Immunology 1989 **68** 365–370

Surface expression of differentiation antigens on lymphocytes in the ileal and jejunal Peyer's patches of lambs

W. R. HEIN, L. DUDLER & C. R. MACKAY *Basel Institute for Immunology, Basel, Switzerland*

Accepted for publication 20 July 1989

SUMMARY

The surface phenotype of lymphocytes in the ileal (IPP) and jejunal (JPP) Peyer's patches (PP) of lambs was compared using flow cytometry and immunohistology with a panel of monoclonal antibodies (mAb). The B-cell markers p220, BAS9A and surface Ig molecules were detected on 70–95% of cells from the IPP. T-cell markers were detected on < 1% of IPP lymphocytes, confirming that the IPP in lambs contains virtually only B lymphocytes. The JPP contained a lower proportion of B cells and 16% T cells, nearly all of which expressed the CD4 molecule. Interestingly, the reactivity of a fourth B-cell marker, BAQ44a, differed from this pattern; only 12% of IPP lymphocytes were positive whereas 70% of JPP lymphocytes expressed this marker. A majority of both IPP and JPP lymphocytes (80–95%) expressed the cell adhesion molecules CD11a (LFA-1) and LFA-3. Other adhesion molecules, such as CD2 and CD44, were expressed by fewer cells from the IPP than from the JPP. MHC class I antigens were detected on more than 95% of lymphocytes from both the IPP and JPP. In the case of MHC class II antigens, more positive cells occurred in the IPP (> 95%) than in the JPP (80%). The *in situ* localization of cell-surface antigens was assessed by immunohistology. $CD4^+$ T cells occurred in the interfollicular T-cell regions and in JPP follicles, whereas $CD8^+$ T cells localized only in the interfollicular regions and were absent from follicles. The pattern of expression of B-cell markers, adhesion molecules and MHC antigens indicated that a gradient of increasing maturity of B cells existed within follicles from the base towards the dome region. The data presented here lend support to the notion that the IPP in lambs represents a novel B-cell lymphoid tissue with a function different from that of the conventional Peyer's patches found in the jejunum.

INTRODUCTION

Several lines of evidence suggest that the IPP in lambs may be a primary B-cell organ similar to the bursa of Fabricius in birds. Removal of the bulk of the IPP before or soon after birth prevents the post-natal expansion of the number of circulating B cells in lambs (Gerber, Morris & Trevella, 1986). Lymphopoiesis in IPP commences during the second half of fetal gestation; the level of lymphocyte production is not affected by the deliberate introduction of antigen and IPP undergo involution around the time of puberty (Reynolds & Morris, 1983, 1984). IPP in lambs have a higher lymphocyte birth rate than either JPP or thymus (Reynolds, 1986). However, relatively few of the lymphocytes produced in lamb IPP leave their site of production and most probably die *in situ* (Pabst & Reynolds, 1986).

Relatively few studies have been done on the phenotypes of cells in the Peyer's patches of sheep. Examination of tissue sections shows that there are clear differences in the distribution of T cells between IPP and JPP: whereas JPP have large interfollicular T-cell areas, IPP contain only small triangular T-cell regions near the apex of follicles (Larsen & Landsverk, 1986). In another study, IPP cells were found to be mainly 'low $sIgM^{+}$' and PNA^+, which suggested that they were distinct from peripheral B cells (Miyasaka *et al.*, 1984). This paper compares by cytofluorometry and immunohistology the expression of a range of lymphocyte differentiation antigens within the IPP and JPP of lambs. The results support the proposition that JPP are very similar to conventional Peyer's patches found in other species, whereas the IPP are different and most likely have a specialized role in B-cell production similar to the bursa of Fabricius in birds.

Abbreviations: FACS, fluorescent-activated cell sorter; FITC, fluorescein isothiocyanate; IPP, ileal PP; JPP, jejunal PP; LCA, leucocyte common antigen; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PNA, peanut agglutinin; PP, Peyer's patch(es).

Correspondence: Dr W. R. Hein, Basel Institute for Immunology, Grenzacherstrasse 487, Postfach CH-4005, Basel, Switzerland.

MATERIALS AND METHODS

Animals and collection of specimens

White Alpine and Black Jura lambs of both sexes up to 4 months of age were obtained from Versuchsbetrieb Sennweid, Olsberg, Switzerland. Pieces of the ileal and several jejunal Peyer's patches were removed from the intestines at necropsy and promptly frozen in liquid nitrogen. The IPP was sampled at a

position 20–40 cm anterior to the ileo-caecal junction. The samples were stored over liquid nitrogen vapour and sectioned on a freezing microtome for immunoperoxidase staining. For the preparation of single cell suspensions, a segment of terminal ileum and several JPP were resected and placed immediately in ice-cold PBS.

