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Histological and immunohistological investigation of alimentary tract lymphoid tissue in the koala (*Phascolarctos cinereus*), brushtail possum (*Trichosurus vulpecula*) and ringtail possum (*Pseudocheirus peregrinus*)

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ABSTRACT

The histological appearance and distribution of T cells, B cells and plasma cells were investigated for oropharyngeal tonsils, small intestinal lymphoid aggregations (Peyer's patches), caecocolic lymphoid patches and mesenteric lymph nodes of koalas (*Phascolarctos cinereus*), common brushtail possums (*Trichosurus vulpecula*) and common ringtail possums (*Pseudocheirus peregrinus*). The histological organisation and distribution of lymphoid cell subpopulations of these tissues were similar to those described in eutherian mammals, although some differences were found in comparison with previous descriptions of American opossum tissues. The main variation among the 3 species was in the structural organisations of the oropharyngeal tonsil and the caecocolic lymphoid patch which were more complex in the koala than in possums. In the koala the extensive crypts of the oropharyngeal tonsils and folding of the mucosa of the caecocolic lymphoid patch increased their surface area and, in addition, both structures had areas of epithelium heavily infiltrated with T and B cells. These features could indicate that these structures are important in immunological surveillance of orally presented antigens in koalas.

Key words: Marsupial; alimentary lymphoid tissue.

INTRODUCTION

There is little published information regarding the histological organisation of the alimentary tract lymphoid tissue of Australian marsupials, including the koala (*Phascolarctos cinereus*). This is despite the fact that it is well accepted that this tissue has a central role in generating immune responses to the many pathogens presented to the mucosal surfaces of the body (Croitoru & Bienenstock, 1994) and that chlamydial disease affecting multiple mucosal surfaces is common in koalas. Detailed histological descriptions of alimentary tract lymphoid tissue in Australian marsupials are limited to a report of the light microscopic appearance of Peyer's patches in the marsupial mice *Antechinus swainsonii* and *A. stuartii* (Poskitt et al. 1984) and of the histological and ultrastructural features of caecocolic lymphoid patches, along with a description of their lymphatic drainage pathways and the anatomical arrangement of the mesenteric lymph nodes, in the koala (Hanger & Heath, 1994).

In humans and many domestic animals the general histological features of the oropharyngeal tonsils, gut associated lymphoid aggregations and mesenteric lymph nodes have been well described and, in addition, the usual general pattern of distribution of lymphocyte subpopulations within these tissues is well investigated (Nicander et al. 1993; Fawcett, 1994*a*, *b*; Raviola, 1994). However, to our knowledge, information for marsupials on lymphocyte subpopulation distribution is limited to studies of American opossum peripheral lymphoid tissues (Mason et al. 1992; Jones et al. 1993) and Peyer's patches (Coutinho et al. 1993, 1994).

The present study was undertaken in order to describe the histological appearance of koala alimentary tract lymphoid tissue and to investigate the

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distribution of T cells, B cells and plasma cells within these tissues. To fulfil the latter aim, antibodies raised against intracytoplasmic peptide sequences of human lymphocyte associated molecules known to have cross-species reactivity (Jones et al. 1993) and an anti-koala serum IgG antibody were utilised. Alimentary lymphoid tissues from common brushtail possums (*Trichosurus vulpecula*) and common ringtail possums (*Pseudocheirus peregrinus*) were examined histologically and immunohistologically for comparative purposes.

MATERIALS AND METHODS

Animals/tissues

Animals included in the study were presented for necropsy because of death or euthanasia for humane reasons following traumatic injury or disease and were considered not to have significant alimentary tract involvement which would influence the size or activity of the local lymphoid tissue. Histological examination was undertaken for oropharyngeal tonsils, small intestinal lymphoid aggregations, large intestinal lymphoid aggregations and mesenteric lymph nodes from 17 fresh (mixed sex, 18 months–14y) and 26 chilled (mixed sex, 10 months–15y) koalas, 3 fresh (mixed sex, adult/aged adult) and 7 chilled (mixed sex, back young-adult) brushtail possums and 2 fresh (male, adult) and 5 chilled (mixed sex, back young-adult) ringtail possums, although a full range of tissues was not taken from all animals. Fresh animals were those that had tissues fixed within several hours of death. Chilled animals were those that had tissues fixed within 1 d (possums) or 3 d (koalas) of death and storage at 4–6 °C. Tissues from 13 koalas, 4 brushtail possums and 5 ringtail possums were used for immunohistological examination but a complete range of tissues was not examined for each animal.

Tissues were fixed in 10% buffered formol saline, embedded in paraffin and stored at 4 °C. Some intestinal tissues had been immersed in 5%, 10% or 50% acetic acid for anatomical studies before being fixed in formalin. Selected paraffin embedded formalin fixed lymphoid tissues obtained from 2 koalas immediately after euthanasia were donated by Fiona Wallace, Australian Institute of Mucosal Immunology, Newcastle, Australia.

Sections for histological examination were cut at 6 μm and stained with haematoxylin and eosin (H&E). Sections for immunohistological staining were cut at 4 μm, mounted on 3-aminopropyltriethoxysilane coated slides, dried at 37 °C and stored overnight at room temperature.

