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Identification of a unique lymphocyte subpopulation in the sheep uterus

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SUMMARY

A panel of monoclonal antibodies was used to define the lymphocyte subpopulations in the sheep uterus at various stages of the oestrous cycle. A striking finding was that the majority of lymphocytes in the uterine and endometrial glandular epithelia belonged to a unique lymphocyte subpopulation that expressed the CD45R antigen but was negative for major histocompatibility complex (MHC) class II molecules and expressed low or undetectable levels of the CD5 antigen. When examined under the electron microscope using the immunogold technique, the CD45R$^+$ lymphocytes were found to have one to three membrane-bound granules in their cytoplasm. Other lymphocyte subpopulations found in the uterus at various stages of the oestrous cycle were localized mainly in the caruncular and intercaruncular stroma. The unique CD45R$^+$ granular lymphocyte subpopulation may be equivalent to the 'natural killer' cells reported in mouse and man, and may have an important role in local immunity of the female reproductive tract.

INTRODUCTION

The mammalian uterus is unique in that it possesses the ability to mount an immune response against pathogenic organisms while not usually responding to 'foreign' antigens on sperm and fetal allografts (Head & Billingham, 1986). In order to understand how this discrimination occurs, an investigation of the lymphocyte subpopulations present within the uterine epithelium and the underlying stroma was undertaken. Lymphocytes have been identified in the uterine epithelium of the non-pregnant cow, and their number does not change significantly throughout the oestrous cycle (Skjerven, 1956; Vander-Wielen & King, 1984). Lymphocytes are also commonly found in the endometrium of the sheep uterus (O'Shea & Wright, 1984). However, no studies have been undertaken in ruminants on the characterization of lymphocyte subpopulations in the uterus.

In this study, monoclonal antibodies directed against seven different sheep lymphocyte antigens, CD45 (SBU-LCA; Maddox, Mackay & Brandon, 1985a), CD5 (SBU-T1; Mackay *et al.*, 1985), CD4 (SBU-T4; Maddox, Mackay & Brandon, 1985b), CD8 (SBU-T8; Maddox *et al.*, 1985b), MHC class II (OLA SBU. II; Puri, Gorrell & Brandon, 1987), CD45R (SBU-20.96; Mackay, Maddox & Brandon, 1987) and a unique sheep-specific T-cell marker SBU-T19 (Mackay, Maddox & Brandon, 1986) have been used to characterize lymphocytes in the uterus.

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MATERIALS AND METHODS

Tissues

Sheep uteri at various stages of the oestrous cycle were obtained from six ewes: two early-stage (Day 5), two mid-stage (Day 11) and two late-stage (Day 16). Day 0 was designated as the commencement of oestrus, and this was determined by the use of a sire sine harness attached to a vasectomized ram. In addition, 15 uteri were collected from an abattoir and were classified as early ($n=5$), mid (5) and late stage (5) of the oestrous cycle. Three non-cycling uteri were obtained from lambs. Uteri collected from an abattoir closely resembled those obtained from sheep at known stages of the oestrous cycle in both gross and histological appearance, and were easy to classify as regards the stage of the oestrous cycle. The early-stage uteri had small haemorrhagic corpora lutea and contained numerous mitotic figures when examined histologically. Uteri were regarded as being at the mid-stage when the ovaries contained large reddish corpora lutea. In all cases histological examination confirmed that the corpora lutea had a mature structure with no sign of regression. Uteri were classed as at the late-stage when corpora lutea were pale-yellowish and histological examination revealed vascular changes in blood capillaries and small venules consistent with early regression (O'Shea, Nightingale & Chamley, 1977).

For immunohistochemical studies uterine tissue from both horns was embedded in OTC compound (Miles Scientific, Naperville, IL), snap-frozen and stored at $-70°$. Some tissues were also fixed in 95% cold ethanol and processed according to

Table 1. Reactivity of monoclonal antibodies to sheep lymphocytes

Antigen	mAb clone number	Antigen distribution	Reference
CD45 (SBU-LCA)	1-28-124	All leucocytes	Maddox *et al.* (1985a)
CD5 (SBU-T1)	25-91	Present on all T lymphocytes, absent from B lymphocytes	Mackay *et al.* (1985)
CD4 (SBU-T4)	44-38 and 44-97	Present on subset of T lymphocytes that are $CD8^-$, $SBU\text{-}T19^-$; absent from B lymphocytes	Maddox *et al.* (1985b), Mackay *et al.* (1986)
CD8 (SBU-T8)	38-65	Present on subset of T lymphocytes that are $CD4^-$, $SBU\text{-}T19^-$, absent from B lymphocytes	Maddox *et al.* (1985b), Mackay *et al.* (1986)
SBU-T19	19-19	Present on subset of T lymphocytes that are $CD4^-$, $CD8^-$, absent from B lymphocytes	Mackay *et al.* (1986)
CD45R (SBU-p220)	20-96	Present mainly on B lymphocytes and small subset of T lymphocytes	Mackay *et al.* (1987)
MHC class II	49-1	Present on B lymphocytes and activated T lymphocytes; 49-1 is monomorphic for *all* four subsets of sheep class II molecules	Puri *et al.* (1987)

the method of Sainte-Marie (1962), and some were fixed in Bouin's fluid.

