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Ontogeny of ovine lymphocytes

III. AN IMMUNOHISTOLOGICAL STUDY ON THE DEVELOPMENT OF T LYMPHOCYTES IN SHEEP FETAL LYMPH NODES

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SUMMARY

The development of T lymphocytes in ovine fetal lymph nodes was studied immunohistologically using a panel of monoclonal antibodies. T lymphocyte subsets appeared within the ovine fetal lymph node in a specific sequence. SBU-T1- and SBU-T8-positive lymphocytes were seen at Day 47 of gestation. LCA, sIg, MHC I, MHC II, SBU-T6 and 46.66 antigens were also seen within the lymph node at this time. The first SBU-T4-positive cells were seen within the fetal lymph node at Day 50 of gestation, along with the first 20.96-, 25.69- and 38.38-positive cells. SBU-T19 lymphocytes appeared later in gestation, being present in fetal lymph nodes from Day 69 of gestation. The appearance and distribution of T and B cells in the developing lymph nodes of the ovine fetus is described and compared with the ontogeny of lymphocytes in the fetal thymus and spleen.

INTRODUCTION

The ability of the fetal lamb to respond to various antigens develops in a sequential fashion during the 150-day gestational period of the sheep (Silverstein *et al.*, 1963; Fahey & Morris, 1974); however, the fetus is capable of responding to a wide range of antigens over a short period of time around mid-gestation (Fahey & Morris, 1974). Lymphocytes are present in the thoracic duct lymph of the sheep as early as Day 75 of gestation (Pearson, Simpson-Morgan & Morris, 1976). The fetal pool of the recirculating lymphocytes increases exponentially over the last third of the gestation period, reflecting the increasing mass of total lymphoid tissue (Cahill *et al.*, 1980).

The fetal and neonatal development of lymph nodes has been investigated by light microscopy in several mammalian species, but very little is known about the development of T and B lymphocyte areas within the mammalian lymph node. Although lymphocytes have been found within ovine fetal lymph nodes at 30–45 days of gestation (Tuboly, Glavits & Bucsek, 1984), it is not known whether these lymphocytes are of the T- or B-lymphocyte lineages.

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Abbreviations: g.a., gestational age; LCA, leucocyte common antigen; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; sIg, surface immunoglobulin.

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This paper reports the results of a study on the ontogeny of T and B lymphocytes using monoclonal antibodies to sheep lymphocytes in the developing ovine fetal lymph node.

MATERIALS AND METHODS

The methods and monoclonal antibodies used in this paper are described in accompanying papers (see pages 97 and 107). Details of the fetuses used are presented in Table 1.

RESULTS

The lymph nodes examined in this study were the prescapular and mesenteric lymph nodes. The anatomy of the lymph node is described in the context of a peripheral cortex, deep cortical units and a medulla (Belisle & Sainte-Marie, 1981). The earliest prescapular lymph node studied was taken from a fetus of 47 days g.a. Although lymph nodes were present before this time, it was difficult to locate them macroscopically. The earliest mesenteric lymph nodes studied were taken from a fetus of 64 days g.a. The mesenteric lymph nodes resembled the prescapular lymph node in overall morphology at all fetal ages studied, except that they were smaller and less developed, possessing smaller deep cortical units. A small number of popliteal lymph nodes were also examined from late term fetuses and neonatal lambs.

Day 47–Day 48

At this stage of gestation, the prescapular lymph node consisted of a loose mesenchymal reticulum which contained sparsely

Table 1. Details of fetuses and lambs used to study the ontogeny of ovine prescapular lymph nodes

Crown-rump length (cm)	Age (days)*	Number of fetuses or lambs
7·7–8·2	47–48	2
9·1–12·5	50–58	7
15–20	64–77†	3
26·5–32	93–106†	4
35–48	113–145†	6
ND	0–14†‡	4

ND, not determined.

* Unless indicated, refers to gestational age. Gestational ages were determined from crown-rump lengths using the formula derived by Barcroft (1946).