Antibodies

The monoclonal antibodies used in this study and their target specificities are shown in Table 1 (reviewed by Mackay, 1988). Fluorescein isothiocyanate (FITC) conjugates of $F(ab')_2$ rabbit anti-sheep Ig and $F(ab')_2$ goat anti-mouse Ig were purchased from Cappel Laboratories (Cochranville, PA).

Table 1. Monoclonal antibodies used and their target specificity

Surface molecules	mAb	Cells marked/function	Reference
CD2	36F	T cells/adhesion molecule	Mackay *et al.* (1988a)
CD4	SBU-T4	T-helper cells	Maddox *et al.* (1985)
CD5	ST-1	T cells, some B cells	Beya *et al.* (1986)
CD8	ST-8	T-cytotoxic cells	Ezaki *et al.* (1987)
CD11a(LFA-1)	F10-150	Adhesion molecule	Mackay *et al.* (submitted)
CD44(Pgp-1)	25-32	Homing receptor	Mackay *et al.* (1988b)
CD45(LCA)	151	Leucocyte common antigen	Miyasaka *et al.* (1988)
CD45R(p220)	20-96	B cells, some T cells	Mackay *et al.* (1987)
B5	B5-5	T and B cells/T-cell activation	Hein *et al.* (1988)
γ/δ TcR	86D	T-cell receptor	Mackay *et al.* (1989)
LFA3	L180/1	Adhesion molecule	Hünig (1985)
BAQ 44a	BAQ 44a	B cells	*
BAS 9A	BAS 9A	B cells	*
IgM	25-69	B cells	Mackay, unpublished data
MHC I	SBU-1	MHC class I antigens	Gogolin-Ewens *et al.* (1985)
MHC II	SW73.2	MHC class II antigens	Hopkins *et al.* (1986)

* From VMRD Inc., Pullman, WA.

