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Histomorphometrical and immunohistochemical study of cecal apex in rabbit (*Oryctolagus cuniculus*) and hamster (*Mesocricetus auratus*) with emphasis on microfolded cell

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Abstract

Fifteen mature rabbits and the same number of mature hamsters were conducted in this study to investigate the differences in the structure of the apical segment of the cecum in rabbits and hamsters, as well as to provide baseline information about the distribution and number of M cells. Microscopic examination of the apex's rabbit and hamster cecal walls revealed four primary tunicae: mucosa, submucosa, muscularis, and serosa. In rabbits, the mucosa of the appendix was thrown with leaf-like folds lined with simple columnar epithelium with many goblet cells. Among these folds, there was a dome shape structure which is constructed from the aggregation of a large number of lymphoid follicles and lined with a special kind of epithelium which was termed follicle associated epithelium (FAE) and characterized by the presence of a large number of M cells, which were larger than the columnar cells and contained several lymphocytes. The immunohistochemical expression of M cells showed a strong reaction against GP2 in the cytoplasm and cell membranes, particularly in the dome epithelium of the rabbit appendix. In contrast, the reaction was weak or limited in hamsters. Concerning FAE of dome shape in the cecum of rabbit, most M cell were concentrated in the basal part of this epithelium, whereas the least expressed in the apex of the FAE. This study concluded that the apex of the rabbit cecum had more well-developed lymphoid tissue than the hamsters. The M cell count in rabbits was higher than that of hamsters, where the most occurrence of M cell was in the appendix of rabbits.

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Introduction

The cecum is generally labeled as the first segment of the large bowel, as a closed-end tubular structure among the ascendant colon and ileum, and divided into three portions: base, body, and apex, namely ampulla coli, corpus ceci, terminal section apex ceci. In mammals, the appendix stated that humans, rabbits, and certain rodents have a similar genetic origin and great lymphatic condensation (1). Therefore, the immune role performed by the appendix of humans and rabbits parallels the immune roles performed by the final parts of the cecum in other animals, missing the vermiform appendix (2). Specialized epithelial cells called Microfolded cells are crucial for the carriage of intra-luminal macromolecules and pathogenic factors from the small intestinal lumen to the underlying mucosal lymphoid tissues, where the immune responses are initiated, and antigen processing takes place (3-7).

This work was conducted because there is a dearth of information about the distribution and number of M cells in the cecum of rabbits and hamsters, as well as the significance of this cell in boosting immunity.

Materials and methods

Ethical approval

All experimental animals were set in the study after the approval of the Scientific Committee, College of Veterinary Medicine, University of Mosul under the reference no. UM.VET.2023.087, issued 20/8/2023.

Animals and study design

This study employed 15 adult rabbits (*Oryctolagus cuniculus*) and 15 adult hamsters (*Mesocricetus auratus*) regardless of gender. All animals were reserved under the lab. environments of 25˚C temp. Moreover, free tap water and food access are permitted for 12 hours daily and 12 hours at night. The animals were bought from the local market in Mosul. A high dose of sodium pentobarbital at 100 mg/kg bw was injected with intracardiac to euthanize animals (8).

Histological and histochemical approaches

Specimens of 1 cm were taken from the apex of the cecum (*appendix vermiformis*)) for both species of animals. All specimens were rinsed gently with normal saline to empty the cecum content and then immersed in 10% neutral buffer formalin directly for 48 hours. The specimens were processed according to the paraffin embedding procedure, where the specimens were embedded vertically to gain all layers of the cecal wall (9,10), and the tissue blocks were sectioned at 5 µm by a rotary microtome. The sections of the tissue were stained with Hematoxylin and Eosin (H&E) and Periodic acid Schiff (PAS) stains for a general description of the cecal wall of the apex (11-13).

Anti GP2 antibody (GP2 Polyclonal Antibody, Catalog Number: E-AB-90418 Elabscience) was used as the primary antibody for identification of M- cells and its manifestation, distribution, and concentration in apex in two species of animals. Tissue sections at 5 µm were prepared to achieve the Immunohistochemical (IHC)technique. The M cell percentage was estimated by counting M -M cells/ 100 nuclei in the cecal epithelium. The histological sections were treated according to the formula stated by the supplier company (14,15). All micromorphometric measurements were achieved using a digital tube microscope camera (OMAX 18MP) in China, which provided image processing software (Toup view). All lenses of the Olympus microscope (CX21) were calibrated using camera software and a stage micrometer to ensure the accuracy of the micromorphometric measurements.

