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Studies on the differentiation of T lymphocytes in sheep

II. TWO MONOCLONAL ANTIBODIES THAT RECOGNIZE ALL OVINE T LYMPHOCYTES

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SUMMARY

Two mouse monoclonal cytotoxic antibodies (ST-1_a and ST-1_b) recognize an antigen present on the large majority of thymocytes and all T cells in the periphery, but not B cells or other haemopoietic cells in sheep. Examination of frozen sections of various fetal tissues revealed that the cells expressing this antigen first appeared in the thymus, and these cells markedly increase in numbers in the peripheral lymphoid tissues after mid-gestation. Large accumulations of positive cells were located in the paracortex of lymph nodes, the periarteriolar lymphoid sheath of the spleen, and interfollicular areas of jejunal Peyer's patches, all of which are known to be T-dependent areas. Treatment of lymphocytes with ST-1_a and complement resulted in the abrogation of T-proliferative responses, but the response to a B-cell mitogen, lipopolysaccharide, was not reduced. Neither ST-1_a nor ST-1_b cross-reacted to lymphocytes obtained from other species of animals (man, monkey, mouse, rat, guinea-pig, chicken, frog, pig, horse, goat and cattle). Based on these findings, it was concluded that the expression of the antigen recognized by ST-1_a and ST-1_b is restricted to the T-cell lineage of sheep, and that all ovine T cells express this antigen. Furthermore, ST-1_a and ST-1_b were determined to recognize the same antigen by reciprocal blocking experiments.

INTRODUCTION

Recirculation of lymphocytes has been studied extensively by the use of sheep (cf. Hay & Cahill, 1982). However, the paucity of reliable cell-surface markers in this species has hampered more detailed studies of the lymphocyte recirculation. While we have previously reported a monoclonal antibody (mAb) T-80 which binds to the majority of T cells in efferent lymph of sheep (Miyasaka *et al.*, 1983), we found that a considerable proportion of peripheral blood lymphocytes (PBL) without detectable amounts of surface immunoglobulin (sIg^- cells) showed no binding of this T-80 mAb. The presence of T cells that are negative for the binding of T-80 mAb was also suggested from the lack of positive cells in the cortico-medullary junction in the thymus (Miyasaka *et al.*, 1983, 1984). Therefore, we set out to produce mAb against all T cells and/or T cells with no demonstrable binding of T-80 ($T\text{-}80^-$ T cells). From a series of fusions, we obtained a panel of mAb against various subpopulations of sheep lymphocytes, and preliminary reports have been made (Miyasaka *et al.*, 1985a; Ezaki *et al.*, 1985).

In a series of papers, we describe the production of two mAb (ST-1_a, ST-1_b) to an antigen expressed by all sheep T cells. In this paper, the specificity of these mAb and the tissue localization, functional properties and ontogeny of the cells recognized by these mAb are presented. In a companion paper (M.-F. Beya and M. Miyasaka, submitted), we deal with biochemical studies of the T-cell antigen, as well as its structural variation in the course of T-cell maturation.

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MATERIALS AND METHODS

Animals

White Alpine and Black Jura sheep of both sexes were obtained from Versuchsbetrieb Sennweid, Olsberg, Switzerland. Fetal lambs were obtained from the timed matings of superovulated ewes. BALB/c mice, outbred rats, guinea-pigs and rabbits were obtained from the Institut für Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland. Chickens and frogs were obtained from breeding colonies kept in our institute. A stumptail monkey was obtained from Hoffmann–La Roche, Basel, Switzerland. Human blood was obtained from one of us (M.M.). Cattle and pig blood were obtained from the abattoir. Horse and goat blood were taken from stock animals of our institute.

Surgical procedures

The method used for the cannulation of the efferent lymphatic duct of the prescapular lymph node has been described in detail elsewhere (Miyasaka & Trnka, 1985).