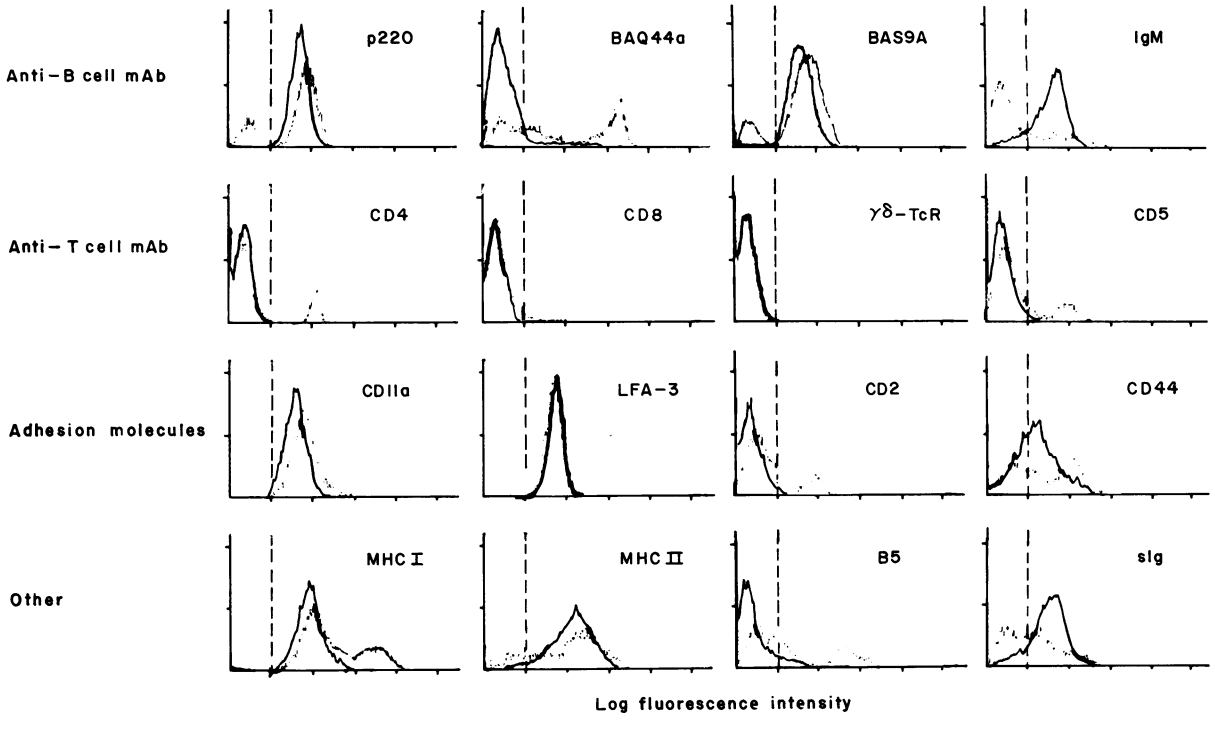

Figure 1. Phenotype of lymphocytes in the ileal and jejunal PP of lambs. Single cell suspensions prepared from the IPP and JPP were reacted with appropriate mAb and analysed using a flow cytometer, as described in the Materials and Methods. Broken lines indicate the point beyond which staining was assessed as positive, i.e. above control staining. The profiles show representative results obtained for lymphocytes from the IPP (dark lines) and JPP (light lines) of one lamb.

Table 2. Percentages of lymphocytes defined by surface markers in the IPP and JPP of lambs

Surface marker	IPP	JPP
CD5	0·7±0·5	15·8±6·0
CD4	0·8±0·6	13·7±5·5
CD8	0·3±0·5	1·5±0·9
CD2	0·8±0·5	17·2±6·3
γ/δ TcR	0·3±0·2	0·9±0·5
p220	98·5±0·5	75·7±4·3
BAQ44a	12·3±4·6	70·4±8·7
BAS9A	97·2±2·8	74·9±6·6
IgM	74·7±8·6	34·8±11·3
sIg	75·3±9·2	49·7±12·7
CD11a	83·6±4·7	97·6±1·6
LFA-3	97·5±1·3	98·2±0·4
CD44	29·8±6·7	70·3±8·6
B5	10·6±4·1	31·5±13·3
MHC I	98·6±0·4	99·0±0·8
MHC II	98·3±0·6	79·6±3·2

Data shown as mean percentage of lymphocytes positive for each marker (±SD) in the IPP and JPP of five lambs.

Immunoperoxidase staining of frozen tissue sections

Monoclonal antibody supernatants were used to stain frozen tissue sections by the immunoperoxidase method as described elsewhere (Beya *et al.*, 1986).

Surface immunofluorescence staining and cytofluorometry

Single cell suspensions of freshly isolated PP cells were prepared as described elsewhere (Miyasaka *et al.*, 1984) and surface labelled using direct (sIg) or indirect (mAb) immunofluorescence staining and analysed with a FACS analyser (Becton-Dickinson, Mountain View, CA) as described previously (Ezaki *et al.*, 1987).

RESULTS

Phenotype of cells from IPP and JPP of lambs

We analysed the phenotype of cells isolated from the IPP and JPP of five lambs, ranging in age from 5 days to 16 weeks, using a wide range of markers. Some of the mAb used identified B-cell or T-cell specific markers, some reacted with specific adhesion molecules, while others identified molecules such as the MHC antigens, which may occur on many cell types (Fig. 1). A summary of the functional relevance of these markers (where known) is presented in Table 1. Over the range of 5 days to 16 weeks, there was no consistent age-related trend in terms of the proportion of cells in the IPP or JPP which expressed a specific marker.

T-cell markers. Cell suspensions prepared from the IPP contained less than 1% T cells as assessed using the T-cell specific markers CD4 and CD8 and a mAb specific for the γ/δ T-cell receptor. In addition, the CD5 molecule, which is expressed on all T cells as well as a small proportion of peripheral B cells, was virtually absent from IPP lymphocytes (Table 2). JPP suspensions contained a mean of 16% T cells as assessed by CD5-bright staining, and nearly all of these were of the CD4 phenotype (14%), whereas only 2% and less than 1%, respectively, expressed CD8 or the γ/δ TcR (Table 2).

B-cell markers. Two B-cell markers, p220 and BAS9A, were expressed by a high proportion of lymphocytes from the IPP (>95%), consistent with the notion that the IPP are comprised almost entirely of B lymphocytes. Interestingly, another B-cell marker, BAQ44a, was detected on very few IPP lymphocytes (12%), although this marker was expressed on all circulating B cells (not shown), as well as 70% of JPP lymphocytes (Table 2). Surface immunoglobulin molecules were detected using a mAb specific for sheep IgM and a rabbit antiserum to sheep Ig. A mean of 75% of IPP lymphocytes was positive for both reagents while, in the case of JPP lymphocytes, a mean of 35% and 50% reacted, respectively, with anti-IgM and anti-Ig reagents (Table 2).

