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Altered expression of MUC2 and MUC5AC in response to *Shigella* infection, an *in vivo* study

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Abstract

Infection of mucosal epithelial cells by *Shigella* species leads to an intense and acute inflammatory bowel disease that is characterized by watery diarrhea and purulent discharge. Mucin production is a common defense mechanism to protect the underlying mucosa against pathogens. The molecular mechanism(s) underlying mucin induction is unknown in Shigellosis. In this study, we have evaluated the relationship between *Shigella* infection, the expression of MUC2 and MUC5AC and the participation of signaling molecules TNF-α, PKC and ERK1/2. *Shigella* infection up-regulated MUC2 and MUC5AC expression in 6–8 h, through activation of TNF-α, PKC and ERK1/2. These results confirm that, in response to *Shigella* infection, the normal expression pattern of MUC-2 and MUC-5AC is altered. This *in vivo* study brings new insights into the molecular pathogenesis of Shigellosis and new potential therapeutic targets for Shigellosis.

Keywords: *Shigella* species; MUC2; MUC5AC; Ileal loop ligation assay; Shigellosis

1. Introduction

Infectious diseases kill about 11 million children each year, and of the 11 million deaths, acute diarrheal diseases account for 3.2 million deaths in children less than 5 years of age. 600,000 of the 3.2 million deaths annually are contributed by shigellosis with 80% of the deaths occurring in the first 2 years of life [1]. Bacillary dysentery, the severest form of which is called Shigellosis, is caused by *Shigella* bacilli which bind and cross the mucus barrier that covers the intestinal epithelium before invading the mucosal epithelial cell [2].

The most common *Shigella* species causing shigellosis in the developing countries are *Shigella flexneri* and *Shigella dysenteriae* 1. *S. flexneri* is responsible for the endemic form of the disease, whereas *S. dysenteriae* accounts for the epidemic form of the disease. Industrialized areas are dominated by *Shigella sonnei* but *Shigella boydii* is rarely encountered and seems essentially associated with cases of shigellosis in the Indian subcontinent [3].

Shigella infection is a common intestinal problem that is usually self-limiting but it can become life threatening in infants as a result of dehydration or chronic malnutrition [4] and also in immunocompromised individuals because of their inability to combat the infection [5].

Adhesion of enteric pathogens to the mucosa of the gastrointestinal tract was recognized as an important early event in the colonization and development of diarrheal diseases [6]. Hence, the infectious diarrheal disease must be controlled to improve the life expectancy and quality of life for the children.

Mucins have been reported to be pivotal to maintaining epithelium homeostasis in inflammatory diseases and cancer. The major component of mucus is mucin, a heavily glycosylated glycoprotein. Mucins protect, lubricate the epithelial surface and trap particles, including bacteria and viruses, for mucociliary clearance. To date, 20 mucin genes (MUC) have been identified and noted as MUC1–2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B,MUC6–13, MUC15–17, and MUC19–20 and they are observed as *O*-linked glycoproteins expressed either at the cell surface or as secreted molecules to

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form a protective gel [7,8]. MUC2, MUC3 and MUC4 are the prominent mucins expressed in normal colonic mucosa [9,10]. MUC5AC is normally expressed in airway and gastric epithelial cells and is highly expressed in colorectal carcinomas [11].

Bacterial infections up-regulate the production of intracellular adhesion molecules (ICAM-1) and mucins (MUC2 and MUC5AC) in infected intestinal mucosa by inducing the secretion of TNFα and Interleukin-1 [12–16]. TNFα, a proinflammatory cytokine, has been shown to induce the over-expression of MUC2 and MUC5AC in airway, biliary and middle ear epithelial cells. Hypersecretion of mucin (MUC2) and ICAM-1 was observed in cultured human epithelial cells [17]. Bacterial lipopolysaccharide induces the over-expression of MUC2 and MUC5AC in cultured biliary epithelial cells through the activation of PKC in a TNFα-dependent manner [16]. Studies on both gram negative and gram positive bacteria demonstrated a relationship between bacterial infections and over-expression of MUC2 and MUC5AC in the respiratory tract and middle ear epithelium [14].

