IN VITRO MODULATION OF ADHERENCE AND INVASION ABILITY OF ENTEROINVASIVE *ESCHERICHIA COLI* BY DIFFERENT VIRUSES

[a]Coralia Bleotu, [b] Mariana Carmen Chifiriuc, [c]Olguta Dracea, [c]Carmen Iordache, [c]Cristina Delcaru, [b]Veronica Lazar

aStefan S. Nicolau Institute of Virology, 285 Mihai Bravu, 030304, Bucharest, Romania
bUniversity of Bucharest, Faculty of Biology, 1-3 Portocalelor, Bucharest Romania
[C]National Institute for Research and Development in Microbiology and Immunology Cantacuzino

carmen_balotescu@yahoo.com, phone: 0040766728315

ABSTRACT: Studies on the relationship between viral and bacterial infections showed that in the context of viral infections the immunity of host organism is reduced temporarily, increasing the incidence of bacterial infections, like faster bacterial colonization of immunocompromised bodies, by increasing the level of expression of epithelial cell receptor for bacterial adesins. Modulation of viruses infected host cells signaling may also induce changes in the cytoscheleton, which may result in the increase / decrease invasion capacity of bacterial cells. Enteroinvasive *Escherichia coli* causes intestinal infections exploiting host cell function, which include the invasion into non-phagocytic eukaryotic cells such as epithelial and endothelial cells and associated host cell actin cytoskeletal rearrangements. One of our aims was to investigate the *in vitro* adherence and invasion capacity induced by an diarhhoeal enteroinvasive *Escherichia coli* strain in the presence of different viral strains*: Vaccinia* virus (Poxviridae), measles virus (Paramyxoviridae II); e*chovirus 32* (Picornaviridae) and *Herpes simplex virus* 1 (Herpesviridae). The viral adsorption on HeLa cells was done for six hours at 37^0C, followed by the evaluation of bacterial adherence and invasion to HeLa cells performed by the adapted Cravioto's method and gentamycin protection assay. Viral preinfection of the cellular substrate induced an increased bacterial adherence index, as well as changes in the adherence pattern from diffuse tu aggregative. In exchange, the general effect of viral infection on invasive bacterial capacity was the decrease of invasive ability. In conclusion, viral pre-infection of the susceptible substrate influenced the adherence and invasion ability of enteroinvasive *E. coli* bacterial strain, as observed by the intensification of the adherence capacity, explaining the increased incidence of bacterial infections after viral infections, as well as faster bacterial colonization of immunocompromised hosts and by reducing the invasive capacity of epithelial cell by bacterial strains, pleading for increased incidence of extracellular pathogenic organisms in post-viral infections.

Key words: Enteroinvasive *Escherichia coli*, viral infection, adherence and invasion

INTRODUCTION

Hosts and microbes associate in a variety of relations along a continuum ranging from symbiotic to pathogenic (Vestad, 2009). The bacterial infections are a major source of human disease, many bacterial pathogens having the ability to adhere to host tissues, to evade innate and acquired immune defenses, to secrete toxins, to overcome tissue barriers, and to invade host cells (Hauck, 2009). Each single pathogen species has developed its own variation of these specific virulence factors allowing to interfere with defined host processes or to recognize particular structures.

The large assortment of fimbrial and afimbrial adhesins of bacteria bind specifically to a wide variety of distinct molecules (carbohydrates, glycolipids, proteins) on their host cells (Cundell and Tuomanen, 1995). Often, these adhesive interactions determine if a pathogen is able to cause disease in a particular organism (Croxen and Finlay, 2009). In many instances, virulence factor expression is tightly regulated, and bacterial pathogens respond with specific gene regulation events during their interaction with different host compartments.

Pathogenic *Escherichia coli* causes a large spectrum of intestunal and extraintestinal infections exploiting host cell function, which include the invasion into non-phagocytic eukaryotic cells such as epithelial and endothelial cells and associated host cell actin cytoskeletal rearrangements (Yatsuyanagi et al., 2002).