Adhesion molecules. The surface expression of four molecules known to mediate cell–cell adhesion was assessed using specific mAb. More than 95% of both IPP and JPP lymphocytes expressed LFA-3 determinants. A majority of lymphocytes from each type of PP also expressed the CD11a (LFA-1) molecule, although the actual proportion was slightly higher in the case of JPP (>95%) than for IPP (>80%). There were clear differences between lymphocytes from these two sources in terms of the expression of the CD44 'lymphocyte homing receptor'. The CD44 molecule was expressed by 30% and 70% of lymphocytes from the IPP and JPP, respectively (Table 2). The number of $CD2^+$ cells in IPP and JPP correlated with the numbers of T lymphocytes, since CD2 in sheep is expressed on T cells but not on B cells. CD2 was expressed on fewer than 1% of IPP lymphocytes but on 17% of JPP lymphocytes (Table 2).

MHC and other molecules. MHC class I antigens were detected on more than 95% of lymphocytes from both the IPP and JPP. In the case of MHC class II antigens, more positive lymphocytes were found in the IPP (>95%) than in the JPP (80%). The B5 molecule, which is expressed by nearly all mature circulating T and B cells in sheep, was detected on only 11% of IPP lymphocytes and 32% of JPP lymphocytes (Table 2).

In situ localization of cell surface antigens

In order to compare the distribution of cell-surface antigens in IPP and JPP, serial cryostat sections were prepared and stained with selected mAb using the immunoperoxidase technique. In JPP, T lymphocytes localized predominantly to the interfollicular regions and extended almost to the base of the follicle. However, a significant number of T cells were also found within the follicle itself; these localized in the dome region in the upper half of the follicle (Fig. 2). As described previously (Larsen & Landsverk, 1986), the interfollicular T-cell regions in IPP were much reduced and were confined to small wedge-shaped areas immediately beneath the muscularis mucosae. The T-cell areas extended for only short distances from the muscularis mucosae, and individual IPP follicles were closely apposed without intervening T-cell regions, as in JPP. There were very few T cells within IPP follicles (Fig. 2).

Virtually all of the T cells found within the dome region of JPP follicles were of CD4 phenotype (Fig. 2). These cells also localized in the interfollicular region. In contrast, CD8 T cells occurred exclusively in the interfollicular region and were not found within JPP follicles. $CD4^+$ and $CD8^+$ T cells showed a