Many studies have reported qualitative as well as quantitative abnormalities of mucin gene expression in infectious gastrointestinal diseases. The abnormal appearance of MUC5AC, MUC6, and MUC5B and disappearance of normal MUC2 gene expression were observed in Crohn's diseases (CD) [10,18]. *In vitro* infection of human colonic epithelial cells with *Shigella* species showed differential expression of mucin genes in a cell type-dependent manner, resulting in a reduction in the secretion of proinflammatory cytokines such as TNFα [10]. However, the precise biological function in host–pathogen interactions has not been shown. Even though significant progress has been made toward identifying the virulence mechanisms of *Shigella* species, little information is available on the molecular mechanism(s) underlying the regulation of mucin expression in *Shigella* infection. Hence, the present study was undertaken to evaluate the relationship between bacterial infections, the hypersecretion of mucin, in particular MUC2 and MUC5AC, and the participation of TNF-α and other signaling molecules like PKC and ERK1/2 in an experimentally induced shigellosis model system using rabbit intestine.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

Clinical isolates of *S. flexneri* and *S. dysenteriae* were obtained from Christian Medical College (CMC) Vellore, India. The strains were routinely grown in Luria–Bertani (LB) broth or Tryptic soy broth (Himedia, Mumbai, India) at 37 °C, overnight.

2.2. Rabbit Ileal loop ligation assay

The rabbit ileal loop ligation assay was done by the method as previously described [19]. Briefly, white New Zealand rabbits (body weight 1–1.5 kg) were fasted for 24 h before being anesthetized with Ketamine hydrochloride (20 mg/kg body weight) [20]. After making a small incision in the abdominal region, inocula of 10^9 colony forming units (CFU) in 0.5 ml of PBS (pH 7.4), was injected into ligated distal ileal loops and the animals were kept in aseptic conditions for 6–8 h. The loops with PBS (vehicle) served as control. After the incubation period, the animals were sacrificed, and the infected loops were washed with PBS containing gentamycin (50 μg/ml). All the experiments were carried out as per the guidelines provided by the Institutional Animals Ethics Committee (IAEC) (IAEC No. 01/016/04), CPCSEA No. 360/01/a CPCSEA. Infected loops were used for standard histological staining, immunohistochemistry (IHC) and western blotting as described below.

2.3. Immunohistochemical analysis

Expression of MUC2 and MUC5AC was examined in the rabbit intestinal tissue sections by immunohistochemistry as described [21]. Briefly, sections designed for neuraminidase treatment were washed twice in phosphate buffered

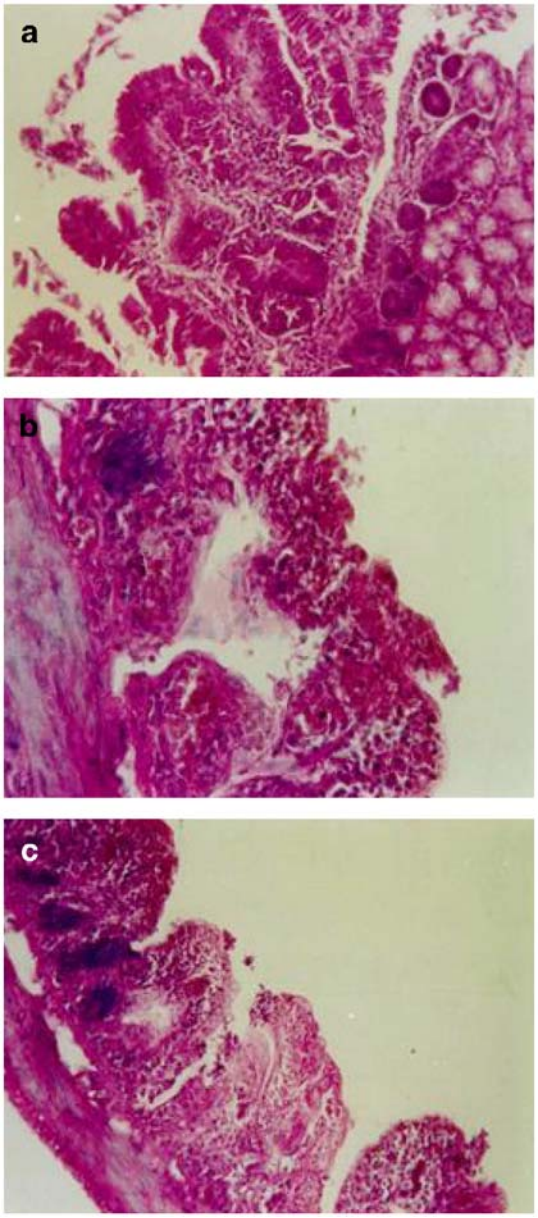

Fig. 1. Morphological changes in rabbit distal ileal loop (original magnification 100×). Histological appearance of normal rabbit intestinal ileal loop shows normal architecture with elongated microvilli (a). Infection of ligated rabbit intestinal loop with clinical isolates of *S. dysenteriae* shows ulceration and inflammatory infiltration of the mucosal surface with hemorrhagic exudates (b). Broadening and congestion of the microvilli with only mild inflammatory infiltration in the lamina propria was observed in *S. flexneri* infected ileal loops (c).