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Effects of Yeast and Bacterial Commensals and Pathogens of the Female Genital Tract on the Transepithelial Electrical Resistance of HeLa Cells

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Abstract: Commensals of the human body can shift to a pathogenic phase when the host immune system is impaired. This study aims to investigate the effect of seven yeast and two bacterial commensals and opportunistic pathogens isolated from blood and the female genital tract on the transepithelial electrical resistance (TER) of human cervical epithelial cell cultures (HeLa). The pathogens *Candida tropicalis*, *C. parapsilosis,C. glabrata, C. krusei, C. albicans* and *Saccharomyces cerevisiae*, caused a significant decrease in TER as compared to the controls; *Lactobacillus* spp caused a significant increase in TER *versus* the controls and *Escherichia coli* had no effect on the TER of the cell monolayers. The above data show that *Candida* spp., *S. cerevisiae* and *Lactobacillus* spp. have a non-selective effect on the TER of HeLa cell monolayers. These results are consistent with the *in vivo* non-selective action of these microorganisms on the various human mucosal epithelia.

Keywords: Candida, *E. coli*, HeLa cells, Lactobacillus, Saccharomyces, Transepithelial electrical resistance.

INTRODUCTION

Barrier-forming cells, such as the epithelial cells, are known to form dense layers with tight cell to cell junctions when cultured *in vitro* on porous membranes, which are also instituted in intact tissues [1]. Measurement of the transepithelial electrical resistance (TER) is an established method to assess the barrier function of these reconstructed epithelia. TER can be determined on vital cell cultures, where the overpassing of a certain threshold is a sign of cell layer confluence and integrity [1].

Bacteria and yeasts are members of the normal flora of the human body epithelia and endothelia, such as the skin and the mucosal epithelia. *Candida* spp. are commensal organisms that colonize the skin, intestinal tract, the oral and vaginal mucosa of healthy individuals rarely triggering infection. However, they can shift from a commensal to a pathogenic phase, when the host local or systematic immune system is impaired, thus they proliferate causing disease [2]. Of the numerous *Candida* spp. few are identified as human pathogens; specifically *Candida albicans, C. parapsilo-*

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sis, C. glabrata, C. tropicalis and *C. krusei* are most frequently identified as causes of mucosal and invasive infections.

Destruction of the mucosal epithelial barrier through heavy colonization and release of diverse virulence factors affecting local immunity leads to dissemination of these yeasts ending up to systemic infection [3, 4]. The effect of *Candida* spp. on TER has been hitherto investigated in experimental models resembling the intestinal mucosa, using cell monolayers of human colon tumorigenic cell lines (Caco-2) and Pig Epithelial Cell Jejunum (IPEC-J2), for studies on host beneficial/yeast pathogen interactions in the intestinal flora [5, 6]. When tested in the IPEC-J2 cell line, *Candida* spp. are reported to decrease the relative TER, as a sign of monolayer integrity and cell to cell junction derangement, whereas, when tested in the Caco-2 cell line some of the strains including *Candidakrusei*, *Kluyveromycesmarxianus*, *C. rugosa* and *Trichosporon asahii* had the opposite effect [5]. Similarly, several reports using Caco-2 cell lines have studied the alterations in TER caused by other intestinal commensal microbial species (*Saccharomyces* spp., *Lactobacillus* spp., *Escherichia coli*), either singly or in mixed cultures, in order to study potential probiotic properties conveying protection on the integrity function of the intestinal epithelium [5, 7 - 13]. There are no reports about the effect of these commensal microorganisms in experimental models using cells from a different tissue than the intestinal epithelium.

The HeLa cell line derives from neoplastic cervical tissue and has been hitherto used in experimental models exploring the virulence properties of microbial pathogens [14 - 18]. The present study investigated the effect of seven yeast and two bacterial commensals and opportunistic pathogens isolated from blood and the female genital tract on the TER of HeLa cell monolayers. To our knowledge it is the first time that the effect of these opportunistic pathogens has been evaluated on the TER of this cell line.

MATERIALS AND METHODOLOGY

Yeast and Bacterial Strains

The study included the following strains isolated from blood (n=4) and from vaginal discharge (n=116): *C. albicans* (n=12), *C. glabrata* (*sensu stricto*, n=13), *C. tropicalis* (n=14), *C. parapsilosis* (*sensu stricto*, n=14), *C. krusei* (n=14), *Saccharomyces cerevisiae* (n=15), *Lactobacillus* spp. (n=20) and *E. coli* (n=18). The number and clinical origin of the isolates are given in Table **1**. All yeast strains were identified by sequencing the D1/D2 variable domains at the 5' end of the large subunit rRNA gene (D1/D2) and stored at -80°C in the UOA/HCPF culture collection (Greece; http://www.eccosite.org). Due to their small number and the absence of any difference in TER (s. section "Results"), strains from blood cultures were added to those of the vaginal discharge.