Primary antibodies for immunohistology

Antibodies used for immunohistological staining of T cells and B cells were anti-intracytoplasmic peptide sequences of human lymphocyte associated molecules which have previously been shown to have broad cross-species reactivity (Jones et al. 1993). These comprised monoclonal mouse anti-CD3 (diluted 1:5–1:10), anti-CD5 (diluted 1:50–1:100) and anti-CD79b (diluted 1:25–1:100) (all donated by M. Jones, LRF Immunodiagnostics, John Radcliffe Hospital, Oxford) and a polyclonal rabbit anti-CD3 (diluted 1:500–1:1000) (Dakopatts, Glostrup, Denmark A452). A polyclonal rabbit antikoala serum IgG antibody was used to stain plasma cells (diluted 1:500–1:3000) (donated by R. Wilkinson, Central Veterinary Laboratories, Dept Agriculture, South Australia). For negative controls for the polyclonal antibodies normal rabbit immunoglobulins (Dakopatts X903) or whole rabbit anti-*Campylobacter jejuni* serum (donated by S. Noor, Department of Veterinary Pathology, University of Sydney) was applied to the sections in place of the primary antibodies. For the monoclonal antibodies normal mouse IgG_1 antichicken immunoglobulin (Silenus, Melbourne 12CONT01), IgG_{2b} anti-*Neisseria gonorrhoeae* (Silenus 12CONT03) or whole mouse serum (Immunodiagnostics, Sydney IDSANC4) was used as a negative control. Omission of primary antibody was also used as a control for some sections.

Staining methods for immunohistology

Mounted and dried sections were deparaffinised in xylene and taken through graded alcohols to water. Endogenous peroxidase activity was quenched by placing the sections in a 0.6% solution of H_2O_2 in 50% methanol/50% phosphate buffered saline (PBS) v/v for 30 min. The slides were washed in PBS, placed in 0.01 M trisodium citrate buffer, microwaved until boiling and were then boiled for 6 min. After allowing the buffer to cool, the sections were washed in PBS and a 1:20 dilution of normal goat serum was applied to the slides for 30 min to block nonspecific protein binding. For biotinylated antikoala IgG, rabbit serum was used instead of goat serum and for unconjugated antikoala IgG, PBS was used instead of goat serum. Excess serum was tapped from the sections and the primary antibody dilutions applied. Primary antibodies were diluted in goat serum diluted 1:20 in PBS

in the case of the monoclonal antibodies and the polyclonal rabbit antihuman CD3. Unconjugated rabbit antikoala IgG was diluted in PBS and biotinylated rabbit antikoala IgG was diluted in normal rabbit serum diluted 1:50 in PBS. Primary antibodies were left in contact with the sections for 1–2 h at 18–25 °C or overnight at 4 °C. Sections were washed in PBS and 1:100 biotinylated goat anti-mouse/rabbit Ig (Dakopatts K492) was applied to the sections for 1–2 h at 18–25 °C. This step was omitted when biotinylated rabbit antikoala IgG was the primary antibody. After washing in of PBS, streptavidin biotin-horseradish peroxidase (Dakopatts K492) was applied to the sections for 30–45 min. The sections were washed in PBS and 3,3′-diaminobenzidine (SK4100, Vector, Burlingame, California) was applied to the sections until optimal brown staining of positive cells occurred (usually 4–8 min) as determined by microscopic examination of the tissues. Sections were then rinsed in tap water, counterstained with haematoxylin, dehydrated through graded alcohols and xylene, mounted and examined microscopically.

RESULTS

Oropharyngeal tonsils

Histology. In koalas both the palatine tonsils and tonsils of the soft palate had crypts lined by stratified squamous epithelium and surrounded by densely packed lymphoid follicles. The palatine tonsils had 1 very deep central crypt surrounded by a large number of densely packed lymphoid follicles. Commonly, multiple lateral crypts could be seen entering the central crypt (Fig. 1). Tonsils of the soft palate had numerous separate crypts opening to the pharyngeal lumen.

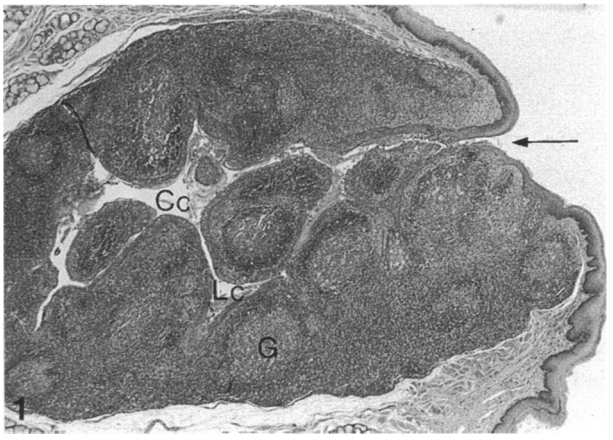

Fig. 1.Palatine tonsil of a koala displaying lymphoid follicles with germinal centres (G) around a deep central crypt (Cc) and some lateral crypts (Lc). H & E, ×6.25

