Introduction

Rabbits are true non-ruminant herbivores and considered as hind gut fermenters. They have a large cecum that can hold up to 40% of the intestinal contents and enables them to eat a primarily fibrous diet (Delaney-Johnson, 2006). The cecum and the vermiform appendix are the places where early lymphoid defense was provided from 30th days of gestation (Dorier et al., 1989). The rabbit contains better developed GALT in comparison with other mammalian species (Cesta, 2006). The GALT in the rabbit are commonly considered to constitute Peyer's patches, sacculus rotundus, appendix, and cecal patches (Haley, 2003); therefore, most of the studies regarding the lymphoid structure in the rabbit are focused on these structures (Gebert and Bartels 1991; Gebert et al., 1992; Jepson et al., 1992, 1993; Regoli et al., 1995). While, in other animals, as in ruminants, pigs, and dogs, the GALT are present in the Peyer's patches region (Haley 2003; Yasuda et al., 2006). There were many studies on the rabbit cecum and its lymphoid structure but the studies on the postnatal development were few so this study aimed to clarify the morphological and morphometrical changes in the cecal GALT during the postnatal stages.

Materials and methods

Thirty five apparent healthy rabbits of both sexes ranging from 1 day old to 16 weeks old were used in this study. For the light microscope, specimens from the ileocecal patch and appendix were collected and fixed in Bouin's fixative and processed for paraffin embedding. Paraffin sections of 5 µm thicknesses were stained with H&E, alcian blue, and PAS stains used for histological structures and micromorphometrical measurements. For scanning electron microscopy, representative tissue samples from different parts of the cecum and appendix were taken, fixed in glutaraldehyde 5% solution in 0.1M phosphate buffer for 18 hours at 4°C. After washing in the same buffer, the specimens were post-fixed in osmium tetraoxide 1% in phosphate buffer for 2 hours followed by washing in the same...
buffer. The samples were then dehydrated in ascending grades of ethanol followed by critical point drying in carbon dioxide then sputter-coated with gold and examined and photographed with a JEOL-5400LV scanning electron microscope.

Results

The rabbit cecum consisted of wide proximal base, narrower body and appendix twisted around itself forming three cecal turns. In all studied ages, the GALT were demonstrated at the ileocecal entrance "ileocecal patch" and in the blind end of the cecum "vermiform appendix" (Fig. 1). The ileocecal patch was irregularly oval-shaped and continued with the saccus rotundus at the end of the ilum.

It measured about 0.3, 0.5, 1.2, 1.5, 2.5 cm in diameter in the 1, 2, 4, 8 and 16 weeks old rabbits respectively. While, the cecal appendix was large and formed terminal blind end of the rabbit cecum. It was bright in color and characterized by thick wall and. It measured about 2.1, 3, 3.6, 4.2, 5.2 and 8.5 cm in 1, 2, 4, 8 and 16 weeks old rabbits respectively. The lymphoid follicles extended around the whole intestinal circumference of appendix (Table 1). In the rest parts of the cecum, the lymphoid follicles were not observed grossly.

Histologically, the gut associated lymphoid tissues were composed of many repeated lymphoid follicles, occupied the whole lamina propria and extended to the submucosa and separated from each other by narrow interfollicular spaces.

In the ileocecal patch, the GALT (Figs. 2a, b & c) consisted of dome- shaped lymphoid follicles separated from each other by low mucosal folds. The follicles were separated from the mucosal folds by clefts. The tips of these follicles became wide and flat with advancement of the age. In the newly born rabbit, they were consisted of small dome region and the ill-developed germinal center and separated from the adjacent by a wide interfollicular areas. With advanced age, the follicles increased in size and became composed of well defined dome region and germinal center up 2nd week of the age, while the interfollicular areas decreased. The dome regions were in direct contact with the lumen. They were covered with special epithelium which characterized by pale cells without well-defined brushed border, numerous intraepithelial lymph cells, a few enterocytes and the lack of goblet cells (Figs. 2d & e).