For electron microscopic examination of uteri, one uterus from each stage of the oestrous cycle was fixed as described by Wooding (1981). Briefly the uterine arteries were cannulated and perfused with 60 ml of 0·1% glutaraldehyde in 0·1 M phosphate-buffered saline (PBS) containing 2% paraformaldehyde. The uterus was then cut open and caruncular and intercaruncular tissues of 1–2 mm slices immersed in the same fixative or in 2% glutaraldehyde for an additional 30 min at room temperature. The tissues were then divided into 'matchsticks' which ran from the top to the bottom of the caruncle and intercaruncular areas. The tissues were fixed for a further 30 min at room temperature and stored at 4° in PBS. Tissues were subsequently embedded in either LR-White or Araldite.

Monoclonal antibodies

All the monoclonal antibodies (mAb) used were produced in our laboratories, and their characteristics are detailed in Table 1. The antigens identified by the mAb have been extensively characterized by immunochemical, one- and two-colour flow cytometry and immunohistological techniques (referenced in Table 1). An additional mAb, 25–69, which binds to sheep IgM as shown by radioimmunoassay, and possibly to other forms of sheep surface immunoglobulin (sIg), was also used in this study (Maddox, Mackay & Brandon, 1987).

Immunocytochemical studies of tissue sections

With the exception of 20-96, 49-1 and 1-28-124, the monoclonal antibodies used in this study reacted only on frozen tissue sections. MAb 20-96 and 49-1 were active on tissues fixed in either 95% ethanol or Bouin's fluid (Fig. 1), and mAb 1-28-124 on tissues fixed in 95% ethanol. For most of the light microscopy studies 6-μm frozen sections were cut, fixed for 10 min in cold ethanol or acetone, air-dried and then stained using the indirect immunoperoxidase technique as previously detailed (Lee *et al.*, 1985). For controls either PBS or 1% normal sheep serum in PBS was substituted for the mAb supernatant. At least one section from each tissue block was routinely stained with haematoxylin and eosin.

In order to determine the phenotype of the lymphocytes stained by mAb 20-96 some sections were stained twice using the indirect immunoperoxidase technique. Sections were firstly stained with mAb 25-91, mounted with water, examined and several areas showing positive cells photographed. Following this the same sections were stained for a second time with the mAb 20-96, mounted with water and the same areas re-photographed. As a control, the mAb 44-38 was used in place of mAb 20-96 in the second staining.

For electron microscope studies, sections on 300-mesh uncoated copper grids were jet-washed with PBS and then floated with the section facing downwards on a drop of undiluted mAb supernatant for 30 min at room temperature.

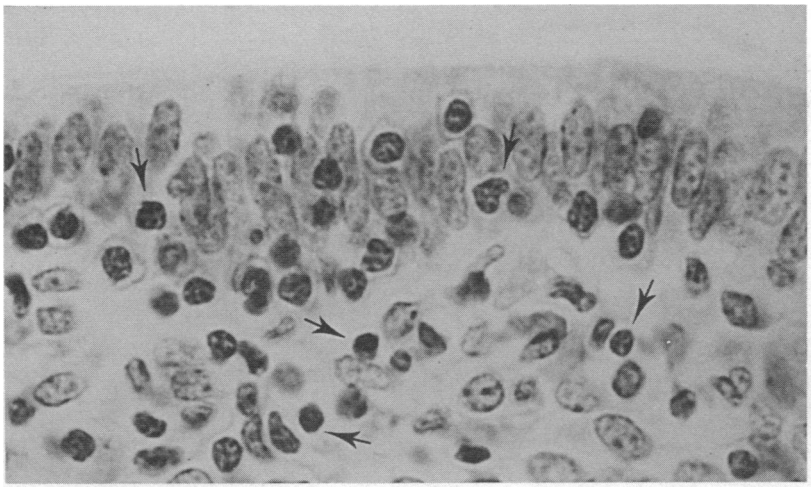

Figure 1. Immunoperoxidase staining of a uterine tissue section from an early cycling ewe with mAb 20-96 showing CD45R$^+$ lymphocytes interspersed between the intercaruncular epithelial cells and also localized at the basal region of the epithelium. Note that some of the lymphocytes in the epithelium (arrows) and those in the stroma (arrows) are unlabelled with mAb 20-96. Magnification × 983.

Following this the grid was jet-washed with PBS before being floated on a drop of 15 nm colloidal gold-labelled goat anti-mouse immunoglobulin (Janssen Pharmaceutica, Beerse, Belgium) diluted 1:20 in PBS for another 30 min at room temperature. The grid was jet-washed with PBS, distilled water, and then stained with uranyl acetate and lead citrate. Control sections were stained as for light microscopy.

