Histochemical and transmission electron microscopic studies of spleen in prenatal stages of goat

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Received: 15-10-2012 Accepted: 15-09-2013

ABSTRACT

A study was conducted on twenty six goat foetuses of different ages to reveal the histochemical and ultrastructural observations of spleen. A strong PAS reaction was seen in all the splenic components of all ages of goat foeti. The APUD cells and mast cells were scattered in the splenic cords. Small and oval argentaffin cells were appeared in white pulp in day old neonates. A reticular cell synsítium carrying sequestered lymphocytes at central parenchyma of spleen of goat aged 106 day of gestation and with advancing age it revealed entrapment of more sequestered lymphocytes at different stages of gestation. In the spleen of day old kids pericapillary macrophage ingesting disintegrating lymphocytes was evident.

Key words: Histochemistry, Prenatal goat, Spleen, Transmission electron microscopy.

INTRODUCTION

The spleen is the largest mass of lymphatic tissue. Three types of spleen appear in different species i.e. defensive, intermediate and storage. Defensive spleen has few trabeculae and muscle fibers and abundant lymphatic tissue (lagomorphs, humans). The storage type has many trabeculae and smooth muscles. It is relatively large with minimum white pulp (horse, dog and cats). The intermediate form occurs in ruminants and swine (Banks, 1993). Histologically the spleen is surrounded by thin capsule in pre-natal life, which gradually becomes thicker in the post-natal life in the Gallus domesticus (Khalil et al., 2009). Ultra structurally the spleen is covered with capsule formed of dense fibroelastic connective tissue and myofibroblasts. The capsule is covered with mesothelial cells of the covering peritoneum in adult albino rabbit (Hegab, 2010). Although the histochemical and ultra-structural studies of post-natal spleen has been studied in several domestic animal but the literature on the developing spleen in goat embryo is very scanty. Hence the present work was undertaken with the objective of studying the histochemical behaviour and cellular changes in developing spleen for better understanding of immune function in neonates to accept environmental challenges.

MATERIALS AND METHODS

The study was conducted on twenty six 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 histochemical and ultrastructural studies on the basis of increasing gestational age (Table, 1). For histochemical study, serial paraffin sections of tissues were stained with PAS reaction for carbohydrates, Alcian blue for acid and strongly sulphated mucopolysaccharides, lead haematoxylin reaction for APUD cells, Masson-Hamperl method for argyrophilic cells, Ferric-ferricyanide reduction reaction for melanin and Thionin for mast cells (Barka and Anderson, 1963; Bancroft and Stevens, 1977).

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, Rajendranagar, Hyderabad, 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, infiltrated 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 thickness were stained with 0.2% Toluidine blue in 0.2m Borax buffer (pH 7.2) for 24 hours. Ultra-thin sections of thickness 50-70 nm

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were mounted on grids and stained with saturated aqueous Uranyl acetate followed by counter stain with 4% lead citrate. Sections were examined at different magnifications under transmission microscope (Hitachi: H-7500, Japan). Photomicrography was made and the electron-graphs were interpreted to analyse the ultrastructural configuration of lymphocytes, lymphoblasts, plasma cells etc. (Bozzola and Russell, 1999).

RESULTS AND DISCUSSION

The spleen of goat foeti aged day 94 of gestation revealed a strong PAS reaction in the endothelium of trabecular arterioles, nodular arterioles and in RBCs of the red pulp. The capsule and trabeculae revealed a strong PAS reaction (Fig. 1). No other changes could be observed with advancing age of the goat foeti. This is an agreement with findings of Waghaye (2007), who reported that the Periodic acid schiff’s reaction for the presence of glycogen was varying from moderate to intense in all the components at different age groups in spleen of goat. Alcian blue reaction was seen in the capsules, trabecular artery and white pulp in the spleen of 99 day old goat foeti. This observations was supported by report of Waghaye, (2007) in spleen of goat.

The mast cells and APUD cells were irregularly scattered in the peripheral part of the PALS, splenic nodules, DLT of the white pulp and red pulp in day old neonates (Fig.2). Melanin granules could not be localized in any structure of the spleen across all age groups of the goat foeti. Few small dense oval argentaffin cells appeared in the white pulp in isolated and scattered fashion. Argyrophilic cells could not be seen in the spleen of the developing goat foeti.

