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Ontogeny of ovine lymphocytes

I. AN IMMUNOHISTOLOGICAL STUDY ON THE DEVELOPMENT OF T LYMPHOCYTES IN THE SHEEP EMBRYO AND FETAL THYMUS

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SUMMARY

The time of appearance of lymphocytes expressing T-cell markers and the subsequent development of the fetal thymus were studied in ovine embryos using a panel of monoclonal antibodies. Leucocyte common antigen (LCA) and major histocompatibility complex class I (MHC I) antigens were seen on a small number of cells within the ovine embryo at Day 19 of gestation. SBU-T6 (CD1)-positive cells were found at Day 22 of gestation, while major histocompatibility complex class II (MHC II) antigens were first observed at Day 25 of gestation. Large basophilic cells, weakly staining for SBU-T1 (CD5), were present in the mesenchyme of the neck and in the dorsal mediastinum and mesentery of embryonic sheep of 33 days gestational age (g.a.); however, no SBU-T1-positive cells were detected in the thymus at this time. No SBU-T4 (CD4)- or SBU-T8 (CD8)-positive cells were detected in any organs of embryos of this age. SBU-T4- and SBU-T8-positive cells were first seen in fetal thymi, and elsewhere within the fetus, at 35–38 days g.a. SBU-T19-positive cells were first seen within the fetal thymus at 50–58 days g.a.

INTRODUCTION

During the embryonic stage of gestation, genesis of the main organs occurs; in sheep this period is before Day 33 of gestation (Green & Winters, 1946). Within the fetal stage of gestation, growth and differentiation of the organs take place. The thymus is the first organ of the immune system to become lymphocytic during histogenesis of mammalian lymphoid tissues (Manning, 1981). The ovine thymus arises as an outgrowth of the ventral diverticulum of the third pharyngeal pouch (Bryden, Evans & Binns, 1972; Jordan, 1976). Large basophilic cells are first seen within the epithelial thymic primordium at 30–31 days gestational age (g.a.), and by 36–38 days lymphopoiesis is well established (Jordan, 1976).

Monoclonal antibodies to ovine lymphocyte antigens have only recently been described (Miyasaka *et al.*, 1983; Gogolin-Ewens *et al.*, 1985; Maddox, Mackay & Brandon, 1985; Mackay, *et al.*, 1985; Puri, Mackay & Brandon, 1985; Mackay, Maddox & Brandon, 1986a). Although detailed studies on the development of the lymphoid system in the ovine fetus have been performed, nothing is known about the role that T cells play in this scheme. This investigation extends a previous study (Mackay, Maddox & Brandon, 1986b) and examines younger ovine embryos, as well as describing additional ovine lymphocyte antigens.

MATERIALS AND METHODS

Embryos and Fetuses

Embryos and fetuses of less than 40 days g.a. were obtained from timed matings of Merino ewes with rams fitted with a crayon and a Sire Sine harness. The day the ewe was marked by the ram was taken as Day 0. Older fetuses were obtained from an abattoir. Fetal age was estimated from the crown-rump length (CRL) according to the formula derived by Barcroft (1946):

$$\text{fetal age (days)} = 2{\cdot}45 \times \text{CRL (cm)} + 27{\cdot}7.$$

Fetal tissues were embedded in OCT (Tissue-Tek, Miles Scientific, Napierville, IL) and snap-frozen. Details of the embryos, fetuses and lambs used for immunohistological study are presented in Table 1.

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Abbreviations: CRL, crown-rump length; g.a., gestational age; HRP-RAM, horseradish peroxidase-labelled rabbit anti-mouse immunoglobulin; HRP-RAS, horseradish peroxidase-labelled rabbit anti-sheep immunoglobulin; HRP-SAR, horseradish peroxidase-labelled swine anti-rabbit immunoglobulin; LCA, leucocyte common antigen; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; PBS, phosphate-buffered saline; sIg, surface immunoglobulin.

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Table 1. Details of embryos, fetuses and lambs used to study the ontogeny of the ovine thymus

CRL (cm)	Age (days)*	Number of fetuses or lambs
ND	19–31	5
2·0–2·5	33	3
3·0	35	1
3·1	37	2
3·3–3·6	35–38	4
4·2–8	39–47	10
9–15	50–64	8
17–32	69–106	7
35–48	113–145	5
ND	7–14†	3

ND, not determined.
* Unless indicated, gestational ages.
† Postnatal age.

Antibodies

The monoclonal antibodies used to detect sheep lymphocyte antigens are listed in Table 2. Three new monoclonal antibodies, which define antigens on the surface of ovine lymphocytes, have been used in this study. The first, 38.38, binds to the majority of peripheral T lymphocytes, medullary thymocytes, a subpopulation of cortical thymocytes, as well as to all granulocytes and monocytes (C. R. Mackay, J. F. Maddox and M. R. Brandon, manuscript in preparation). Currently, no molecular weight has been obtained for this antigen. The second, 46.66, binds to members of a new, low molecular weight family (16,000–20,000 MW) of leucocyte antigens (J. F. Maddox, C. R. Mackay, W. R. Mercer, M. D. Gorrell and M. R. Brandon, manuscript in preparation). The third, 20.96, binds to an epitope on the p220 component of LCA, and reacts with the majority of ovine B cells, as well as a small T-cell subpopulation. This epitope is absent from forms of LCA found on other leucocytes (Mackay, Maddox & Brandon, 1987).