RESULTS

Distribution of lymphocytes in the endometrium

For ease of description the endometrium was divided into (1) caruncular epithelium, (2) caruncular stroma, (3) intercaruncular epithelium, (4) intercaruncular stroma and (5) endometrial glandular epithelium (Fig. 2a and b). In tissues stained with haematoxylin and eosin, lymphocytes were characterized by cells with a dark-staining rounded nucleus that occupied virtually the whole cell (Fig. 2a and b). Irrespective of the stage of the oestrous cycle, lymphocytes were found to infiltrate the caruncular and intercaruncular epithelium (Fig. 2a and b). Lymphocytes were also frequently found in the connective tissue immediately beneath these epithelia as well as in the intercaruncular and caruncular stroma. Occasionally these lymphocytes appeared in clusters and in three sheep (one non-cycling lamb, one early- and one late-cycle ewe) collections of lymphocytes in the form of nodules were observed in the intercaruncular stroma (Fig. 2c). Plasma cells were found around these nodules. In addition to lymphocytes, macrophages were also frequently observed in the caruncular and intercaruncular stroma. The epithelium of the endometrial glands and their ducts were frequently infiltrated by lymphocytes, especially around the basal region (Fig. 2b). Both lymphocytes and macrophages were also seen in the loose connective tissue in the vicinity of the endometrial glands and ducts.

Distribution of lymphocyte subpopulations in the endometrium

With the exception of CD8$^+$ lymphocytes, the pattern of distribution of lymphocytes positive for the markers CD5, CD4, CD45, MHC class II, CD45R or SBU-T19 was generally consistent both within and between the different stages of the oestrous cycle.

CD45 (SBU-LCA). Numerous CD45$^+$ lymphocytes were observed in the caruncular, intercaruncular and endometrial glandular epithelium and in the subepithelial regions. A very high proportion of the cells in the stroma were CD45$^+$. The majority of these were lymphocytes, while others resembled macrophages or had a fibroblast-like appearance.

CD5(SBU-T1). CD5$^+$ cells were observed scattered in the caruncular and intercaruncular stroma and in the loose connective tissue associated with the endometrial glands. Most of these cells were lymphocytes but an occasional 'fibroblast-like' CD5$^+$ cell was found in the stroma immediately beneath the intercaruncular and caruncular epithelium. Some positive cells were also found in the basal region of the caruncular, intercaruncular and endometrial glandular epithelium (Fig. 2d).

CD4 (SBU-T4). Only an occasional CD4$^+$ cell was observed in the caruncular and intercaruncular stroma and the loose connective tissue in the vicinity of the endometrial glands. Some of these cells, presumably macrophages, were large and had a nucleus that varied from ovoid to kidney-shaped. Other CD4$^+$ cells were considered to be lymphocytes.

CD8 (SBU-T8). The pattern of distribution of positively stained CD8 cells was inconsistent as only 12 of the 24 sheep examined had positively stained cells in the caruncular, intercaruncular and endometrial glandular epithelium, and these cells were observed at all stages of the oestrous cycle. The positive cells tended to cluster in patches in the epithelium. There were more positive cells in the stroma than in the epithelium.

SBU-T19. Only an occasional lymphocyte-like SBU-T19$^+$ cell was seen distributed randomly in the stroma of the intercaruncular and caruncular areas. SBU-T19$^+$ cells were not seen in the epithelium or around the endometrial glands.

CD45R (SBU-p220). Lymphocytes positively stained by mAb 20-96 were consistently observed in the caruncular, intercaruncular and endometrial glandular epithelium and in the connective tissue lying immediately beneath these epithelia, in frozen sections as well as in tissues fixed either in Bouin's fluid