Preparation of various cell populations

Peripheral blood mononuclear cells (PB-MNC) were obtained from adult sheep by Percoll density gradient centrifugation of fresh blood buffy-coat cells (Miyasaka & Trnka, 1985). EDTA was used as anticoagulant. Approximately 1–10% of the mononuclear cells were stained positive for alpha-naphthyl acetate esterase (ANAE) (Yam, Li & Crosby, 1971), and these were considered to be monocytes. Efferent lymph lymphocytes were obtained by cannulation of efferent lymph duct of a prescapular lymph node. T cells were obtained by depleting cells adherent to nylon wool (Cahill *et al.*, 1978) or by 'panning' out cells expressing surface immunoglobulin (sIg) (Miyasaka & Trnka, 1985). Populations enriched for T cells contained less than 5% surface immunoglobulin-positive (sIg^+) cells based on staining with fluoresceinated $F(ab')_2$ rabbit anti-sheep Ig (Cappel Lab., Cochranville, PA), and less than 3% monocytes based on staining with ANAE.

$T\text{-}80^-$ cells were selected by complement-mediated killing of purified T cells with T-80 mAb. Peripheral blood T cells were treated with T-80 hybridoma-derived ascites (1:1000) at 4 for 30 min, washed once and then incubated with fresh guinea-pig serum (1:3) at 37 for 45 min. The dead cells were removed by discontinuous Percoll gradient (35%/75%) (Miyasaka & Trnka, 1985). $T\text{-}80^-$ T cells were recovered from the interface between 35% and 75% Percoll layers. The viability was determined by trypan blue dye exclusion. Bone marrow cells, platelets and granulocytes from the peripheral blood were isolated by methods previously described (Miyasaka & Trnka, 1985). PB-MNC from human, monkey, rabbit, chicken, cattle, pig, horse and goat blood were isolated using the method described above for sheep. Frog spleen cells were prepared as described previously (Du Pasquier *et al.*, 1985). Spleens obtained from the mouse, rat and guinea-pig were gently teased, minced, and viable cells were obtained by centrifugation on a 35%/75% Percoll gradient.

Production and screening of mAb

Since an initial aim of the study was to obtain mAb against $T\text{-}80^-$ T cells, BALB/c mice were immunized with T-80 T cells obtained as described above. Eight-week-old female BALB/c mice were immunized i.p. twice with 2×10^7 $T\text{-}80^-$ T cells with a 7-day interval. An intravenous booster injection of 2×10^7 $T\text{-}80^-$ T cells was given 1 week later, and the spleen was obtained 3 days later. A series of cell fusions was performed using X63Ag8.653 myeloma cells (Kearney *et al.*, 1979) according to the method of Köhler & Milstein (1975). The supernatants from the fused hybridoma cells were screened first by a radioimmunoassay with paraformaldehyde-fixed sheep efferent cells and efferent T cells as target cells (Stocker & Heusser, 1979). Positive reactions were developed by use of ^{125}I-$F(ab')_2$ sheep anti-mouse Ig (Amersham International, Amersham, Bucks, U.K.). The proportion of cells stained by positive hybridoma supernatants was determined by indirect immunofluorescence (IF) with fluorescence-activated cell sorter (FACS II, Becton-Dickinson, Mountain View, CA) analysis (2×10^4 cells/assay).

The immunoglobulin isotype of each mAb was initially determined by Ouchterlony analysis, and the results were confirmed by IF assay on acetone–alcohol fixed hybridoma cells (Kearney & Lawton, 1975) using goat anti-mouse Ig class-specific antibodies (Southern Biotechnology Associates, Birmingham, AL). The IF assay to detect mAb binding with FITC-$F(ab')_2$ goat anti-mouse Ig has been described elsewhere (Miyasaka & Trnka, 1985). All antibodies used were titrated for optimal immunofluorescence, and the stained cells were examined with a Leitz Orthoplan fluorescence microscope equipped with Ploem epi-illumination with discriminating sets of excitation and barrier filters or a FACS II equipped with argon-ion laser. The FACS was set to exclude dead cells by forward light scatter.