Table 1. Transepithelial electrical resistance (TER) in Ohm/cm^2 of the HeLa cell monolayers of controls and after incubation with yeast and bacterial strains.

Microbial Species	Number of Strains	Clinical Origin		TER Ohm/cm^2 (SD)[1]
		Blood	Vagina	
Candida albicans	12	1	11	384 (14)
Candida glabrata	13	1	12	384 (15)
Candida tropicalis	14	-	14	382 (11)
Candida parapsilosis	14	1	13	391 (20)
Candida krusei	14	1	13	377 (16)
Saccharomyces cerevisiae	15	-	15	389 (14)
Lactobacillus	20	-	20	543 (36)
Escherichia coli	18	-	18	487 (10)
Cell line (controls)	81	81		497 (28)

[1]SD: standard deviation

Bacterial identification was performed with conventional microbiological procedures (API E, API 50 CHL, bioMérieux Marcy l'Etoile, France) and the isolated strains were stored at -80 °C until use. For the inoculation of the HeLa monolayers, yeasts and bacteria were subcultured and suspended in RPMI medium in a density of McFarland standard 0.5 (approximately 1.5 X 10^8 CFU/ml for bacteria and 1-5 X 10^6 CFU/ml for yeasts).

Cells in Culture

HeLa cells (CCL-2, American Tissue Culture Association Lot # 58242684) were grown in RPMI 1640; supplemented with 5% FBS and 1% P/S. Cells were split at 80% confluence until passage 20. Cells were maintained in 25 cm^2 flasks (Corning, NY).

Cell Seeding into Transwell Plates

HeLa cells were grown to 80-90% confluence in the appropriate vessels (flasks). Cells were detached and resuspended in growth medium (RPMI 1640, 10% FBS, 1% P/S) and in suspension were counted to determine cell number per ml. For cell culture, cells were plated at a density of 1 X 10^4 cells/well into the filter wells of the 96-well cell culture insert plates (Millicell, Cat. N.: PSHT004R5). Growth medium (250µl, RPMI 1640, 10% FBS, 1% P/S) was added into each of the 96 wells of the receiver plate (Millicell 96-Well Receiver Tray, Cat. N.: MACAC0RS5). One day before inoculation with microbial strains, cells were fed with medium devoid of P/S (RPMI 1640 plus 10% serum). After the addition of 50 µl microbial suspension (or plain culture medium for control) to each well to the cell monolayer, the plates were incubated for seven days at 37°C at 5% CO_2 atmosphere. All strains were tested in duplicate. Cell vitality was everyday checked by trypan blue exclusion of monolayers in control wells with or without microorganisms.

Transepithelial Electrical Resistance (TER) Measurement

Determination of TER was performed by the use of an $EVOM^2$ Epithelial Volt-ohmmeter equipped with an STX100 electrode (World Precision Instruments). As a blank, it was used the resistance reading (Ohm) from the filter membranes without cells. The resistance value (Ohm) of the monolayer was obtained from each well individually. From the resistance reading value for each well, the blank value was subtracted and this value was multiplied by the area of the filter (0.12 cm^2). Thus, values were expressed in Ohm x cm^2. After the TER measurement, the cultures were microscoped in order to examine any morphological changes of the cell monolayers, as well as of the inoculated yeasts and bacteria.

Statistical Analysis

For the analysis of data we used t-tests and ANOVA. The latter was used to determine the differences among several population means. The mean difference is significant at the 0.05 level. All the results were confirmed using non-parametric tests.

RESULTS

The results of TER after seven days incubation of the infected HeLa cell monolayers are shown in Table **1**. When compared to the controls (plain HeLa cells), a significant decrease (*P*-value: <0.001) was identified in the TER of the following pathogens: *C. tropicalis, C. papapsilosis, C. glabrata, C. krusei, C. albicans* and *S. cerevisiae*.

Lactobacillus spp. increased the TER of the cell monolayers significantly, *versus* the baseline of the controls, as well as *versus* all the *Candida* spp., *S. cerevisiae* and *E. coli* (*P*-value: <0.001 for all). There was neither a significant difference in the TER decrease among the various yeast species, the isolates from blood being added to those from vaginal discharge, due to their small number (data not shown).

The *E. coli* strains isolated from vaginal secretions included in the study did not influence the TER of the HeLa cell monolayers. Microscopic examination of the cell cultures during and at the end of the incubation period (seven days) and the final TER measurement did not reveal any apparent morphological changes on the cell monolayers. The inoculated yeasts displayed both yeast and the hyphal forms, with the exception of *C. glabrata*, which was only present in the yeast form. No morphological alterations were recorded in the inoculated bacteria. All the cell monolayers were vital until completion of the experiments, as controlled by trypan blue exclusion.

DISCUSSION

In the present study, all of the *Candida* spp. and *S. cerevisiae* significantly decreased the TER of HeLa cell monolayers (Table **1**). For *E. coli* no TER alterations were detected, whereas, *Lactobacillus* spp. increased the TER, indicating that they can induce a protective effect on the cell monolayer integrity.