In transmission electron microscopic study the lymphocytes close to the sinusoids of the splenic red pulp of prenatal goat aged day 99 revealed dense chromatin condensation. The chromatin content was more compact and dense in the nuclei of some other lymphocytes lying in red pulp. The lymphocytes revealed cytoplasmic processes (Fig. 3). These findings corroborate the report of Bessis (1964) except the presence of vacuoles in the cytoplasm of the lymphocytes in spleen.

The sinusoidal wall lined with endothelial cells revealed dense elongated nucleus with heterochromatin. This observation was similar to findings of Burke and Simon (1970), Polak et al. (2009) and Hegab (2010). A reticular cell synsultium carrying the sequestered lymphocytes was evident in the central parenchyma of the spleen of goat fetus aged 106 days of gestation. The synsultium appeared to be invaded frequently by a profuse capillary bed. The sequestered lymphocytes revealed nuclear condensation, fragmentation, liquifaction and vacuolation (Fig. 4). The nuclear fragments or the apoptic bodies appeared in the cytoplasm of the macrophage and the macrophages were located in the strategic position of the reticular synsultium (Fig. 5). Reticular cells with oval elongated nuclei and processes were also evident on 112 days of gestation (Fig. 6). Similar findings were reported by Hegab (2010) in the spleen of adult albino rabbit.

By 130 days of gestation this reticular synsultium revealed entrapment of more lymphocytes at different stages of extensive apoptotic body formation particularly in the

<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>

FIG 1: Photomicrograph of spleen of 94 day old goat foetus showing alcian blue reaction (→) in the capsule, trabecular artery and white pulp. Note moderately strong PAS reaction (→) in the red pulp, capillary endothelium and inner part of capsule. Periodic acid Schiff Alcian blue x 400

FIG 2: Photomicrograph of spleen of day old neonate kid showing the distribution of APUD cells in the red pulp. Lead hamatoxylin x 1000

TABLE 1: Showing the number of goat foeti with their crown-rump length and calculated age
FIG 3: Electron micrograph of a section of splenic red pulp of 99 day old goat foetus showing dense and compact chromatin in the indented nuclei and thin cytoplasmic processes (CP).

LY- Lymphocyte    N-Nucleus    C- Cytoplasm
Uranyl acetate x 8950

pericapillary region of the reticular net. The arteriolar endothelial cells had elongated and indented nuclei containing diffusely scattered dense chromatin material. In the spleen of day old neonatal kid lymphocytes of different size with either euchromatic or heterochromatic nuclei were alined on the sinusoidal wall. The dense chromatin material was scattered in the periphery revealing distinct nuclear pores. Typical mature lymphocytes appeared in the periarteriolar lymphatic sheath of the spleen. The lymphoblasts revealed a more bulky cytoplasm with few

FIG 4: Electron micrograph of a section of splenic parenchyma of 106 day old goat fetus showing nuclear hypertrophy, chromatin-condensation, fragmentation, liquefaction and vacuolation in disintegrating lymphocytes trapped within the reticular synsitium.
Uranyl acetate x 7160

FIG 5: Electron micrograph of a section of splenic parenchyma of 106 day old goat fetus showing, amacrophage ingesting the nuclear fragments (Top right) of degenerating lymphocytes and capillary endothelial cells (left) of the reticular synsitium.
Uranyl acetate x 7160

FIG 6: Electron micrograph of a section of splenic white pulp of 112 day old goat fetus showing the ultra-structural configuration of the reticular cell. Note the vesicular, elongated and indented nucleus (NC) with an apparent nucleolus (NL). The cytoplasm (C) is scanty.
Uranyl acetate x 12530

FIG 7: Electron micrograph of a section of spleen of day old kid showing lymphoblasts (LB) with bulky cytoplasm. Note few rough endoplasmic reticulum (ER) and Golgi vesicles (→).
Uranyl acetate x 7160
rough endoplasmic reticulum and Golgi vesicles (Fig. 7). Plasma cells were oval in shape with eccentric nucleus at the junction of red pulp and white pulp in day old neonates.

The cytoplasm was rich in rough endoplasmic reticulum and Golgi vesicles (Fig. 8) similar observation was also made by 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. Bloom and Fawcett (1968) made similar observations on developing plasma cells.

REFERENCES