Both 44.38 and 44.97 were used to detect SBU-T4, as it was found that the combination gave stronger staining. Peroxidase-labelled rabbit anti-mouse Ig (HRP-RAM), peroxidase-labelled rabbit anti-sheep Ig (HRP-RAS) and peroxidase-labelled swine anti-rabbit Ig (HRP-SAR) were purchased from Dako (Copenhagen, Denmark).

Immunohistological staining

Cryostat sections, 3–5 μm thick, were air-dried at 4° overnight before fixation in acetone for 10 min at 4°. Sections tested for SBU-T19 reactivity were fixed in absolute alcohol rather than acetone. Antigens were localized on tissue sections using an indirect immunoperoxidase method, essentially as described by Barclay (1981). To increase the sensitivity of detection of SBU-T1, SBU-T4, SBU-T8 and 38.38 antigens, a three-step immunoassay was used. The tissue sections were incubated with culture supernatant in a humidified chamber for 1 hr at room temperature. After washing the sections in phosphate-buffered saline (PBS), they were incubated with HRP-RAM (1/25 dilution in 10% normal sheep serum in PBS) for 1 hr at room temperature, washed again in PBS and incubated with HRP-SAR (1/30 dilution in 10% normal sheep serum in PBS) for 30 min. Control slides were reacted with HRP-RAM alone, and HRP-RAM followed by HRP-SAR.

A single-step immunoassay was used to detect sheep immunoglobulins, using HRP-RAS. The sections were washed

Table 2. Reactivity of monoclonal antibodies to sheep lymphocytes

Antibody clone name	Antigen	Reactivity	Reference
25.91	SBU-T1 (CD5)	All T lymphocytes and thymocytes	(1)
44.38/44.97	SBU-T4 (CD4)	80% thymocytes, 40% lymph lymphocytes*	(2)
38.65	SBU-T8 (CD8)	80% thymocytes, 20% lymph lymphocytes*	(2)
19.19	SBU-T19	1% thymocytes, 5% lymph lymphocytes*	(2)
20.27	SBU-T6 (CD1)	Cortical thymocytes, some B cells	(1)
46.66		All T lymphocytes and thymocytes, subpopulation granulocytes and monocytes	†
20.96	p220 (CD45R)	All B and subpopulation of T lymphocytes	(6)
38.38		Medullary thymocytes, T lymphocytes, granulocytes and monocytes	†
1.11.32	LCA (CD45)	All leucocytes	(3)
41.19	MHC I		(4)
28.1	MHC II		(5)

* SBU-T4, SBU-T8 and SBU-T19 are present on mutually exclusive populations of peripheral T lymphocytes, and are absent from B lymphocytes.
† Manuscripts in preparation.
(1) Mackay *et al.* (1985).
(2) Mackay *et al.* (1986a).
(3) Maddox *et al.* (1985).
(4) Gogolin-Ewens *et al.* (1985).
(5) Puri, Mackay & Brandon (1985).
(6) Mackay, Maddox & Brandon (1987).

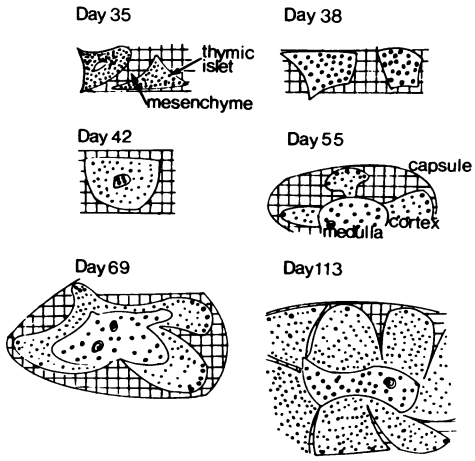

Figure 1. Development of the ovine thymic cortex and medulla during gestation. Day 35, a small number of aggregates of basophilic cells are found within the mesenchyme of the neck. Day 38, discrete areas of thymic tissue are found within the mesenchyme of the neck. Some of these areas contain cells that strongly express MHC I antigens and weakly express MHC II antigens, while other areas contain cells that strongly express MHC II antigens and weakly express MHC I antigens. Day 42, the thymus has separated into primitive medullary and cortical areas. Medullary areas contain cells that strongly express MHC I antigens. Day 55, the thymus is delineated by a capsule. Cortical tissue is joined to the medulla by narrow tracts and does not surround the medulla. Primitive Hassall's corpuscles are found within the medulla. Day 69, the thymus contains many Hassall's corpuscles. The medulla is surrounded by cortical tissue. Day 113, there is more cortical tissue than medullary tissue within the thymus and the structure resembles that of the postnatal thymus.

in PBS before development of the colour reaction with 0·06% diaminobenzidene tetrahydrochloride, 0·01% hydrogen peroxide in 0·025 M Tris, 0·14 M sodium chloride, pH 7·6.