Studies on the relationship between viral and bacterial infections showed that in the context of viral infections the immunity of host organism is reduced temporarily (Mackowiak, 1978), increasing the incidence of bacterial infections, like faster bacterial colonization of immunocompromised bodies, by increasing the level of expression of epithelial cell receptor for bacterial adesins. Modulation of viruses infected host cells signaling may also induce changes in the cytoscheleton (Nørregård Nielsen et al., 1987; de la Torre and Borrow, 2007) which may result in the increase / decrease invasion capacity of bacterial cells.

Viral infection and subsequent bacterial colonization can pose serious and potentially fatal problems to the host (Nichol and Cherry,1967). The attachment of pathogenic bacteria to mucosal surfaces is the first step in many infections (Lu and Walker, 2001)**,** it also seems possible that viral infection of epithelial cells may render them more susceptible to bacterial adherence.

Our purpose was to study the influence of viral preinfection on colonization capacity of cellular substrate by the enteroinvasive *E. coli* strain using HeLa cells.

MATERIAL AND METHODS

Bacterial strains: The pathogenic strain was one **enteroinvasive *Escherichia coli* O28** strain isolated from diarrhea. The biochemical identification was performed by classical biochemical tests and serological confirmation.

Viral strains: ***Vaccinia*** virus (Poxviridae), **measles** virus (Paramyxoviridae II); **e*chovirus 32*** (Picornaviridae) and ***Herpes simplex virus* 1** (Herpesviridae) from collection of Institute of Virology were used.

Experimental model: The study was performed on HeLa cell line**.** Briefly, 2 x 10^5 cells were seeded in 35 mm Petri dish and then incubated at 37ºC for 24 hrs. The medium was throughout and viral adsorption was done for six hours at 37^{0}C. Thereafter the bacterial adherence and invasion assays have been performed.

Evaluation of adherence to HeLa cells: For the adherence assay, Cravioto's adapted method was used: The HeLa cell monolayers were washed 3 times with PBS; 1 ml of fresh medium without antibiotics was added to each well (Cravioto et al., 1979). Suspension of *E. coli* from bacterial mid-logarithmic phase cultures grown in nutrient broth was adjusted at 10^7 CFU/ml and 1 ml was used for the inoculation of each well. The inoculated plates were incubated for 2 hours at 37ºC.

After incubation, the monolayer were washed 3 times with PBS, briefly fixed in cold ethanol (3 min), stained with Giemsa stain solution (1:20) and left to incubate for 30 min. The plates were washed, dried at room temperature overnight, and examined microscopically (magnification, ×2500) with immersion and photographed with a Contax camera adapted for Zeiss microscope for evaluate the adherence index and patterns.

Invasion assay: Bacterial suspension were inoculated on two HeLa cell plates and incubated at 37^0C for two hours. After that, in 1 plate 100 µg/ml gentamycin solution 1mg/ml was added to kill extracellular bacteria, and incubated for 1 hour. After incubation, plates were washed 3 times with PBS and permeabilized with 0.1% Triton X-100. Serial dilutions were seeded on solid media in order to establish the adhesion plus invasion indexes (C.F.U./ml) in plate without gentamycin, and invasion index in plate where gentamycin solution was added (Lazar et al., 2004; Smarandache et al., 2004).

RESULTS

Qualitative assay of bacterial adherence was performed using Giemsa staining and optical examination. This type of method evaluates adherence of pathogenic strains to the cellular substrate and the pattern of adherence. Three distinct patterns of adherence have been investigated during this study: localized adherence (LA), in which bacteria attach to and form microcolonies in distinct regions of the surface; diffuse adherence (DA), in which bacteria adhere evenly to the whole cell surface, and aggregative adherence (AggA), in which aggregated bacteria attach to the cell in a stacked-brick arrangement (Cravioto et al., 1979). The adherence index was expressed as the ratio between the number of the eukaryotic cells with adhered bacteria : 100 eukaryotic cells counted on the microscopic field. Our EIEC strain exhibited diffuse and aggregative adherence pattern (Figure. 1).