† Mesenteric lymph nodes were also studied.

‡ Postnatal age.

located large lymphoid cells (Fig. 1). The outer area of the lymph node contained more lymphocytes than the inner area, which consisted mainly of blood vessels and lymphatics. There was no obvious capsule and the subcapsular sinus delineated the lymph node from the surrounding mesenchyme. Many cells within the lymph node expressed LCA antigens and some of these cells appeared dendritic. Some lymphocytes were seen which expressed T-cell markers, and a smaller number of sIg-positive lymphocytes were identified. The T and B lymphocytes were found just below the subcapsular sinus in the primitive cortex. The antigens which were observed on cells within the lymph node at this time were SBU-T1, SBU-T6, SBU-T8, 46.66, MHC I, MHC II and LCA. No cells were found which expressed the SBU-T4, SBU-T19, 20.96 or 38.38 antigens. MHC I antigens were seen on all cells within the lymph node and the staining pattern was predominantly reticular.

Day 50–Day 58

By Day 50 of gestation, ovine fetal lymph nodes consisted of a very thin lymphocytic cortex surrounding a large medulla which contained few lymphocytes. The first SBU-T4-, 20.96-, 25.69- and 38.38-positive cells were observed, and the majority of these cells, along with cells expressing other lymphocyte markers, were located in the cortex of the lymph node and the area immediately beneath it (Fig. 2a). Small numbers of intense SBU-T6 cells were found within the lymph node (Fig. 2b) and, as in younger and older fetuses, MHC I antigens were seen on all cells within the lymph node (Fig. 2c). No SBU-T19-positive cells were found in fetal lymph nodes of this age.

Primitive deep cortical units began to appear as ovoid accumulations of lymphoid cells in the cortex (Fig. 1). In contrast to lymph nodes from postnatal lambs which contained many deep cortical units, there were usually only one or two of these units per lymph node. The majority of sIg-positive cells were located within the peripheral cortex while occasional sIg-positive lymphocytes were also scattered randomly within the remainder of the lymph node.

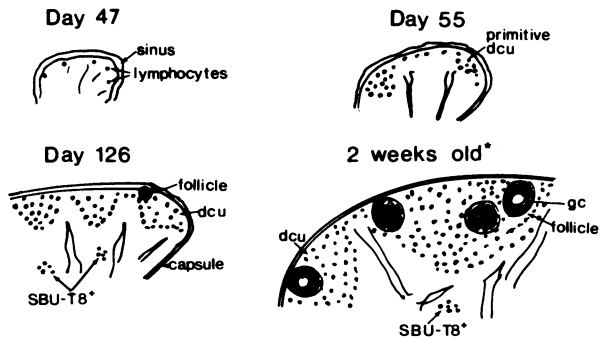

Figure 1. Development of the ovine fetal lymph node. Day 47, the subcapsular sinus separates the lymph node from the surrounding mesenchyme. Day 55, primitive deep cortical units are apparent within the lymph node. Trabeculae have started to appear. Day 126, the lymph node contains follicles and deep cortical units and is surrounded by a well-developed capsule. 2 weeks of age, germinal centres are apparent within some follicles. Clusters of SBU-T8-positive cells are found within the medulla. gc, germinal centre; dcu, deep cortical unit. * Postnatal age; † positive.

Day 64–Day 77

The peripheral cortex of the prescapular lymph node was thicker in lymph nodes of 64 days g.a. than in lymph nodes from younger fetuses. The deep cortical units were more developed, and the first signs of a capsule were apparent.

Lymph nodes contained intense MHC I- and MHC II-positive cells, which were particularly common in the cortex. The numbers of positive SBU-T1 (Fig. 3a), SBU-T4 (Fig. 3b), SBU-T8 (Fig. 3c), sIg, 20.96, 25.69, 38.38 and 46.66 cells had increased. A small number of SBU-T6-positive cells were found in the cortex of the lymph node. Most of the T lymphocytes were located within the cortex, although there were a few scattered SBU-T1- and SBU-T8-positive cells within the medulla. Very few, if any, T lymphocytes were found in the mesenchyme surrounding the node, although many LCA-positive cells were seen in this region.