RESULTS

Embryos from Day 19 to Day 33 of gestation

The earliest embryos examined were of 19 days g.a. LCA was seen on occasional cells within the embryonic liver, and MHC I antigens were found on a small number of cells scattered throughout the Day 19 embryo. The SBU-T6 antigen was first seen at Day 22 of gestation on a small number of cells within the embryo, mainly situated within the aorta.

By Day 25 of gestation many more cells in the embryonic liver, as well as elsewhere within the embryonic body, expressed LCA, MHC I and SBU-T6 antigens. The first cells to express MHC II antigens were found in the liver of embryos of 25 days g.a.

No cells expressed any of the T-cell antigens tested for in fetal livers of less than 37 days g.a., with the exception of 38.38 which was detected on occasional cells from Day 22 g.a. At 39 days of gestation, a small number of cells within the fetal liver expressed SBU-T1, SBU-T4, SBU-T8 and 46.66 antigens. These cells were large and found mainly within the hepatic blood vessels.

Throughout the remainder of the gestational period small numbers of both T and B cells were found in the fetal liver. Many LCA-positive cells were seen, and it is probable that this antigen was being expressed on immature cells of haemopoietic lineages as well as on Kupffer cells and circulating leucocytes.

In fetuses of 33 days g.a., scattered cells were seen within the mesenchyme of the neck and in the dorsal mediastinum and mesentery which expressed the SBU-T1 antigen weakly. In these sites, positive cells were located adjacent to blood vessels, and SBU-T6, MHC I, MHC II and LCA antigens were also seen on some cells within these areas. Cells expressing SBU-T4 and SBU-T8 antigens were first seen within the dorsal mediastinum and mesentery of ovine fetuses of Day 35 gestation (J. F. Maddox, C. R. Mackay and M. R. Brandon, manuscript in preparation).

Thymic development

It was found that the early stages of fetal thymus development correlated more closely with CRL than with the age determined from timed matings. The CRL measurements probably reflect the physiological age of the fetus more accurately than do the timed matings; this could be due to the different lengths of time between fertilization and mating or time of implantation. Fetal thymi were histologically detected in the neck of fetuses of 31 days g.a. and older. None of the antigens studied was found in the thymus at this time.

Thymic development is described in terms of the stages that were observed.

Stage 1: embryo of 2·0–2·4 cm CRL, 33 days g.a. At this stage of development, the thymus consisted of a collection of large basophilic cells ventral to the trachea and in close association with the anterior cardinal vein (internal jugular vein) just rostral to the thoracic inlet. MHC I antigens were expressed very weakly on some cells of the Day 33 thymus, but more strongly on cells adjacent to the thymus. None of the cells within thymi of fetuses of 33 days g.a. expressed any of the T-cell antigens studied, nor any of the other antigens for which we tested.

Although SBU-T1-positive cells were not found in the thymus at this age, they were found elsewhere in the embryo. A small number of scattered cells expressing SBU-T1 were found within the mesenchyme of the neck, and in the dorsal mediastinum and mesentery of fetuses of 33 days g.a. Positive cells were located adjacent to blood vessels in all of these sites. These cells had very large nuclei and a sparse, wispy cytoplasm. Some of the cells located in the same areas of the neck, mediastinum and mesentery expressed SBU-T6, MHC I, MHC II and SBU-LCA. There were many more cells that expressed SBU-LCA than there were cells staining for the other antigens studied. It would be interesting to perform double-labelling studies to determine which antigens are co-expressed. Much of the staining for MHC I antigens in these areas appeared reticular, as did some of the staining for MHC II antigens. There were very few SBU-T1-positive cells found elsewhere in the embryo, although some cells within the developing kidney were found to express SBU-T1. MHC I antigens were also found on the endothelium lining blood vessels, as were SBU-T6 antigens. Many more cells stained for MHC I and MHC II antigens than expressed SBU-T1 antigens.

Stage 2: fetus of 3·0 cm CRL, Day 35. At this stage, the fetus showed great variation in the antigens expressed in different regions of the thymus. The thymus extended from just below the thyroid gland to the thoracic inlet, in the form of several clusters

of large cells with big nuclei and sparse cytoplasm. Some of these cell clusters contained a central lumen (Fig. 1).

