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Original article

Neisseria gonorrhoeae induced disruption of cell junction complexes in epithelial cells of the human genital tract

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Abstract

Pathogenic microorganisms, such as *Neisseria gonorrhoeae*, have developed mechanisms to alter epithelial barriers in order to reach sub-epithelial tissues for host colonization. The aim of this study was to examine the effects of gonococci on cell junction complexes of genital epithelial cells of women. Polarized Ishikawa cells, a cell line derived from endometrial epithelium, were used for experimental infection. Infected cells displayed a spindle-like shape with an irregular distribution, indicating potential alteration of cell–cell contacts. Accordingly, analysis by confocal microscopy and cellular fractionation revealed that gonococci induced redistribution of the adherens junction proteins E-cadherin and its adapter protein β-catenin from the membrane to a cytoplasmic pool, with no significant differences in protein levels. In contrast, gonococcal infection did not induce modification of either expression or distribution of the tight junction proteins Occludin and ZO-1. Similar results were observed for Fallopian tube epithelia. Interestingly, infected Ishikawa cells also showed an altered pattern of actin cytoskeleton, observed in the form of stress fibers across the cytoplasm, which in turn matched a strong alteration on the expression of fibronectin, an adhesive glycoprotein component of extracellular matrix. Interestingly, using western blotting, activation of the ERK pathway was detected after gonococcal infection while p38 pathway was not activated. All effects were pili and Opa independent. Altogether, results indicated that gonococcus, as a mechanism of pathogenesis, induced disruption of junction complexes with early detaching of E-cadherin and β-catenin from the adherens junction complex, followed by a redistribution and reorganization of actin cytoskeleton and fibronectin within the extracellular matrix.
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Keywords: Neisseria gonorrhoeae; Epithelial cells; Adherens junctions; Pathogenesis

1. Introduction

Neisseria gonorrhoeae is the infectious agent of the sexually transmitted disease known as gonorrhea. The most important targets of infection in human females are tissues of the genital tract, i.e., cervix, vagina, urethra, uterus and fallopian tubes, while in men the most common target of infection is the urethra. The bacterial membrane components, Pili and Opacity-associated proteins (Opa), are key ligands to establish the initial contact and tight adherence to epithelial host cells, respectively [1]. After binding, *N. gonorrhoeae* requires overcoming the epithelial barrier of the target tissues in order to invade.

The epithelium is a well-organized structure of mucosal tissue that acts as a barrier to protect underlying structures from external pathogenic microorganisms. For their part, microorganisms have evolved mechanisms to cross epithelial barriers and reach sites for host colonization. Going across the epithelium, microorganisms can also access receptors hidden on the basal–lateral epithelial membrane to attach and enter cells where they can replicate [2–6]. Mechanisms of transport across the epithelium include transcytosis, by which microorganisms

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are incorporated into vesicles and then delivered on subepithelial tissues without disassembly of the epithelial barrier. Another mechanism is the disruption of the epithelial barrier by the induction of direct breakdown of tight (TJ) and adherens junctions (AJ) and further paracellular migration of the bacteria. Several studies of gonococcal infection have shown that the bacterium is endocytosed by male urethral cells and epithelial cell lines derived from cervical and fallopian tube tissues, suggesting that transcytosis is a mechanism of gonococcal invasion [7–12]. On the other hand, studies have shown that gonococci also cross the epithelial barrier in male urethra through paracellular spaces to reach the subepithelial connective tissue on the third day post-infection [10]. Transmission electronic microscopy (TEM) has also shown that piliated gonococci migrate between epithelial cells and travel in lateral direction, disrupting the integrity of the tissue [13]. In addition, some observations suggest that paracellular migration also occurs in the infected female genital tract because epithelium is modified during infection. For example, TJ and AJ among Fallopian tube epithelial cells appear lost after five days of gonococcal infection [14] and many ciliated cells lose their activity and are sloughed from the epithelium, suggesting that cell adhesion complexes might be at least temporarily disassembled during the infection [14,15].

If paracellular migration of gonococcus occurs during the infection of the genital tract, a delocalization of intercellular junctional complexes must take place. The impact of gonococcal infection on TJ and AJ protein interactions has been studied in colonic polarized epithelial cells [16], but there are no previous studies in male and female genital tissues, the natural targets of gonococcal infection in human. Thus, the aim of this study was to examine the effect of gonococcus on cell junction complexes of both an endometrial epithelial cell line and primary culture of Fallopian tube epithelial cells. This study seeks to understand the molecular mechanisms by which gonococci attach to, damage or invade the reproductive mucosa.

