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Relative Distribution of M Cell in the Follicle-associated Epithelium of Peyer's Patch and Solitary Lymphoid Nodule of Porcine Intestines

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ABSTRACT

Background: Gut-associated lymphoid tissue (GALT) in pig comprises the Peyer's patches (PP) in the small intestine and solitary lymphoid nodules (SLN) in the large intestine. The dome epithelium that lines the lymphoid follicles of PP and SLN is specially designed for the uptake of gut luminal antigen where M cell is the principal cell type responsible for antigen uptake and processing.

Methods: In porcine, the M cells were detected in cryosections with the help of FITC-labelled anti-cytokeratin-18 antibody.

Result: The relative distribution pattern of this cell type reflected the possible difference in the microbial environment and, necessity of protective or tolerogenic response against the gut luminal antigens in small and large intestines. It also revealed the preferred location of uptake of mucosal antigen. The number of M cells was highest in the ileum and rectum in the small and large intestine respectively. These cells were mainly confined to the follicle-associated epithelium of the intestinal lymphoid aggregated. However, M cells were also observed in absorptive villi epithelium and in the villi epithelium where lamina propria lymphocytes were more in number.

Key words: Cytokeratin, Immunohistochemistry, M-cell, Peyer's patch, Solitary lymphoid nodule.

INTRODUCTION

Microfold cells or M cells are important cells present in the follicle-associated epithelium of gut-associated lymphoid tissue *e.g.*, Peyer's patch and solitary lymphoid nodule. These cells are specialized for the uptake and processing of particulate antigen from the gut lumen; hence responsible for immune stimulation or tolerance in the host. The property of sampling of particulate antigen has made this cell lineage a preferred target for delivery of mucosal vaccine. Very little information is available regarding the significance of the relative distribution pattern of M cells in small and large intestines in porcine. Hence, this study was attempted to elucidate the relative distribution pattern of M cells in FAE of PP and SLN in different segments of small and large intestines of crossbred growing piglets. This information was assumed to help to select the preferred site for mucosal vaccine administration.

MATERIALS AND METHODS

The study was conducted in the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India. The study was conducted during the period October, 2022 to December, 2023. Twelve (12) numbers of samples of intestines (both small and large) were collected from freshly slaughtered crossbred piglets irrespective of age and sex and were utilized for the study. The samples were snap frozen and preserved in liquid nitrogen maintained at -196°C. Cryosections of 7-9-micron thickness was obtained from the tissue samples in a cryotome. Cryosections of segments of intestines containing PP and SLN were utilized for the detection of M-cells and Cytokeratin-18 was taken as a marker for porcine M-cells (Tohno *et al*., 2006).

1. The cryostat sections were washed in 0.2 M PBS (pH 7.4) for 5 minutes.
2. After washing in PBS, the sections were incubated for 20 minutes in 1% bovine serum albumin (BSA) at room temperature to block non-specific binding sites.

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3. The sections were now incubated in appropriate dilutions (1:200) of Monoclonal Anti-cytokeratin peptide 18-FITC Ab, Clone CY-90 procured from Sigma for 0.5 hours in a dark humidified chamber inside temperature being 37°C.
4. Sections were washed in PBS three times, mounted in glycerin jelly and examined under the fluorescent microscope.