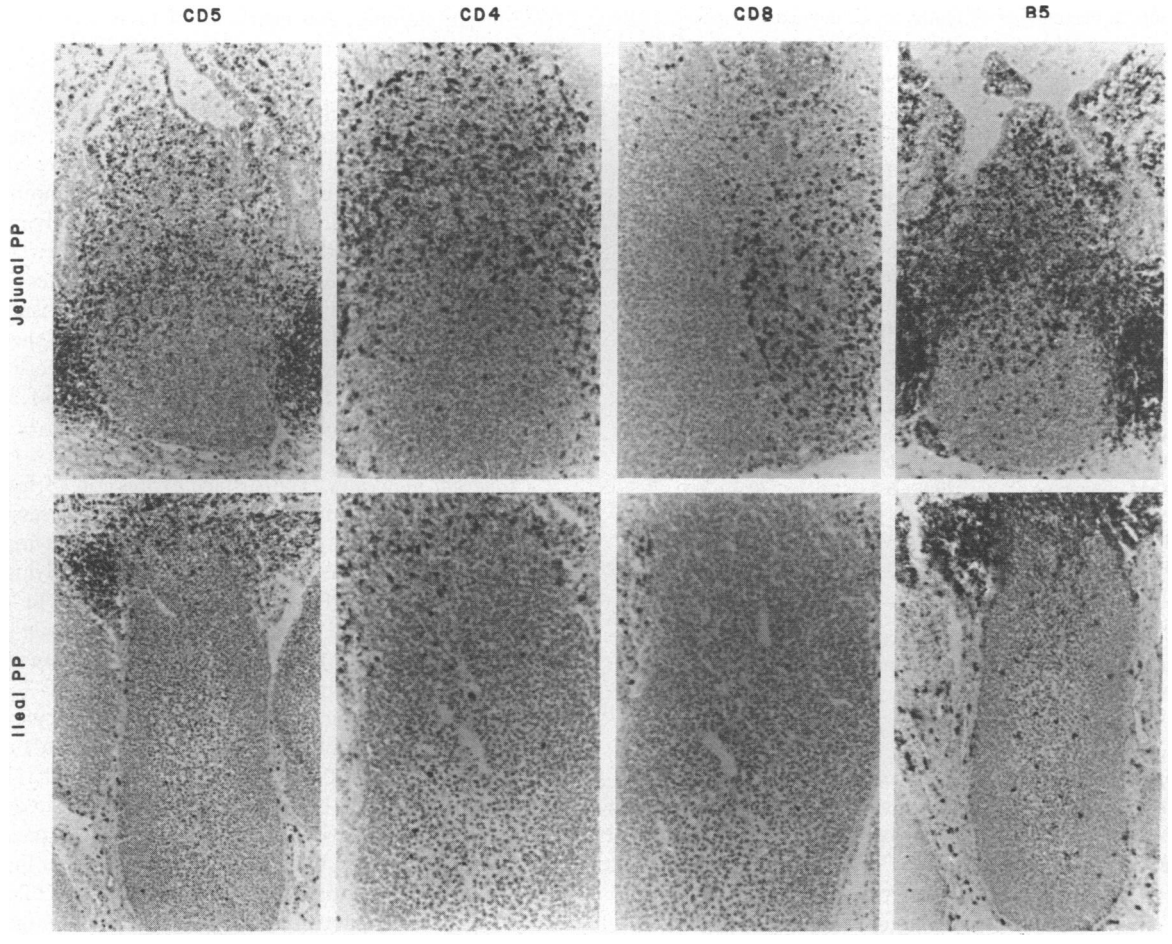

Figure 2. Immunoperoxidase staining of ileal and jejunal Peyer's patches of lambs. Lymphocyte differentiation antigens were demonstrated on serial frozen sections of lamb PP as indicated. CD5, B5 × 45; CD4, CD8 × 70.

similar distribution in IPP, although their numbers were strikingly fewer. The distribution of the B5 antigen in JPP and IPP was comparable to that of T cells, although more lymphocytes within the dome regions of the follicles were positive (see Fig. 2).

There were differences between JPP and IPP in the localization of lymphocytes which expressed MHC class I and II antigens. In JPP, the cells occupying a crescent-shaped region at the base of follicles appeared to be MHC I-negative–low, while the remainder of follicular lymphocytes were positive (Fig. 3). In IPP, the MHC I-negative–low cells were more prominent and occupied a subcapsular region around the entire periphery of the follicle. Lymphocytes in the central core of IPP follicles appeared MHC I positive (Fig. 3). Most of the cells in JPP follicles appeared to express MHC class II antigens, although the intensity of staining varied and was most intense in the dome region. In IPP, there was also an increasing gradient in the intensity of MHC II expression towards the dome region (Fig. 3).

The majority of JPP follicular lymphocytes expressed the p220 component of the sheep leucocyte common antigen. Positive cells also occurred in the interfollicular region. In IPP the p220 antigen was expressed intensely by cells in the dome region of the follicle. When stained with mAb 151, which identifies a 'framework' epitope of the LCA of sheep, virtually all lymphocytes in JPP and IPP were positive. A zone of intense staining was evident around the periphery of IPP follicles (Fig. 3).

DISCUSSION

The observations presented here demonstrate that the IPP and JPP in lambs contain quite different cellular constituents. Furthermore, if the percentages of B cells, T cells and T-cell subsets ($CD4^+$ or $CD8^+$) found in the PP of lambs are compared with those reported in the PP of pigs, mice and rats (Table 3), it becomes apparent that the IPP of lambs have a unique composition insofar as they contain virtually only B lymphocytes. This suggests that the continuous PP located in the terminal part of the ileum in lambs has a function that is different from conventional discrete PP and lends support to the notion that this tissue is a primary site of B-cell production.

The JPP in lambs contained more T cells and in this respect resembled more closely the cellular composition of the JPP in pigs and that of the PP found in the small intestine of mice and rats (Table 3). Interestingly, there is a considerable variation between species in the reported numbers of T cells in PP and in the relative proportion of $CD4^+$ and $CD8^+$ lymphocytes. Pigs