SBU-T19-positive cells were first found in prescapular lymph nodes from fetuses of 69 days g.a. These cells were few in number, and were mainly situated within the cortex of the node. Lymphocytes expressing sIg were found scattered singly within the peripheral cortex as well as within the small follicles in the cortex of Day 69 lymph nodes. The cells within these follicles were also stained by 20.96 and 25.69. More cells were stained by 20.96 within the cortex of the lymph node than expressed sIg; however, within the medulla the reverse situation was found with more cells expressing sIg than were stained by 20.96. More cells expressed MHC II antigens than expressed sIg, reflecting the expression of MHC II antigens on non-lymphoid cells.

Day 93–Day 106

With increasing gestational age, the peripheral cortex of the fetal lymph node increased in thickness, although it still remained very thin in comparison to the medulla. The number of deep cortical units per lymph node increased as did the size of these units. The B lymphocyte follicles also slowly increased in size and these follicles protruded into the subcapsular sinus. The capsule was more distinct than in lymph nodes from younger fetuses, and was several cells deep. Reticular staining for MHC II was most intense within the cortical units and was weaker elsewhere within the lymph node.

As well as SBU-T8-positive cells being observed in the cortex of the lymph node, small foci of SBU-T8-positive lymphocytes

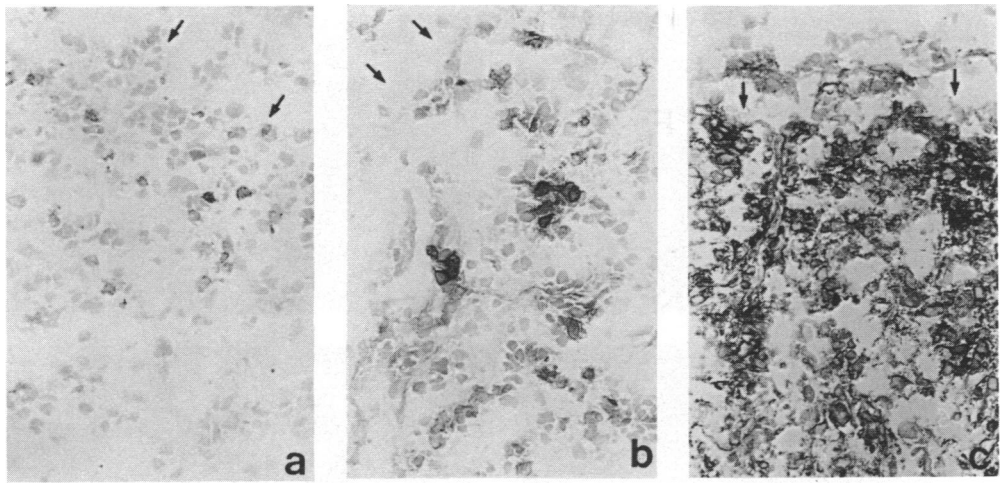

Figure 2. Distribution of lymphocyte populations within ovine prescapular lymph nodes at Day 50 of gestation. (a) SBU-T1; (b) SBU-T6; and (c) MHC I. Arrows indicate the subcapsular sinus. Magnification × 150.