Selected hybridomas were subcloned three times by limiting dilution, and then grown in ascitic form in pristane-primed mice. MAb were purified from ascitic fluid or culture supernatant by affinity chromatography using Protein A–Sepharose (Pharmacia, Uppsala, Sweden) or Affi-Gel Protein A (Bio-Rad, Richmond, CA), and subsequently conjugated with fluorescein isothiocyanate (Goding, 1976) or biotinylated with biotin succinimide ester (Beyer & Wilchek, 1978). Two mAb secreted from T-81 and MF-160 hybridomas were purified and called ST-1_a and ST-1_b, respectively.

Blocking experiments

Binding of St-1_a and ST-1_b was tested on cells pretreated with an appropriate antibody (ST-1_b or ST-1_a). Efferent lymph cells, 5×10^6, were incubated with varying concentrations of purified ST-1_a or ST-1_b or medium alone for 30 min at 4 and washed three times. Preincubated cells were subsequently stained with 50 μg FITC-conjugated ST-1_a or ST-1_b for 30 min at 4 and examined with a Leitz fluorescence microscope. The extent of inhibition of antibody binding was expressed as percentage inhibition calculated as follows:

$$\%\ \text{inhibition of ST-}1_a\ \text{binding with ST-}1_b = \frac{\text{proportion (\%) of ST-}1_a\ \text{positive cells in ST-}1_b\text{-pretreated cell preparation}}{\text{proportion (\%) of ST-}1_a\ \text{positive cells in medium-pretreated cell preparation}} \times 100.$$

Inhibition of ST-1_b binding with ST-1_a was calculated in a similar way.

Immunoperoxidase staining of frozen sections.

Eight-micron cryostat sections were cut, stored overnight at room temperature (RT) and fixed in acetone for 5 min. After drying, sections were incubated with optimal dilutions of mAb for 30 min at RT, followed by washes in three changes of Tris-buffered saline, pH 7·6, containing 0·25% bovine serum albumin and 0·05% Tween 20 (TBS + BSA + Tween 20). A second antibody, peroxidase-conjugated goat anti-mouse Ig (KPL, Gaithersburg, MD) was placed on sections for 30 min at RT. After washes in two changes of TBS (+BSA + Tween 20) followed by a wash in TBS without BSA (TBS + Tween), the sections were immersed in methanol for 1 min and dried. They were subsequently rehydrated in TBS + Tween and incubated with 0·1% diaminobenzidine tetrahydrochloride (DAB), 0·02% hydrogen peroxide in PBS for 10 min at RT. Preparations were subsequently washed in TBS + Tween, refixed in methanol for 5 min, dried and incubated with 0·5 M copper sulphate in 0·9% NaCl for 10 min. After a further washing, slides were counterstained with Giemsa and coverslipped.

Proliferative responses to mitogens and alloantigens

These are described in detail elsewhere (Ezaki *et al.* 1985). In order to examine whether the cells recognized by the mAb are

involved in proliferative response to mitogens and alloantigens, PB-MNC (2×10^7 cells) were treated with mAb at 4° for 30 min, washed once and then incubated with rabbit complement (C) (Cedarlane, Hornby, Ontario) at 37° for 30 min. This treatment was repeated once more to kill antigen-positive cells completely. The optimal concentrations of ascitic fluid containing the mAb (1:500 ~ 1000) and C (1:2) for the maximum killing were determined by trypan-blue dye exclusion and FACS using propidium iodide exclusion. After the treatment, the cells were washed once, resuspended in an appropriate volume without adjusting the number of remaining viable cells, and used for proliferation assays.

In some experiments, serial dilutions of ascites containing mAb were directly added to the culture and proliferative assays were carried out subsequently. No complement was used in these experiments.

RESULTS

Reactivity of ST-1_a and ST-1_b monoclonal antibodies to viable cells in suspension

Out of 632 hybridomas resulting from the fusion of mouse myeloma X63Ag8.653 with mouse splenocytes immunized against sheep lymphocytes, two hybridomas that produce IgG2a mAb against sheep T cells were isolated. As shown in Table 1, these mAb, ST-1_a and ST-1_b, reacted with approximately 49% of peripheral blood mononuclear cells, 77–80% of efferent lymph cells and 84–90% of thymocytes when analysed by a FACS. In the ileal Peyer's patches, a major site for B-cell production in sheep, only 0·4–0·8% of cells were positive for these mAb. More than 70% of the cells in the ileal Peyer's patches expressed surface Ig (sIg^+).