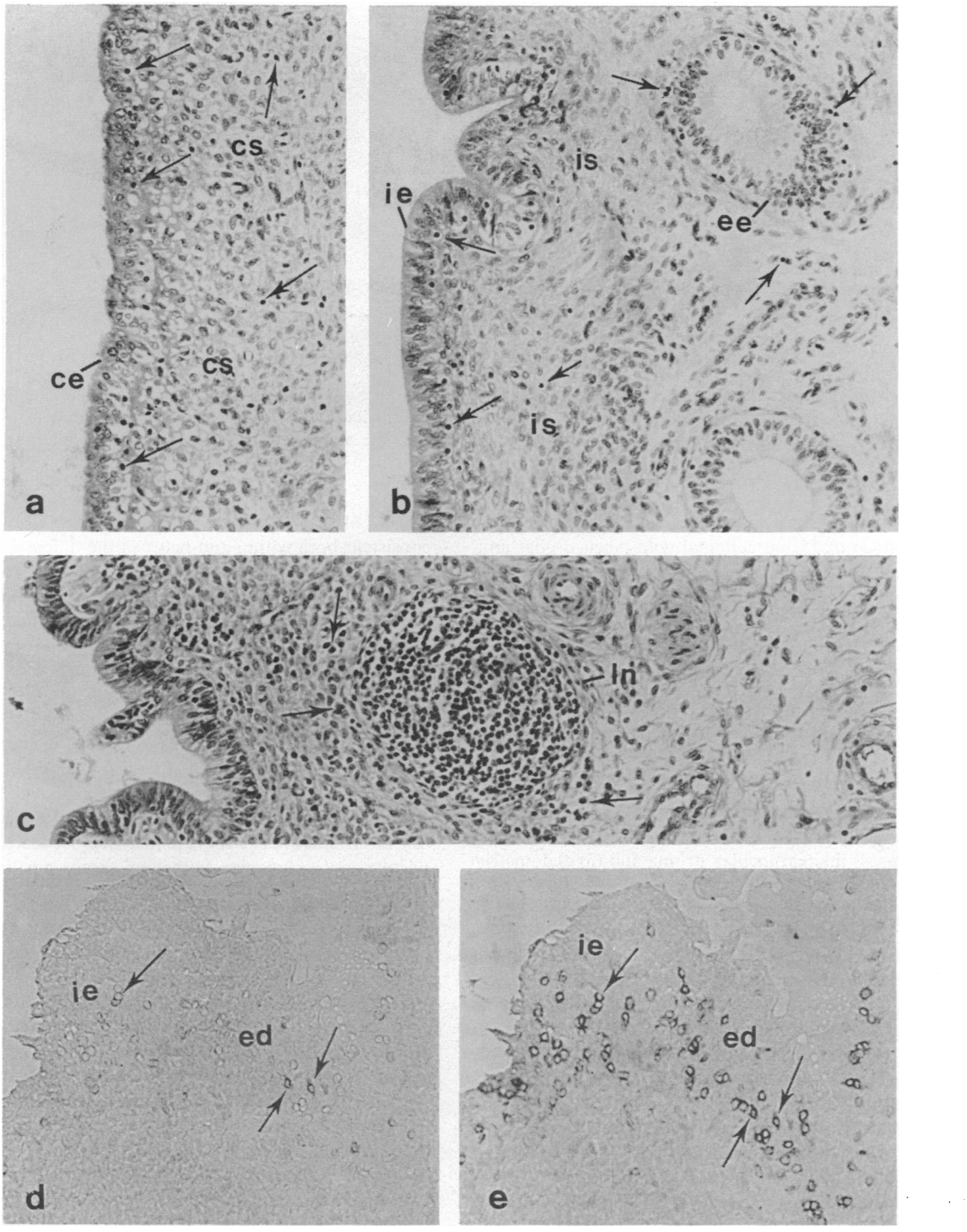

Figure 2. (a, b) Haematoxylin and eosin-stained section showing lymphocytes (arrows) in the (a) caruncular epithelium (ce) and caruncular stroma (cs), (b) intercaruncular epithelium (ie), intercaruncular stroma (is) and endometrial glandular epithelium (ee). (c) A lymphatic nodule (ln) in the intercaruncular stroma with plasma cells (arrows) located in the vicinity (haematoxylin and eosin). (d, e) Immunoperoxidase staining, first with mAb 25-91 (d) showing lymphocytes stained positively for CD5 antigen (arrows) infiltrating the intercaruncular (ie) and endometrial ductal (ed) epithelium. (e) This same section was washed and restained with mAb 20-96. Note that most of the lymphocytes in the epithelium not labelled or weakly labelled in (d) by mAb 25-91 are now labelled by mAb 20-96. This indicates that most of the $20\text{-}96^+$ lymphocytes in the epithelium express very little or undetectable levels of $CD5^+$ antigen. Arrows indicate the same $CD5^+$ cells shown in (d). Magnification (a–e) × 200.