LCA staining within the thymus was variable. In some areas only small numbers of cells expressed LCA, whereas in other areas the majority of thymocytes expressed LCA (Fig. 2a). MHC I antigens were seen on most of the cells of the thymus (Fig. 2b), and the staining was more intense than seen in the thymus of 33 days g.a. MHC II antigens were also present (Fig. 2c) but were seen on fewer cells than MHC I antigens. The expression of SBU-T1 was highly variable (Fig. 2d). In the neck near the thoracic inlet single SBU-T1 positive cells were seen within the thymus, while higher up in the neck groups of weakly staining SBU-T1-positive cells resided. SBU-T8-positive cells were occasionally found in sections of the thymus, while SBU-T4-, SBU-T6-, and 46.66-positive cells were absent.

Stage 3: fetus of 3·1 cm CRL, Day 37. LCA was expressed on approximately half to two-thirds of the cells found within thymi of fetuses of 3·1 cm CRL, while SBU-T1 was seen on a slightly smaller population of cells. This is probably due to the presence of SBU-LCA on cells of the macrophage and granulocytic lineages as well as on lymphocytes. As it is hard to determine in frozen sections which of the cells within the fetal thymus are epithelial, it is not known what proportion of thymocytes expressed SBU-T1 or SBU-LCA antigens at this time. SBU-T4 cells were absent from thymi of this gestational age, whereas a few SBU-T8-positive cells were found within the thymi.

SBU-T6 and 46.66 antigens were very weakly expressed on some cells within the thymus, with 46.66 staining more cells than SBU-T6. The staining of these antigens took the form of discrete spots on the surface of the cell, rather than forming a continuous rim around the cell. No cells within the thymus were stained by 20.96 or 38.38, nor did any cells express SBU-T19.

Stage 4: fetus of 3·3–3·6 cm CRL, Days 35–38. More SBU-T8-positive cells (Fig. 3b) were seen in thymi from fetuses of this age than in thymi from younger fetuses. Occasional weakly stained SBU-T4-positive cells (Fig. 3c) were also seen. SBU-T6 (Fig. 3a) and 46.66 (Fig. 3d) expression on thymic cells was still very weak in comparison to the strong staining seen on thymocytes of older fetuses. These antigens were present on fewer cells than were SBU-T1-positive; however, staining of these antigens was stronger than that seen for SBU-T1. SBU-T1 (Fig. 3e) and LCA (Fig. 3f) antigens were seen on most thymocytes, with SBU-T1 being seen on slightly fewer cells than were LCA-positive. MHC I antigens (Fig. 3g) were seen on some thymocytes as well as on some epithelial cells, while MHC II antigens showed a predominantly reticular staining pattern (Fig. 3h). MHC I antigens were present on most cells within the thymus, while MHC II antigens were expressed on fewer cells (Fig. 3g, h). Serial sections showed that, in general, parts of the thymus which stained strongly for MHC I antigens weakly expressed MHC II antigens, whereas parts that stained more strongly for MHC II antigens had weaker expression of MHC I antigens (Fig. 1).

The thymus contained a mixture of small, medium and large cells, with most cells being medium to large in size. The few small cells that were present tended to be in clusters. The SBU-T8-positive cells were small or large in size. Many of the 46.66-negative cells were small.

MHC II antigens were present on relatively fewer cells in the thymi of these fetuses than in earlier fetal thymi. Some of the positive cells were obviously dendritic in nature, and most of the cells were medium to large in size. MHC I antigens were still strongly present on the majority of cells within the fetal thymus and some of the positive cells appeared to be dendritic.

