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Histology and Histochemical Distribution of Goblet Cells in the Descending Colonic Epithelium of the Swamp Buffalo (*Bubalus bubalis*)

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ABSTRACT

Predominant glycoconjugates in the descending colon are synthesized and secreted by goblet cells. Changes in the chemical composition of the intestinal mucous affect the functioning of the large intestine. The expression of glycoconjugates in the colonic epithelium of buffaloes was studied using histochemical methods. Paraffin sections 3 μm thick were stained with the following reagents: 1) haematoxylin and eosin (H&E), 2) alcian blue (AB) pH 2.5, 3) periodic acid –Schiff (PAS), 4) AB pH 2.5 – PAS, 5) high iron diamine (HID), 6) HID–AB pH 2.5, and three lectins, *7) Arachis hypogeae* (PNA), 8) *Ulex europaeus* (UEA-I) and 9) *Triticum vulgaris* (WGA). Neuraminidase was used before staining with PNA.

The histology of the descending colonic epithelium is characterized by an absence of villi; the epithelium have numerous simple columnar crypts. The crypts consist of columnar absorptive cells and goblet cells. The goblet cells in the descending colon of buffaloes contained acid and neutral glycoconjugates, whose expression was increased from the lower crypts to the upper crypts. There were more sulphated glycoconjugates in the goblet cells of lower crypts than in upper crypts. The crypts of the upper areas showed more intense expression of carboxylated glycoconjugates and sialic acid together with D- galactose and N- acetylglucosamine. The expression of α -L- fucose was found in the goblet cells throughout all crypts. The results indicated that sulfomucin and α -L- fucose played a lubricating role, while sialic acid and sialoderivatives provided protection against pathogenic infection.

Key words: histochemical, glycoconjugates, descending colon, goblet cells, swamp buffalo

INTRODUCTION

The swamp buffalo has been bred both for meat and for farming, particularly in tropical regions. Recently, buffalo populations have decreased in many countries, thus an improvement in breeding and nutritional management is required. Many studies have shown that dietary factors may affect goblet cell numbers and modulate the secretory activity of goblet cells in rats. (Satchithanandam *et al*., 1990; Lien *et al*,, 2001). However, there is no information on the histology and histochemistry of the large intestine in swamp buffalo.

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The main functions of the large intestine are to propel undigested food and waste product, and to reabsorb electrolytes and water. Therefore, protection of the mucosal epithelial surface from mechanical damage is required. Goblet cells are abundant in the large intestinal epithelium and play an important protective role by synthesizing and secreting mucous (Specian and Oliver, 1991). Histochemical studies have shown that the chemical properties of secretory products from goblet cells vary among species. (Sheahan and Jervis, 1976). Lectins are a group of proteins that can bind carbohydrate in a very specific way (Goldstein *et al.,* 1980) and their specificity in binding carbohydrates is higher than that obtained by other histochemical techniques. Many lectins are used to study glycoconjugates in tissues, including intestines (Alroy *et al*., 1984; Colony and Steely, 1987). In this study, histochemical observations revealed the distribution of secretory glycoconjugates in the colonic goblet cells of the swamp buffalo.

MATERIALS AND METHODS

Histochemical staining procedures

The descending colons of three adult buffaloes were used for this study. Tissue samples of the descending colon, extending from the flexure coil to the rectum were dissected and fixed in Carnoy's fluid for 4 h at room temperature. The samples were prepared using a paraffin technique and the paraffin blocks were cut into 3 μ thick sections. Section were de-paraffinized in xylene, hydrated in a graded ethanol series and then subjected to the following staining procedures. Hematoxylin and Eosin (H&E) were used to stain the histological structure (Spicer *et al*., 1967). Alcian blue (AB) pH 2.5 was used to detect acid glycoconjugates (Pearse, 1985). Periodic acid-Schiff (PAS) was used to reveal the binding of vicinal diol groups, AB pH 2.5–PAS was used to demonstrate binding of acidic and neutral mucosubstances (Spicer *et al*., 1967), High iron diamine (HID) was used to reveal the binding of sulfated mucins (Pearse, 1985), HID-AB pH 2.5 differentiated neutral from sulfated and non-sulfated acid mucosubstances (Spicer, 1965).