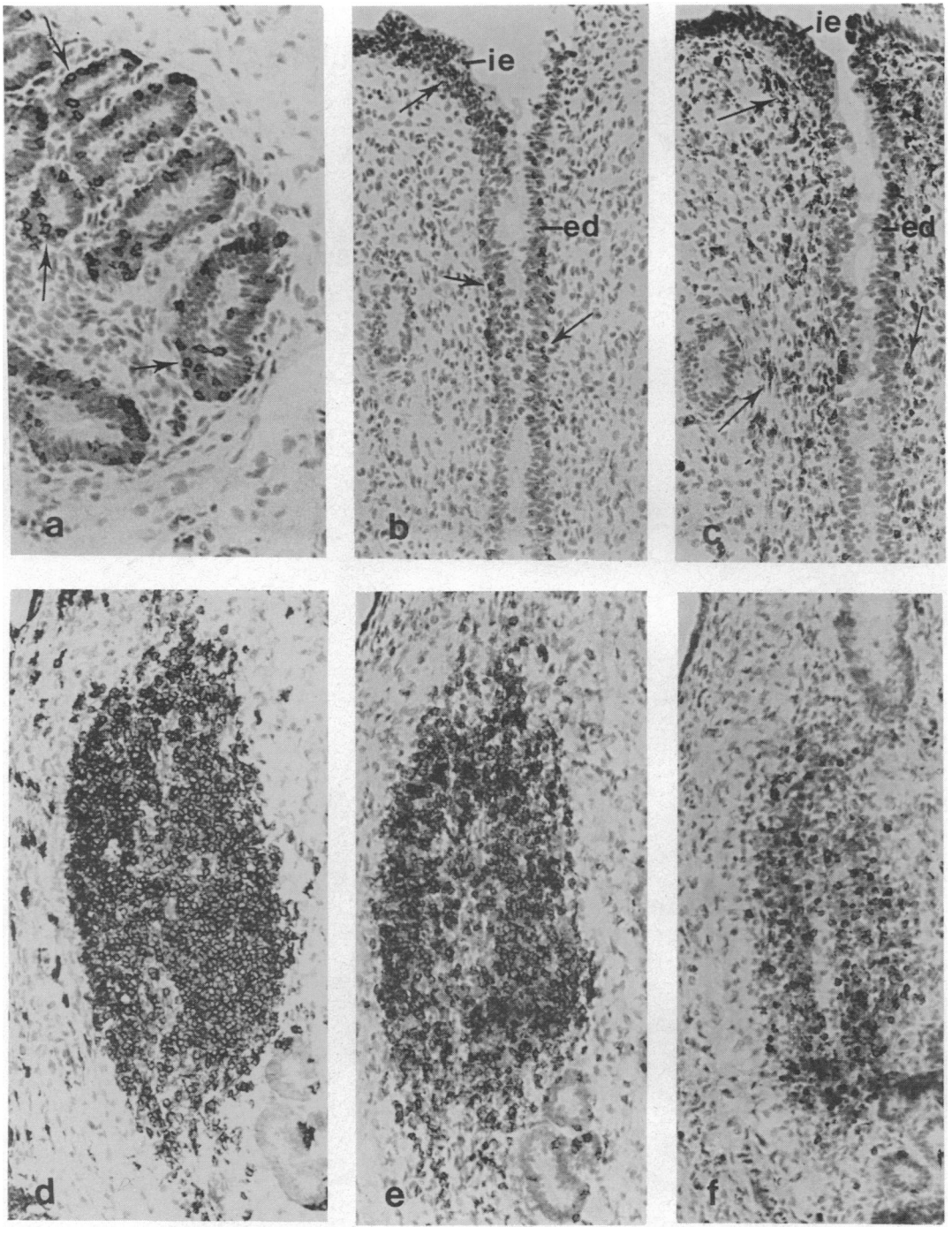

Figure 3. (a, b) Immunoperoxidase staining of uterine tissue section from a mid-cycling ewe with mAb 20-96 showing extensive infiltration of $CD45R^+$ lymphocytes (arrows) into the (a) endometrial glandular epithelium and (b) intercaruncular epithelium (ie) and endometrial ductal epithelium (ed). (c) Immunoperoxidase staining with mAb 49-1 of an area in close proximity to the area shown in (b). Note that hardly any lymphocytes in the epithelium are MHC class II^+, indicating that they are not B lymphocytes. Staining of lymphocytes and spindle-shaped cells in the stroma is evident (arrows). (d–f) Immunoperoxidase staining of sections from a lymphatic nodule in the intercaruncular stroma stained with (d) mAb 49-1, (e) mAb 20-96 and (f) mAb 25-91. Virtually all the cells are MHC class II^+ (d) and $CD45R^+$ (e), indicating that most of them are B lymphocytes. Very few $CD5^+$ cells are present (f). Magnification (a) × 200, (b-f) × 144.