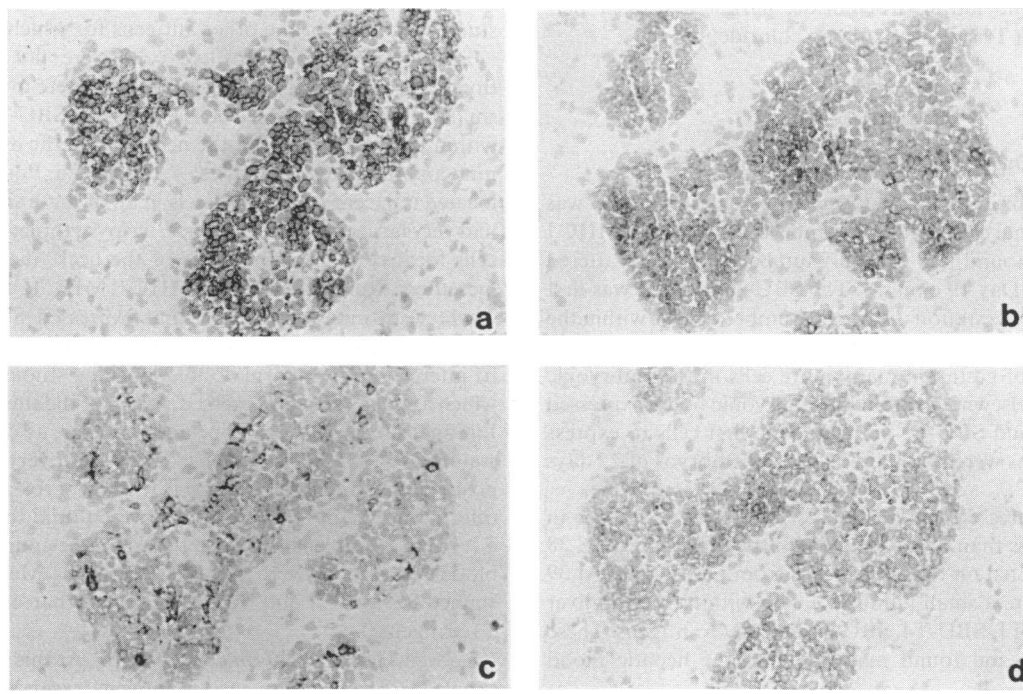

Figure 2. Expression of antigens on Day 35 (Stage 2) ovine fetal thymocytes. (a) SBU-LCA; (b) MHC class I; (c) MHC class II; and (d) SBU-T1. Magnification × 150.

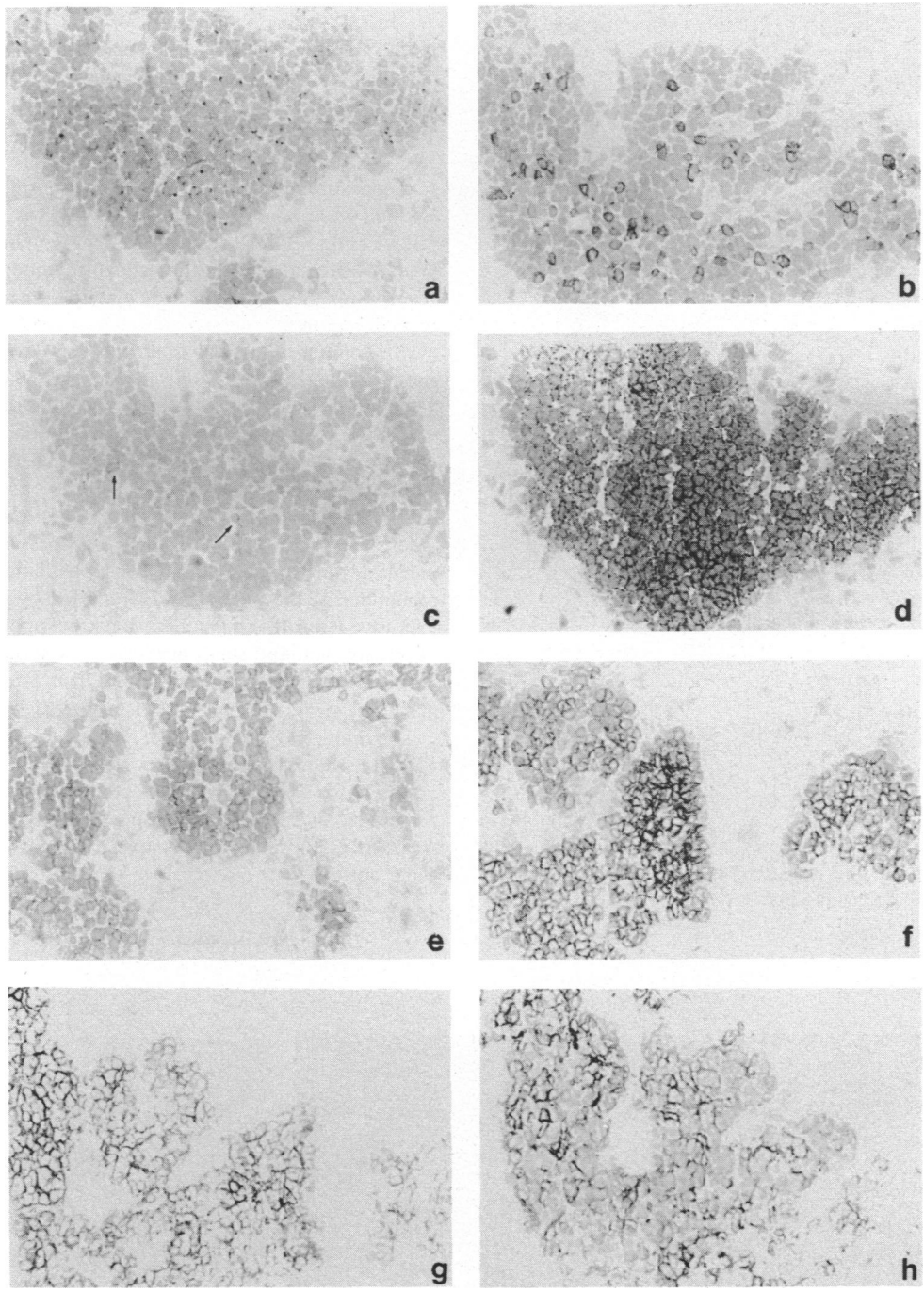

Figure 3. Distribution of lymphocyte populations within the ovine fetal thymus at Day 38 (stage 4) of gestation. (a) SBU-T6; (b) SBU-T8; (c) SBU-T4; (d) 46.66; (e) SBU-T1; (f) SBU-LCA; (g) MHC I; and (h) MHC II. Arrows show weakly stained cells. Magnification × 150.

