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Antagonistic effect of *Candida albicans* and IFNγ on E-cadherin expression and production by human primary gingival epithelial cells

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ABSTRACT

Caused mainly by *Candida albicans*, oropharyngeal candidiasis is the most common oral complication associated with HIV disease worldwide. Host defenses against *C. albicans* essentially fall into two categories: specific immune mechanisms and local oral mucosal epithelial cell defenses. Since oral mucosa is the first line of defense in the form of a physical barrier against *C. albicans* invasion, and since epithelial cells are involved in anti-*Candida* innate immunity through different cytokines, we wanted to determine whether *C. albicans* alters E-cadherin expression and production, and whether interferon-γ (INFγ), a TH1 cytokine, is involved in the anti-*Candida* defense. Using primary human gingival epithelial cells, we demonstrated that *C. albicans* significantly decreased E-cadherin mRNA expression and protein production. This effect was basically obtained at later infective periods (24 and 48 h). Interestingly, when IFNγ was added to *C. albicans* infected epithelial cell cultures, it prevented the side effect of *C. albicans* on E-cadherin mRNA expression and protein production and deposition. All together, these results suggested concomitant interactions between oral epithelial cells and IFNγ against *C. albicans* infection.

1. Introduction

Mucosal candidiasis, especially the oropharingeal type (OPC), is a common opportunistic infection in both immunocompromised and immunocompetent persons [1]. The leading cause of candidiasis is *Candida albicans*, a commensal dimorphic yeast that colonizes up to 60% of normally healthy individuals [2]. Symptomatic OPC appears under a number of predisposing conditions [3,4] involving the adhesion, growth and invasion of *C. albicans* causing inflammation and tissue damage [5–7].

Host defenses against *Candida* infections are complex and imply cellular and humoral factors. For instance, the respective roles of host and fungus constituents in the pathogenesis process are still being discussed. Following its adhesion and growth, *C. albicans* cells must migrate across epithelial and endothelial layers leading to systemic *Candida* infection [8]. To go through the tissues, *Candida* will encounter multiple key proteins involved in cell–cell contact including E-Cadherin [9–11,29]. In the oral cavity, as a structural protein, E-cadherin maintains a continuous epithelial attachment around the tooth, providing a structural barrier against noxious agents passing from the oral cavity into the tooth-supporting tissue [12,13]. Abnormal degradation of E-cadherin may contribute to tissue weakness [14,15] and susceptibility caused by *C. albicans*, provoking oral candidiasis. It is then important to investigate whether *C. albicans* affects the structural integrity of the mucosal tissue leading to OPC.

In addition to their role as a structural barrier against noxious agents, epithelial cells may be involved in innate immunity against bacterial and yeast infections via an active inflammatory process [6,16,17].

Cell-mediated immunity (CMI) plays a major role against mucosal candidiasis as proven by the high incidence of candidiasis in immunocompromised patients [18–20]. Experimental models revealed that Th2-type responses are associated with susceptibility to gastrointestinal and systemic candidal infections in animals, whereas Th1-type responses are associated with resistance to infections [18–20]. Indeed, several features of the live vaccine strains of *C. albicans* suggest that T-cell-dependent anticandidal protection is a typically polarized Th1 response [21,22]. This requires IFNγ and CD4+ cells for induction [23]. It also involves INFγ-releasing CD4+ and CD8+/CD4+ cells in the effector phase [24]. IFNγ is also produced by keratinocyte cell lines [25] and gingival epithelial cells under *C. albicans* stimulated, or non-stimulated, conditions [6]. Thus, IFNγ may play a role in anti-candidal defense mechanisms. Given the biological role of oral mucosa as a barrier [26] and the role of epithelial cells as active immunocytes through pro-inflammatory cytokines [6,27], we hypothesized that oral epithelial cells, through E-cadherin and IFNγ, maintain the dynamic equilibrium between the oral microbial community (free

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microorganisms and dental plaque bacteria) and the host. Thus, the objective of this study was to investigate the effect of *C. albicans* on the expression of E-cadherin by normal human gingival epithelial cells, and to shed light on the role of the IFNγ on this E-cadherin protein following cell contact with *C. albicans*.

2. Materials and Methods

2.1. Oral epithelial cell isolation and culture

Small pieces of palatal mucosa were biopsied from gingival graft patients after obtaining their informed consent. The biopsies were treated with thermolysin (500 μg/ml) to separate the epithelium from the lamina propria [28]. Epithelial cell suspensions were obtained by treating the tissue with a 0.05% trypsin-0.01 M EDTA solution. Freshly isolated epithelial cells (9×10^3 cells/cm^2) were cultured in a 3:1 mixture of the Dulbecco–Vogt modification of Eagle's (DME) medium and Ham's F12 (H) (Invitrogen Life Technologies, Burlington, ON, Canada) supplemented with 25 μg/ml adenine, 10 μg/ml human epidermal growth factor (Chiron Corp., Emeryville, CA, USA), 0.4 μg/ml hydrocortisone (Calbiochem, La Jolla, CA, USA), 5 μg/ml bovine insulin, 5 μg/ml human transferrin, 2×10^{-9} M 3,3′,5′,triiodo-L-thyronine (Schwarz/Mann, Cleveland, OH, USA), and 10% fetal calf serum (NCS, fetal clone II, Hyclone, Logan, UT, USA). After being characterized, the oral epithelial cells were used at passage three to perform this study.