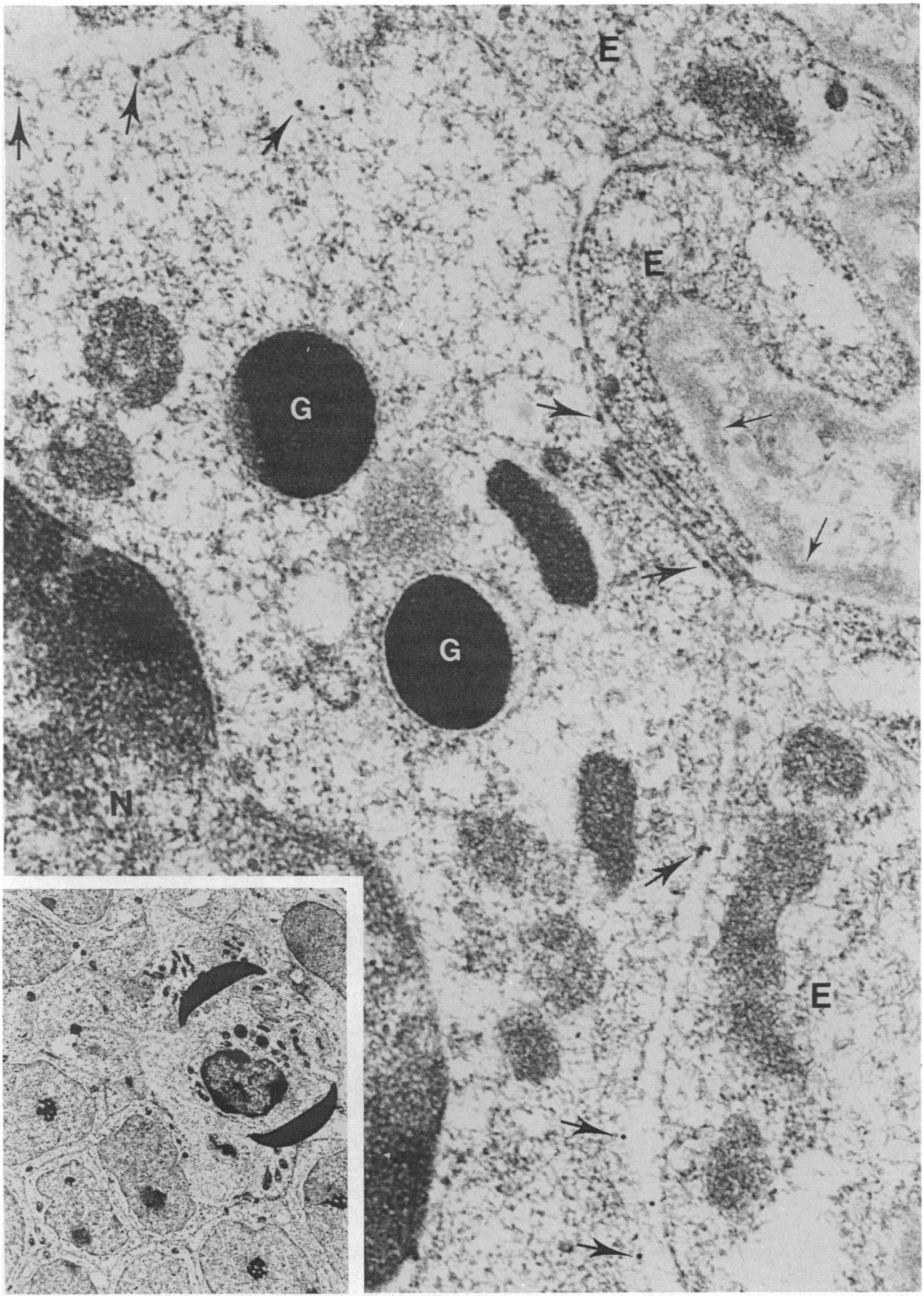

Figure 4. Electron micrograph of a granular lymphocyte located at the basal region of the uterine epithelium stained with mAb 20-96 using the immunogold technique (inset magnification × 14,800). Higher magnification (× 46,000) of the bracketed area in the inset clearly shows gold particles (large arrows) localized almost exclusively on the surface of the cell. Two membrane-bound granules (G) are also present. Small arrows indicate basement membrane, E = uterine epithelial cell; uranyl acetate and lead citrate.

or 95% ethanol (Figs 1, 2e, 3a and b). Positive cells sometimes appeared as clusters of 16–30 cells. Intermingled with them were some unstained lymphocytes (Fig. 1). Very few CD45R$^+$ lymphocytes were seen scattered among the caruncular and intercaruncular stromal cells. Sequential staining of tissue sections, firstly with mAb 25-91 and then with 20-96, revealed that the CD45R$^+$ lymphocytes were weakly stained or negative for the CD5 antigen (Fig. 2d and e). Serial sections revealed that CD45R$^+$ lymphocytes in the caruncular, intercaruncular and endometrial glandular epithelium did not express MHC class II antigens (Fig. 3b and c) or sIg.

As the CD45R antigen was consistently present on the surface of most of the lymphocytes residing in the epithelia of the endometrium, and it also survived all the fixatives used, electron microscopy was employed to localize the CD45R antigen and to examine the morphology of the CD45R$^+$ cells. Gold particles marking the presence of the CD45R antigen were uniformly distributed on the surface of most of the lymphocytes (Fig. 4) interspersed between the basal region of the epithelial cells. These cells were usually seen with a pale cytoplasm containing a few mitochondria, some rough endoplasmic reticulum and occasionally a centriole. However, in some of the CD45R$^+$ lymphocytes, a striking feature was the presence of one to three dark-staining membrane-bound cytoplasmic granules (Fig. 4). Occasionally CD45R$^-$ non-granulated lymphocytes were seen, but the granulated lymphocytes were always CD45R$^+$. Of the tissue sections examined, no granular lymphocyte was detected in the stroma and virtually all the lymphocytes observed in this area were CD45R$^-$. Gold particles were occasionally found over other cell organelles and connective tissues, but they were never above the background levels observed in the controls. As the MHC class II and CD5 epitopes identified by the monoclonal antibodies used in this study do not survive the electron microscope fixation techniques, it was not possible to determine whether the granulated CD45R$^+$ lymphocytes were positive for either of these antigens using electron microscopy.