ST-1_a and ST-1_b mAb did not recognize erythroblasts and myeloid cells obtained from the bone marrow, nor monocytes, granulocytes, platelets and erythrocytes obtained from the peripheral blood.

FACS analysis on separated T and B cells stained with mAb clearly revealed that both mAb reacted with T cells but not B cells (Fig. 1). These results suggest that both ST-1_a and ST-1_b mAb recognize only thymocytes and peripheral T cells among haemopoietic cells of the sheep. It was a consistant observation that the staining intensity was relatively weak with thymocytes, intermediate with peripheral blood T cells, and strongest with efferent lymph T cells (data not shown).

Table 1. Binding of ST-1_a and ST-1_b monoclonal antibodies to various cell types of sheep

	ST-1_a	ST-1_b	Anti-Ig
PB-MNC*	48·8 ± 10·6†	48·7 ± 10·2	46·1 ± 8·5
Efferent lymph lymphocytes	76·8 ± 1·5	79·9 ± 4·4	19·8 ± 1·5
Thymocytes	90·6 ± 2·3	83·5 ± 8·0	0·3 ± 0·1
Ileal Peyer's patch cells	0·4 ± 0·1	0·8 ± 0·3	72·9 ± 7·2
Erythroblasts	0	0	0
Erythrocytes	0	0	0
Myeloid cells in bone marrow	0	0	0
Peripheral blood polymorphonuclear cells	0	0	0
Platelets	0	0	0

* Peripheral blood mononuclear cells.

† Expressed as a percentage of cells positive for the binding of respective antibody determined by FACS.

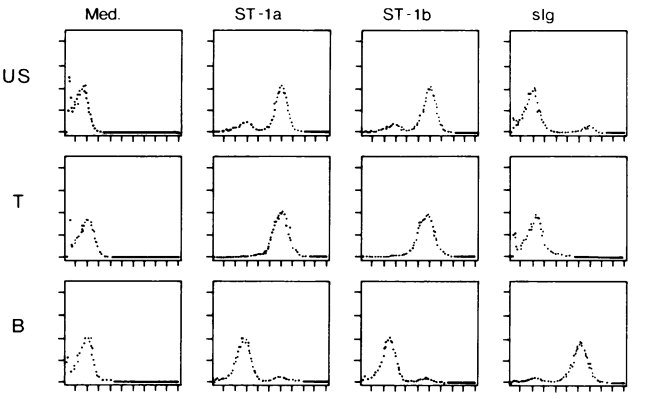

Figure 1. ST-1_a and ST-1_b bind to ovine T cells. Efferent lymph lymphocytes were obtained by the cannulation of efferent lymph duct of a prescapular lymph node. These cells (US) were separated into T cells (T) and B cells (B) by the 'Ig panning' method. Subsequently, they were stained for antigens detected by ST-1_a and ST-1_b mAb, and rabbit anti-sheep immunoglobulin. Negative control was obtained by incubating cells with medium (Med.) alone, and subsequently with FITC-F(ab')$_2$ goat anti-mouse Ig. FACS profiles of stained cells are illustrated by dotted lines. Logarithmic fluorescence intensity is plotted on the abscissa in arbitrary units, and linear cell frequency is given on the ordinate.

In order to test whether or not the specificities of ST-1_a and ST-1_b mAb are identical, blocking experiments were performed. It was found that preincubation of efferent lymph cells with ST-1_b mAb inhibited the binding of ST-1_a mAb in a dose-dependent manner, and that saturating amounts of ST-1_b mAb completely blocked subsequent binding of ST-1_a (Fig. 2). In the reverse experiment, ST-1_a mAb was found to block ST-1_b mAb binding in an identical manner, suggesting that these two mAb recognize identical antigens.

