MORPHOLOGICAL STUDIES OF THE PEYER'S PATCH OF THE GOAT (Capra hircus). II. MICROSCOPIC CHANGES WITH AGE.

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SUMMARY

Light microscopic studies were performed on the Peyer's patches of neonatal, three and 12 months old goats. Neonatal kids had mature lymphoid follicles but with immature intervening villi epithelium. The jejunal, jejuno-ileal and colic Peyer's patches (JPP, JIPP and CPP, respectively) differed in their follicular histology and life history. The JIPP showed some properties of a primary lymphoid organ.

INTRODUCTION

Studies in the sheep and bovine have revealed the existence of two distinct types of Peyer's patches which may be different functionally (Reynolds, 1980; Reynolds and Morris, 1983; Miyasaka *et al.*, 1984). The rabbit has three types of Gut-associated lymphoid tissue follicles (Waksman et al., 1973).

Histological studies were undertaken to show the types and structure of the Peyer's patches of the caprine.

MATERIALS AND METHODS

Forty four goats were used in the experiment *viz* twelve hours-old kids (neonates which had not suckled and weighing 1.9 ± 0.3 (mean ± standard deviation) (n = 10) and four which suckled weighed 2.1 ± 0.2kg. Three months old kids weighing 5.0 ± 1.3 kg (n = 15) and twelve months old goats weighing 20.5 ± 2.9 kg (n = 15).

After marking the intestinal levels according to established boundaries (Habel, 1975 and Schummen *et al.*, 1979), the Peyer's patches were located by observing through the serosae of the intestines. About 2cm. long intestine section with a Peyer's patch randomly selected in the kid jejunum were ligated after squeezing out the contents. Bouin's fixative was injected into the ligated segments until mild distension. A similar procedure was done on the cranial, mid and caudal parts of JIPP as well as in the colon Peyer's patch.

About 1.5cm length of the Bouin's injected intestine segments were resected and fixed in Bouin's fixative. Similar specimens were also taken from the four kids which had suckled.

Specimens were processed for paraffin embedding, sectioned at 5um and stained with Harris-Haematoxylin-Eosin using a standard procedure (Stevens, 1982).

From each sample, five stained sections were selected for the study. On each section, the following parameters were studied.
(a) cortex - medulla differentiation of the lymphoid follicles
(b) depth and breadth of the follicles
(c) interfollicular area breadth and (d) dome size. The last three were measured by using a calibrated eye-piece. On each section, five of the above parameters were measured and the means recorded.

RESULTS

Peyer's patches at birth:

Ninety two percent of the Peyer's patches of the neonatal kids had follicles clearly differentiated into cortex and medulla. Connective tissue capsules separated the follicles from each other and from the interfollicular area.

The JPP follicles were short and pear-shaped, while of the JIPP were long cylindrical and tightly packed (Figs. 1 and 2). Follicles of the colon Peyer's patches were short, round or oval shaped. The follicles had an outer cortical zone of mainly small lymphocytes mixed with few large lymphocytes, tinge-body macrophages and reticular cells.

On the luminal side of the follicles, between the follicle center and the dome, the cortical layer form the corona. True germinal centers were not observed, but in the four kids which had suckled, the follicles showed a tremendous increased population of lymphocytes (compare Figs. 1 and 2).

The interfollicular areas were bordered on the luminar side by the lamina muscularis mucosa. Those of the JPP and CPP were broader than that of the JIPP. Post-capillary high-endothelial venules (PC-HEV) were present in the interfollicular areas. Eosinophilic globules were observed in the small intestine villi epithelium, lamina propria and occasionally in the lacteals of the kids which had suckled but not on the dome epithelium. These had similar staining with the lumenal contents (Fig.3).

Epithelial cells of the villi of kids which had not suckled were columnar and their nuclei were near the apices (Fig.4). In the kids which had suckled also in the three and 12 months old goats, the villi columnar epithelial cells had their nuclei near their bases.

Histological changes with age:

At three months age, 96% of the follicles were differentiated into cortex and medulla. The Peyer's patches follicles architecture was as in the neonatal kids, and they had the basic morphologic characters of dome, corona and interfollicular zone. In few observations, however, some follicles showed invagination on the domes. Nevertheless, only 20% of JPP and JIPP follicles showed typical germinal centers, but all the CPP follicles had germinal centers. Mitotic cells were frequently observed in the periphery of the germinal centers and PC-HEV were evident in the interfollicular areas.

In the goats of 12 months age group, all the Peyer's patches had distinct cortices, medulla and germinal centers, and their shapes were as for the neonatal and three months old kids. The interfollicular areas were generally widest in the JPP. Some follicles in the JIPP were smaller than the adjacent ones and they showed an increased infiltration with reticular cells. PC-HEV were evident in the interfollicular areas.