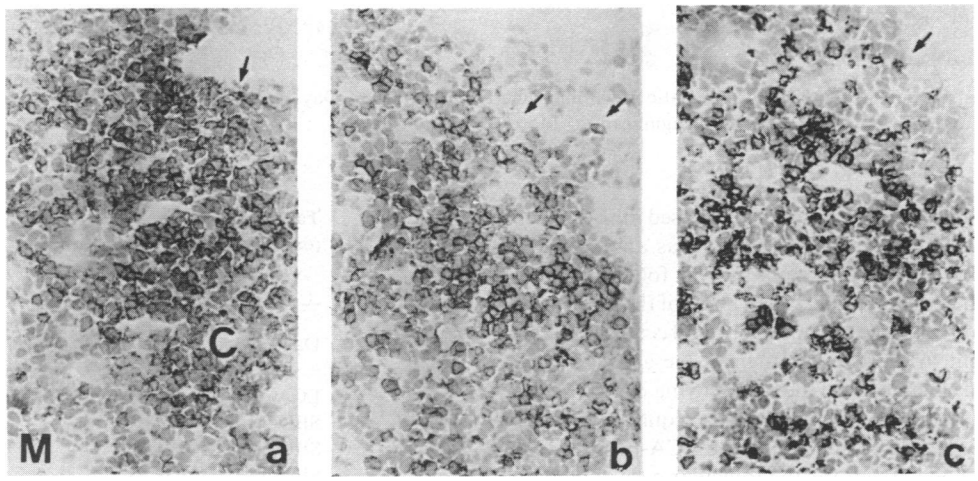

Figure 3. Distribution of lymphocyte populations within ovine prescapular lymph node at Day 69 of gestation. (a) SBU-T1; (b) SBU-T4; and (c) SBU-T8. Arrows indicate the subcapsular sinus. C, Cortex; M, Medulla. Magnification × 150.

were found in the medullary areas just beneath the cortex. Many SBU-T8-positive cells were also found scattered throughout the medulla. The SBU-T8 foci, which also expressed SBU-T1 and 46.66 antigens, increased in size with increasing gestational age. Few SBU-T4- or 38.38 positive cells were found in the medulla. The SBU-T4-positive cells in the peripheral cortex and deep cortical units outnumbered the SBU-T8-positive cells.

Day 113–Day 145

Fetal lymph nodes of this age were very similar in structure to lymph nodes from younger fetuses (Fig. 1). The ratio of cortical to medullary tissue increased slightly, and this was more apparent in the mesenteric lymph nodes than in the prescapular lymph node. More B lymphocyte follicles were found within the cortex than in lymph nodes from younger fetuses. The number of SBU-T4-positive cells in the cortex was about twice that of the SBU-T8-positive cells (Figs 4b–c). The foci of SBU-T8-positive cells within the medulla increased in size. The number of SBU-T4 cells within the medulla increased as gestation approached term. Antibodies MHC II showed intense staining of the follicles with less intense inter-follicular and medullary staining (Fig. 4d).

The size of the follicles within lymph nodes from fetuses at Day 113 of gestation was approximately double the size of follicles at Day 77 of gestation, while the size of follicles within lymph nodes of fetuses at Day 145 of gestation was twice as large as found at Day 113 of gestation. The follicles contained some SBU-T1- (Fig. 4a) and SBU-T4-positive cells in addition to lymphocytes which expressed sIg, 20.96 and 25.69. A small number of scattered sIg- and 20.96-positive cells were also seen within the peripheral cortex.

Small clusters of small cells with very intense basophilic nuclei were found in the medulla of the lymph node at this stage of gestation. These cells were reminiscent of cells seen in the fetal liver and spleen which were stained by the monoclonal antibodies 17.3 and 46.57 (Maddox, Mackay & Brandon, 1986).