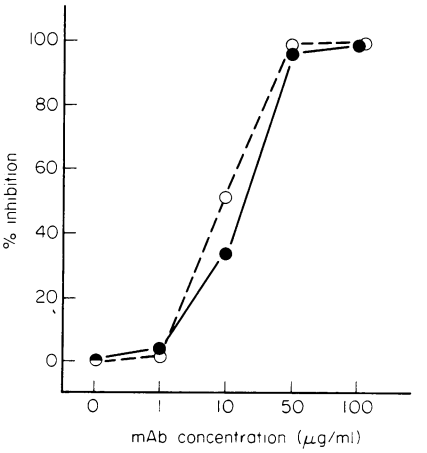

Figure 2. Reciprocal inhibition of mAb binding. Efferent lymph lymphocytes (5×10^6 cells) were incubated with varying concentrations of purified ST-1_a (●——●) or ST-1_b (O- - - -O) for 30 min at RT, washed and subsequently stained with 50 μg FITC-ST-1_b (solid line) or FITC-ST-1_a (dotted line). The extent of inhibition of antibody binding was calculated as described in the Materials and Methods.

Tissue localization of cells recognized by ST-1_a and ST-1_b

Frozen sections of various organs were stained by an immunoperoxidase method. Figure 3 shows ST-1_a staining of the thymus, spleen, mesenteric lymph node and jejunal Peyer's patches. In the thymus, both cortex and medulla had diffuse staining, the latter being more intensely stained. In the spleen, positively stained cells accumulated in the periarteriolar lymphoid sheath (PALS) of the white pulp and also scattered throughout the red pulp. Within lymph nodes, major accumulations of positive cells were found in the paracortex. Positive cells were found only sporadically in germinal centres. In jejunal Peyer's patches positive cells were concentrated in interfollicular areas, while occasional cells were stained within the follicles. The lamina propria also contained a large number of positive cells. The liver, kidney and brain were almost completely negative for ST-1_a positive lymphocytes. Staining obtained with ST-1_b mAb was in every respect identical to that with ST-1_a, in