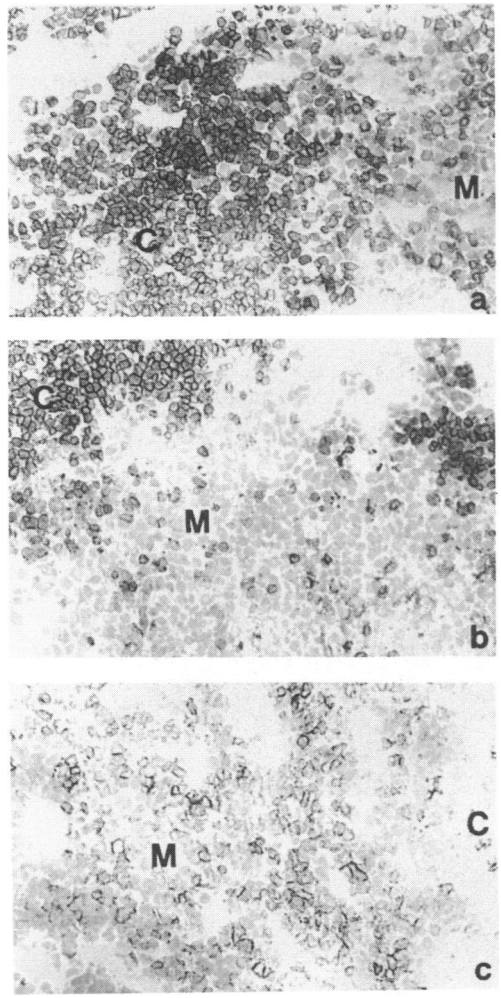

Figure 4. Distribution of lymphocyte populations within the ovine fetal thymus at Day 50 of gestation. (a) SBU-T4; (b) SBU-T6; and (c) 38.38. C, cortex; M, medulla. Magnification ×150.

As for earlier fetuses, large cells expressing T-cell antigens were also found at other sites in the fetal body. Some of the staining of the SBU-T4- and SBU-T8-positive cells appeared cytoplasmic, but most of the positive cells appeared to express SBU-T4 and/or SBU-T8 antigens on their surfaces.

Day 39–Day 47. Although very few thymocytes expressed SBU-T4 and/or SBU-T8 antigens in the Day 39 fetal thymus, there was a rapid increase in the proportion of fetal thymocytes expressing SBU-T4 and/or SBU-T8 antigens soon after this time, so that by Day 43 of gestation most of thymocytes were SBU-T4 and/or SBU-T8 positive.

Separation of the thymus into a primitive cortex and medulla was apparent in fetuses of 42/43 days g.a. and older. SBU-T4 and SBU-T8 antigens were present on some medullary thymocytes and on most cortical thymocytes. Thymocytes negative for these antigens were mainly located within the outer cortical region.

Staining for 46.66 was stronger on thymocytes of this gestational age range than in thymi from younger fetuses. The first staining by 38.38 was seen on a small number of medullary thymocytes in fetuses at Day 42 of gestation.

Day 50–Day 64. A distinct cortex and medulla was apparent within the fetal thymus from this time, as defined by both lymphoid and epithelial antigens. In contrast to thymi from older fetuses or lambs, the cortex does not surround the medulla, but is joined to it by narrow channels (Fig. 1). The remainder of the medulla was bounded by connective tissue. Primitive Hassall's corpuscles were first apparent in fetal thymi of 50 days g.a. and small numbers of well-developed Hassall's corpuscles were found within thymi from fetuses of 55–60 days g.a.

Thymocytes from fetuses of 50 days g.a. and older expressed SBU-T1 in an adult manner. The medullary cells stained intensely for SBU-T1, as did scattered cortical cells, while the remainder of cortical cells stained weakly for SBU-T1. Very weak SBU-T1 staining was apparent on the cells within the outer cortex.