RESULTS AND DISCUSSION

Various methods have been employed to detect and quantify the M cells in the FAE of the GALT. However, due to differences in the morphology and immunological characteristics it was difficult to find out a universal and handy method to identify this cell type in various species (Casteleyn *et al*., 2013). In the current study, the FITC-labeled anticytokeartin-18 antibody was used to detect the M cells. Gebert *et al*. (1994) studied the dome epithelium of PP of minipig immunohistochemically and demonstrated the presence of cytokeratin-18 in the M cells' cytoskeleton. His experimental results revealed that porcine M-cells were different from the normal enterocytes in composition of the cytoskeleton and cytokeratin-18 could be regarded as a useful marker to detect porcine M-cells. Tohno *et al*. (2006) also did double immunostaining for cryosections of ileal PP for the detection of M-cells and used cytokeratin-18 as a marker of M cells. In the present study, it was reconfirmed that cytokeratin-18 is specific for M cells of FAE of PP of crossbred growing piglets. In the present study, FITC labeled anti-cytokeratin-18 antibody was utilized and yielded reliable results (Fig 1 to 8). Earlier, double immunostaining, using two antibodies (primary and secondary) along with a fluorescent or enzyme tag was utilized; being tedious, time-consuming and costly. In the present study, it was confirmed that FITC-labeled anti-cytokeratin-18 antibody could be used effectively to detect porcine M cells which were, indeed a cost-effective and time-saving technique.

In the current study, M cells were found specifically in the FAE of PP and the crypt overlying SLN/LGC of small and large intestines respectively (Fig 1 to 8). Hsieh *et al*. (2012) discussed about the distribution pattern of M cells and opined that these cells were interspersed among normal intestinal epithelium in the FAE. In this study, the M cells were found to be located more towards the flank of the dome region while the tip of the FAE had fewer M cell (Fig 2). This finding might be indicative of the generation and maturation of M cells from normal crypt epithelial cells. More number of M cells in the crypt might be suggestive of a preferred site for antigen uptake in the PP.

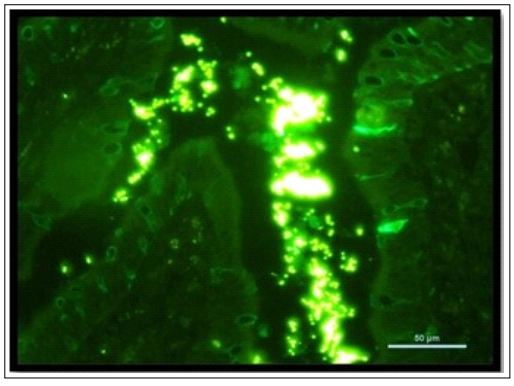


Fig 1: Photomicrograph showing section of dome of PP (arrow), with m-cell, ×400.

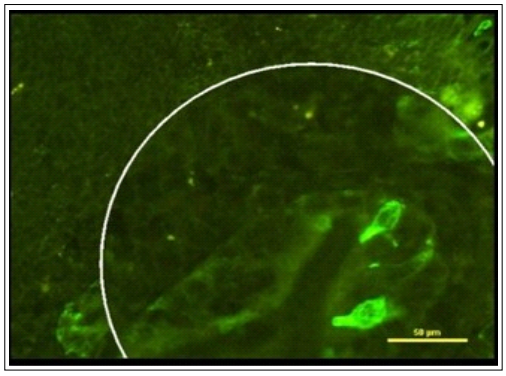


Fig 2: Photomicrograph showing section of dome of PP, ×400.

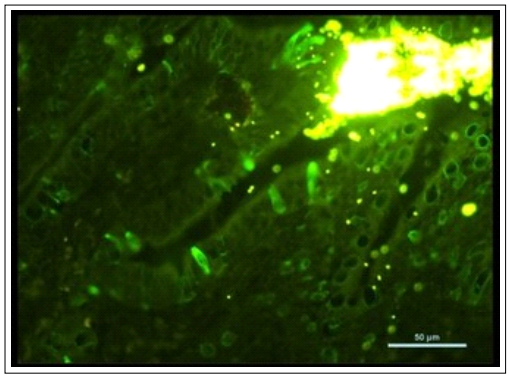
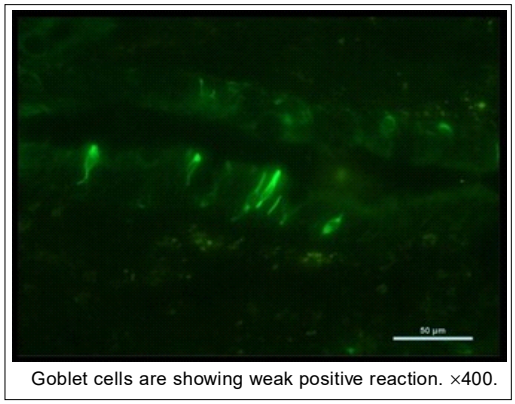


Fig 3: Photomicrograph showing section of dome of PP, villi having intraepithelial lymphocytes (arrow), ×400.