Birth–14 days of age

In the first week after birth there was a vast increase in the size of

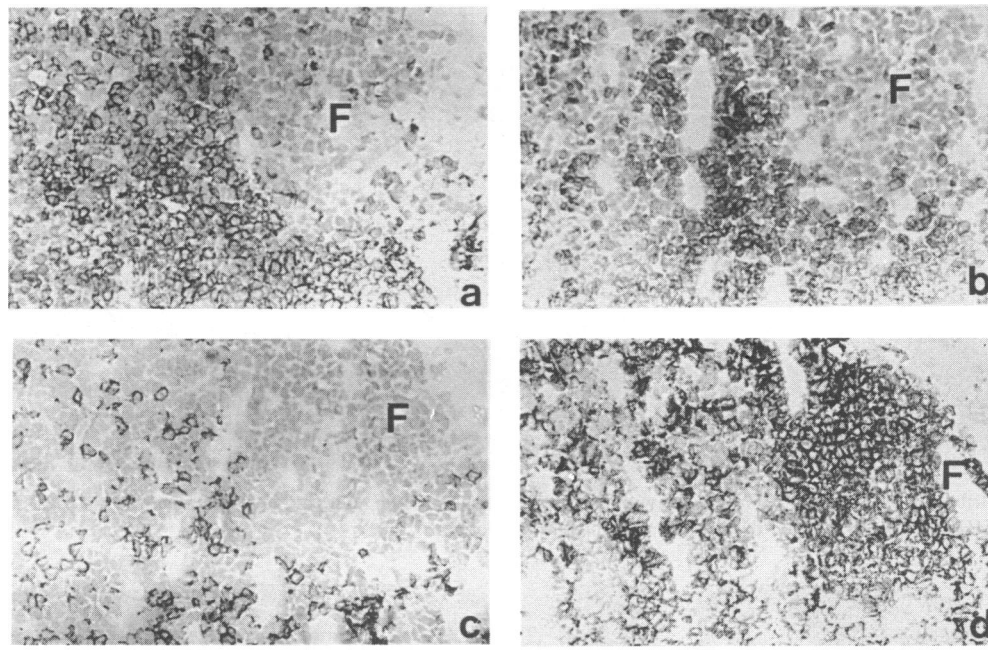

Figure 4. Distribution of lymphocyte populations within ovine lymph nodes at Day 113 of gestation. (a) SBU-T1; (b) SBU-T4; (c) SBU-T8; and (d) MHC II. F, Follicle. Magnification × 150.

the lymph nodes. The deep cortical units increased in size, as did the follicles (Fig. 1). The peripheral cortex was absent in the areas between the deep cortical units. The follicles of the prescapular lymph node were larger than those of the mesenteric lymph nodes. Some follicles contained germinal centres and this was more apparent in prescapular than in mesenteric lymph nodes. Trabeculae extended into the parenchyma of the prescapular lymph node and the thickness of the connective tissue capsule increased. The 46.66 stained all the LCA-positive cells within the lymph node except for the follicular cells.

The SBU-T19 antigen was present on many cells situated within the medulla and within the cortex of the lymph node. In the deep cortical units SBU-T19-positive cells were found mainly between the follicles and the medulla.

The 20.96 stained many cells within the deep cortical units and most cells within the follicles, as well as many cells within the medulla. This contrasted with earlier stages of development of the lymph node where staining by 20.96 appeared to be predominantly restricted to B cells.

By two weeks after birth, the structure of the lymph nodes had undergone a change. Lymph nodes had increased substantially in size, and the medulla was no longer much larger than the cortex. This increase in cortical size was due to both an increase in the number and size of the B-lymphocyte follicles. Instead of most follicles being located just beneath the subcapsular sinus, as in earlier lymph nodes, follicles were scattered throughout the cortex. This is especially apparent in the mesenteric lymph nodes. Germinal centres were present in some of the follicles (Fig. 1), with this being more apparent in the prescapular lymph node than in the mesenteric lymph nodes. The 20.96 strongly stained cells located within the follicular mantle, and stained cells within the follicular germinal centres weakly or not at all.

SBU-T4- and 38.38-positive cells were found mainly in the peripheral cortex and deep cortical units. There were fewer SBU-T8-positive cells than SBU-T4-positive cells in the cortex, however there were many more SBU-T8-positive cells in the medulla. SBU-T1- and 46.66-positive cells were found in both the medulla and the cortex. Fewer SBU-T19-positive cells were seen within the lymph nodes of 14-day-old lambs and these cells were found mainly adjacent to follicles in the cortex and within the medulla. This pattern resembled that found in older lambs and adult sheep (Mackay, Maddox & Brandon, 1986).

