Light and ultra structural study of ileal peyer’s patches in goat foetuses


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ABSTRACT

By 94th day rudimentary form of Peyer’s Patches (PPs) appeared in the ilealsubmucosa and with advancing age it revealed lymphocytes, macrophage and few eosinophilic cells. In day old neonates PPs revealed secondary nodules with a predominance of lymphocyte, plasma cells, macrophages, reticular cells, RBCs etc. The collagen fibers and elastic fibers did not appear in outer thin fibrous covering of the ileal PP by 94 days of gestation. By 112 days few weakly reactive collagen fibers appeared at said location. With advancing age prominent and abundant reticular fibers were evident in the wall of arteriolar, capillaries and venules of the PP follicles and iliac submucosa as well as in lamina propria. Ultrastructurally ilealpeyer’s patches (PPs) revealed lymphocytes with elongated and ovoid nuclei. With advancing age medium and large lymphocytes appeared in a loose fashion. In day old neonates numerous and rapidly developing plasma cells were seen in the PP.

Key words: Goat foetus, Ileal payer’s patches, Light microscopy, Transmission electron microscopy.

INTRODUCTION

Intestinal immunity is made up of several types of lymphoid tissue that produce and store immune cells that carry out attacks and defend against pathogens. Aggregated lymphoid nodules or Peyer’s patches spread all over the three segments of small intestine and their concentration is more in the ileum (Dellmann,1993). They are usually confined in the submucosa on the side opposite to mesenteric attachment Popo et al. (2004). The diffuse and discrete lymphoid aggregation constitute the primary host defence immune component of the gut wall to respond the enteric antigens and even to eliminate them through local antibody secretion. The peyer’s patches in the ileum of calves are clearly developed at the birth and grow rapidly during the first week of life (Heilmann and Steinbach, 1978). Morphologically they contain three basic elements i.e. dome epithelium, follicle and thymus dependent area (Pospischil et.al, 1986). Light and electron microscopic studies showed the ontogeny of reticular cells in the ilealpeyer’s patches of sheep to start from day 70 of gestation (Nicander et.al. 1991). A thorough knowledge of the cell recruitment of Peyer’s patches is very essential to gain a comprehensive knowledge on the gut immunology and to form a basis for the interpretation of various pathological conditions of the gut. Hence, the present work has been undertaken to explore the prenatal development of theilealPeyer’s patches in goat.

MATERIALS AND METHODS

The study was conducted on goat foetuses of different ages. The approximate age was calculated using the “CRL- Gestation age” correlation of Norden and De Lahunta (1985) in sheep. The foetuses were grouped into 94, 99,112,130 and day old neonates for light microscopy and electron microscopy, on the basis of increasing gestational age. For light microscopy, the tissue pieces were collected in 10 percent neutral buffered formalin and were processed routinely to obtain 5 to 6μm thick serial paraffin sections. For transmission electron microscopy samples were fixed in 2.5% Glutaraldehyde (EM grade, Sigma) in 0.05m phosphate buffer (pH 7.2) for 24 hour at 4°C (during this lapse of 24 hours, the specimen were dispatched under ice pack to RUSKA Lab, College of Veterinary Science, SVVU, Rajendra Nagar, Hyderabad-30, India). Tissues were post fixed in 0.5% Osmium tetra oxide in the same buffer for 2 hours. Following fixation samples were dehydrated in a series of graded alcohol, infiltered and embedded in Araldite 6005 resin. Both semi thin and ultra-thin sections were cut with a glass knife on Leica Ultra Cut (Ultra Microtome UCT-GA-D/E-1/100). Semi thin sections of 200-300nm thick were...
TABLE 1: Showing the number of goat foeti with their crown-rump length and calculated age

<table>
<thead>
<tr>
<th>Number of foeti</th>
<th>Crown-rump length (cm)</th>
<th>Calculated gestational age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>26.5</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>130 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>Day old neonate</td>
</tr>
</tbody>
</table>

As the fetal age advanced, the PPs got conspicuous, compact and enlarged to become elliptical, pyramidal oval or elongated in outline, and occupied the 2/3rd of the submucosa in a cross section (Fig.2). By 112 days lymphocytes, macrophages and few eosinophilic cells appeared in the PP. Numerous capillaries also invaded the follicles. The lamina propria revealed few lymphocytes and granulocytes (Fig.3).