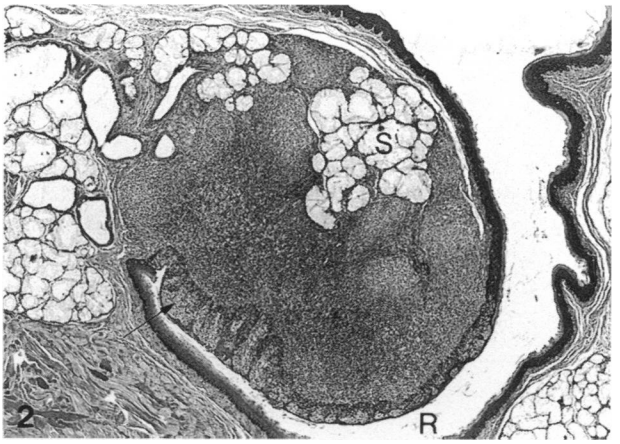

Fig. 2. Palatine tonsil of a brushtail possum showing lymphoid tissue bulging into the pharyngeal lumen and covered by surface pharyngeal stratified squamous epithelium. This has created a shallow recess (R). Extensive lymphoid infiltration of the epithelium can be seen (arrow). Salivary gland tissue (S) is present amongst the lymphoid tissue. H & E, ×10.

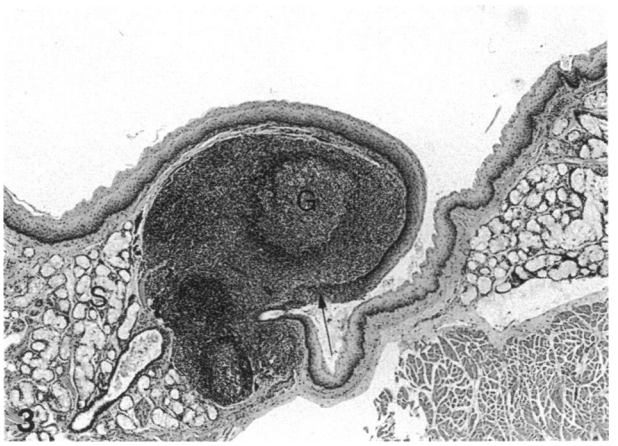

Fig. 3. Palatine tonsil of a ringtail possum displaying an apparent large germinal centre (G) and epithelium heavily infiltrated with lymphoid cells (arrow). Salivary gland tissue (S) is present adjacent to, but separate from, the lymphoid tissue. H & E, ×10.

In contrast, the palatine tonsils of possums consisted of an ovoid body of lymphoid tissue composed of follicles bulging into the oropharyngeal lumen and covered by stratified squamous pharyngeal epithelium. The bulging gave the appearance of a shallow recess. In brushtail possums salivary gland tissue was sometimes present within the body of the tonsillar lymphoid tissue (Fig. 2) whilst in ringtail possums it was closely associated with, but separate from, the lymphoid tissue (Fig. 3).

In all 3 species the tonsillar lymphoid follicles almost invariably had large germinal centres with dense, distinct caps oriented towards the crypts (koalas) or oropharyngeal lumen (possums). The

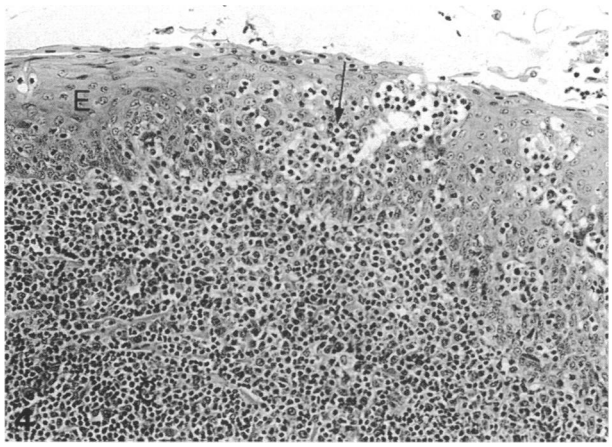

Fig. 4. Palatine tonsil of a brushtail possum showing surface epithelium (E) containing clusters of, and individual, lymphocytes (arrow) above the cap (C) of a lymphoid follicle. H & E, × 50.

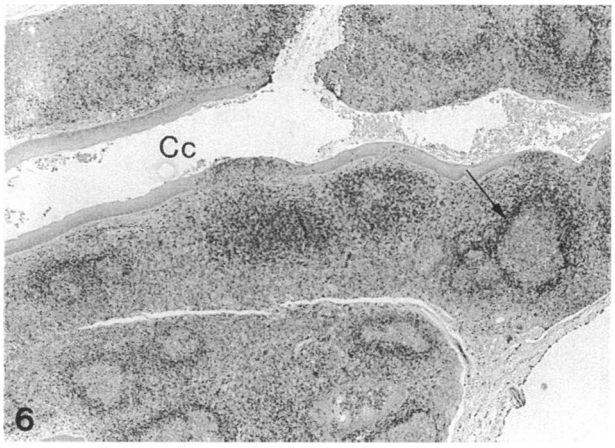

Fig. 6. The same palatine tonsil of a koala as in Figure 5 treated with antihuman CD79b (1/25). Dense positive cells are primarily associated with follicular mantles (arrow). Cc, central crypt. Immunoperoxidase, × 10.