2. Material and methods

2.1. Neisseria gonorrhoeae

Strains of *N. gonorrhoeae* MS11 ($Pili^{+}/Opa^{-}$) and FA1090 ($Pili^{-}/Opa^{-}$) were obtained from the *Neisseria* strain collection of Dr. Igor Stojiljkovic's laboratory. Bacteria were cultured for 18–24 h at 37 °C and 5% CO_2 in GC agar (Difco, Becton Dickinson, Maryland, USA) supplemented with IsoVitaleX (Becton Dickinson) and the morphology of the colonies was confirmed by stereomicroscopy.

2.2. Cell cultures

The endometrial adenocarcinoma Ishikawa cell line was kindly donated by Gareth Owen (Pontificia Universidad Católica de Chile, Santiago, Chile). Cells were grown and maintained in DMEM medium containing 5% (v/v) fetal calf serum (FCS; Gibco, Invitrogen Co., Carlsbad, CA, USA), 1 mM glutamine (Gibco), 1 mM pyruvate (US Biological, Swampscott, MA, USA), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco), at 37 °C and 5% CO_2. To obtain human oviductal epithelial cells, Fallopian tubes were collected after informed consent from donors undergoing hysterectomy for reasons unrelated to this study. The University's Ethics Committee approved the procedure and tissue use. Samples were transported in DMEM containing high glucose (Gibco), and oviduct epithelial cells were obtained using a previously described procedure [17]. Briefly, longitudinal strips of mucosal folds were dissected and washed in DMEM. Small pieces were obtained and treated for 10 min at 37 °C with 0.25% (w/v) trypsin (Gibco). The cell suspension was centrifuged and the pellet was resuspended in TC-199 containing 10% v/v FCS, 1 mM glutamine, 1 mM pyruvate, 5 mg/ml insulin (Novo Nordisk A/S, Bagsvaerd, Denmark) and antibiotics (50 IU/ml penicillin and 50 μg/ml streptomycin). Cells were seeded and incubated for 1 h at 37 °C in 5% CO_2. Adherent cells were removed and non-adherent epithelial cells were cryopreserved until use [17]. Cultured cells were routinely tested for viability and immunostaining of cytokeratin 18 and vimentin (Chemicon International, Millipore, Billerica, MA, USA). Four to six days after culture, monolayers usually contained more than 95% of oviduct epithelial cells. For immunofluorescence assays, cells were seeded on collagen-pretreated round cover slips in 24-well tissue culture plates and incubated until 80–90% confluence.

2.3. Antibodies

Rat anti-E-cadherin (DECMA-1), mouse anti-β-catenin (E–5), goat anti-occludin (N-19), rabbit anti-ZO-1 (H-300), mouse anti-EGFR (528), mouse anti-fibronectin (P1H11), mouse anti-Vimentin (VI-RE/1), mouse anti-p120 (6H11), goat anti-Actin (I-19), mouse anti-phospho ERK (E–4), goat anti-ERK2 (C-14), mouse anti-phospho-p38 (D-8), rabbit anti-IκBβ (C-20) and Golgi marker (AE-6) antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Donkey anti-goat-HRP, donkey anti-mouse-HRP and goat anti-mouse-HRP secondary antibodies were from Santa Cruz Biotechnology Inc. Alexa Fluor-488 donkey anti-rabbit IgG antibody, Alexa Fluor-488 goat anti-mouse IgG antibody, and Alexa Fluor-488 donkey anti-mouse antibody, were from Molecular Probes (Invitrogen Co.).

2.4. Infection of epithelial cells

Ishikawa cells were grown in 6 or 24-well tissue culture plates until 80% confluence. One day before infection, culture medium was removed and replaced by medium without antibiotics. Bacteria were suspended in DMEM without supplements or phenol red. The optical density at 600 nm was measured and colony-forming units (CFU) estimated. Bacteria were then added to cell cultures at a multiplicity of infection (MOI) of 100 and cultures were maintained at 37 °C and 5% CO_2. At the indicated time points, incubation was stopped by

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