With age the capillary invasion increased, the nodules got compact and dense with strong basophilia and diffuse lymphatic tissue (DLT) were evident between the PP follicles. Stinson and Calhoun (1993) reported ileocaecal PPs to grow to a maximum size and maturation before birth and disappeared by 15 months of age of the sheep. The present observations are in agreement with the Beyaz and Asti (2004) who reported the development of ileal Peyer’s patches in bovine fetuses. In day old neonates PP revealed secondary lymphatic nodules (i.e. germinal center, mantle, ...
marginal zone) with a predominance of lymphocytes, plasma cells, macrophages, reticular cells, RBCs (Fig. 4). Present findings is tally with observation of Cesta (2006). The capillaries were abundant in the mantle of the nodules. The lamina propria revealed both dense and diffuse lymphoid aggregation (DLT).

The collagen fibers and elastic fibers did not appear in the outer thin fibrous covering of the ileal PP by 94th day of gestation. By 112th day the outer fibrous capsule of the ileal PP revealed a weak tinctorial affinity for the aniline blue of Masson’s trichrome stain. Reticular fibers appeared in the parenchyma of ileal PP. By 99 days the fibers were meager and with advancing age the reticular fibers got the continuity and appeared as thin fibers forming a meshwork around the lymphocytes of PP by 130 days of age. This is in agreement with the observations of Nicander et al. (1991) in sheep. The reticular fibers emanated from the capsule of the PP into peripheral region of the follicles. Prominent and abundant reticular fibers were evident in the wall of arterioles, capillaries and venules of the PP follicles and lamina propria (Fig. 5).

Under transmission electron microscopy ilealpeyer’s patches (PP) revealed the lymphocytes with elongated nuclei appeared in the pericapillary location. By 112th day the lymphocytes of the peripheral zone revealed ovoid nuclei of different sizes (Fig. 6).

With advancing age medium and large lymphocytes appeared in a loose fashion and involved the capillary network. Condensed chromatin was dispersed throughout the nuclear sap and the nuclear pores were distinct. The cytoplasm was moderately electron dense. The lymphoblasts revealed a large circular or ovoid central nucleus and it contained peripherally scattered chromatin material. These findings corroborate the report of Bessis (1964). The pores on the nuclear membrane were very distinct. A thin circular rim of granular cytoplasm around the nucleus was evident and it revealed small stakes of rough endoplasmic reticulum (Fig. 7). These fine structural features support the idea that

**FIG 4:** Photomicrograph of ileum of day old kid showing secondary lymphatic nodules (GC- Germinal centre, M- Mantle & MR- Marginal zone) and diffuse infiltration of lymphocytes into lamina propria.

H & E x 400

**FIG 5:** Photomicrograph of ileum of 130 day old goat foetus showing the distribution of reticular fibers (RF) in the lamina propria and submucosa.

Gomori’s silver reticular Stain x 400

**FIG 6:** Electron micrograph of a section of ilealpeyer’s patches of 112 day old goat fetus showing the ultra-structural configuration of peripheral zone of lymphocytes. (L- Lymphocyte, C- Cytoplasm, N- Nucleus)

Uranyl acetate x 7160

**FIG 7:** Electron micrograph of a section of ilealpeyer’s patches of one day old kid showing lymphoblasts (LB) with bulky cytoplasm. Note few rough endoplasmic reticulum and Golgi vesicles.

Uranyl acetate x 7160
the cells are still able to undertake proliferation and differentiation through protenacioussecretion. The euchromaticnucleus indicates its function capability to undertake karyokinesis.

In day old neonates numerous and rapidly developing plasma cells were seen in the PP. These cells were mostly round or ovoid in shape. The nucleus was spherical, eccentric and contained coarse masses of heterochromatin arranged radially in a cartwheel like configuration. The bulky cytoplasm was rich in rough endoplasmic reticulum, Golgi vesicles and sometimes fewelectron dense Russel’s bodies (Fig.8).

These findings are in agreement with the reports of Bessis (1964) and Banks (1993). On the other hand the plasmoblasts revealed a thicker cytoplasm with almost similar organelles that are contained in mature plasma cells, but the nuclear chromatin material did not appear to exhibit the typical cartwheel configuration(Fig.9).

The appearance of plasma cells/plasmoblasts in the ilealpp in day old neonates indicates the possession of antigenic challenge most likely through colostrum suckling by the kids.

In conclusion it was observed that during prenatal development the Peyer’s patches first appeared as aggregations of lymphocytes at 94 days of gestation. Distinct follicles with dome like structures appeared at 112 days. In day old neonates secondary lymphatic nodules and ultra-structurally plasma cells/plasmoblasts was observed. Hence, the kids acquires immunity by birth itself due to the well-developed Peyer’s patches in the gut. These findings will contribute to detect the pathological conditions and ascertain the immune responses in neonatal goat at birth.

REFERENCES