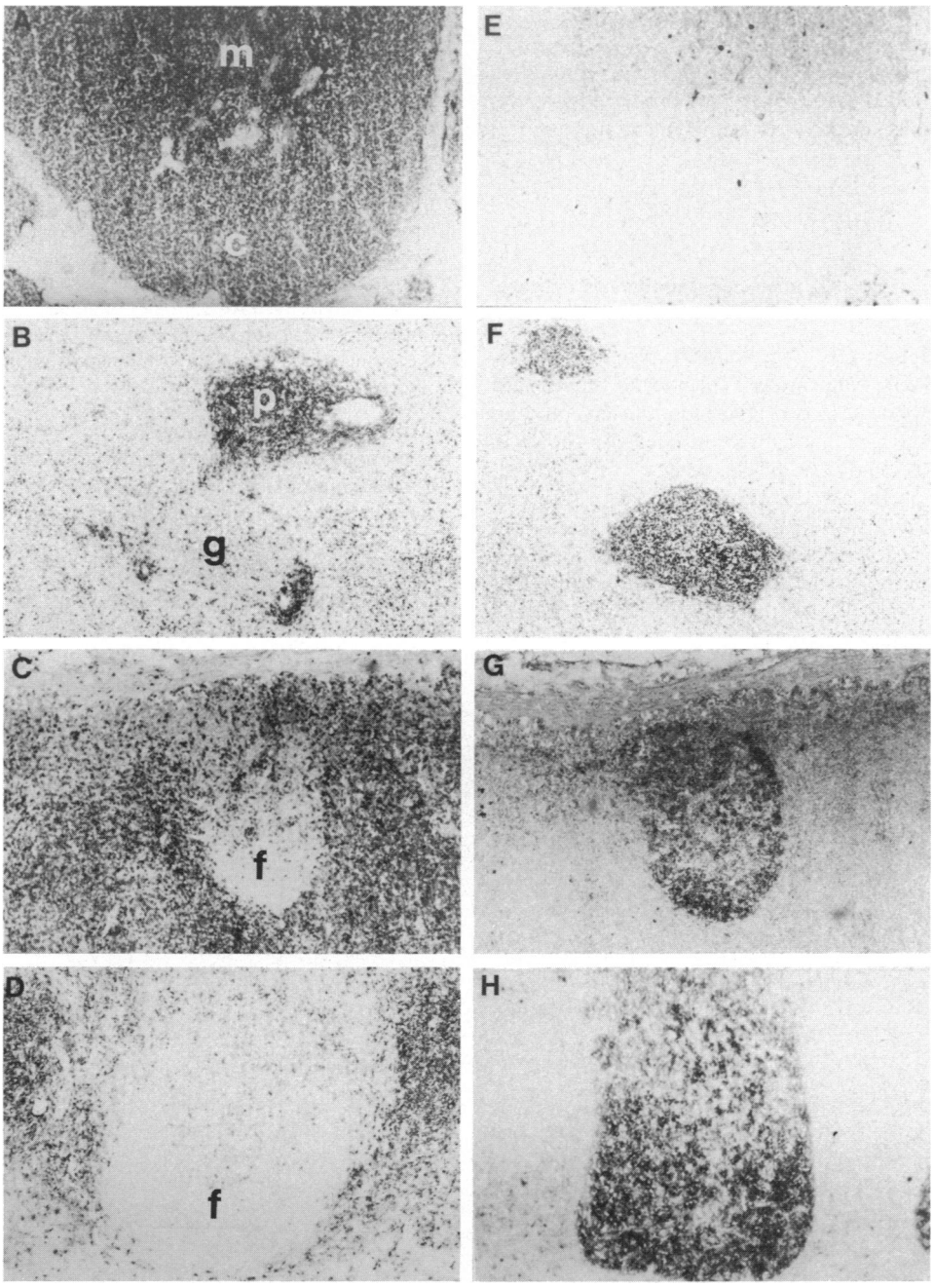

Figure 3. Frozen sections of ovine thymus (A,E), spleen (B,F), mesenteric lymph node (C,G) and jejunal Peyer's patches (D,H). Serial sections were stained with ST-1_a (A,B,C.D) or E-53 (E,F,G,H). E-53 stains B cells selectively (Miyasaka *et al.*, 1985a). Thymus: diffusely positive for ST-1_a (A) and negative for E-53 (E). The medulla (m) is more heavily stained than the cortex (c). Spleen: periarteriolar lymphoid sheath (p) is ST-1_a positive and germinal centres (g) are E-53 positive. Mesenteric lymph node: ST-1_a positive cells are abundant in the cortex. Occasional cells are present in the follicle (f). Jejunal Peyer's patches: interfollicular areas are ST-1_a positive, whereas follicles (F) are E-53 positive. (Magnification $\times 32$).

that positive cells were most abundant in the T-dependent area (data not shown). These results again suggest that ST-1_a and ST-1_b mAb recognize the same cells, and that the cells belong to the T-cell lineage.

Functional studies.

In order to examine whether antigens recognized by ST-1_a and ST-1_b mAb are associated with any major T-cell functions, PB-MNC were treated with mAb in the presence of complement and subsequently tested to determine their ability to respond to mitogens and alloantigens. In some experiments cells were incubated with mitogens in the presence of varying doses of mAb in the culture. No complement was added in the culture.

Incubation of cells with ST-1_a in the absence of complement did not result in the alteration of T-proliferative functions (Table 2), suggesting that the determinant recognized by the mAb is not involved in any of the T-cell functions tested. However, when cells were treated with ST-1_a and complement, it was found that the subsequent proliferative responses to PHA, Con A and PWM were reduced by more than 75% (Table 2). Alloreactivity was also diminished by around 90%. In contrast, the proliferative response to lipopolysaccharide, a B-cell mitogen, was not reduced by this treatment, suggesting that only T-cell activity was affected.

When the phenotype of lymphocytes proliferating in response to PHA and Con A was examined, it was found that the blast cells were strongly positive for ST-1_a and ST-1_b binding (data not shown), indicating that the antigen recognized by the mAb is also present on sheep T-blast cells, and is not lost from T cells during a 3–4-day culture period *in vitro*.