Goblet cells are showing weak positive reaction. ×400.

Fig 4: Photomicrograph showing dome villi (arrow) and absorptive villi, note the presence of m cells in dome FAE.

In crossbred growing piglets, these cells were elongated, columnar with a basolateral pocket and were interspersed in between the absorptive enterocytes in the FAE (Fig 1). M cells in most of the sections were seen with one or two numbers of fluorescent particles. Fluorescent particles were seen in different positions of the cell depending upon the time of incubation (Fig 1). Although the M-cells were predominantly found in the FAE of PP and LGC; variable numbers of M cells were also present in the absorptive villi epithelium in between the lining columnar cells and in the crypts of the large intestine (Fig 7,8). In the large intestine such cells were mainly seen in the crypts overlying the LGC or SLN. M cells were also seen in the villi epithelium where numerous lymphocytes were located intra-epithelial and, in the lamina propria. The cells were more in number towards the base of the crypts.

In the present study, a smaller number of M cells was also observed in the normal absorptive villi as well as in the ileocecal junction (Fig 3,6). Although most of the authors (Gebert *et al.*,1996 and Nicolette, 2000) emphasized that M cells were specific for FAE of PP, still the presence of M cells in the normal absorptive epithelium might be indicative of the possible role of the absorptive epithelium in the uptake and processing of particulate antigen. It might also be due to the transformation of absorptive enterocytes to M cells by certain agents to facilitate entry of antigen into the system. This possibility of transformation of absorptive epithelium into FAE-like epithelium was demonstrated by Tahoun *et al.* (2012). They experimented on *in vivo* and *in vitro* model of infection by *Salmonella* Typhimurium and found that it specifically targets antigen-sampling microfold (M) cells to translocate across the gut epithelium.

In the present study, M-cells were found to be present in more number in per unit stretch of FAE of ileum than that of duodenum and jejunum (Table 1). The presence of a greater number of M cells in the ileal FAE may be suggestive of a preferred site for antigen uptake in the small intestine.

The presence of M cells in the large intestinal mucosa was recorded in the present study. These cells were fewer in number in the normal crypt epithelium. However, the number of such cells was abundant near the pitcher-like invagination of LGC of colon and rectum (Fig 7,8). The M cells were also observed in the epithelium of the pitcher-like invagination, which might be regarded as the FAE of PP. The presence of M cells in these locations might be a strategic target for antigen uptake and processing by the lymphoid tissues of the large intestine.

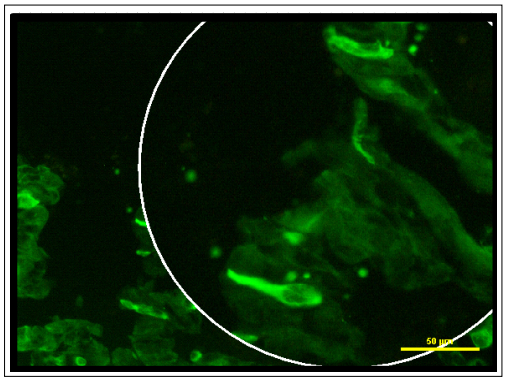


Fig 6: Photomicrograph showing presence of M-cell in the ileo-cecal junction. ×400.