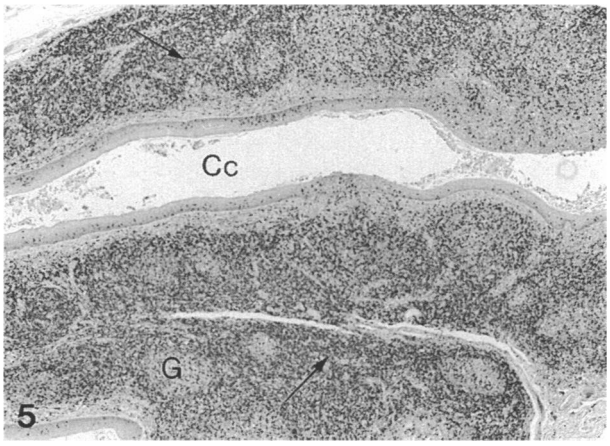

Fig. 5. Palatine tonsil of a koala treated with antihuman CD3 (1/1000) displaying dense positive cells in interfollicular areas (arrows). There are scattered positive cells in germinal centres (G) and mantles. Cc,central crypt. Immunoperoxidase, × 10.

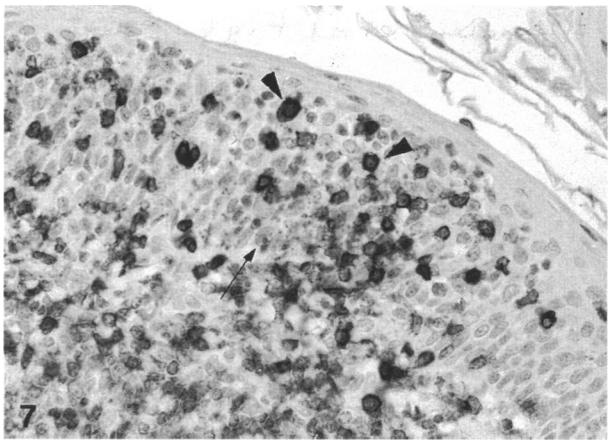

Fig. 7.Palatine tonsil of a brushtail possum treated with antihuman CD3 (1/500) showing positive cells (arrowheads) within the epithelium. Not all the intraepithelial lymphocytes are positive. Arrow denotes base of epithelium. Immunoperoxidase, × 100.

interfollicular lymphoid tissue was usually densely packed with small lymphocytes. Plasma cells and pigment containing cells with the morphological appearance of macrophages were scattered amongst all lymphoid components. In all species individual and clusters of lymphocytes were frequently present within the epithelium overlying the caps of lymphoid follicles (Fig. 4). In possums this primarily occurred in the epithelium of the recess formed by bulging of the lymphoid tissue whilst in koalas it occurred in the deeper part of the central crypt and in the lateral crypts. Some parts of the epithelium were so heavily infiltrated that they lost the characteristic appearance of stratified epithelium. Small numbers of neutrophils were also sometimes present within the epithelium. Within the lumen of the crypts of koala tonsils, squamous epithelial cells, lymphocytes, neutrophils, mucin and bacteria were variably seen.

Immunohistology. CD3 and CD5 positive cells were very dense in the interfollicular parts of the tonsils. Some positive cells were also present scattered in the mantles and germinal centres, between and beneath the overlying epithelium and within crypt lumens (Fig. 5). CD79b positive cells were most numerous in the follicular mantles and caps. Positive cells were also seen in the interfollicular areas, in germinal centres, within the covering epithelium and within the lumen of the crypts (Fig. 6). Where groups of lymphocytes were present in the overlying epithelium, CD79b, CD3 and CD5 positive cells were present (Fig. 7), but there appeared to be predominantly CD79b positive cells when the epithelium was directly over a follicle. Plasma cells commonly formed a thin rim around the periphery of the tonsils. They were also present in the submucosa and within the epithelium, in germinal centres, surrounding the periphery of follicles, in the

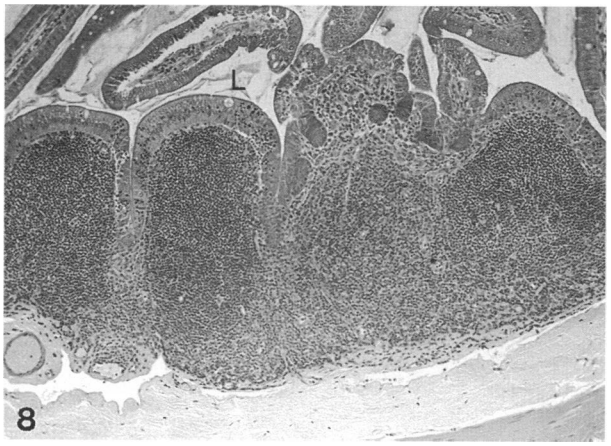

Fig. 8. Peyer's patch of a koala showing orientation of lymphoid follicle caps towards the intestinal lumen (L). H & E, × 10.

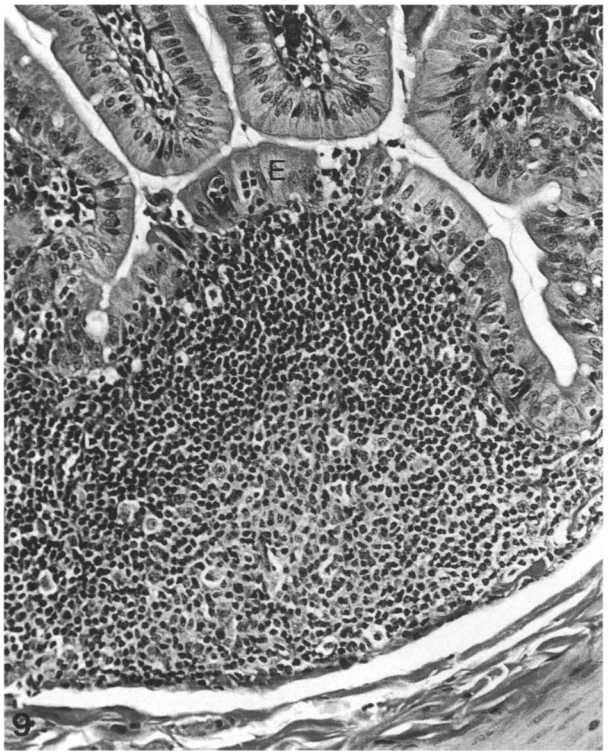

Fig. 9. Peyer's patch of a brushtail possum displaying lymphocytes within nonvillous epithelium (E) overlying a follicle. H & E, × 75.

interfollicular areas and in the lumen of the tonsillar crypts.