Lectin staining procedures

Three lectins were used to specify various sugar moiety; *Arachis hypogaea* (PNA) reveals the specificity binding of terminal galactose, while *Ulex europaeus* agglutinin I (UEA-I) binds to L-a-fucose and *Triticum vulgaris* (WGA) binds to N-acetylglucosamine. Sections were de-paraffinized then hydrated and rinsed two times in PBS pH 7.4 for 5 min, followed by incubation with a lectin for 30 min at room temperature. Sections were rinsed twice in PBS pH 7.4 for 5 min. The sections were treated with an avidin-biotin complex (Vector lab.) followed by a wash in PBS for 5 min. The sections were immersed in DAB for 10 min, then washed in tap water and counterstained with haematoxylin, dehydrated in graded ethanol and mounted with Permount. Desialylation with neuraminidase was performed before counterstaining with PNA.

Scoring of tissue staining

To score positive staining of goblet cells the histochemical method was modified. The sectional appearance under a microscope was divided into lower crypts and upper crypts. Six non overlapping 200x fields with longitudinally section mucosa were randomly chosen from three stained tissue sections per animal. Evaluation of the histochemical change in the goblet cells was scored numerically by the staining intensity where; 0 = negative, 1 = weak, 2 = moderate, 3 = strong and 4 = very strong.

RESULTS

General histology

The H&E stained sections showed tunica

mucosa, which were composed of three parts: epithelium, laminar propria and muscularis mucosae. The colonic epithelium consisted of simple columnar absorptive cells and goblet cells. Crypts were without villi, but contained numerous goblet cells, which were glandular simple columnar epithelial cells and were found scattered among vacuolated cells and absorptive cell in the crypts. Muscularis mucosae was present as a single, smooth muscle layer. Tunica submucosa contained loose connective tissue with blood vessels.

Tunica muscularis consisted of two smooth muscle layers, with the inner circular layer as a completely encircling band and the outer longitudinal layer being thicker than the inner layer.

Tunica serosa was a thin layer of loose connective tissue covered by mesothelium.

Histochemical study

Goblet cells throughout a crypt were stained densely with AB pH 2.5, PAS staining, indicating the strong expression of acidic glycoconjugates and vicinal diol, respectively. The deep purple color in the goblet cells resulting from AB pH 2.5-PAS was due to the mixed presence of acid and neutral glycoconjugates, which showed as deep blue and magenta, respectively. The results of AB pH 2.5-PAS staining of goblet cells from the upper and the lower crypts were the same.

HID-AB pH 2.5 double staining differentiated the expression of sulfomucins, which were positive (black) for HID staining in contrast to non-sulfomucin expression, which was blue by AB staining. The pattern of HID staining in the goblet cells showed that sulfomucin was expressed to a greater extent in the lower part of crypts, whereas non-sulfomucin was denser in the upper part of crypts. The UEA-I and WGA staining patterns expressing α -L- fuc and N-acetylglucosamine were displayed equally in goblet cells of both the upper and the lower crypts. This result revealed that the expression of α-L-fuc was higher than N-acetylglucosamine. PNA-bound Gal β 1, 3–GalNAc was not found in any goblet cells throughout the descending colon. The upper part of crypts showed moderate Gal β 1, 3-GalNAc (Table1) after digestion of neuraminidase.

DISCUSSION

The descending colon of the buffalo consists of numerous goblet cells in the upper and the lower part of crypts, which contain acid and neutral glycoconjugates. Acid mucosubstances

Table 1 Intensity of histochemical reactions of goblet cells in the descending colon of swamp buffalo.