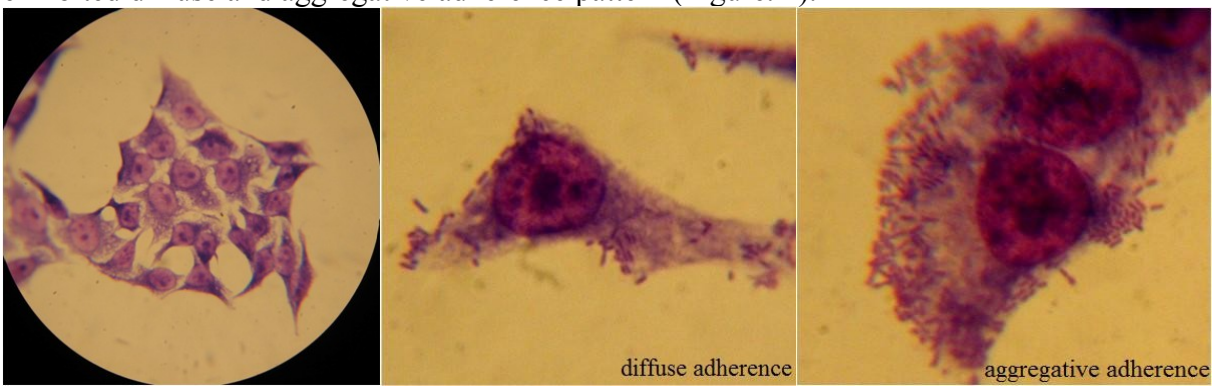

Figure. 1. The bacterial adherence pattern of EIEC strain to HeLa cell line

Viral preinfection of the cellular substrate induced an increased bacterial adherence index (Table 1), as well as changes in the adherence pattern from diffuse tu aggregative (Table 2).

Table no. 1. The qualitative evaluation of bacterial adherence to the viral preinfected cellular substrate.

Bacterial strains	**Adherence index value**				
	HeLa	HeLa +HSV1	HeLa +measles	HeLa+ Echo	HeLa+ Vaccinia
Enteroinvasive E.coli O28 8074	**10**	**100**	**100**	**100**	**100**

Table no. 2. The qualitative evaluation of bacterial adherence patterns to the viral preinfected cellular substrate.

Bacterial strains	***Adherence pattern***				
	HeLa	HeLa+ HSV 1	HeLa + measles	HeLa + Echo	HeLa + Vaccinia
Enteroinvasive E.coli O28 8074	diffuse	aggregative	diffuse	diffuse	aggregative

Quantitative determination

Quantitative determination of adherence and invasion capacity of pathogenic bacterial strains to the cellular substrate (HeLa line) as well as the viral preinfected substratum was performed using modified Cravioto methods in order to estimate intensity of adherence and invasion process.

Related to the influence of viral preinfection on bacterial adherence to cellular substratum, all viruses induced increased adherence capacity of the tested bacterial strain in the quantitative assay (Figure. 2). In exchange, the general effect of viral infection on invasive bacterial capacity was the decrease of invasive ability.

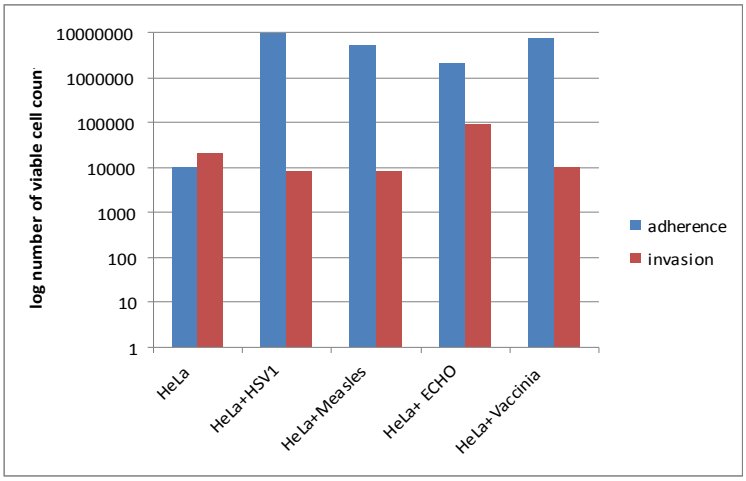

Figure. 2. The influence of viral preinfection on bacterial adherence and invasion of cellular substrate