Small intestinal lymphoid aggregations (Peyer's patches)

Histology. The histological appearance of Peyer's patches in the small intestinal lamina propria was similar for koalas and possums and consisted of multiple, closely associated lymphoid follicles with well developed germinal centres and well defined caps oriented towards the intestinal lumen (Fig. 8). Intra-

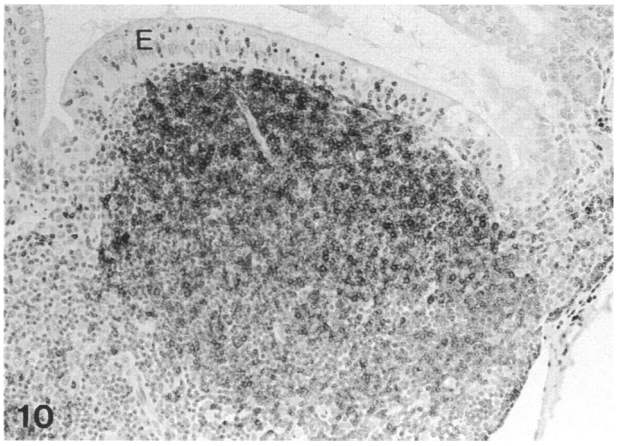

Fig. 10. Cap of a lymphoid follicle within a Peyer's patch of a koala treated with antihuman CD79b. There are numerous positive cells in the cap whilst scattered positive cells are in the overlying nonvillous epithelium (E). Immunoperoxidase, × 50.

follicular invaginations of dome epithelium were seen in some patches. Elevated dome regions with nonvillous epithelium overlay the follicles. Intraepithelial lymphocytes were present as individual cells and as small clusters (Fig. 9). Dense interfollicular lymphocyte accumulations were present.

Immunohistology. CD3 and CD5 positive cells were predominantly seen in high density in the interfollicular areas of Peyer's patches. Scattered positive cells were also present within the overlying epithelium, in all components of follicles, and in the lamina propria of the entire circumference of the gut. Intraepithelial lymphocytes in the nonfollicular areas of the villous epithelium were invariably CD3 or CD5 positive. High densities of CD79b positive cells were present in the follicular mantles, especially in the cap region and extending through the dome region (Fig. 10). Scattered cells within germinal centres were also CD79b positive. CD79b positive cells were present individually and as clusters within the follicle associated epithelium (Fig. 10). Some intraepithelial lymphocytes did not stain for CD3, CD5 or CD79b. Plasma cells were concentrated in the dome region and the lamina propria of the villi adjacent to follicles. They were also present in smaller numbers in the lamina propria of the villi of other parts of the intestine (Fig. 11) and in small numbers within and adjacent to follicles.

Caecocolic lymphoid patches

Histology. Aggregated lymphoid tissue located at the caecocolic junction in koalas and possums had similar structural organisation to that of the small intestinal Peyer's patches, with many closely associated lymphoid follicles located in the lamina propria

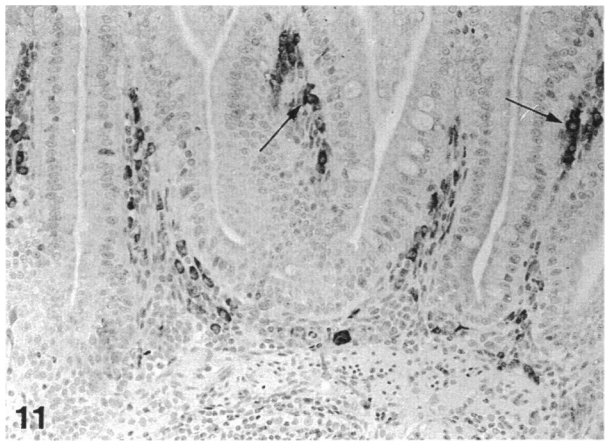

Fig. 11. Small intestinal villi of a koala treated with antikoala IgG (1/1000). Plasma cells (arrows) are present primarily within the lamina propria. Immunoperoxidase, × 50.

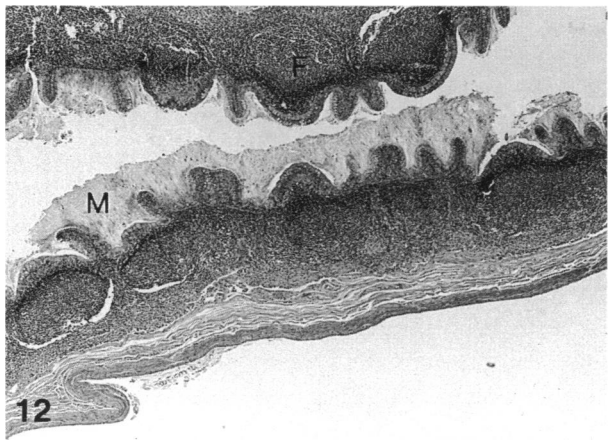

Fig. 12. Caecocolic lymphoid patch of a koala displaying a surface mucobacterial layer (M) and numerous follicles (F) with well developed caps oriented towards the intestinal lumen. H & E, × 10.