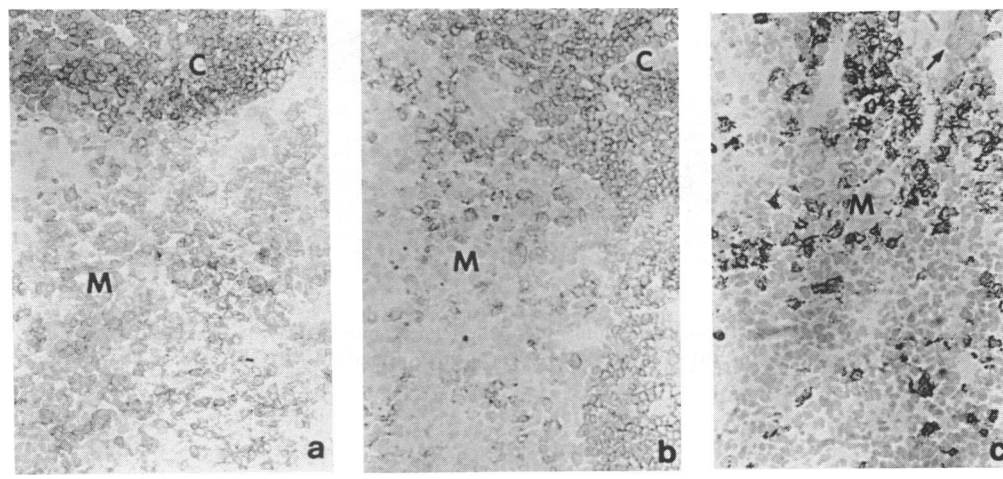

Figure 5. Distribution of lymphocyte populations within the ovine fetal thymus. (a) Day 78, SBU-T4; (b) Day 78, SBU-T8; and (c) Day 113, SBU-T19. C, cortex; M, medulla. The arrow in (c) indicates a Hassall's corpuscle. Magnification ×150.

The majority of Day 50 thymic cortical cells strongly expressed SBU-T4 (Fig. 4a) and/or SBU-T8 antigens. In the medulla, about one-quarter to one-third of the cells were SBU-T8 positive, while half to two-thirds were SBU-T4 positive. When SBU-T4 and SBU-T8 were stained for in concert, a population of large cells found just beneath the capsule lacked both these antigens.

Most cells in thymi of this age expressed LCA and 46.66 antigens. The cortical cells stained less intensely than the medullary cells for LCA, whereas the cortical cells showed stronger 46.66 staining. Staining for MHC antigens revealed a picture similar to that found in postnatal thymi. SBU-T6 antigens were seen on most cortical thymocytes as well as occasional thymocytes within the medulla (Fig. 4b) while the cells stained by 38.38 were mainly located in the medulla (Fig. 4c).

Occasional sIg-positive cells were seen in the medulla of thymi from fetuses of 50 days g.a. The number of sIg-positive cells was greater in thymi of 55 days g.a. The antibody 20.96 also stained cells located within the medulla of thymi from fetuses of 50 days g.a. and older. More medullary cells were stained by 20.96 than were sIg positive. Small numbers of SBU-T19-positive cells were first seen in the medulla of fetal thymi between 50 days and 58 days of gestation.

Day 69–Day 106. Hassall's corpuscles were very prominent at this stage of gestation. Fewer Hassall's corpuscles were found in thymi from fetuses older than 100 days g.a. but the Hassall's corpuscles were larger in size than those found in thymi from younger fetuses. SBU-T4 and SBU-T8 antigens were present on most cortical thymocytes and some medullary thymocytes (Fig. 5a, b). The ratio of cortical to medullary thymic tissue increased during this time.

The 20.96 stained a smaller proportion of cells located within the thymic medulla than was found in thymi from younger fetuses, while an increasing number of cells within the thymic medulla were stained by 38.38 and SBU-T19. There were many more 38.38-positive cells than cells expressing SBU-T19. Most of the cells that expressed SBU-T19 were located in the medulla, with only occasional scattered positive cortical cells. Many cortical cells were very weakly stained by 38.38.

Day 110–Day 145. At 110 days g.a. the thymus contained many SBU-T19 lymphocytes, most of which were present in the medulla, although some SBU-T19-positive cells were found in the cortex (Fig. 5c).

The thymus at this time structurally resembled the thymus from postnatal lambs (Fig. 1). Fewer and smaller Hassall's corpuscles were seen. The outer-most cortical cells lacked SBU-T4 and SBU-T8 antigens, but these double-negative cells constituted only a small proportion of the cortical cells. Approximately four times as many medullary thymocytes expressed SBU-T4 as compared to SBU-T8.

Lambs 7–14 days of age. The thymus of lambs at 7–14 days of age was very similar to that found in the late-term fetus, with a high ratio of cortical to medullary thymic tissue and a similar distribution of antigens on the surface of thymocytes. This appearance was very similar to that seen in older lambs (Mackay *et al.*, 1986b).

Table 3. The times of first appearance of antigens within the embryo during ovine development*

	Gestational age (days)	
Antigen	Body	Thymus
LCA	19†	35
MHC I	19†	33
SBU-T6	22	35–37
MHC II	25	35
SBU-T1	33	35
SBU-T4	35	35–38
SBU-T8	35	35
46.66	35–37	35–37
sIg	39	50
38.38	22	42
20.96	50	50
SBU-T19	50–58	50–58

* The ages in this table represent the first time an antigen was observed on cells. For the non-lymphocyte specific markers this does not mean that the cells expressing this marker at this time were lymphocytes.

† Earliest age studied.