Statistical analysis

A computer program (Sigma stat V13.2 / SYSTAT) was used for the micromorphometric investigation. The Chi-square test was used to compare the M cell percentage between rabbits and hamsters (16).

Results

Histological findings

In rabbits, the surface epithelium of the apex(appendix) was thrown with leaf-like folds or villi-like folds with narrow bases and wide apical portions. A simple columnar epithelium with many goblet cells lines these folds. The core of these folds is constricted from loose connective tissue infiltrated with many lymphocytes. Each fold filled with cross sections of intestinal glands or crypts of Lieberkühn (Figure 1). These folds were lined by simple columnar epithelium constructed mainly from columnar enterocytes and many goblet cells (Figure 2). Among these folds, there was a dome shape structure which is constructed from the aggregation of a large number of lymphoid follicles; these follicles were covered with a special kind of epithelium, which was termed dome epithelium called follicle-associated epithelium (FAE); this type of epithelium consisted of enterocytes with numerous modified cells called M cell (microfolded cells). This FAE epithelium appeared different from the adjacent epithelium lining the mucosal folds, which were thrown with numerous goblet cells but not M cells (Figure 3).

The goblet cells in the dome epithelium appeared absent but, in certain positions, had a few goblet cells. On the other hand, an enormous quantity of intra-epithelial lymphocytes occupies the epithelium covering domes. In certain sites, they seem like bunches of lymphocytes, specifically those close to M cells that form pouches for enclosing such bunches. The M cell is characterized by its large size compared with columnar cells and its pocket that houses several lymphocytes. The lamina propria in the dome shape is very scant, while it appears clearly within the core of the mucosal fold. Muscularis mucosa appeared as a thin, interrupted sheet of smooth muscle fibers separating lamina propria from the underlying submucosal layer. Tunica submucosa appeared as a thin layer of vascularized areolar connective tissue containing lymphocytes. A ganglionic nerve plexus in the submucosa was characterized by its pale basophilic cytoplasm and prominent nucleus, defined as Meissner plexus (Figure 4). Tunica muscularis is constructed from two layers: the circular inner layer and the longitudinal outer layer; among the two layers, there were numerous Auerbach's nerve plexus (myenteric) in different sizes, most of these ganglionic nerve plexuses surrounded with connective tissue fibers (Figure 5). The outermost layer, the tunica serosa, consisted of one layer of mesothelium covering a tinny layer of loose collagen fibers.

In hamsters, the wall of the apex of the cecum had a histological structure different from that of rabbits. The mucosa of this part was thrown with many folds of different sizes, and there were no lymphoid follicles nor related follicle-associated epithelium. The mucosal folds in hamsters appeared larger than in rabbits (Figure 1). Generally, the wall of the cecum of the apex had the same

layer of tunica as a rabbit. However, the surface epithelium was thrown with many crypts of Lieberkühn (intestinal glands) more than that of a rabbit, lined by enterocytes and many goblet cells (Figure 3). Few M cells were detected in this epithelium. The general thickness of the apex wall is less than that of the rabbit because of the absence of lymphoid follicles, but the tunica mucosa appeared slightly thicker than that of the rabbit. The muscularis mucosa in the apex of the hamster appeared well-defined and constricted from numerous layers of smooth muscle fibers. It appeared continuous and thicker than that of a rabbit. The crypts were numerous, deep, and simple columnar epithelium with goblet cells lining it. Different levels of mitotic Figures were noticed in the crypts, especially within the basal part of the crypts. Muscularis mucosa is a continuous bundle of smooth muscle fibers. It appears relatively thicker than a rabbit and extends within the core of folds. The submucosa of the apex of the hamster is occupied with numerous blood vessels and aggregation of lymphocytes, plasma cells, and well-defined connective tissue more than that of rabbits. Also, the submucosa has a large number of ganglionic nerve cells.