(Fig. 12). The follicles had well developed caps oriented towards the intestinal lumen but the dome regions were not raised as much as those of the small intestinal lymphoid aggregations. The epithelium overlying the lymphoid follicles was not usually organised in distinct crypts (Fig. 13). Single and grouped intraepithelial lymphocytes were present, and in some koalas large numbers of intraepithelial lymphocytes disrupted epithelial architecture. Intrafollicular invaginations of the overlying epithelium were sometimes seen in ringtail possums. In koalas the caecocolic lymphoid patches took the form of mucosal folds along both sides of which many lymphoid follicles were densely packed whereas in possums the aggregations were flat to the intestinal surface. In koalas, a mucobacterial layer was closely adherent to the lumenal surface of the whole large intestinal region, including the lymphoid patches (Fig. 12). Pigment laden macrophages were commonly scattered through

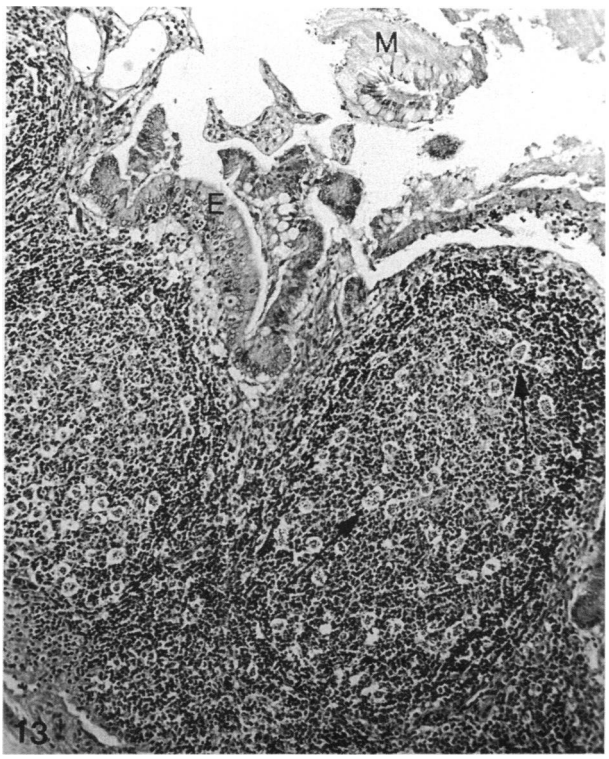

Fig. 13. Caecocolic lymphoid patch of a koala showing prominent germinal centres, numerous macrophages (arrows), and overlying epithelium (E) infiltrated with lymphocytes. The mucobacterial layer has separated (M). H & E, × 40.

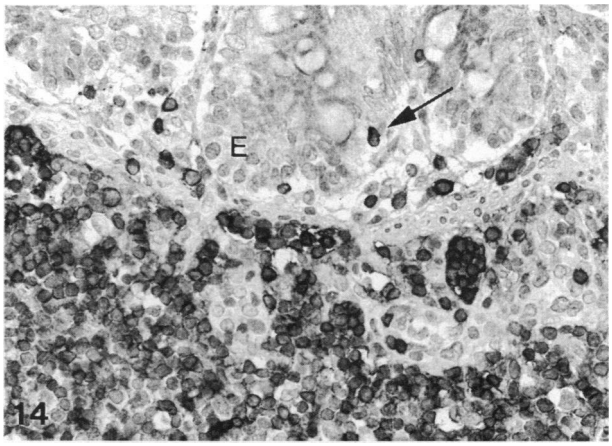

Fig. 14. Caecocolic lymphoid patch of a brushtail possum treated with antihuman CD3 (1/1000). An interfollicular area just below surface epithelium (E) has numerous positive cells. Positive intraepithelial cells are present (arrow). Immunoperoxidase, × 100.

koala caecocolic patches, especially interfollicular regions.

Immunohistology. The interfollicular regions of the caecocolic lymphoid patches were densely populated with CD5 and CD3 positive cells. Individual and clusters of positive cells were also seen within the overlying epithelium and in the lamina propria (Fig. 14). CD3 and CD5 positive cells were scattered in

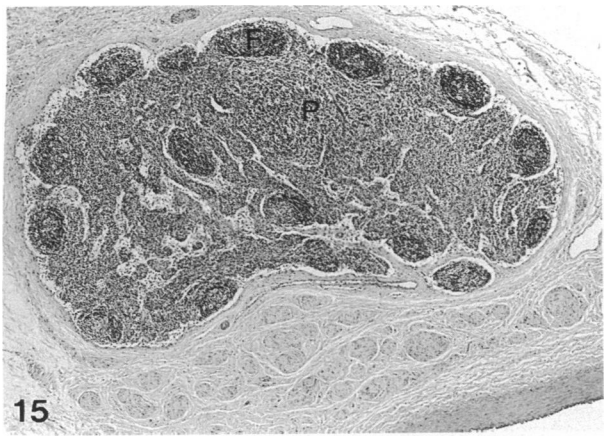

Fig. 15. Mesenteric lymph node of a koala displaying well dispersed, small follicles (F) and extensive interfollicular and paracortical areas (P). H & E, × 10.