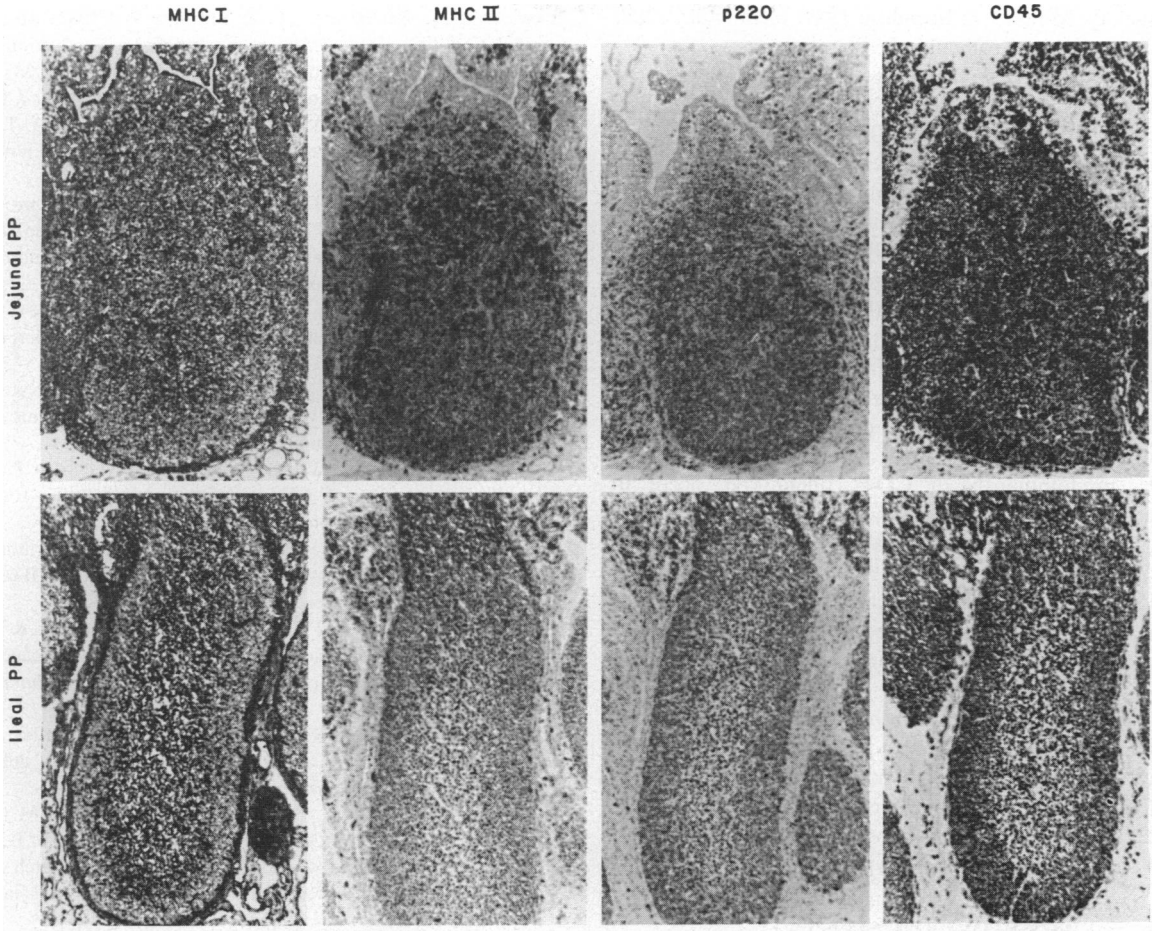

Figure 3. Immunoperoxidase staining of ileal and jejunal Peyer's patches of lambs. Lymphocyte differentiation antigens were demonstrated on serial frozen sections of lamb PP as indicated; ×45.

and mice have more T cells in the discrete PP than do either rats or lambs, and this appears to be due to a far greater number of $CD8^+$ lymphocytes. In addition to species differences such as these, the composition of the PP may also vary with the age of the animal. In pigs, the numbers of all T cells increased with advancing age (Rothkotter & Pabst, 1989) and a relatively higher percentage of $CD8^+$ lymphocytes were found in the PP of aged mice (Vetvicka *et al.*, 1987). Age-related changes in the proportions of lymphocyte subsets in PP could represent a normal developmental process or may reflect a local immune response mechanism caused by long-term exposure to gut antigens.

Table 3. Percentages of B cells, T cells and T-cell subsets ($CD4^+$ or $CD8^+$) in the PP of different species

		Lambs (1–16 weeks)	Pigs* (6 weeks)	Mice†		Rats	
				Adult	Aged	Adult*	Adult‡
B	IPP	<95	63	36	36	64	55
	JPP	75	46				
T	IPP	<1	6	55	57	15	18
	JPP	16	45				
$CD4^+$	IPP	<1	3	24	26	14	10
	JPP	14	23				
$CD8^+$	IPP	<1	2	32	17	4	6
	JPP	2	20				

* Rothkötter & Pabst (1989).
† Vetvicka *et al.* (1987).
‡ Lyscom & Brueton (1982).