Table 1. Showing the measurements of the cecal patch and appendix

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>One day</th>
<th>Two weeks</th>
<th>Four weeks</th>
<th>Eight weeks</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall thickness (μm)</td>
<td>0.3 ± 0.08</td>
<td>0.5 ± 0.09</td>
<td>1.2 ± 0.1</td>
<td>1.5 ± 0.15</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Height of the lymph follicle (μm)</td>
<td>173 ± 30.3</td>
<td>231 ± 16.4</td>
<td>342 ± 29.7</td>
<td>423 ± 32.9</td>
<td>512 ± 56.2</td>
</tr>
<tr>
<td>Wall thickness of lymph follicle (μm)</td>
<td>50 ± 36.6</td>
<td>146 ± 90.6</td>
<td>185 ± 13.1</td>
<td>285 ± 12.2</td>
<td>246 ± 12.6</td>
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</tbody>
</table>

Fig. 1. The rabbit cecum consisted of base (B), body (Bo) and appendix (ap). The cecal patch (cp) situated at the ileocecal junction continued with the saccus rotundus (sr): (As) ascending colon, (Inf) intestinal folds.

In the appendix (Figs. 3a, b & c), the GALT were in the form of small lymph follicles in the newly born rabbits and increased rapid in size with age to become pyramidal lymph follicles at 15 days old. And up to 30 days old, these follicles enlarged and became barrel-shaped with round mucosal tip.

Table 1. Showing the measurements of the cecal patch and appendix
and fused with each other under the epithelial invagination. In the newly born rabbit, each lymphoid follicle consisted of dome region and germinal center, while up to the 2nd week of age the follicles presented four different regions: dome region, germinal center, coronal area and a wide interfollicular area between neighboring follicles. The covering mucosa of the appendix formed tongue shaped mucosal folds in the newly born rabbit. With the advancement of the age, these folds increased in size became elongated projections with wide luminal tips and appeared as umbrella like structures over the tips of the dome lymphoid follicles. Their covering epithelium (Figs. 3d &e) was cuboidal or short columnar contained many intraepithelial lymphocytes and lacked the goblet cells. The tips of the lymphoid follicles were covered with columnar epithelium while the sides covered with cuboidal epithelium with interepithelial lymphocytes.

Morphometrically (Table 1), the dimensions of the lymphoid follicles of the cecal GALT increased in size with the advancement of the age. But the increase differed from the cecal patch to the appendix and also in the dimension of the same structure. The height of the lymphoid follicles of the cecal patch in the adult rabbit was three folds that in the newly born rabbit, while their width at the former rabbits was 5 folds at the later ones. The height of the lymphoid follicles of the appendix in the adult rabbits was 6 folds that in newly born ones, while their width in the former was 3 folds that in the newly born rabbits.

By Scanning electron microscope, the mucosal surface of the ileocelecal patch presented dome shaped lymphoid follicles surrounded by mucosal ridges separated by deep grooves. In the newly born rabbits (Fig. 4a), the lymphoid structures were small irregular oval or rounded in shape and not separated completely from the surrounding ridges.
With the advancement of age, they increased gradually in size and became irregular rounded or dome shaped at two weeks old (Fig. 4b) and appeared as button like structures which completely separated from the surrounding ridges up to 4 weeks old (Fig. 4c). In all studied ages, the covering epithelium of the lymphoid structures consisted of cells with variable sizes, shapes, and surface structure (Fig. 4d & e). It consisted of large polygonal cells with dense, uniformly long microvilli (enteroabsorptive cells), smaller cells with less dense, thicker microvilli of irregular length (M cells). Goblet cells could not be observed on the surface of the lymphoid follicles. The epithelium of mucosal ridges and the intestinal crypts were consisted of large polygonal enteroabsorptive cells with uniformly long microvilli and goblet cells in addition to many round openings of the intestinal crypts. From the cut wall, the dome areas and lymphoid follicles showed a similar appearance, with tightly packed lymphoid-like cells generally with a smooth surface, but we were unable to find differences between germinal centers and mantle areas of the follicles.

At the appendix, the surface mucosa appeared in form of irregular mucosal folds separated by deep grooves contained many dome-shaped lymphoid structures (Figs. 5a, b, c & d). The surface epithelium of the mucosa folds consisted of microvilliated cells and goblet cells and showed many round openings of the intestinal glands in all studied ages (Fig. 5h). While that of the lymphoid structures consisted of irregular polygonal mi-
crovillated cells at their tips and consisted of two types of cells at their sides: small polygonal cells with dense, uniformly short microvilli and large irregular with micro-folded surface and lack the microvilli (Figs. 5e, f & g). The microvillated cells were similar to that of deep structures of the intestinal crypts.