Staining procedures	Staining intensities of goblet cells	
	Lower part of crypts	Upper part of crypts
AB pH 2.5	3	3-4
PAS	3	3-4
AB pH 2.5-PAS	3-4	3-4
HID	3-4	2-3
HID-AB pH 2.5	2-3	2-3
PNA	0	0
UEA-I	3-4	3-4
WGA	1-2	1-2
Neuraminidase-PNA	0	1-2

Note: Staining intensity reaction was indicated numerically where: 0 = negative; 1 = weak; 2 = moderate; 3 = strong; 4 = very strong.

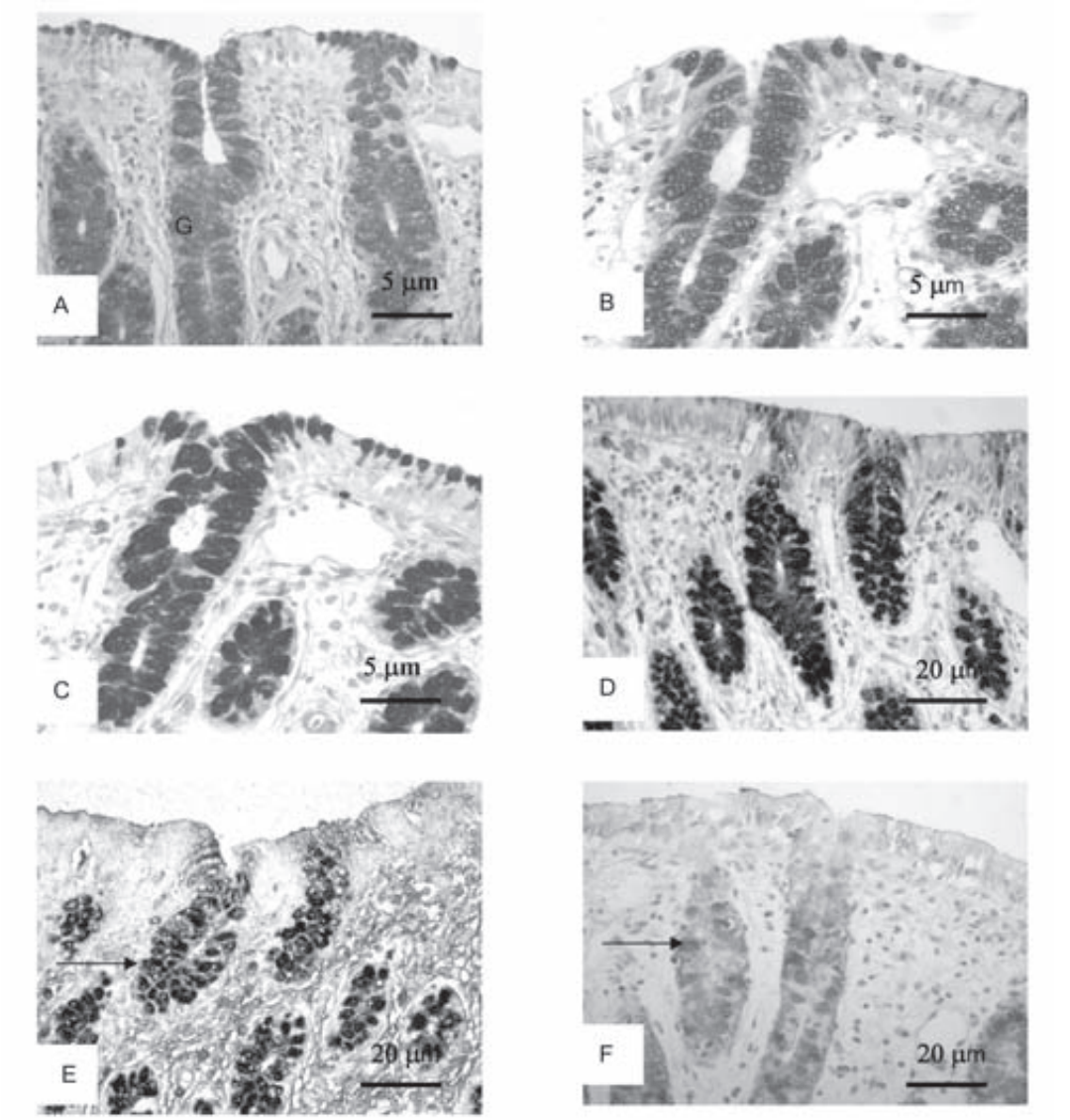

Figure 1 Photomicrographs showing glycoconjugates expression of goblet cells within crypts of the descending colonic epithelium. (A) AB pH 2.5 staining showing the presence of acid glycoconjugates in goblet cells (G); (B) A vicinal diol group was evident in goblet cells, using the PAS technique; (C) AB pH 2.5- PAS staining revealing a high intensity of acid and neutral glycoconjugate expression; (D) HID staining of goblet cells of the lower part crypts showing stronger staining than those of the upper part crypts; (E) HID-AB pH 2.5 staining showing goblet cells in the lower part of crypts containing sulfomucin (arrow), whereas goblet cells of the upper part of crypts had carboxylated glycoconjugates; (F) UEA-I stained α- L-fucose (arrow) in goblet cells throughout all of crypts. Figures A, B and C, scale bar = 5 µm. Figures D, E and F, scale bar=20 µm.

have been found predominantly in the large intestine of mammals (Sheahan and Jervis, 1976; Chen *et al*., 1993). HID staining showed more intensity sulfated glycoconjugates in parts of the lower crypts than the upper parts, which was different from the colon of a rabbit, in which sulfomucin was expressed in the upper part crypts, while lower part crypts contained sulfomucin plus sialic acid. (Reid *et al*.,1988). The results of the current study were similar to those reported for the colonic epithelium in other ruminants (Pongket *et al*., 2001), while the rabbit is a herbivore and monogastric and so different glycoconjugates were correlated with changes in morphogenesis and physiological functions. HID-AB staining showed a moderate intensity of carboxylated and sulfated glycoconjugates compounds. However, the possibility of masked sialomucin expression could not be excluded, since the HID-AB staining showed