2.2. C. albicans growth

The *C. albicans* SC5314 strain was grown on Sabouraud dextrose agar (SAB; Becton–Dickinson) at 30 °C. *C. albicans* suspensions were produced by inoculating 10 ml of phytone-peptone (PP) broth medium (Becton–Dickinson) supplemented with 0.1% glucose with one colony. The culture was grown to the stationary phase for 18 h in a shaking water bath. The blastoconidia were collected, washed with PBS, and enumerated using a hemacytometer. Cell suspensions were adjusted to 10^5 *C. albicans*/ml and used to infect gingival epithelial cell cultures.

2.3. Epithelial cells culture in the presence of C. albicans and INFγ

Epithelial cells were detached from 75 cm^2 culture flasks using trypsin. They were washed twice in culture medium, then counted and seeded into six-well tissue culture plates (Falcon, Becton Dickinson, Lincoln Park, N.J.) and incubated in a 5% CO2 atmosphere at 37 °C. The epithelial cells were cultured to about 90% confluence. They were then put in contact with *C. albicans* (10^3 cells/cm^2) for 4 h then non-attached *Candida* to epithelial cells were washed out by discarding culture medium and its replacement with fresh one. At this step *Candida* infected and non infected epithelial cells cultures were pulsed or not with IFNγ (20 ng/ml of purified protein, Cedarlane Laboratories Limited, Hornby, Ontario), or with *C. albicans* and IFNγ. Non-stimulated gingival epithelial cell cultures were used as negative controls. Stimulated and non-stimulated epithelial cell cultures were incubated 4, 24 and 48 h. To prevent *Candida* overgrowth and its damage to epithelial cell monolayers, culture medium was replaced each 8 h to washout *Candida* suspended in the culture medium. At the end of each incubation period (4, 24 and 48 h), epithelial cells were used to extract either total RNA or total protein as previously described [28].

2.4. RNA extraction and quantification

Total cellular RNA was extracted using the Illustra RNAspin Mini (GE Health Care UK Limited, Buckingham, UK). The concentration, purity, and quality of the isolated RNA were all determined using the Experian system and RNA StdSens analysis kit according to instructions provided by the manufacturer (Bio-Rad, Hercules, CA, USA).

2.5. Quantitative real-time RT-PCR

RNA (1 μg of each sample) was reverse transcripted into cDNA using Maloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen Life Technologies, Mississauga, ON, Canada) and random hexamers (Amersham Pharmacia Biotech, Inc., Baie d'Urfé, QC, Canada). The conditions for the preparation of the cDNA templates for PCR analysis were 10 min at 65 °C, 1 h at 37 °C and 10 min at 65 °C. Amounts of mRNA transcripts were measured using the Bio-Rad CFX96 real-time PCR detection system. Reactions were performed using a PCR supermix from Bio-Rad (iQ SYBR Green supermix). Primers (Table 1) were added to the reaction mix at a final concentration of 250 nM. Five microlitres of each cDNA sample was added to a 20 μl PCR mixture containing 12.5 μl of iQ SYBR Green supermix (Bio-Rad) and 0.5 μl of specific primers (E-Cadherin or GAPDH) (Medicorp, Inc., Montréal, QC, Canada) and 7 μl of RNase/DNase-free water (MP Biomedicals, Solon, OH, USA). Each reaction was performed in a Bio-Rad MyCycler Thermal Cycler. For the qPCR, the CT was automatically determined using the accompanying Bio-Rad CFX manager. The thermocycling conditions for the E-Cadherin and GAPDH were 10 min at 95 °C, denaturation for 10 s at 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C, with each reaction performed in triplicate. E-Cadherin mRNA was amplified by PCR with a maximum of 23 cycles, and GAPDH mRNA was amplified by PCR with a maximum of 16 cycles.The specificity of each primer pair was verified by the presence of a single melting temperature peak. GAPDH produced uniform expression levels varying by less than 0.5 CTs between sample conditions and was therefore used as a reference gene for this study.

2.6. Western blotting and immunodetection of E-cadherin

Western blotting analyses were performed as described previously [28]. Briefly, non-stimulated and stimulated gingival epithelial cells were lysed in Tris buffer containing 200 mM Tris pH 6.8, 20% glycerol, 2% SDS, and 5% β-mercaptoethanol and equal amounts of total protein (100 μg) were loaded onto a 7.5% SDS–PAGE gel. After electrophoretic separation, the proteins were transferred to a nitrocellulose blotting membrane (Pall Gelman Laboratory, Ann Arbor, MI, USA), blocked for 1 h with TBS-T (100 mM Tris pH 7.5, 0.9% NaCl, and 0.05% Tween 20) supplemented with 5% non-fat skim milk. The membrane was incubated overnight at 4 °C with a mouse anti-human E-cadherin monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, Calif., USA) diluted to 1:200. This antibody was anti-E-Cadherin G-10, sc-8426. It is a mouse monoclonal antibody raised against amino acids 600–707 mapping within an extracellular domain of E-cadherin of human origin. Following incubation the anti-E-cadherin antibody, the membrane were washed in TBS-T (1×15 min and 3×5 min)

Table 1
Primers used for quantitative Polymerase chain reaction (qRT-PCR) analysis.

Gene name	GenBank no. #	Primers sequences
E-cadherin	NM_001039258.1	Sense: 5′-GCAGAAGATCACGTACCGCAT-3′ Antisense: 5′-AAGGACCTGCCCCCACATA-3′
GAPDH	NM_002046	Sense: 5′-GGTATCGTCGAAGGACTCATGAC-3′ Antisense: 5′-ATGCCAGTGAGCTTCCCGTTCA GC-3′