The mean depth and breadth of the Peyer's patches follicles are shown in Table 1. Generally, the neonatal kids had the smallest depth and breadth. However, an interesting observation was that the mid JIPP of the 12 months old goats had a depth which was significantly ($P<0.05$) lower than that of the neonatal and three months old kids. Its mean breadth was also significantly ($P<0.05$) lower than that of three months old kids

Table 1: Mean depth and breadth (cm.) of Peyer's patches follicles of neonate, 3 months and 12 months old goats (Mean ± Standard deviation).

Age	Follicle 2	3	4	5	6
Neonate					
Depth	0.65±0.14	0.72±0.12	0.73±0.10	0.65±0.07	0.29±0.02
Breadth	0.42±0.04	0.45±0.03	0.28±0.01	0.31±0.02	0.27±0.04
3Months					
Depth	0.68±0.01	0.84±0.12	1.06±0.12	0.96±0.06	0.81±0.08
Breadth	0.62±0.07	0.45±0.03	0.39±0.02	0.47±0.02	0.56±0.06
12Months					
Depth	0.87±0.01	0.76±0.12	0.68±0.15	0.99±0.07	0.76±0.07
Breadth	0.60±0.04	0.45±0.05	0.31±0.23	0.52±0.08	0.72±0.07

2= Jejunal Peyer's patch; 3= Proximal JIPP;
4= Mid-JIPP; 5= Distal JIPP; 6= Colic Peyer's patch.

Figure 1: JPP follicle of a neonatal kid which had not suckled. Note its shape, height of the dome and the lymphoid cells population. (H. & E. 125x).

Figure 2: Mid JIPP follicles from a neonatal kid which had suckled. Note the shape of the follicles, height of the domes and the lymphoid cells population in contrast to Fig. 1. (H.&E. 125x).

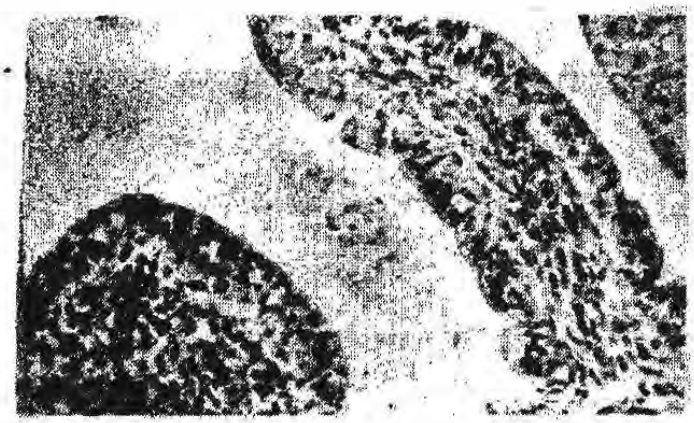

Figure 3: Dome (D) and villi (V) of JPP from a neonatal kid which had suckled. Note the eosinophilic globules in the epithelial cells of the villi but not on the dome (H.&E. 250x).

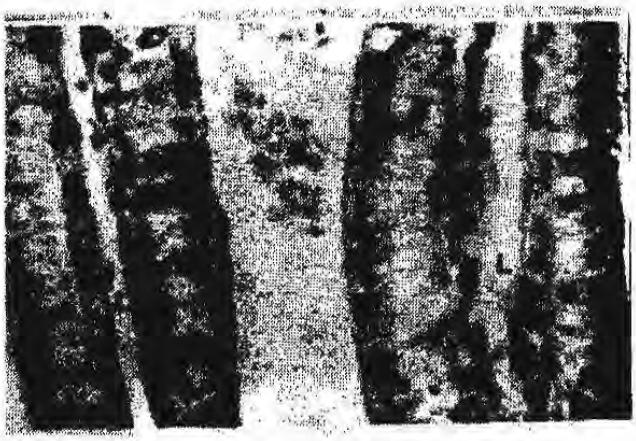

Figure 4: Villi from a neonatal kid which had not suckled. Note the apical position of the epithelial cells' nuclei. L, lacteals (H.&E. 500x).

and about equal to that of neonatal kids.

DISCUSSION

Peyer's patch at birth:

Peyer's patches are prenatally formed in the dog, cattle and sheep (Bryant and Shilfrine, 1972; Doughri *et al.*, 1972; Reynolds and Morris, 1983). Kids were killed within twelve hours after birth. It is therefore very likely that, like the sheep, cattle and dog, the observed maturity of the patches occured during the prenatal period.

The nature of the caprine placenta which is of epithelio-chorial and partly syndesmochorial makes the environment in which the foetus develops antigen-free both from exterogenic antigens and maternal immunoglobulins (Brambell, 1970). Therefore, the histological maturity of the Peyer's patches follicles observed in the neonatal kids most likely had occured in the absence of antigenic stimulation. However, there could have been autogenous enterogenic antigens, but these have not been shown to exist at least in the sheep which have same type of placenta and equal gestation period as the goat (Reynolds, 1980). Factors other than antigens must have caused the lymphopoietic activity in the Peyer's patches of the kids in a similar manner as in the avian bursa.