The Meissner nerve plexus was found in fewer numbers than in rabbits and was located mainly in the upper zone of the submucosa (Figure 4). The tunica muscularis in hamsters appeared thicker than in rabbits and was constricted by the thinner outer and thicker inner layers. Many Auerbach (myenteric) nerve plexus was observed between the inner and outer layers of tunica muscularis, and some blood vessels invested the inner thick layer. The density and sizes of Auerbach's nerve plexuses in a hamster appeared more than that of a rabbit (Figure 5). Tunica serosa was a very slim coat of collagen fibers with mesothelial cells.

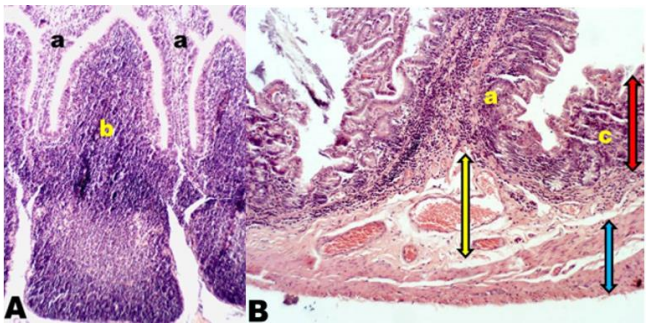

Figure 1: Microphotograph of cecal apex in (A)rabbit, (B) hamster showed the (a) cecal fold, (b) dome shape of the lymphoid follicle, (c) crypt of Lieberkühn, (red double heads arrow) tunica mucosa, (yellow double heads arrow) tunica submucosa, (blue double heads arrow) tunica muscularis. H&E, 100X

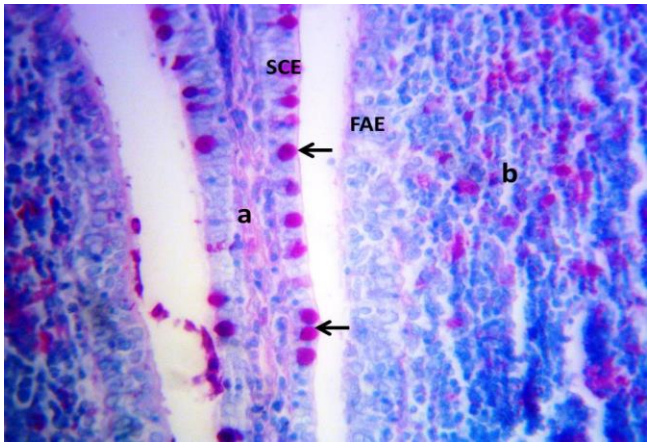

Figure 2: Microphotograph of cecal apex in rabbit, showed the (a) cecal fold, (b) lymphoid follicle, (FAE) follicle associated epithelium covering the dome shape, SCE) simple columnar epithelium lining the fold, (arrows) goblet cells. PAS stain, 400X

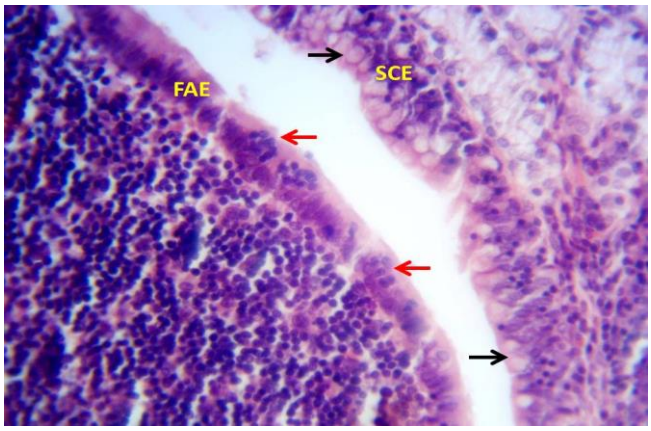

Figure 3: The microphotograph in the appendix of the rabbit showed the M cell (red arrow), follicle-associated epithelium (FAE), simple columnar epithelium (SCE), and goblet cell (black arrow). H&E stain, 400X