In experimental models, determination of the TER is considered a good indicator for assessing the anatomical and functional integrity of epithelial cell monolayers [1]. The effect of various microbial pathogens corresponds to the *in vivo* effect on epithelial tissue such as the mucosa barrier.

The decrease in TER reflects the degree to which ions move paracellularly through the tissue. Many pathogens invading and traversing the mucosal epithelium cause a derangement of the intercellular junctions, thus decreasing the TER as a result of an increased total passive ion flow [19 - 21]. The strong TER decrease recorded for the *Candida* strains from vaginal discharge provides initial evidence for its contribution in invading the mucosal epithelium.

Various microbial commensals of the normal mucosal flora, including bacteria and yeasts can penetrate the mucosa barrier of hosts with altered physiological or immunological responses and enter the bloodstream causing severe septicaemia and sepsis [22]. *Candida albicans* interacts with the host through an array of virulence factors [22, 23] with the host epithelial cells as a commensal, and as an invasive pathogen. It can enter and traverse the epithelial cells either by adherence and invasion through endocytosis or active penetration, or it can pass through the intercellular spaces due to disruption of the interepithelial cell junctions [6, 24], which causes the decrease in the TER of cell monolayers. This effect of *C. albicans* has been previously reported on human intestinal epithelial monolayers (Caco-2 cells) [6, 24] where the resulting intercellular cleavage event translates into an increase in monolayer permeability and a subsequent decrease in the TER. Decrease in TER was also observed in the present study among *C. tropicalis, C. papapsilosis, C. glabrata, C. krusei* using HeLa cells. Indeed the aforementioned common *Candida* species bear a multitude of virulence factors [25]. The detected decrease in TER with *C. albicans* and non-albicans *Candida* species, during the fungal-host interaction, confirms that virulence potential is multi-factorial.

For *S. cerevisiae*, there are reports with increasing evidence in the literature, that it can cause severe infectious disorders, like fungemia, endocarditis, infections of the lower respiratory tract, the urogenital tract and the skin, in immunocompromised individuals, such as AIDS patients, transplant recipients and cancer patients [26 - 31]. Using intestinal epithelial cells (IEC) and Caco-2 cells, it has been reported that *S. cerevisiae* was able to decrease the TER, inducing IL-8 secretion, a sign of mucosal immune system stimulation [5, 7]. The same effect of *S. cerevisiae* on TER was observed in the present study with HeLa cells, confirming the ability of this yeast to invade the epithelial tissue through disruption of the intercellular tight junctions. This property, in addition to an endocytic machinery reported previously, seems to be a very important virulence determinant for *S. cerevisiae* pathogenicity [32]. There are very few reports about the effect of *E. coli* strains on the TER of cell monolayers. Enteropathogenic *E. coli* are found to decrease the TER in Caco-2 and MDCK cell cultures [10], while a synergistic effect of non-pathogenic *E. coli* and *C. albicans* has been described elsewhere [12]. We used an *E. coli* strain isolated from vagina, in a case of asymptomatic post-menopausal colonization and we did not find any effect on the TER of the HeLa monolayers.

The most interesting issue arising from the present study is the increasing effect on TER of HeLa cells exerted by *Lactobacillus*. The probiotic activity of lactobacilli, based on their regulatory and antimicrobial role in the maintenance of the normal vaginal flora and their use in restoring it after insult, is already known [33 - 35]. Alteration of the normal vaginal microbial flora from lactobacilli to coliform uropathogens may be the result of hormone deficiency, sexual activity, use of contraceptives or antibiotic treatment [36]. More than 80 different *Lactobacillus* spp. are found to be able to restore the imbalance caused by these factors [37]. This preventive and therapeutic activity of *Lactobacillus* includes the ability to keep the acidic pH ≤ 4.5, the production of bacteriocins and hydrogen peroxide, the production of biosurfactants and the blockade of adhesion through co-aggregation with uropathogens [38 - 40]. Along with these properties, previous studies report an increasing effect on the TER of Caco-2 and HT-29 cell cultures [8, 9, 11]. These reports are in agreement with the present study using HeLa cell monolayers, and show a protective action of these bacteria on the interepithelial cell integrity.

The next step toward this research direction could be the investigation of the effect on TER of various cell lines, through an experimental model using mixed microbial populations (yeasts and bacteria). However, such results would be difficult to interpret with respect to their relevance *in vivo*, because of the very individualized balance of the commensal microbial flora among the various human organisms.

CONCLUSION

Overall, the *Candida* spp., *S. cerevisiae* and *Lactobacillus* spp. tested in this study seem to have a non-selective effect on the TER of HeLa cell lines. These results are consistent with the *in vivo* non-selective, either harmful or protective, action of these microorganisms on the various human mucosal epithelia.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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