NOTE *Anatomy*

Expression of Mucins in the Mucosal Surface of Small Intestines in 1 Week-Old Pigs

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(Received 14 July 2009/Accepted 18 October 2009/Published online in J-STAGE 27 November 2009)

ABSTRACT. The aim of this study was to determine the immunoexpression of mucins in jejunal and ileal villous epithelium using six antibodies against MUC1, MUC2, MUC4 MUC5AC, MUC5B and MUC6. The immunohistochemical score for MUC1 has significantly intense staining compared with MUC2 (P=0.008) and the immunohistochemical socre for MUC4 and MUC 6 has significantly intense staining compared with MUC2 (P=0.032) in ileal villous surface. The immunohistochemical score for MUC4 (P=0.008), MUC5AC (P=0.016) and MUC6 (P=0.016) in ileal villous surface has significantly intense staining compared with ileal cryptic surface. The results of this study demonstrated that six mucins gave distinctly different expression patterns throughout the 1 week-old porcine small intestinal tract.

KEY WORDS: intestine, mucin, swine.

J. Vet. Med. Sci. 72(2): 245–247, 2010

Mucins are considered as the first line of defense for epithelial tissues since they act as physical barriers between the extracellular milieu and the mucosal surface. At least 20 genes that code for mucin proteins have been identified in humans and have been designated as MUC1–2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6–13, MUC15–17 and MUC19–20 [5]. 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 specifically by goblet cells and form the gel that covers and protects mucosal surfaces. Membrane-anchored mucins consist of the small mucin, MUC1 and the two large mucins, MUC3 and MUC4, which are anchored in the glycocalyx [6]. MUC1, 2, 3 and 4 are the major mucins expressed by the normal intestinal epithelium in human [3, 4].

Preweaning piglets are usually raised intensively, providing greater opportunity for spread of enteric pathogens. As a consequence diarrhea is the major disease problem of pig production [2]. A wide variety of etiological agents is involved, several of which may be present in the preweaning piglets at 1-week-old [10]. The mucin expression may be altered in intestine from pigs naturally infected with etiological agents such as *Escherichia coli, Clostridium perfringens* and for viral pathogens such as porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and rotavirus.

Although the mucin expression was well characterized in human intestines, no one has reported expression of mucins in porcine intestine. Therefore, several secreted (MUC2, MUC5AC, MUC5B and MUC6) and membrane-anchored (MUC1 and MUC4) mucins were selected to characterize the expression profile of mucins in mucosal surface of porcine normal small intestinal epithelium by immunohistochemistry.

Ten 1-week-old conventional Landrace-Large White cross-bred pigs were used in this study obtained from 5 farms (two pigs per farm). For euthanasia, all pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution as previously described [1]. These pigs were negative for bacterial pathogens such as *E. coli, Clostridium perfringens* and for viral pathogens such as PEDV, TGEV and rotavirus by fecal samples. The small intestines were collected from 2 areas as follows: (i) jejunum (15 cm distal to the duodenomesocolic fold) and (ii) ileum (10 cm proximal to the ileocecal junction at the ileocecal fold). The samples were fixed with neutral buffered 10% formalin. After fixation overnight, tissues were processed for paraffin embedding. The methods were approved by the Seoul National University Institutional Animal Care and Use Committee.

Six different primary antibodies were used for immunohistochemical analysis: monoclonal mouse anti-human MUC1 (No. 182298), MUC2 (No. 182299), MUC4 (No. 182322), MUC5AC (No. 182261), MUC5B (No. 376300) (Zymed Laboratories, Invitrogen Corporation, Carlsbad, CA, U.S.A.) and monoclonal mouse anti-human MUC6 (No. VP-M658, Vector Laboratories Inc., Burlingame, CA, U.S.A.). According to the manufacturer, all mucin antibodies used in this study were cross-reactive to porcine mucins by western blotting method (data not shown).