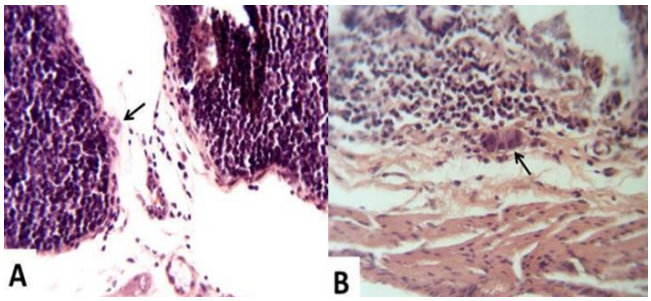

Figure 4: Microphotograph showed Meissner's nerve plexus (black arrow) in the submucosa of the apex in (A) rabbit and (B) hamster. H&E, 400X.

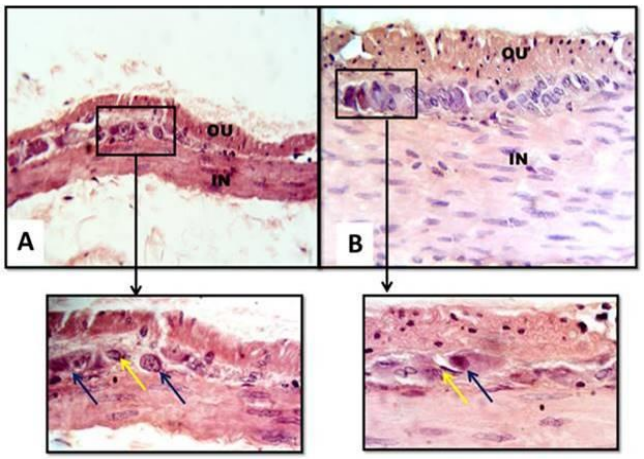

Figure 5: Microphotograph showed the Auerbach's nerve plexus in the apex of (A)rabbit and (B)hamster. Neuron (black arrows), glial cell (yellow arrow). H&E, 400X and 1000X.

Immunohistochemical findings

Immunohistochemical expression of M cell showed a strong reaction against GP2 (brown color), which appeared in the cytoplasm and on the apical and lateral cell membrane of M cell (mature M cell) in the dome epithelium, especially in the appendix of rabbit (Figures 6 and 7), as well as in the cecal epithelium of hamster (Figures 8 and 9). The highest number of M cells was found in the appendix of rabbits, 11%. while it was 4% in hamster. Concerning FAE of dome shape in rabbit cecum, most M cells were concentrated in the basal part of this epithelium (74%), then in dome mid-edge (20%), whereas the least number was expressed in the apex of the FAE (5%). Notably, we noticed that goblet cells reacted moderately against GP2, which only appeared on the apical cell membrane.

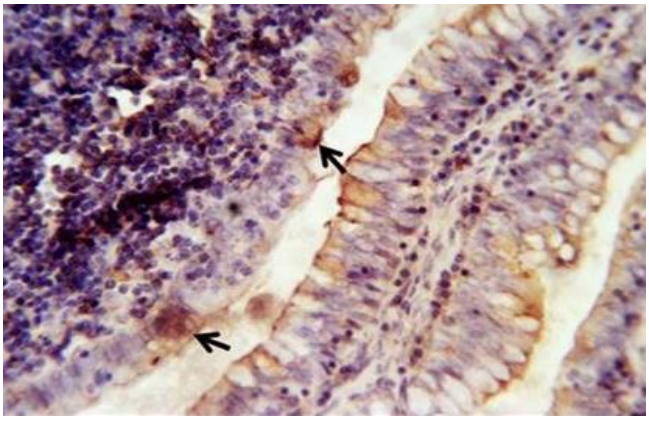

Figure 6: Microphotograph of cecal apex in rabbit showed the immunohistochemical staining with GP2 marker. M cell (black arrows) in the follicle-associated epithelium. GP2 IHC.400X.