Table 2. The times of first appearance (days of gestation) of antigens on cells within the ovine foetal lymph node

Day 47*	Day 50	Day 69
LCA	SBU-T4	SBU-T19
SBU-T1	20.96	
SBU-T6	25.69	
SBU-T8	38.38	
MHC I		
MHC II		
46.66		
sIg		

* Earliest age examined.

DISCUSSION

The colonization of lymph nodes by T and B lymphocyte subpopulations has been followed from Day 47 of gestation in fetal sheep. Summaries of when the lymph node is first colonized by lymphocytes and a diagrammatical representation of lymph

node development are presented in Table 2 and Fig. 1, respectively. As cells expressing T and B markers were found in the earliest lymph nodes studied, it is unknown which lineage appears first within the node, although there were greater numbers of T lymphocytes. As some of these antigens are not T-cell specific, it is possible that some of these cells are actually of other leucocyte lineages (Maddox, Mackay & Brandon, 1987a). Substantial development of ovine lymph nodes takes place during gestation, with the appearance of all the elements of adult lymph nodes except the follicular germinal centres. Lymph nodes are relatively small in fetuses, as compared to the size they reach following antigenic stimulation in neonates. Stimulation of the fetal lamb with antigen accelerates the development of the fetal lymphoid immune system (Fahey & Morris, 1978); while removing the thymus from fetal lambs of 60 days g.a. retards the subsequent development of the lymphoid system and reduces the size of the recirculating pool of lymphocytes (Cole & Morris, 1973).

No germinal centres were found in ovine lymph nodes during gestation. Germinal centres started to appear in ovine lymph nodes 2 weeks after birth. Antigenic stimulation during gestation has been reported to produce germinal centres and increase the rate of development of fetal lymph nodes (Fahey, 1976).

B-cell development within ovine fetal lymph nodes resembles that reported for human fetal lymph nodes. Lymphocytes are first seen in human lymph nodes during Week 12 of gestation (Markgraf, von Gaudecker & Muller-Hermelink, 1982), and follicles are first detected at 17 weeks of gestation (Bofill *et al.*, 1985).

It is difficult to compare development of lymph nodes between sheep and rodents, as rodents are exposed to antigen before significant development of their lymph nodes has occurred. New-born mouse mesenteric lymph nodes have many T lymphocytes but few B lymphocytes (Friedberg & Weissman, 1974). By the eighth day after birth the number of B lymphocytes has increased and these cells are found in follicles, although no germinal centres are present (Friedberg & Weissman, 1974). It is possible that the development of lymph nodes in germ-free conditions represents development observed in fetal lambs. Studies comparing animals raised in germ-free conditions with those raised normally have shown that the development of lymph nodes within germ-free animals is much slower than in normal animals, proceeds to a lesser extent, and then undergoes involution (Kovaru *et al.*, 1979).

Table 3. Summary of the times of first appearance (days of gestation) of T lymphocyte antigens within the ovine embryo and fetus

	Body	Thymus	Spleen	Lymph node
SBU-T1	33	35	43–44*	47*
SBU-T4	35	35–38	50–50	50
SBU-T8	35	35	43–44*	47*
SBU-T19	50–58	50–56	57	69
38.38	22	42	50–55	50

* Earliest age examined.

The sequence of appearance of the lymphocyte subpopulations within the ovine fetus was the same for the lymph node as for the spleen and thymus (Table 3). The major difference was the delay before colonization of the lymph node and spleen as compared to the thymus. SBU-T4-positive cells were seen within the fetal thymus and dorsal mesentery 15–20 days before they were seen within the fetal lymph node and spleen. SBU-T8-positive cells were always seen before SBU-T4-positive cells within lymphoid organs, and SBU-T4-positive cells were themselves found before SBU-T19-positive cells. SBU-T19-positive cells were seen within the fetal spleen and thymus at similar times, but were not observed in the fetal lymph node until later in gestation. The similarity of the times of first appearance of SBU-T19-positive cells within the thymus and spleen raises the question of whether SBU-T19 cells pass through the thymus on their way to the spleen.

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