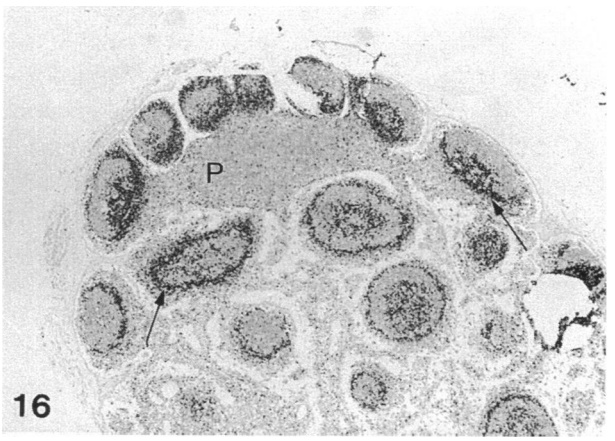

Fig. 16. Mesenteric lymph node of a brushtail possum treated with antihuman CD79b (1/50) showing numerous positive cells in follicular mantles (arrows) and scattered positive cells in germinal centres and in interfollicular and paracortical areas (P). Immunoperoxidase, × 10.

germinal centres and surrounded the periphery of follicles, particularly in the dome region. CD79b positive cells were most dense in the follicular mantles, especially in the caps of the follicles and in the dome region. CD79b positive cells were seen within the overlying epithelium in koalas, usually as clusters. Some positive cells were also present in germinal centres and the interfollicular areas. Plasma cells were most commonly found around the perimeter of follicles and in the lamina propria overlying the follicles. Scattered plasma cells were present in germinal centres, in the interfollicular areas and surrounding the periphery of the patch adjacent to the smooth muscle and serosa.

Mesenteric lymph nodes

Histology. The mesenteric lymph nodes had prominent medullary and paracortical areas. Lymphoid follicles were often separated by extensive interfollicular regions, and were frequently small and did not always have germinal centres, especially in koalas and ringtail possums (Fig. 15). Pigment laden macrophages were scattered throughout koala lymph nodes but were often concentrated in medullary cords and sinuses.

Immunohistology. Cells positive for CD3 and CD5 were densely packed in the interfollicular cortex and deep cortex. In addition, positive cells were scattered in the mantles and germinal centres, and were present in the medullary cords, sometimes in large numbers. CD79b positive cells were consistently and densely present in follicular mantles and throughout primary follicles (Fig. 16). Scattered positive cells were also seen in the interfollicular and deep cortex, germinal centres and medullary cords. Plasma cells were scattered throughout the lymph nodes but were usually more numerous at the periphery of follicles and in the medullary cords.

DISCUSSION

The antibodies against T and B cell determinants used in this study have been shown previously to react against human lymphocyte associated molecules (Mason et al. 1989, 1992; Jones et al. 1993). The CD3 antigen is considered to be the most reliable marker for the identification of T cells although the CD5 antigen is also expressed by most tissue T cells (Lanier & Jackson, 1992). In this study the antihuman CD5 marker provided little additional information over the antihuman CD3 as both had similar distributions in all the lymphoid tissue studied. The CD79b antigen is associated with the β-chain of the transmembrane heterodimer associated with surface immunoglobulin of B lymphocytes and is expressed by precursor and mature lymphocytes but not plasma cells (Mason et al. 1992). In this study, antihuman CD79b provided good visualisation of the distribution of B lymphocytes but not committed plasma cells. Antikoala IgG was used as a plasma cell marker in the present study, however this is not an ideal antibody for this purpose as it cannot be excluded that the anti-IgG antibody was binding to IgG which was bound in vivo to the Fc receptors of other cell types. In other species anti-IgM and anti-IgD are regarded as superior antibodies for marking surface immunoglobulin (Lanier & Jackson, 1992). Subclasses of immunoglobulin other than IgG have not, however, been isolated and identified so far

in the koala. In the present study only positively staining cells with plasma cell morphology were counted but it was noted that all cells staining strongly with anti-IgG did have the appearance of plasma cells.

The histological and immunohistological appearance of the tissues examined in koalas and possums was similar to that of eutherian mammals (Nicander et al. 1993; Fawcett, 1994*a*, *b*; Raviola, 1994) and was consistent with the limited number of histological descriptions of gut associated lymphoid tissue in Australian marsupials (Poskitt et al. 1984; Hanger & Heath, 1994). Within the present study the most obvious variation was the greater structural complexity of oropharyngeal tonsils and caecocolic lymphoid patches in koalas compared with the 2 species of possum. However, the histological and immunohistological features of lymphoid tissue within these structures was essentially the same for all 3 species.