Intestinal samples were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. Sections (4

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μm) of each sample were placed on positively charged slides (Superfrost/Plus slide, Erie Scientific Company, Portsmouth, NH, U.S.A.). Sections were dewaxed, rehydrated, and processed for antigen retrieval by citrate pretreatment (0.1 mol/*l* sodium citrate buffer, pH 6.0) followed by microwave heating for 10 min [8, 9]. Endogenous alkaline phosphatase was quenched with 20% glacial acetic acid for 2 min at 4°C. Slides were digested at 37°C for 30 min in 100 μg/m*l* proteinase K (Gibco BRL, Grand Island, NY, U.S.A.) in phosphate buffered saline (PBS). Slides were then incubated with power block (BioGenes, San Ramon, CA, U.S.A.) for 30 min at room temperature to saturate nonspecific protein-binding sites. All antibodies used in this study were diluted 1:50 in PBS containing 0.1% Tween 20 (optimized dilution). The slides 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 flooded and incubated for 1 hr at 36°C with biotinylated goat anti-mouse IgG (No. E0433, Dako, Glostrup, Denmark) diluted 1:250 in PBS containing 0.1% Tween 20 then washed in 0.1% Tween 20. Next, sections were flooded and incubated for 1 hr at 36°C with streptavidin-alkaline phosphatase conjugate (Roche Molecular Biochemicals, Mannheim, Germany), 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, U.S.A.) for 10 min at room temperature. The sections were lightly counterstained with Mayer's hematoxylin.

Mucosal surface of small intestine was given staining scores in respect of the estimated percentage of the cells stained. Samples were also considered "score 1" if the surface membranes of at least 10% of the cells stained completely with the specific antibody, regardless of intensity. Samples were also considered "score 2" if the surface membranes of at least 10–50% of the cells stained completely with the specific antibody, regardless of intensity. Samples were also considered "score 3" if the surface membranes of at least 50–100% of the cells stained completely with the specific antibody, regardless of intensity.

A nonparametric Kruskal-Wallis test and Mann-Whitney test were carried out for comparison of the immunohistochemical staining score among 6 MUCs between villous surface and cryptic surface, and between jejunal surface and ileal surface among 6 MUCs. Statistical significance was accepted as $P<0.05$.

Immunohistochemical staining was identical or very similar to intestinal epithelium in conventional pigs examined. However, the distribution and intensity of the immunoreactivity were heterogeneous in a given specimen whatever the antibody, with a decrease or an increase in the intensity of the staining depending on the area examined. There is no significant different staining for immunohistochemistry in jejunal villous and cryptic surface among 6 MUCs examined. However, in ileal villous surface, the immunohistochemical score for MUC1 has significantly intense staining compared with MUC2 (P=0.008) and the immunohistochemical score for MUC4 and MUC 6 has significantly intense staining compared with MUC2 (P=0.032) (Fig. 1). Immunoreactivity was predominantly at the mucosal surface of jejunal and ileal villous surface compared to jejunal and ileal cryptic surface in MUC2 (P=0.008). In addition, the immunohistochemical score for MUC4 (P=0.008), MUC5AC (P=0.016) and MUC6 (P=0.016) in ileal villous surface has significantly intense staining compared with ileal cryptic surface (Fig. 2).

Distinct mucin binding pattern may have been identified among different species. In human, normal ileal mucosa shows main expression of MUC2 and MUC3, lesser expression of MUC1 and MUC4, and no expression of MUC5AC, MUC5B, MUC6 and MUC7 [3]. However, in pigs at 1 week old, MUC1 is the main secreted mucin making up the mucus layer and gel forming mucins (more particularly MUC5AC and MUC6) were also observed in conventional jejunal and ileal mucosa of pigs. In mice, MUC1 and MUC2 show strong expression in jejunal and ileal cryptic surface [11] while, in the present study, MUC1 and MUC2 show week expression in jejunal and ileal cryptic surface in pigs at 1 week old.