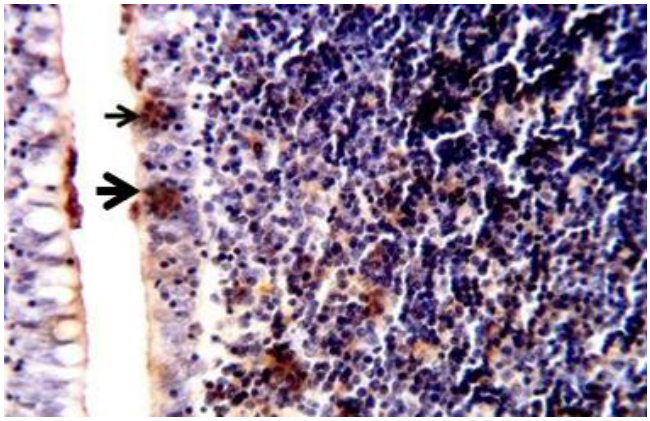

Figure 7: Microphotograph of cecal apex in rabbit showed the immunohistochemical staining with GP2 marker. It showed an M cell (black arrow) in the follicle-associated epithelium. GP2 IHC. 400x.

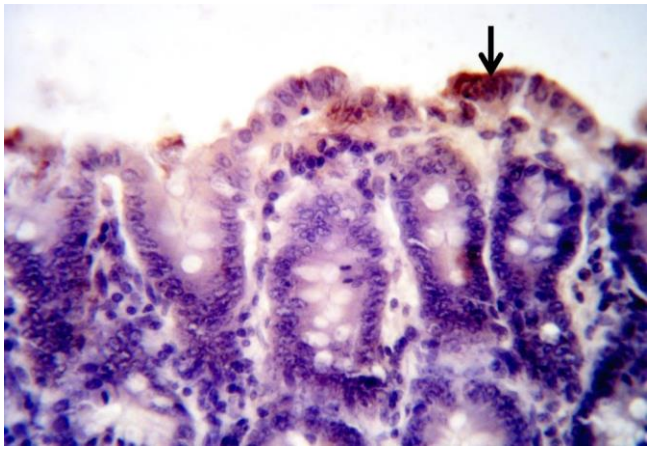

Figure 8: Microphotograph of cecal apex in hamster showed the immunohistochemical staining with GP2 marker. M cell (black arrow) in the lining epithelium of the fold. GP2 IHC. 400x.

Discussion

The FAE was present in the rabbit appendix and absent in the hamster because there were no lymphatic follicles underneath the epithelium. This made the epithelium lining the folds appear different from the adjacent epithelium lining the dome-shaped structure (17-21). The role of the M cells has been formerly established (22), with antigen sampling considered critical for initiating immune responses specific to gastrointestinal environmental antigens (23). The current study records the presence of M cells in the apex of the cecum in both species, with the highest occurrence in rabbits, but other researchers cited that only the follicular-related epithelium and, infrequently, the villi next to the lymphoid follicles were found to contain M cells (24,25). Various

markers were used to express M cells, such as lectin UEA-1, claudin 4, Glycoprotein 2, vimentin, and cytokeratin (26); in our study (the GP2) marker was used, anyway.

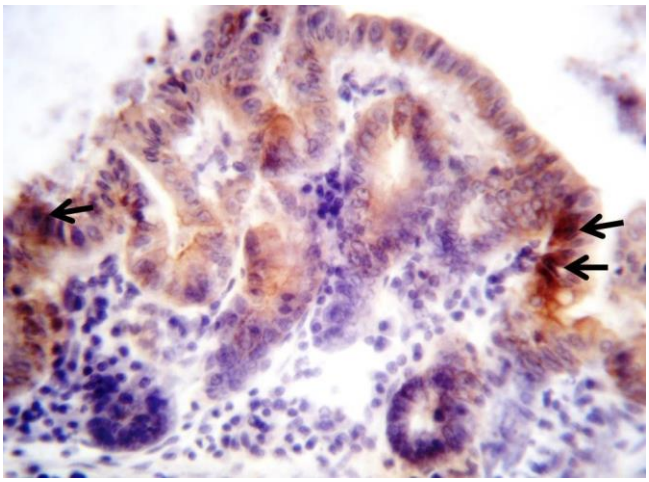

Figure 9: Microphotograph of cecal apex in hamster showed the immunohistochemical staining with GP2 marker. It showed an M cell (black arrow) in the lining epithelium of the fold. GP2 IHC. 400x.