Discussion

Gut associated lymphoid tissues were presented throughout the gastrointestinal tract in mammals and consisted of aggregated and solitary lymph nodes (Macdonald 2003; Newberry 2008). The rabbits presented better developed GALT in comparison with other mammalian species (Cesta, 2006). They were commonly considered to constitute Peyer's patches, sacculus rotundus, appendix, and ileoceleal patches (Haley, 2003). Whereas, cecum of the ruminants, pigs, and dogs, the GALT presented only in the ileoceleal patches (Haley, 2003; Yasuda et al., 2006). In the human, the GALT were found in the ileoceleal patches and appendix (Gebert and Bartels, 1991; Gebert et al., 1992; Jepson et al., 1992, 1993; Regoli et al., 1995). In the present study, GALT were macroscopically observed through both mucosal and serosal surfaces of the cecum at the ileoceleal patch and at the blind end vermillon appendix. The appearance and organization of these GALT were consistent with previous reports regarding classical Peyer's patches (Haley, 2003; Cesta, 2006).

The ileoceleal lymphoid tissue were composed of numerous aggregated lymph follicles, each of them have dome region, germinal center and separated by narrow inter-follicular areas in different studied ages. While, in the appendix, the lymphoid structures were composed of one lymphoid follicle with germinal center in the newly born rabbit, but up to the 2nd week of age the follicles present four different sites: a dome region, a germinal center, a coronal area, and a wide interfollicular area between neighboring follicles.

The lymph follicles were covered by low columnar epithelium containing intraepithelial lymphocytes and leukocytes, the same results have

Fig. 4 (a - e), 4a): SEM of cecal patch in one day old rabbits, showing the lymphoid follicles were small irregular oval or rounded in shape and not separated completely from the surrounding ridges. 4b & c) SEM of the cecal patch in rabbits, the lymphoid follicles were irregular rounded or dome shaped at two weeks old and appeared button like structures and completely separated from the surrounding ridges by deep clefts at 16 week old rabbits. 4d & e): SEM showing the surface epithelium of the cecal patch of newly born rabbit (4d) and adult rabbit (4e) respectively. It consisted of enteroabsorptive cells (Em) and M cells (M).
been reported by Snipes (1978) and Macdonald (2003) and Newberry (2008). The ileocecal lymphoid follicles were in direct contact with the lumen, while those of the appendix were covered by the interval mucosal folds. The dome epithelium provided both a protective barrier over lymphoid follicles and a route for antigen uptake from the gut, develops in rabbit cecum during the first week of neonatal life (Roy and Varvayanis, 1987). By SEM, the epithelium covered the lymphoid follicles was cuboidal or short columnar with two types of cells; enterocytes with homogeneous and tightly packed microvilli and another cell type, of reduced number with thicker and longer microvilli, the so-called M cells. These result are similar to that described in Pyre's patches of the pig (Torres-Medina, 1981 and Jose et al., 1990). So we can concluded that the ileocecal lymphoid patches are important in the immunity of the region. Where they were well situated functionally in that they are exposed both to unfermented ingesta from the ileum, and to the fermenting contents of the caecum and proximal colon. They seem to imply some effect on luminal flow which may create changes in the microenvironment. Although the functional significance of the recesses is not known, their structure would seem

Morphometrically; the lymphoid follicles of the cecal GALT increased in size with the advancement of the age. The height of the lymphoid folli-
icles of the cecal patch in the adult rabbit reach three folds that in the newly born rabbit, while their width at the former rabbits was 5 folds at the later ones. On other hand, the height of the lymphoid follicles of the appendix in the adult rabbits was 6 folds that in newly born rabbits, while their width in the former was 3 folds that in the newly born rabbits.

In the appendix, the mucosa presented two types of epithelium: a short columnar with goblet cells form tongue shaped mucosal ridges in the newly born rabbits. They increased in size and differ in shape with advancement of the age and became elongated projections (umbrella like villi) up to the 4th week of ages. Another short cuboid, almost smooth, at the level of the short conic projections or dome. The villus epithelium may have digestive functions of absorption and secretion, while the second, with smooth cells, similar to “M” cells, described for Peyer’s patches, could be antigen receiver and presenter cells (Shreedhar et al., 1996; Montcourrter et al., 2001). The specialized follicular associated epithelium containing M cells might represent induction sites for the mucosal immune system in (Pabst et al., 2005). Similar findings has been established in rabbit (Keren et al., 1978; Dasso et al., 2000; Iwatsuki et al., 2002) mice (Hamada et al., 2002), guinea pig (Rosner and Keren 1984), human (Moghaddami et al., 1998), and. Also the dome epithelium provides both a protective barrier over lymphoid follicles and a route for antigen uptake from the gut, develops in rabbit cecum during the first week of neonatal life (Roy and Varvayanis, 1987).

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References