Ontogeny of cells detected by ST-1_a and ST-1_b mAb

In order to investigate the ontogeny of cells recognized by these mAb, FACS analysis of single-cell suspensions obtained from

Table 2. Effect of ST-1_a monoclonal antibody on proliferative functions of sheep peripheral blood lymphocytes

	Treatment		[^{3}H]TdR incorporation ($\times 10^3$ c.p.m.)					
Cells	ST-1_a	Complement	PHA	Con A	PWM	LPS	Autologous PBL	Allogeneic PBL
I. PB-MNC pretreated*	–	–	131·0	157·9	80·0	43·4	0·7	18·9
	–	+	146·0	164·2	93·9	80·0	0·6	17·8
	+	+	20·2	34·3	35·5	63·7	0·5	1·3
II. PB-MNC co-cultured†	–	–	119·1	107·0	94·0	ND‡	ND	
	+	–	115·9§	103·2	106·7			

* PB-MNC were pretreated with or without antibody and C, and subsequently tested for their proliferative functions.

† PB-MNC were tested for their proliferative functions in the presence or absence of serially diluted (1:50 ~ 2000) ascites containing ST-1_a.

§ C.p.m. obtained at an antibody concentration of 1:100 are given. Very similar c.p.m. were obtained at other antibody concentrations examined.

‡ ND, not determined.

Table 3. Proportion of ST-1_a positive cells in various organs of the sheep fetus

	Days of gestation*					
	56	78	84	98	103	132
Thymus	97·5† (0·1)‡	98·9 (0·3)	ND§	94·5 (0)	ND	99·1 (0)
Spleen	ND	22·1 (26·6)	9·8 (22·0)	25·9 (8·9)	21·8 (9·5)	59·1 (10·8)
PB-MNC	0·2 (0·1)	7·2 (2·7)	1·5 (1·2)	ND	26·4 (8·5)	48·2 (9·1)
Prescapular lymph node	ND	ND	69·8 (4·5)	70·2 (4·3)	ND	82·5 (7·4)
Mesenteric lymph node	ND	ND	ND	44·9 (8·0)	ND	68·4 (36·0)
Liver	0·2 (0·2)	0·5 (0·2)	0·1 (0)	0·2 (0)	0·2 (0·4)	2·6 (0)
Bone marrow	ND	ND	ND	0·2 (6·7)	ND	0·2 (0·2)
Ileal Peyer's patches			ND			1·5 (75·5)
Jejunal Peyer's patches						9·5 (78·5)

* Six fetuses of various ages (one fetus for each individual age) were obtained from timed matings of superovulated ewes.

† Expressed as a percentage of cells positive for ST-1_a mAb binding determined by FACS. Almost identical results were obtained with ST-1_b mAb.

‡ Proportion of surface Ig-positive cells.

§ ND, not determined.

various organs of fetal lambs of various ages were made (Table 3). An immunoperoxidase staining of frozen sections obtained from various fetal organs was also carried out to obtain information on the exact topology of positive cells within the given organ. The youngest fetus examined using FACS analysis was 56 days of gestation. At this age approximately 98% of thymocytes were stained positively by ST-1_a and ST-1_b. With an immunoperoxidase staining method, it was found that cells in both the cortex and the medulla were positively stained. In a young fetus (42 days), upon which only immunoperoxidase staining of transverse whole-body sections was performed, it was found that only the thymus contained demonstrable accumulation of positive cells. At this stage no cortico-medullary difference was observed, and the great majority of the cells within the thymus were positive for ST-1_a and ST-1_b. Positive cells subsequently appeared in the spleen and the prescapular lymph node, followed by the mesenteric lymph node. By 100 days of gestation, a significant proportion of PB-MNC became positive for the mAb binding. Both the liver and the bone marrow contained very few positive cells throughout gestation. The jejunal Peyer's patches contained a few positive cells, and they were restricted to interfollicular areas. Only a few cells were positive in the ileal Peyer's patches.

Reactivity of ST-1_a and ST-1_b to xenogeneic lymphocytes

Both ST-1_a and ST-1_b antibodies reacted with only sheep lymphocytes. No reactivity was observed with lymphocytes obtained from the human, monkey, mouse, rat, guinea-pig, chicken, frog, pig, horse, goat and cow (data not shown), suggesting that the antigen appeared after the divergence of sheep and other ruminants from a common ancestor.