Human palatine tonsillar crypt epithelium is usually heavily infiltrated with mixed populations of lymphoid cells along with smaller numbers of plasma cells, macrophages and polymorphonuclear leucocytes, in patches known as reticular epithelium (Perry, 1994). This epithelium is considered to be important for the immunological functions of the tonsil and is regarded as a physiological response to constant antigen exposure. Similar patches of heavy infiltration with mixed lymphoid cells, and sometimes small numbers of neutrophils, were seen in the epithelium overlying lymphoid follicles in the present study, most extensively in the koala. Although effects of stress or disease on lymphocyte migration and tissue distribution cannot be excluded, there were no consistent differences in histological appearance or lymphocyte subpopulation distribution among animals presented for a variety of diseases or injuries and which had been held in captivity for variable periods of time prior to death. In addition, some animals included in the study died soon after a traumatic injury and had no evidence of significant underlying organic disease. No animals with large numbers of neutrophils present in the mucosa, as an indication of acute inflammation, were included in the study. It would seem likely therefore that the appearance of the tonsils in the present study represents a range of physiological, rather than pathological, states. Other structural features of note were the multiple crypts of the tonsils of the soft palate and the large and sometimes branching crypt system of the palatine tonsils of the koala which would provide a large surface area for interaction with orally presented antigen. This, considered in conjunction with the presence of reticulated epithelium, suggests that the oropharyngeal tonsils of the koala could play an important role in immunological surveillance of the oral cavity; but functional studies are required to confirm this. Although the appearance of small intestinal Peyer's patches in koalas and possums was similar to that described for eutherian mammals it was somewhat at variance with that previously described for American opossums (Coutinho et al. 1993, 1994). In the opossum an obvious dome region is not present and the lymphoid follicles are enveloped by lymphatic sinuses basally and laterally. In addition, Coutinho et al. (1994) utilised anti-CD79a and anti-CD79b antibodies successfully as B cell markers and showed that opossum lymphoid follicles lack a well defined mantle zone. In contrast, koala and possum follicles had distinct mantle zones consisting almost entirely of B cells.

In eutherian species the vast majority of intraepithelial lymphocytes are T cells (Kato & Owens 1994). The small percentage of B cells which do occur are preferentially distributed in the dome epithelium overlying the Peyer's patch lymphoid follicles, often in clusters (Spencer et al. 1985, 1986*a*; Ermak & Owen, 1986; Press et al. 1991). T cells also occur within the dome epithelium, and in humans they may be arranged in clusters (Bjerke et al. 1988). The findings for T and B cell identification of intraepithelial lymphocytes for koalas and possums are consistent with the previous description for eutherian mammals but differ from those reported for the American opossum where CD3 positive cells could not be demonstrated in the follicle associated epithelium of Peyer's patches (Coutinho et al. 1993, 1994). In the opossum CD3 positive cells were present in the lamina propria, interfollicular areas and epithelium of non-follicular associated intestinal villi and immunoglobulin positive cells were detected in association with M cells of the follicle associated epithelium. M cells are known to be important for sampling antigens from the intestinal lumen and transporting them to the underlying lymphoid tissue, where they can induce an immune response (Croitoru & Bienenstock, 1994). M cells typically have an invaginated basal surface with the resulting recesses occupied by lymphocytes (Fawcett, 1994*b*). It is possible that the clusters of intraepithelial lymphocytes seen in follicle associated epithelium in the present study were associated with M cells, but electron microscopy is necessary to confirm their presence.

In rodents a proportion of intraepithelial lymphocytes are CD8 positive but negative for pan T cell markers (Lefrancois, 1994; Lyscom & Brueton, 1982; Lefrancois, 1994), and in humans a small percentage of intraepithelial lymphocytes are non-B, non-T cells

(Selby et al. 1981). Individual and clusters of intraepithelial lymphocytes which did not stain for either CD3 or CD79b were detected in some intestinal sections of koalas and possums. Although individual variation in staining may account for this result, it is also possible that these cells do represent a distinct subset of T lymphocytes similar to the situation found in rodents and humans. Unfortunately, the lack of availability of markers for CD4 and CD8 lymphocyte subsets in marsupials means that at present this phenomenon cannot be investigated further.

In the human appendix, high densities of lymphocytes infiltrate the dome epithelium over lymphoid follicles (Spencer et al. 1985). Similarly, the caecocolic lymphoid patches of koalas, and to a lesser extent of possums, had extensive infiltration of the follicle associated epithelium with a mixed population of T and B cells. It has been suggested previously that the central recess and anatomical location of the koala caecocolic lymphoid patch may increase the opportunity for antigen sampling at this site (Hanger & Heath, 1994). The presence of large numbers of intraepithelial lymphocytes, similar to the Peyer's patches and oropharyngeal tonsils, could be expected to further enhance this opportunity.

It is well accepted in eutherian species that IgA is the predominant immunoglobulin in mucosal secretions. Plasma cells staining for all isotypes of antibody can be found within the organised mucosal tissues, although their pattern of distribution varies with species (Sminia & Plesch, 1982; Spencer et al. 1986*a*, *b*; Hogenesch & Felsburg, 1992). This is thought to be related to the migration and maturation patterns of IgA producing plasma cells. In the current study, similar investigation of the pattern of distribution of plasma cells producing different immunoglobulin isotypes could not be done because only antikoala IgG was available to stain plasma cells for cytoplasmic immunoglobulin. Recently, however, a polyclonal antihuman IgA antibody has been successfully used for immunocytochemical staining of opossum plasma cells and this may have applications to Australian marsupials (Coutinho et al. 1993, 1994). In addition, these authors successfully utilised a commercially available monoclonal antihuman HLA-DR antibody to detect MHC class II-like antigens in opossum Peyer's patches.

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