DISCUSSION

Colonization of the vertebrate thymic rudiment by extrinsic stem cells is necessary for the development of thymic lymphocytes (Moore & Owen, 1967; Le Douarin & Jouterau, 1975; Tompkins, Reinschmidt & Volpe, 1979; Vasse, 1983). These stem cells are thought to be derived from an intra-embryonic source (Dieterlen-Lievre, 1975), and foci of basophilic cells have been identified in avian embryos (reviewed in Le Douarin, Dieterlen-Lievre & Oliver, 1984). Stem cells colonizing the mammalian thymic rudiment have previously been thought to be derived from the embryonic liver, with the source of these liver stem cells being unknown; it is possible they come from the yolk sac and other derivatives of the mesodermal blood islands (Stutman, 1978). The dorsal mesentery of the ovine embryo could represent a source of pre-thymic haemopoietic stem cells. Pyke & Bach (1979) showed the existence of a discrete population of murine fetal liver cells that were attracted by the murine fetal thymus. No similar studies have been done in sheep.

One reason that the yolk sac was initially considered to be the source of the stem cells invading the avian and murine thymus was that, at the time of thymic colonization, it was thought to be the only major site of blood production (Moore & Owen, 1967). At the time of colonization of the ovine thymic rudiment the ovine yolk sac has involuted and, hence, is unlikely to be a direct source of stem cells. Large basophilic cells expressing T-cell antigens are found in other sites of the ovine embryo at, or before, the time that they are first seen within the thymus. These cells are particularly obvious within the dorsal mediastinum near to the aorta, but are also found near to blood vessels within the mesenchyme of the neck, and within the dorsal mesentery of the abdomen.

It is unknown if pre-thymic stem cells migrating into the thymus are already committed to the T-cell lineage. The presence of T-cell markers on extra-thymic cells prior to their appearance on cells within the ovine thymus supports the concept that cells migrating into the thymus are already

committed to the T-cell lineage. The possibility that these markers, SBU-T1, SBU-T4 and SBU-T8, are present on leucocytes of other lineages at this time must be considered, as in other species CD5 antigens are also found on a small population of B cells (Hayakawa *et al.*, 1983; Bofill *et al.*, 1985). The CD4 antigen is also found on macrophages (Wood, Warner & Warnke, 1983; Pilkington *et al.*, 1984) and the CD8 antigen is also found on NK cells (Cantrell *et al.*, 1982; Perussia, Fanning & Trinchieri, 1983). We feel that it is unlikely that the SBU-T1-positive cells are primitive B cells, given the lack of other B-cell markers on these cells and the subsequent presence of SBU-T4 and SBU-T8 antigens on cells in these areas. It is possible that the early SBU-T1-positive cells already express other T-cell markers but that the sensitivity of the immunohistochemistry technique is too low to detect them. The human pan-T antigen 3A1 (CD7) has also been found on lymphoid cells prior to thymic colonization (Lobach *et al.*, 1985).

After the initial thymic colonization, the development of the ovine fetal thymus resembles the development of the murine thymus. Expression of SBU-T1 on thymocytes precedes that of SBU-T8 and SBU-T4. One difference between mice and sheep is that MHC I antigens are detected before MHC II antigens in the ovine fetal thymus, whereas the converse situation is found in the mouse (van Ewijk *et al.*, 1982). How ovine ontogeny relates to the human situation is unknown as there have been no reliable studies published on antigen expression during early thymic development.

SBU-T8-positive cells are detected slightly earlier within the fetal thymus than are SBU-T4-positive cells, although in the latter stages of gestation there are more SBU-T4 thymocytes than SBU-T8 thymocytes. The reason for the different times of appearance of SBU-T4 and SBU-T8 cells within the thymus is unknown; one possibility is that they represent different lineages of thymocytes. An alternative theory is that SBU-T4 gene expression is switched on later than SBU-T8. If two separate lineages of cells colonize the thymus, this begs the question of why double-positive cells then appear, and from what cells are they derived? While the medulla initially comprises a large proportion of the thymus, as gestation proceeds its relative size decreases, so that by Day 96–106 of gestation the double-positive small cortical cells constitute the dominant thymus inhabitants.

The delay before the appearance of 38.38 cells and subsequent delay before the appearance of SBU-T19-positive cells within the thymus is interesting. The emergence of 38.38-positive cells may be due to expression of this antigen on thymocytes already present within the thymus. In contrast, as the SBU-T19 antigen defines a discrete T-cell lineage (Mackay *et al.*, 1986a), and SBU-T19-positive cells were first found within the thymus at the same time as cells expressing sIg and staining for 20.96, it is probable that SBU-T19 cells migrate to the thymus along with 20.96 and sIg-positive lymphocytes.

It is concluded that a specific sequence of appearance (summarized in Table 3) of cells expressing T lineage and other markers is seen within the ovine embryo and fetal thymus.

Availability of Monoclonal Antibodies

The monoclonal antibodies described in this publication, except for those described as in preparation, are available for research. Please direct written enquiries to Ms Kaye MacRae, Department of Veterinary Preclinical Sciences.

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