Mucins play a role in the animal alimentary tract by pro-

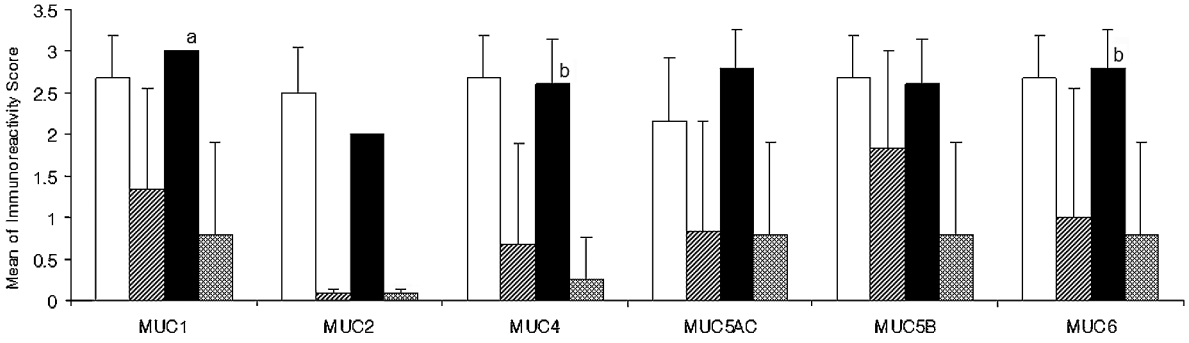

Fig. 1. The results of immunohistochemical staining score for 6 different MUC in jejunal villous (□) and cryptic surface (▨), and ileal villous (■) and cryptic surface (⊠). a) The immunohistochemical score for MUC1 has significantly intense staining compared with MUC2 in ileal villous epithelium (P=0.008). b) The immunohistochemical score for MUC4 and MUC6 has significantly intense staining compared with MUC2 in ileal villous epithelium (P=0.032).

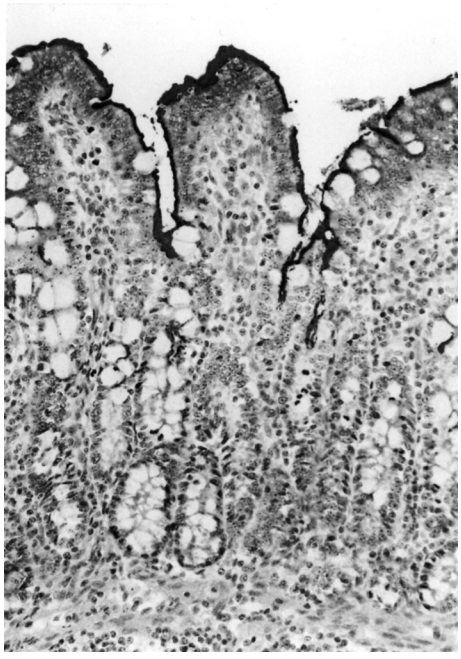

Fig. 2. Ileum from a 7 days old pig. There is prominent expression of MUC4 in the ileal villous epithlium surface. Immunohistochemistry, alkaline phosphatase, red substrate.

tecting the epithelial surface from injury and infection and by facilitating removal of pathogens that enter the intestine. In the present study, six mucins gave distinctly different expression patterns throughout the porcine small intestinal tract. These observations suggested that they are differentially regulated and are likely to make different functional contributions to the mucosal barrier. Although the relationship between mucin expression and functional role are needed to establish, MUC1 appears to play a significant role in host defense in acute infection [7]. In addition, gel forming mucins have a role in epithelial wound healing after mucosal injury in inflammation [3]. Further study is needed to determine the functional role of mucins expressed in porcine intestine.

ACKNOWLEDGEMENT(S). The research was supported by contract research funds of the Research Institute for Veterinary Science (RIVS) from the College of Veterinary Medicine and by Brain Korea 21 Program for Veterinary Science in Republic of Korea. The research was also supported by Brain Korea 21 Program for Veterinary Science and the Korea Research Foundation Grant (KRF-2006–J02902–2) in Republic of Korea.

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