The PP in lambs produce vast numbers of lymphocytes each day. Virtually all of the lymphopoiesis occurs in the follicles, while only occasional cells in the interfollicular regions divide (Reynolds, 1986; Pabst & Reynolds, 1986). The staining patterns seen on frozen sections of IPP and JPP provide evidence that a gradient of B-lymphocyte maturity exists within individual follicles. The peripheral zone of MHC class I-negative–low cells in IPP suggests that this region contains the most immature cells. The expression of IgM was also found to be lower in this region of the follicle (Larsen & Landsverk, 1986). These observations accord well with the finding that the mitotic rate in the outer or cortical zone is two to three times greater than in the inner or medullary zone (Reynolds, 1987). In the remainder of the IPP follicle there appeared to be a gradient of increasing cellular maturity towards the dome region, since the intensity of staining for MHC II, p220 and B5 molecules, all of which are expressed at high levels on most mature circulating

B cells (Mackay, Maddox & Brandon, 1987; Hein *et al.*, 1988) was greater towards the follicular dome.

In JPP follicles, a less extensive crescent-shaped area of MHC I-negative cells was located at the base of follicles, suggesting that, when compared to IPP, JPP contain relatively fewer immature cells. Other studies employing the *in vivo* perfusion of [^{3}H]thymidine and the metaphase arrest technique have shown that the labelling index and rate of entry into mitosis is lower in JPP than in IPP (Pabst & Reynolds, 1986; Reynolds, 1986). There was a less obvious gradient of increasing maturity towards the dome of JPP follicles, although many cells in the dome region itself were strongly positive for MHC II, p220 and B5. However, it is certain that not all of these cells are recently produced B cells, since there is an extensive extravasation and recirculation of T lymphocytes and perhaps other cells in the dome region of JPP.

T cells, predominantly of CD4$^+$ phenotype, occur in germinal centres of lymph nodes in rodents (Rouse, Ledbetter & Weissman, 1982), humans (Poppema *et al.*, 1981) and in sheep (Maddox, Mackay & Brandon, 1985). Indeed T cells are essential for the germinal centre reaction to occur. Similar cells occur in mouse PP and it has been proposed that they influence gene rearrangements by B cells leading to the expression of other Ig isotypes (Kawanishi, Saltzman & Strober, 1983). Their extensive presence in the JPP of lambs raises the question as to whether similar processes might be occurring in this tissue. In direct contrast, the extreme paucity of these cells in IPP suggests that B-cell maturation in this organ is unlikely to be influenced significantly by T-cell interactions and implies a further physiological difference between the two types of PP in lambs.

ACKNOWLEDGMENTS

We thank Drs T. Landsverk, O. Vainio and B. Imhof for their critical review of the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche and Co. Ltd, Basel, Switzerland.

REFERENCES

Beya M.-F., Miyasaka M., Dudler L., Ezaki T. & Trnka Z. (1986) Studies on the differentiation of T lymphocytes in sheep. II. Two monoclonal antibodies that recognize all ovine T lymphocytes. *Immunology*, **57,** 115.

Ezaki T., Miyasaka M., Beya M.-F., Dudler L. & Trnka Z. (1987) A murine anti-sheep T8 monoclonal antibody, ST-8, that defines the cytotoxic T lymphocyte population. *Int. Archs. Allergy Appl. Immunol.* **82,** 168.

Gerber H.A., Morris B. & Trevella W. (1986) The role of gut-associated lymphoid tissues in the generation of immunoglobulin-bearing lymphocytes in sheep. *Aust. J. exp. Biol. Med. Sci.* **64,** 201.

Gogolin-Ewens K.J., Mackay C.R., Mercer W.R. & Brandon M.R. (1985) Sheep lymphocyte antigens (OLA). I. Major histocompatibility complex class I molecules. *Immunology*, **56,** 717.

Hein W.R., McClures S., Beya M.-F., Dudler L. & Trnka Z. (1988) A novel glycoprotein expressed on sheep T and B lymphocytes is involved in a T cell activation pathway. *J. Immunol.* **140,** 2869.

Hopkins J., Dutia B.M. & McConnell I. (1986) Monoclonal antibodies to sheep lymphocytes. I. Identification of MHC II molecules in lymphoid cells in resting lymph and after *in vivo* antigen inoculation. *Immunology*, **59,** 433.