the expression of both sulfated and non-sulfated acid mucosubstances, seen as black and blue staining, respectively. The sulfated mucin in the lower parts of crypts has the role of a lubricant, while upper parts of crypts contained sulfomucin plus more carboxylated glycoconjugates that play an important role in resisting the invasion of potential pathogens. Furthermore, in rats, the presence of sialomucin in goblet cells has been revealed by lectin histochemistry (Freeman *et al*., 1980; Caldero *et al*., 1988) and was also shown in this study.

Lectin methods have been used as a useful technique to identify sugar residues in glycoconjugate histochemistry. The results obtained by the binding of UEA-I and WGA suggest that goblet cells of the colonic crypts contained α-L-Fucose residues, which were predominant throughout crypts, while all of crypts contained moderate N-acetylglucosamine. Digestion with neuraminidase to display galactose in goblet cells of the upper part of crypts was found at the penultimate position of the carbohydrate chain. Colonic epithelia are often terminated with sialic acid or sulfate groups, which increase the charge of the mucus and therefore its tenacity, which in turn increases its potential to resist attacks by bacterial enzymes. (Rhodes, 1989).

Finally, the goblet cells of the descending colon of the swamp buffalo consist of acid glycoconjugates with sulfomucin, which is considered to be an indicator of mucin maturity and is associated with increased protection of the epithelium (Specian and Oliver, 1991). α-L-fucose was found to be the dominant sugar residues in the descending colonic epithelium of swamp buffalo. Fucose has a high molecular mass with hydrophobic o-link glycoprotein and have been implicated in several important biological process, including inflammation and lubrication (Hollingsworth and Swanson, 2004). N-acetylglucosamine, galactose terminated with sialic acid appeared to be rather common. This study demonstrated the pattern of the normal distribution of colonic mucosubstances in swamp buffalo which may simplify the study of pathological conditions and alimentation.

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