In conclusion, viral pre-infection of the susceptible substrate influenced the adherence and invasion ability of enteroinvasive *E. coli* bacterial strain, as observed by the intensification of the adherence capacity, explaining the increased incidence of bacterial infections after viral infections, as well as faster bacterial colonization of immunocompromised hosts and by reducing the invasive capacity of epithelial cell by bacterial strains, pleading for increased incidence of extracellular pathogenic organisms in post-viral infections.

Acknowledgements

This research was financially supported by the CNCSIS (National Council for Reasearch in Higher Education Institutions) Research Project, Ideas no. 295/2007

REFERENCES

1. Cravioto A, Gross R J, Scotland SM, Rowe B. 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. 3:95–99
2. Croxen M, Finlay BB. 2009. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology*, advance online publication, Published online 7 December 2009 | doi:10.1038/nrmicro2265
3. Cundell D.R., Tuomanen E. 1995. Attachment and interaction of bacteria at respiratory mucosal surfaces. În: "Virulence mechanisms of bacterial pathogens. Cap. I. Bacterial adherence, colonization and invasion on mucosal surfaces" (Roth, J.A., Bolin, Carole A., Brogden, Kim A., Minion, F.C., Wannemuehler, M.J., Eds.). Sec. Ed., Washington, D.C., Asm Press, 3 – 20, 1995.
4. de la Torre J.C. and Borrow P., Chapter 22 Virus-induced alterations in cells, in Principles of Medical Biology, Volume 9, Part 2, 1998, Pages 365-379 Hauck C.R. Bacterial Pathogens, in Host Pathogen Interactions, p. 3-57, Edited by Rupp S., and Sohn K., Humana Pres 2009.
5. Lazar V., Balotescu M.C., Smarandache D., Vassu T., Sasarman E., Petrache L.M., Orasanu M., Cernat R. *In vitro* study of the interference of some Enterococcus strains with the adhesion of human and poultry enteropathogens to HeLa cells. Roum. Biotech. Lett. 2004; *9* (3): 1675 – 1681.
6. Lu L., Walker W.A. 2001. Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium, Am J Clin Nutrition, 73, 6, 1124S-1130S.
7. Nichol K.P., and Cherry J.D. 1967. Bacterial-viral interactions in respiratory infections of children. New Engl. J. Med. 277:667-672.
8. Mackowiak P.A. 1978. Microbial Synergism in Human Infections, N Engl J Medş 298:21-26.
9. Nørregård Nielsen L., Forchhammer J., Dabelsteen E., Jepsen A., Stubbe Teglbjærg C., Norrild B. 1987. Herpes Simplex Virus-induced Changes of the Keratin Type Intermediate Filament in Rat Epithelial Cells. J Gen Virol, 68, 737-748.
10. Smarandache D., Lazar, V., Balotescu M.C., Vassu T., Ghindea R., Sasarman E., Petrache L.M., Orasanu M., Cernat R. Characterization of adhesion properties to the cellular substratum of some *Enterococcus strains* selected for potential use in probiotic products or food products. RBL. 2004; *9* (3): 1669-1674
11. Vestad E. 2009. Cooperation and conflict in host–microbe relations, APMIS, 117: 311–322.ş
12. Yatsuyanagi J., Saito S., Sato K., Miyajima Y., Amano K.I., Enomoto K. 2002. Characterization of enteropathogenic and enteroaggregative *Escherichia col*i isolated from diarrheal outbreaks. J. Clin. Microbiol., 40: 294-297.

****