The presence of HE-PCV in the interfollicular areas of the neonatal kids implies that the avenues for lymphocytes recirculation are established either prenatally or within twelve hours after birth. In lambs, lymphocytes recirculation starts in the foetal period (Cole and Morris, 1973 and Reynolds, 1976).

In the four kids which had suckled, the eosinophilic globules which were observed on the villi epithelium, lamina propria and lacteals were most probably colostral proteins. Similar globules have been reported in piglets of less than five days of age and similarly, they were not observed on the dome epithelium (Chu *et al.*, 1979). Maternal antibodies in the colostrum are transferred across the

intestinal epithelium a few hours after birth (Simpson-Morgan and Smeaton, 1972). The findings in this study indicate that colostral proteins are absorbed by villi epithelium but not probably by the dome epithelium. The latter probably have other immunological functions as suggested by other workers (Bockman and Cooper, 1973; Owen and Jones, 1974; Landsverk, 1981; Chu and Liu, 1984). The invaginations which were occasionally observed on the dome epithelium and the significantly higher domes in the JIPP could have a function of increasing the surface area for antigen contact.

The difference in the position of the nuclei of the villi epithelial cells in kids which had not suckled on one hand, those which had suckled and three and 12 months old goats on the other hand is also of interest. It is possible that kids are born with immature villi epithelium which matures when the gut function of absorption is initiated. Whether it is the absorption *per se* or other unknown factors which are involved in eliciting this change remains to be clarified.

Microanatomy of the Peyer's patches:

The caprine dome epithelium was columnar and had few goblet cells like that of pigs (Chu *et al.*, 1979). It is noncolumnar in sheep (Reynolds and Morris, 1983). On the dome epithelium of rabbits and calves Peyer's patches and avian bursa, goblet cells are lacking (Hess *et al.*, 1973; Schaffner *et al.*, 1974; Landverk, 1981; Burns, 1982). So there seem to be variation between species on the microanatomy of the dome epithelium.

Similar to observations in sheep and calves (Reynolds and Morris, 1983; Landsverk 1984) the caprine shows differences between the microanatomy of the JPP and that of JIPP. The rabbits have three types of follicles (Waksman *et al.*, 1973). The histology of the rabbit appendix resembles the caprine JIPP, that of the Peyer's patches resembles the JPP follicles while the histology of the sacculus rotundus resembles the CPP follicles. The difference between the microanatomy of the JPP, JIPP and CPP raise a possibility that these three types of Peyer's patches may also be different functionally.

The rabbit appendix (which histologically resembles the JIPP) shows some features in common with the avian bursa (Archer *et al.* 1964; Stramignoni and Mollo, 1968; Perey *et al.*, 1970; Cooper and Lawton, 1972). In the sheep the ileocecal (or ileal) Peyer's patch (equivalent to the JIPP) is the major site for B-lymphocyte production (Reynolds, 1976; Gerber, 1989; Miyasaka *et al.*, 1984). On the other hand, the JPP is the major site for T-lymphocytes recirculation (Kagnoff, 1981). The histological resemblance of the caprine JIPP to the rabbit appendix and ileal Peyer's patches of sheep, which are structures with some primary lymphoid organ properties is worth noting. Whether the caprine JIPP is also a major site for B-lymphocytes production is yet to be determined. However, the variation in the extent of germinal centers development in the JPP, JIPP and CPP of the three months old kids is worth noting. Reasons as to why they were poorly developed in the JPP and JIPP follicles are not known. But probably, the JPP and the JIPP have a different immunological function around the three months age but at 12 months age they may together with the CPP be performing the same immunological function. However, the large interfollicular areas with HE-PCV in the JPP implies that the JPP may as in the sheep, calf and rabbit, probably serve as the major site for T-

lymphocytes recirculation (Waksman, 1973; Kagnoff, 1981; Larsen and Landsverk, 1985).

Gross atrophy of the JIPP was supported by histological observations and it seemed to have started at the mid-region of this long patch. The JPP and CPP did not show signs of atrophy. The thymus and avian bursa are maximally developed early in postnatal life and involute later in life (Cole and Morris, 1973; Chaffner, *et al.*, 1974). The caprine JIPP seems to be maximally developed at about three months age. The histological maturity of the JIPP in the neonatal kids, its maximal development in early postnatal life, its proximity to the gut lumen and its involution with advancing age resembles a primary lymphoid organ.

ACKNOWLEDGEMENTS

The author wishes to thank Professors Per Kjaersgaard and Nils Bjorkman for their supervision and DANIDA for financing the project.

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