Notably, the highest number of M cells was in the lowest part on the edges of the dome, while they were almost absent in the apex. This is because M cells arise from stem cells of intestinal crypts (27,28). In the current study, concerning mature M cells, GP2 immuno-expression was seen in each cytoplasmic area near intraepithelial lymphocytes IEL and perinuclear cytoplasm. In contrast, the perinuclear cytoplasm reaction was seen in immature M cells. Immune reactions were undetected throughout the cells' apical cytoplasm (29).

Miyazawa *et al*. (24), in their study on pigs, stated that young M cells, due to adjacent connection with lymphocytes in the margin related to FAE, differentiate firstly into mature M cells and far along into abortive columnar cells close to the apex of the dome. Other scientists stated that pig's Peyer's patches M cells, the same as M cells of the mouse (30) and rabbit (31), developed into enterocytes and then, in the apex, suffered from apoptosis, then expelled to the lumen. In the current study, depending on the existence of developed M cells with a tinny cytoplasm that borders intra-epithelium lymphocytes with their pouches in FAE in the margin of the area of the dome and demonstration of young M cells missing IEL pouches in the FAE close the crypts by GP2 immuno-histochemical staining, the views proposing that M cells arise from crypts have been favored. Additionally, according to many observations of several GP2-positive M cells in the apex FAE of some dome areas in an appendix in the current study thus, our research does not prove that M cells differentiate into enterocytes and are expelled into the apex lumen (32-34).

Conclusion

This study concluded that rabbits' apex of the cecum had well-developed lymphoid tissue compared to hamsters. Rabbits' M cell count was also higher, and most M cells were found in the rabbit's appendix.

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Conflict of interest

The author declares that there are no conflicts of interest.

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دراسة نسيجية شكلية قياسية وكيميائية نسيجية مناعية لقمة الأعور في الأرنب والهامستر وأهمية الخلية المطوية الدقيقة

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فرع التشريح، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أجريت هذه الدراسة على خمسة عشر أرنبا بالغا والعدد نفسه من الهامستر البالغ للتحري عن الاختلافات في تركيب الجزء القمي من الأعور لدى الأرانب والهامستر بالإضافة إلى توفير معلومات أساسية حول توزيع عدد الخلايا المطوية الدقيقة. كشف الفحص المجهري لجدران قمة الأعور لدى الأرانب والهامستر عن أربعة غلالات أساسية: الغلالة المخاطي، والغلالة تحت المخاطية، والعضلية والمصلية. كان الغشاء المخاطي للزائدة الدودية في الأرنب مليئا بطيات تشبه الأوراق مبطنة بظهارة عمودية بسيطة مع عدد كبير من الخلايا الكأسية. وبين هذه الطيات توجد تراكيب على هيئة قباب مكونة من تجمع عدد كبير من الجريبات اللمفاوية ومبطنة بنوع خاص من الظهارة يسمى الظهارة المرتبطة بالجريبات والتي تتميز بوجود عدد كبير من الخلايا المطوية الدقيقة التي كانت أكبر من الخلايا العمودية وتحتوي على العديد من الخلايا اللمفاوية. أظهر التعبير المناعي الكيميائي للخلايا المطوية الدقيقة تفاعلا قويا ضد الكلايكو بروتين الثاني في السيتوبلازم والأغشية الخلوية وخاصة في ظهارة قبة الزائدة الدودية للأرانب بينما كان التفاعل ضعيفا أو محدودا في الهامستر. فيما يتعلق بالظهارة المرتبطة بالجريبات في أعور الأرانب، تركز أكبر عدد من الخلايا المطوية الدقيقة في الجزء القاعدي من هذه الظهارة بينما تم التعبير عن أقل عدد في قمة الظهارة. استنتجت هذه الدراسة أن قمة الأعور في الأرنب تحتوي على نسيج لمفاوي عقيدي متطور أكثر مما في الهامستر وأن أعداد الخلايا المطوية الدقيقة كانت أكثر أيضا.