Immunocytochemical staining of stroma lymphoid follicles

Lymphoid follicles were seen in the intercaruncular stroma of three sheep. Most of the cells in the follicles were CD45R$^+$ (Fig. 3e), whereas only some cells at the periphery of the follicles were CD4$^+$ and CD5$^+$ (Fig. 3f). No CD8$^+$ or SBU HT19$^+$ cells were observed. MHC class II (Fig. 3d) and CD45 were present on virtually all of the cells in these follicles. CD45R$^+$ plasma cells were also identified in the vicinity of these nodules.

DISCUSSION

Histological studies of paraffin sections revealed that, irrespective of the stage of oestrus, lymphocytes were consistently found in the caruncular, intercaruncular and endometrial glandular epithelium and in the intercaruncular and caruncular stroma. By using monoclonal antibodies specific for sheep lymphocytes, both T and B cells were identified in the endometrium. Of the lymphocytes characterized on haematoxylin and eosin-stained sections or identified as being CD45$^+$, few possessed the T-cell antigens CD4, CD8 and SBU-T19. The largest population of cells present in the caruncular, intercaruncular and endometrial glandular epithelium and in the subepithelial regions were CD45R$^+$, but negative for sIg and MHC class II antigens, suggesting that they are not B cells. These lymphocytes were stained by mAb 20-96, which has been shown to detect the p220 component of leucocyte common antigen present on B cells and on a small subset of weakly positive CD5, sIg$^-$ cells found in peripheral blood but absent from macrophages and granulocytes (Mackay *et al.*, 1987). However, since the 20-96$^+$ cells present in the endometrium express barely detectable levels of CD5 antigen, these lymphocytes appear to be similar to the CD45R$^+$ cells present in peripheral blood of sheep that are also weakly CD5$^+$ using FACS analysis (Mackay *et al.*, 1987). Since these cells do not express the B-cell markers MHC class II antigens and sIg, they are phenotypically distinct from the small number of CD5$^+$ B cells present in the peripheral blood and spleen of man (Caligaris-Cappio *et al.*, 1982) and mouse (Hayakawa *et al.*, 1983).

The stability of the epitope on the CD45R antigen identified by mAb 20-96 in the presence of various fixatives permitted the morphological definition of these CD45R$^+$ lymphocytes in the uterus by electron microscopy. The general morphology of CD45$^+$ lymphocytes closely resembled that of a typical lymphocyte except for the presence in their cytoplasm of one to three membrane-bound granules, which is a characteristic of this lymphocyte subpopulation.

Immunocytochemical studies on either fixed or frozen tissue sections of the uterus showed that only a small percentage of lymphocytes in the epithelia were not CD45R$^+$. This agreed with the observation that only a few unlabelled lymphocytes were seen within the epithelium under electron microscopic examination. These observations confirmed that other subpopulations of T lymphocytes are present, but the predominant lymphocyte subpopulation in the caruncular, intercaruncular and endometrial glandular epithelium is the CD45R$^+$ granulated lymphocyte, which does not appear to express MHC class II antigens, but expresses only low levels of CD5 antigen.

Lymphocytes having similar granular morphological features to those that we have defined with the monoclonal antibody 20-96 in the sheep uterus have been described in man (Timonen *et al.*, 1979, 1980) and mouse (Luini *et al.*, 1981; Croy *et al.*, 1985; Silvennoinen, Renkonen & Hurme, 1986; Slapsys, Richards & Clark, 1986). It has been suggested that these granular lymphocytes of man and mouse are 'natural killer' cells having an important role in the first line of defence against viral infections and lymphatic tumours (Herberman & Holden, 1978). It has also been shown that natural killer cells have the ability to lyse a variety of tumour cells growing *in vitro* without prior immunization (Roder, Karre & Kiessling, 1981). In view of the finding that the CD45R$^+$ granulated lymphocytes in the sheep uterus are consistently present in the epithelium of the endometrium, a site that is bathed by the external milieu, we suggest these cells may act as a first line of defence against foreign pathogens. If this is true, it would be expected that the epithelia of the mucous membranes of other organs also harbour a similar subpopulation of lymphocytes. Studies are under way to test this hypothesis.

The lymphoid follicles found in the endometrium of three sheep are thought to be primary B-cell regions as most of the cells were both MHC class II$^+$ and CD45R$^+$. The plasma cells in the vicinity of the follicles were probably derived from the B cells in the lymphoid follicles. These findings suggest that the uteri of these sheep may have been exposed to antigenic stimulation.

The data obtained in this study reveal that the endometrium of the sheep is endowed with a cellular population, particularly lymphocytes, that favours the elicitation of a local immune response. The data also suggest that there is 'compartmentalization' of CD45R$^+$ lymphocytes in the endometrial epithelia and subepithelial regions. This phenomenon could be due to a selective migration of this cell type through the capillaries localized in the subepithelial regions. However, the precise role that the CD45R$^+$ granulated lymphocytes identified in this study play in local immunity is unknown, and further studies are necessary to reveal their importance in immunity.

