolayer without polarization (Kazmierczak et al., 2004), but they are widely used as in vitro laboratory model. Due to the unpolarized state of the cells, the villin signal was found throughout the cytoplasm. Incubation with *E. coli* O157:H7 did not affect the signal distribution, while the fluorescence intensity after co-cultivation seems to be low (Figure 2), probably as a result of antigen depletion. ZO-1 displayed different behavior: significant changes in allocation after 3 hours co-cultivation (Figure 3B), which are restored during 6 hours incubation (Figure 3C). This observation allows suggesting the early and reversible influence of epithelial integrity by EcO157:H7 interaction.

**Discussion**

Our previous results showed that both studied bacterial strains displayed significant differences in the adhesion...
pattern to epithelial cells – *E. coli* O157: H7 established mainly large groups, typical of LA, while the *E. coli* O157: H- forming rather LAL (Topouzova-Hristova et al., 2012). Generally, the LA is associated with redistribution of host-cell actin cytoskeleton and formation of compact pedestals (detectable by FAS+ test), but recently (Abu-Ali et al., 2010) ascertained significant differences in attachment patterns of various bacterial strains of the same serotype. Taking into consideration the above fact we focus our study on redistribution of the actin microfilaments in HeLa cells after co-cultivation with *E. coli* O157: H- и *E. coli* O157:H7. Although two strains had different impact on the F-actin allocation, both of them did not form detectable pedestals at the site of bacterial-epithelial cells interaction. Surprisingly, the response of two actin-binding proteins, ZO-1 and villin, after bacterial infection was different. While ZO-1 displayed reversible redistribution, villin did not change location, but its fluorescence intensity was low. In vivo studies the effect of EHEC infection on intestinal epithelial barrier function, detected reduction of trans-epithelial resistance, redistribution of transmembrane tight junction proteins occludin and claudin, but no alterations in position of ZO-1 5-10 days after infection (Roxas et al., 2010). Our results reveal an earlier effect of bacterial infection on epithelial actin-binding proteins without formation of typical A/E lesions.

Fig. 2. Villin distribution was not affected by co-cultivation of HeLa cells with *EcO157:H7*. A – control cells, B – after 3 hours co-cultivation. Bright dots are bacteria. Bar – 5 μm

Fig. 3. Rearrangement of ZO-1 after incubation with HeLa cells with *EcO157:H7*. A – control cells, B – after 3 hours co-cultivation, C – after 6 hours co-cultivation. Bright dots are bacteria. Bar – 5 μm
In conclusion, our data demonstrate that both *E. coli* O157: H- and *E. coli* O157:H7 affect actin microfilament distribution in non-polarized HeLa cells. In addition, *E. coli* O157:H7 caused reversible rearrangement of ZO-1, but not villin.

**Acknowledgements**

This study was supported by the National Research Fund, Republic of Bulgaria, Grant DO02-301/08.

**References**