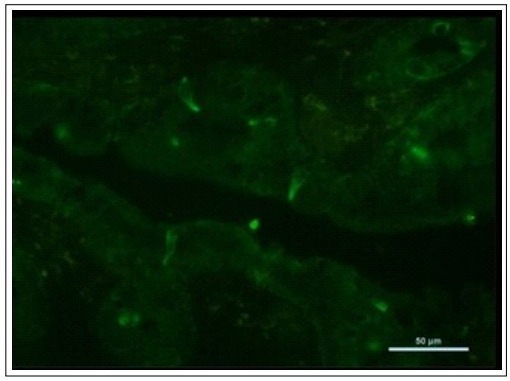


Fig 7: Photomicrograph showing presence of M-cell in the invagination leading to colon LGC. ×400.

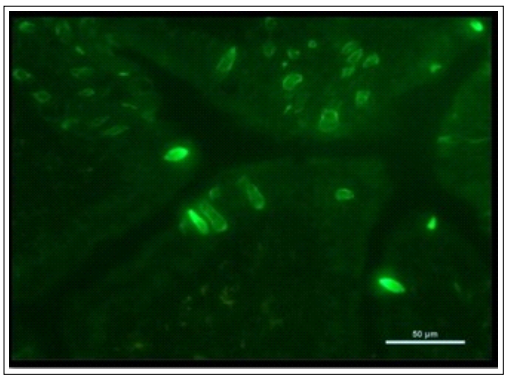


Fig 5: Photomicrograph showing different sectioning plans of m cells. ×400.

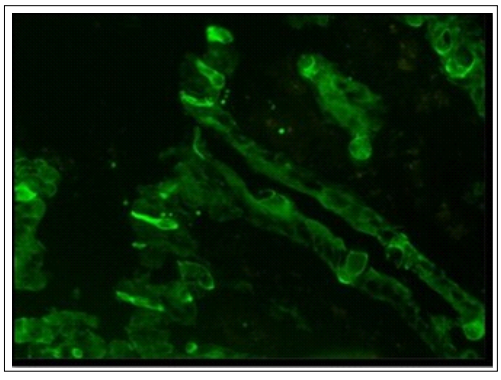


Fig 8: Photomicrograph showing presence of M-cell in the invagination (arrow) leading to rectal LGC. ×400.

Table 1: Table depicting segment-wise average numbers of M cells present in per unit stretch of different compartments of PP and SLN.

Region	Segments	Compartments	Average
Small Intestine	Duodenum	FAE (PP)	4.00±0.365
		FAE (IEL)	0.67±0.121
		Villi epithelium	0.11±0.007
	Jejunum	FAE (PP)	4.50±0.428
		FAE (IEL)	0.89±0.342
		Villi epithelium	0.21±0.012
	Ileum	FAE (PP)	5.33±0.333
		FAE (IEL)	0.91±0.098
		Villi epithelium	0.50±0.224
Large Intestine	Ileo-cecal Junction	FAE	1.67±0.211
		Villi epithelium	0.11±0.001
		Crypt epithelium	4.17±0.307
	Colon	FAE	2.67±0.333
		Depression epithelium	1.67±0.333
		Normal crypt	0.33±0.211
	Rectum	FAE	2.83±0.307
		Depression epithelium	2.67±0.211
		Normal crypt	0.33±0.211

CONCLUSION

M cell is the principal cell type of the gut-associated lymphoid tissue responsible for the uptake and processing of the particulate antigen. The relative distribution of M cells as well as their concentration in the follicle-associated epithelium has a direct bearing on the effectiveness of antigen uptake at that particular site. In crossbred growing piglets, information on the distribution pattern of this cell type will help determine the most effective target for mucosal vaccine delivery. Especially, the difference in concentration M cells in the small and large intestines might indicate the difference in the antigen uptake pattern at both these locations which might be due to different circulatory patterns and different microbial environments.

Conflict of interest

We, the authors, hereby declare that we have no conflict of interest of any form pertaining to the publication of proposed manuscript.

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