Hunig T. (1985) The cell surface molecule recognized by the erythrocyte receptor of T lymphocytes. *J. exp. Med.* **162,** 890.

Kawanishi H., Saltzman L.E. & Strober W. (1983) Mechanisms regulating IgA class-specific immunoglobulin production in gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells *in vitro*. *J. exp. Med.* **157,** 433.

Larsen H.J. & Landsverk T. (1986) Distribution of T and B lymphocytes in jejunal and ileocaecal Peyer's patches of lambs. *Res. Vet. Sci.* **40,** 105.

Lyscom N. & Brueton M.J. (1982) Intraepithelial, lamina propria and Peyer's patch lymphocytes of the rat small intestine: isolation and characterization in terms of immunoglobulin markers and receptors for monoclonal antibodies. *Immunology*, **45,** 775.

Mackay C. (1988) Sheep leukocyte molecules: a review of their distribution, structure and possible function. *Vet. Immunol. Immunopathol.* **19,** 1.

Mackay C.R., Beya M.-F. & Matzinger P. (1989) $\gamma\delta$ T cells express a unique surface molecule which appears late during thymic development. *Eur. J. Immunol.* in press.

Mackay C.R., Hein W.R., Brown M.H. & Matzinger P. (1988a) Unusual expression of CD2 in sheep: implications for T cell interactions. *Eur. J. Immunol.* **18,** 1681.

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.* **110,** 46.

Mackay C.R., Maddox J.F., Wijffels G.L., Mackay I.R. & Walker I.D. (1988b) Characterization of a 95,000 molecule on sheep leucocytes homologous to murine Pgp-1 and human CD44. *Immunology*, **65,** 93.

Maddox J.F., Mackay C.R. & Brandon M.R. (1985) Surface antigens SBU-T4 and SBU-T8 of sheep lymphocyte subsets defined by monoclonal antibodies. *Immunology*, **55,** 739.

Miyasaka M., Dudler L., Bordmann G., Leiserson W.M., Gerber H.A., Reynolds J. & Trnka Z. (1984) Differentiation of B lymphocytes in sheep. I. Phenotypic analysis of ileal Peyer's patch cells and the demonstration of a precursor population for sIg$^+$ cells in the ileal Peyer's patches. *Immunology*, **53,** 515.

Miyasaka M., McClure S., Hein W.R. & Trnka Z. (1988) Differentiation antigens in lymphohemopoietic system of the sheep. In: *Differentiation Antigens in Lymphohemopoietic Tissues* (eds M. Miyasaka and Z. Trnka), p. 499. Marcel Dekker, N.Y.

Pabst R. & Reynolds J.D. (1986) Evidence of extensive lymphocyte death in sheep Peyer's patches. II. The number and fate of newly-formed lymphocytes that emigrate from Peyer's patches. *J. Immunol.* **136,** 2011.

Poppema S.A., Bhan A.K., Reinherz E.L., McCluskey R.T. & Schlossman S.F. (1981) Distribution of T cell subsets in human lymph nodes. *J. exp. Med.* **153,** 30.

Reynolds J.D. (1986) Evidence of extensive lymphocyte death in sheep Peyer's patches. I. A comparison of lymphocyte production and export. *J. Immunol.* **136,** 2005.

Reynolds J.D. (1987) Mitotic rate maturation in the Peyer's patches of fetal sheep and in the bursa of Fabricius of the chick embryo. *Eur. J. Immunol.* **17,** 503.

Reynolds J.D. & Morris B. (1983) The evolution and involution of Peyer's patches in fetal and postnatal sheep. *Eur. J. Immunol.* **13,** 627.

Reynolds J.D. & Morris B. (1984) The effect of antigen on the development of Peyer's patches in sheep. *Eur. J. Immunol.* **14,** 1.

Rothkötter H.J. & Pabst R. (1989) Lymphocyte subsets in jejunal and ileal Peyer's patches of normal and gnotobiotic minipigs. *Immunology*, **67,** 103.

Rouse R.V., Ledbetter J.A. & Weissman I.L. (1982) Mouse lymph node germinal centers contain a selected subset of T cells—the helper phenotype. *J. Immunol.* **128,** 2243.

Vetvicka V., Tlaskalova-Hogenova H., Fornusek L., Rihova B. & Holan V. (1987) Membrane and functional characterization of lymphoid and macrophage populations of Peyer's patches from adult and aged mice. *Immunology*, **62,** 39.