DISCUSSION

In this paper, we described two mAb, ST-1_a and ST-1_b, that are produced by two different hybridomas, T-81 and MF-160, respectively. These mAb showed specific binding to sheep thymocytes and T cells, but not B cells or other haemopoietic cells. Endothelial cells of blood vessels or other tissue parenchyma were not recognized by these mAb. The first appearance of the positive cells was found in the fetal thymus, and the number of positive cells increased markedly in the peripheral lymphoid organs after mid-gestation. In the blood and lymph of postnatal animals, sIg^- cells were almost all positive for the binding of these mAb, and these positive cells were identified in T-dependent areas of various lymphoid organs; this suggests that expression of the antigen recognized by these mAb is restricted to the T-cell lineage, and that all T cells express this antigen. Furthermore, an observation that lymphocytes responding to T-cell mitogens, such as PHA and Con A, remained positive for the mAb binding indicates that the antigen recognized by these mAb is a stable T-cell marker. Since these mAb can lyse T cells in the presence of complement, they can be used as an effective means to remove contaminating T cells from non-T-cell preparations (Miyasaka *et al.*, 1985b). No indication was obtained to suggest that antigens recognized by these mAb mediate any major T-cell functions. Unlike some mAb reported in other species (Van Wauwe, De May & Goosener, 1980; Julius, Heusser & Hartmann, 1984), neither of our mAb are mitogenic, which allows one to separate T cells from heterogeneous cell populations without affecting their proliferative functions.

Although ST-1_a and ST-1_b are produced by two different hybridomas, antigen specificity of these two mAb appears to be identical. Almost complete blocking of mAb binding was demonstrated in a reciprocal manner. The ontogeny and distribution of positive cells in various lymphoid organs are also identical. In a companion paper (M.-F. Beya and M. Miyasaka, submitted), we demonstrate the biochemical identity of the antigens (67,000 MW glycoprotein). The only recognizable difference between these two mAb is the ease of ascites production. Hybridoma T-81, which secretes mAb St-1_a, consistently produced large amounts of ascites in pristane-treated mice, whereas hybridioma MF-160, which produces mAb ST-1_b, formed, in most cases, only solid tumours intraperitoneally in the recipient mice.

When various types of cells were stained with ST-1_a or ST-1_b and analysed with a FACS, it was consistently observed that fluorescence intensity increased in the following order: thymocytes, peripheral blood T cells, efferent lymph T cells, possibly reflecting the extent of maturation of each cell population.

Almost all cells in the fetal thymus stained positive with the ST-1_a and ST-1_b, so the target antigen appears early in the development of T cells. However, it is unknown whether or not pre-T cells or cells which colonize the thymus already express this marker. Around the time when the thymus appears [~36 days (Jordan, 1976)], the bone marrow is still absent in the sheep fetus. The major haemopoietic organ at this stage is the liver; however, clusters of cells positive for ST-1_a or ST-1_b could not be found on frozen liver sections from fetuses of 42–132 days using an immunoperoxidase method. This finding, together with the observation that neither mAb recognizes determinants on xenogeneic lymphocytes, including those obtained from other ruminants, suggest that the target antigen is a species-specific product generated by the interaction of fetal lymphocytes with the thymic microenvironment.

The tissue distribution of cells positive for ST-1_a and ST-1_b binding is very similar to those reported for Lyt-1 positive cells in the mouse (Van Ewijk *et al.*, 1981) and Leu-1 positive cells in the human (Engleman *et al.*, 1981). In the following paper (M.-F. Beya and M. Miyasaka, submitted), suggestive evidence is presented to indicate that antigen recognized by ST-1_a and ST-1_b is homologous to the mouse Lyt-1 antigen and the human Leu-1 antigen.

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Note added in proof

Since we submitted this manuscript, Makay *et al.* (1985) have reported a monoclonal antibody similar to ST-1_a and ST-1_b.