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Escherichia coli K12: An evolving opportunistic commensal gut microbe distorts barrier integrity in human intestinal cells

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A B S T R A C T

Commensal enteric microbes under specific conditions viz. immunocompromised system, altered microbiota or uncompetitive niche induce their otherwise dormant pathogenic phenotype to distort host cellular functioning. Here we investigate how under *in vitro* environment established by using Caco-2 cells, commensal gut microbe *E. coli* K12 (ATCC 14849) disrupt intestinal epithelial barrier function. Caco-2 cells exposed to *E. coli* showed the time dependent significant ($P < 0.01$) decrease in transepithelial electrical resistance (TEER) and concomitantly increased phenol red flux across cell monolayer in contrast to non infected control cells. *E. coli* infected intestinal cells were observed with suppressed ($p < 0.05$) mRNA levels of *ZO-1*, *Claudin-1*, *Occludin* and *Cingulin-1* in contrast to significantly ($p < 0.05$) higher *PIgR* and *hbd*-2 mRNA fold changes. Immunofluorescent and electron micrographs revealed the disrupted distribution and localisation of specific tight junction proteins (Zo-1 and Claudin-1) and actin filament in *E. coli* infected Caco-2 cells that ultimately resulted in deformed cellular morphology. Taken together, *E. coli* K12 under compromised *in vitro* milieu disrupted the intestinal barrier functions by decreasing the expression of important tight junction genes along with the altered distribution of associated proteins that increased the intestinal permeability as reflected by phenol red flux and TEER values.

1. Introduction

In the human gut, a large number of commensal gut microbes help in maintenance of gut physiology including the critical gut barrier integrity that avert the passage of pathogenic microorganisms, noxious luminal toxins and antigens into the systemic circulation [1]. The intestinal barrier integrity under healthy conditions is maintained by a single layer of epithelial cells that separate the microbiota and enteric pathogens from host tissues and immune cells. These intestinal cells are interconnected by a set of apical multi-protein complexes called tight junctions in association with intracellular plaque proteins that establishes the intact cellular monolayer. The tight junction proteins including claudins, occludin and tricullulin form mechanical links between epithelial cells and the intra cellular adapter proteins zonula occludens (ZO) and cingulin facilitate the linkage between tight junction protein complexes and the intra cellular actin filaments that adds further rigidity to this barrier. On the other hand, polymeric immunoglobulin receptor (PIgR) and human-beta defensin 2 (hBD-2) are mucosal defence proteins of innate immune system that together function to counteract pathogen adherence and invasion [2]. Under dysbiosed microbiota composition or pathogen and toxin exposure, the integrity of these intricate barriers is compromised that subsequently lead to the induction or aggravation of intestinal inflammation [3]. Impaired intestinal microbiota not only set in the gut diseases but also leads to development and progression of metabolic disorders and brain dysfunction [4]. Unluckily opportunistic pathogens also make full use of this compromised milieu which enables them to break this barrier by changing the protein junction complexes and leads to leaky intestinal tract characterised with reduced transepithelial/transendothelial electrical resistance (TEER), which is the measure of integrity and permeability of cellular barriers in cell culture models and reflects the ionic conductance of the paracellular pathway in the epithelial monolayer. Leaky barrier has a critical etiologic role in triggering and perpetuating chronic intestinal disorders such as inflammatory bowel diseases and many others [5].

Escherichia coli is one of the predominant facultative commensal anaerobes that is generally considered safe under normal health conditions but has also been associated with development of some chronic intestinal conditions when the host gut milieu is altered by some genetic or environmental factors [6]. *E. coli* K-12 strain is a one of the

Abbreviations: cfu, Colony forming unit; DMEM, Dulbecco's modified Eagle's medium; TEER, Transepithelial electrical resistance

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