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REFERENCES

CALIGARIS-CAPPIO F., GOBBI M., BOFILL M. & JANOSSY G. (1982) Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. *J. exp. Med.* **155,** 623.

CROY B.A., GAMBEL P., ROSSANT J. & WEGMANN T.G. (1985) Characterization of murine decidual natural killer (NK) cells and their relevance to the success of pregnancy. *Cell Immunol.* **93,** 315.

HAYAKAWA K., HARDY R.R., PARKS D.R. & HERZENBERG L.A. (1983) The 'Ly-I B' cell subpopulation in normal, immunodefective and autoimmune mice. *J. exp. Med.* **157,** 202.

HEAD J.R. & BILLINGHAM R.E. (1986) Concerning the immunology of the uterus. *Am. J. reprod. Immunol.* **10,** 76.

HERBERMAN R.B. & HOLDEN A.T. (1978) *Advances in Cancer Research* (ed. R. B. Herberman), p. 305. Academic Press, Orlando, FA.

LEE C.S., GOGOLIN-EWENS K., WHITE T.R. & BRANDON M.R. (1985) Studies on the distribution of binucleate cells in the placenta of the sheep with a monoclonal antibody SBU-3. *J. Anat.* **140,** 565.

LUINI W., BORASCHI D., ALBERTI S., ALEOTTI A. & TAGLIABUE A. (1981) Morphological characterization of a cell population responsible for natural killer activity. *Immunology*, **43,** 663.

MACKAY C.R., MADDOX J.F. & BRANDON M.R. (1986) Three distinct subpopulations of sheep T lymphocytes. *Eur. J. Immunol.* **16,** 19.

MACKAY C.R., MADDOX J.F. & BRANDON M.R. (1987) A monoclonal antibody to the p220 component of sheep LCA identifies B cells and a unique lymphocyte subset. *Cell. Immunol.* (in press).

MACKAY C.R., MADDOX J.F., GOGOLIN-EWENS K.J. & BRANDON M.R. (1985) Characterization of two sheep lymphocyte differentiation antigens SBU-T1 and SBU-T6. *Immunology*, **55,** 729.

MADDOX J.F., MACKAY C.R. & BRANDON M.R. (1985a) The sheep analogue of leucocyte common antigen (LCA). *Immunology*, **55,** 347.

MADDOX J.F., MACKAY C.R. & BRANDON M.R. (1985b) Surface antigens, SBU-T4 and SBU-T8 of sheep T lymphocyte subsets defined by monoclonal antibodies. *Immunology*, **55,** 739.

MADDOX J.F., MACKAY C.R. & BRANDON M.R. (1987) Ontogeny of ovine lymphocytes. II. An immunohistological study on the development of T lymphocytes in the sheep fetal spleen. *Immunology*, **62,** 107.

O'SHEA J.D., NIGHTINGALE M.G. & CHAMLEY W.A. (1977) Changes in small blood vessels during cyclical luteal regression in sheep. *Biol. Reprod.* **17,** 162.

O'SHEA J.D. & WRIGHT P.J. (1984) Involution and regeneration of the endometrium following parturition in the ewe. *Cell Tiss. Res.* **236,** 477.

PURI N.K., GORRELL M.D. & BRANDON M.R. (1987) Sheep class II molecules. I. Immunochemical characterization. *Immunology*, **62,** 567.

RODER J.C., KARRE K. & KIESSLING R. (1981) Natural killer cells. *Prog. Allergy*, **28,** 66.

SAINTE-MARIE G. (1962) A paraffin embedding technique for studies employing immunofluorescence. *J. Histochem. Cytochem.* **10,** 250.

SILVENNOINEN O., RENKONEN R. & HURME M. (1986) Characterization of natural killer cells and their precursors in the murine bone marrow. *Cell. Immunol.* **101,** 1.

SKJERVEN O. (1956) Endometrial biopsy studies in reproductively normal cattle. *Acta Endocrin.* (suppl.) **26,** 1.

SLAPSYS R.M., RICHARDS C.D. & CLARK D.A. (1986) Active suppression of host-versus-graft reaction in pregnant mice. VIII. The uterine decidua-associated suppressor cell is distinct from decidual NK cells. *Cell. Immunol.* **99,** 140.

TIMONEN T., SAKSELA E., RANKI A. & HAYRY P. (1979) Fractionation, morphological and functional characterization of effector cells responsible or human natural killer activity against cell-line targets. *Cell. Immunol.* **48,** 133.

TIMONEN T., SAKSELA E., VIRTANEN I. & CANTELL K. (1980) Natural killer cells are responsible for the interferon production induced in human lymphocytes by tumour cell contact. *Eur. J. Immunol.* **10,** 422.

VANDER-WIELEN A.L. & KING G.J. (1984) Intraepithelial lymphocytes in the bovine uterus during the oestrous cycle and early gestation. *J. reprod. Fert.* **70,** 457.

WOODING F.B.P. (1981) Localization of ovine placental lactogen in sheep placentomes by electron microscope immunocytochemistry. *J. reprod. Fert.* **62,** 15.