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Expression of Mucins and Trefoil Factor Family Protein-1 in the Colon of Pigs Naturally Infected with *Salmonella typhimurium*

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Summary

The expression patterns of different mucins (MUC1, MUC2, MUC4, MUC5AC, MUC5C and MUC6) and trefoil factor family protein-1 (TFF1) in the colon of healthy pigs and pigs naturally infected with *Salmonella typhimurium* is reported. Twenty infected pigs approximately 80–160 days of age from 20 different herds were studied. These animals had similar microscopical change in colonic tissue characterized by mucosal erosion or sloughing and acute inflammation. *S. typhimurium* was cultured from all lesions and the identity of each isolate was confirmed by serotyping. Immunohistochemical studies of colonic tissue revealed reduced expression of MUC4 on the surface of the cryptal epithelium of *S. typhimurium*-infected pigs compared with non-infected pigs ($P < 0.001$). By contrast, colon from infected animals had increased expression of MUC5AC ($P < 0.0001$) and TFF1 ($P = 0.0095$) relative to controls and there was a significant positive correlation between expression of these two molecules (Spearman coefficient 0.64, $P < 0.0001$). Further studies are needed to evaluate the functional relationship between altered expression of these molecules and inflammation in *Salmonella*-infected pigs.

Keywords: colon; mucin; pig; *Salmonella typhimurium*; trefoil factor family

Introduction

In many parts of the world, salmonellosis is considered one of the most economically important diseases of swine. *Salmonella typhimurium* is the most widespread of all salmonellae and is the second most-frequently isolated serotype in swine (Griffith *et al.*, 2006). *S. typhimurium* infection is associated with enterocolitis that is characterized microscopically by shallow erosions of the mucosal epithelium, with an overlying fibrino-necrotic membrane and foci of deep mucosal haemorrhage. More severe lesions consist of focal deep mucosal ulcers that can coalesce (Moxley and Duhamel, 1999; Wills, 2000; Griffith *et al.*, 2006).

Mucins are high molecular weight glycoproteins that constitute the major component of the mucus layer that protects the intestinal epithelium from infectious and non-infectious agents. At least 20 genes encoding mucin proteins have been identified in humans and designated as MUC1–2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6–13, MUC15–17 and MUC19–20 (Hollingsworth and Swanson, 2004). These mucins are broadly divided according to whether they are secreted or membrane anchored. Secreted mucins consist of the small mucin MUC7, and the large gel-forming mucins MUC2, MUC5AC, MUC5C and MUC6 that are synthesized and secreted by goblet cells to form the gel that covers and protects mucosal surfaces.

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Membrane-anchored mucins consist of the small mucin MUC1, and the two large mucins MUC3 and MUC4, which are anchored in the glycocalyx (Lesuffleur *et al*., 1994). MUC1, MUC2 and MUC4 are the major mucins expressed by the normal large intestine (Corfield *et al*., 2000). Mucus-secreting cells also release trefoil factor family (TFF) proteins into the gastrointestinal tract (Hoffmann *et al*., 2001). TFF peptides such as TFF1, TFF2 and TFF3 have a protective effect on mucosal epithelium by actively triggering the repair process. In this regard, TFF1 expression is documented to occur in areas of intestinal ulceration in patients with inflammatory bowel disease (Shaoul *et al*., 2004).

The aim of this study was to determine immunohistochemically whether *S. typhimurium* infection was associated with altered expression of mucins (MUC1, MUC2, MUC4, MUC5AC, MUC5C and MUC6) and TFF1 in the porcine colon.

Materials and Methods

Samples

Samples of colon were obtained from pigs undergoing necropsy examination at the Department of Veterinary Pathology of Seoul National University from January 2005 to December 2006. Tissues from 20 *S. typhimurium*-infected pigs approximately 80–160 days of age and from 20 different herds were selected. These all displayed lesions of mucosal erosion or sloughing, and acute inflammation characterized by infiltration of the lamina propria and crypts of Lieberkuhn by neutrophils and accumulation of mucus within the crypts. *S. typhimurium* was cultured from all lesions and the identity of the organism was confirmed by serotyping. All 20 infected pigs were negative for *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *B. pilosicoli* as determined by polymerase chain reaction (PCR; Kim *et al*., 1998; Choi *et al*., 2002).

Negative control samples were obtained from 20 pigs that were negative on testing for bacterial pathogens including *Salmonella* spp., *L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli* and for viral pathogens such as porcine reproductive and respiratory syndrome virus and porcine circovirus as determined by isolation and PCR (Cheon and Chae, 2000; Kim and Chae, 2004). Ten of these animals were 80 days of age and 10 were 160 days old. The animals were weight-matched to the infected pigs and were obtained from farms that housed their pigs under similar conditions to those experienced by the infected animals.

Immunohistochemistry

Seven different primary antibodies were employed for immunohistochemistry (IHC). These included monoclonal mouse anti-human MUC1, MUC2, MUC5AC, MUC5B and TFF1 (Zymed Laboratories, Invitrogen Corporation, Carlsbad, CA), monoclonal mouse anti-rat MUC4 (Zymed Laboratories) and monoclonal mouse anti-human MUC6 (Vector Laboratories Inc., Burlingame, CA). According to the manufacturer's data sheets, all antibodies had proven cross-reactivity with the equivalent porcine molecules.

Samples of colon were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were cut onto positively charged slides (Superfrost/Plus™, Erie Scientific Company, Portsmouth, NH). Sections were dewaxed through graded alcohols, rehydrated and processed for antigen retrieval by microwaving (2.4 GHz, 700 W power output; LG Electronics, Korea) in sodium citrate buffer (pH 6.0, 0.1 M) for 10 min (Taylor *et al*., 1994; O'Connell *et al*., 2002). Endogenous alkaline phosphatase was quenched by incubation with glacial acetic acid 20% for 2 min at 4°C. Slides were then incubated with Power Block™ (BioGenes, San Ramon, CA) for 30 min at room temperature to saturate non-specific protein binding sites. All primary antibodies were diluted 1 in 50 in PBS containing 0.1% Tween 20. The sections were incubated with primary antibodies overnight at 4°C in a humidified chamber.

After washing three times in PBS containing 0.1% Tween 20, sections were incubated with biotinylated goat anti-mouse IgG (Dako, Glostrup, Denmark) diluted 1 in 250 in PBS containing 0.1% Tween 20 for 1 h at 36°C, and then washed in 0.1% Tween 20. Sections were subsequently incubated with streptavidin–alkaline phosphatase conjugate (Roche Molecular Biochemicals, Mannheim, Germany) for 1 h at 36°C. Following this stage, sections were equilibrated with Tris buffer (pH 9.5) for 5 min at room temperature, and immersed in a solution of Red Substrate™ (Boehringer Mannheim, Indianapolis, IN) for 10 min at room temperature. Finally, the sections were lightly counterstained with Mayer's haematoxylin.

Samples were considered positive if the surface of at least 5% of the cells (either epithelial cells or goblet cells) was labelled with the specific antibody, regardless of intensity. Samples were also considered positive if the surface membranes of at least 10% of the cells were labelled with the specific antibody, regardless of intensity.

Statistical Analysis

Fisher's exact test was used to evaluate differences in expression of mucins and TFF1 between *S. typhimurium*-infected pigs and non-infected control animals. Spearman's rank correlation method was used to assess