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Distribution of porcine CD4/CD8 double-positive T lymphocytes in mucosa-associated lymphoid tissues

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SUMMARY

The present study describes the distribution of CD4/CD8 double-positive (DP) T cells in lymph nodes and mucosa-associated lymphoid tissues of pigs at 6–7 months of age. Proportions of lymphocytes isolated from peripheral, bronchial and mesenteric lymph nodes expressing CD4 and/or CD8 molecules were similar and ranged from 10–13% CD4/CD8 DP cells, 25–27% CD4 single-positive (SP) cells, and 27–32% CD8 SP cells. Mucosa-associated lymphoid tissues had significantly smaller proportions of $CD4^+$ and/or $CD8^+$ T cells than lymph nodes and CD4/CD8 DP cells accounted for a larger proportion of the total $CD4^+$ lymphocytes than in lymph nodes. Compared to the lymph nodes, the predominant $CD4^+$ and/or $CD8^+$ T-cell subset in tonsils was the CD4/CD8 DP population (18%), because of both a higher proportion of these cells and a lower proportion of CD4 SP (12%) and CD8 SP (14%) lymphocyte subsets. Jejunal Peyer's patches were comprised of 12% CD4 SP, 28% CD8 SP and 10% CD4/CD8 DP lymphocytes. In contrast, the mid-section of the continuous Peyer's patch in the ileum contained 7% CD4 SP, 8% CD8 SP and 4% CD4/CD8 DP lymphocytes. The distribution of T lymphocytes in porcine mucosal lymphoid aggregates agrees with the reported correlation between high and low rates of lymphocyte entry into these tissues and the abundance or scarcity of T cells, respectively. Defining the role of CD4/CD8 DP lymphocytes and their unique distribution in mucosa-associated lymphoid tissues in the pig may reveal novel T-cell-mediated regulatory pathways.

INTRODUCTION

Our understanding of T-lymphocyte contributions to host immunity and self-tolerance has been aided significantly by the identification of distinct phenotypic and functional T-cell subsets in most mammalian species. Mechanisms contributing to T-cell subset diversity and anatomic distribution, however, remain unclear. Unique aspects of T-cell biology in the pig make this species particularly suitable for studying the generation of T-cell subset diversity and distribution.[1] For example, in addition to the presence in the peripheral blood of normal adult pigs of the typical $CD2^+$ T-cell subsets expressing either CD4 (characteristic of T helper cells) or CD8 (characteristic of cytotoxic/suppressor T lymphocytes), there is a sizeable population (10–60%) of $CD2^+$ lymphocytes which are CD4/CD8 double-positive (DP).[2–5] While circulating CD4/CD8 DP T lymphocytes are present only in small proportions in human peripheral blood ($< 3\%$),[6,7] this T-cell subset has been found in greater proportions ($> 15\%$) in the peripheral blood of patients suffering from neoplasia,[8] in the joint fluid of rheumatoid arthritis patients,[9] and among lymphocytes infiltrating human[10] and swine[11] kidney allografts. In addition CD4/CD8 DP cells are well represented among intestinal intraepithelial lymphocytes (iIEL) of both mice and rats.[12–14] Another major lymphocyte subset present in the peripheral blood of young swine is comprised of null cells.[15] These lymphocytes are thymus-dependent cells, which are identified by the lack of expression of CD2, CD4, CD8, and surface immunoglobulin, and by the expression of the recently characterized MAC320 antigen and the $\gamma\delta$ T-cell receptor.[15–17]

The palatine tonsils and Peyer's patches are major inductive sites of the mucosa-associated lymphoid tissue, which play a major role in both the development of protective immunity against infectious agents and in the regulation of the immune response to environmental and dietary antigens.[18,19] Knowledge of the cellular components of these organs are essential to understand how these immune mechanisms are effected. In the young pig the discrete jejunal Peyer's patch (jejPP) differ from the morphologically distinct continuous ileal Peyer's patch (ilPP) in its lower incidence of T cells, lack of interfollicular areas and later in its early regression.[20,21] The histological distribution of T and B lymphocytes in the Peyer's patches is characterized by the presence of $CD4^+$ and $CD8^+$ cells in the

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Abbreviations: DP, double positive; iIEL, intestinal intraepithelial lymphocytes; ilPP, ileal Peyer's patches; jejPP, jejunal Peyer's patches; SP, single positive.

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interfollicular and dome areas, and the distribution of B cells in the corona of the follicles and dome area.[22,23] The presence of CD4/CD8 DP cells in the Peyer's patches of 12-month-old swine has been inferred from the observation that $CD4^+$ plus $CD8^+$ lymphocytes outnumbered the $CD2^+$ cells.[24] Null cells have been identified in the porcine ilPP and jejPP,[24] and are thought to belong to the same heterogeneous null lymphocyte family present in the peripheral blood of young swine.[15] In the tonsil, $CD4^+$ and $CD8^+$ lymphocytes are mostly distributed in the perifollicular areas comparable to their distribution pattern in lymph nodes.[23] The presence of DP in the porcine tonsils and lymph nodes has been demonstrated directly by two-colour cytofluorometry.[4] Despite the abundance of CD4/CD8 DP lymphocytes and null cells in the porcine immune system, a direct comparative analysis of the presence of these cell subsets in the Peyer's patches, tonsils and lymph nodes has not been reported.

The present study focuses on the distribution of porcine CD4/CD8 DP, CD4 single-positive (SP) and CD8 SP lymphocyte populations in inductive sites of the mucosal immune system. The analysis was conducted by two-colour cytofluorometry of cell suspension stained with monoclonal antibodies (mAb) specific for CD4 and CD8. Because of this, the CD4/CD8 double-negative (DN) population in this study includes both surface immunoglobulin positive lymphocytes as well as null cells. No attempts were made to evaluate the null cell subset which is an important component of the T-cell population present in these organs. Of particular interest, however, was the distribution of CD4- and/or CD8-positive lymphocyte subsets in the two types of Peyer's patches; i.e. the discrete patches in the jejunum (jejPP) and upper ileum and the long continuous patch in the middle ileum (ilPP), as the rates of lymphocyte entry differ in these discrete lymphoid aggregates.[20] In addition, the distribution of these T-cell subsets in the palatine tonsil, a lymphoid aggregate exposed continually to antigens, was compared to sites of less antigenic exposure, peripheral lymph nodes and blood. The data provide the first direct evidence for the presence of CD4/CD8 DP lymphocytes in gut-associated lymphoid tissues in the pig, and extend previous observations on the abundance of this lymphocyte population in the palatine tonsil. The possibility that CD4/CD8 DP lymphocytes are generated after antigen stimulation in inductive sites of the mucosal immune system is discussed.

MATERIALS AND METHODS

Experimental animals and tissues

Tissues were collected from healthy 6–7-month-old pigs at a local abattoir. Peripheral blood, peripheral (mandibular, retropharyngeal) and mesenteric lymph nodes, tonsils and small intestine were collected from six pigs. Peripheral blood was collected in heparinized tubes and mononuclear cells isolated by density gradient centrifugation using a cushion of Histopaque 1077 (Sigma, St. Louis, MO). Lymph nodes and Peyer's patches from the small intestine were identified and dissected based on their anatomical location.[25] Proximal, middle and distal Peyer's patches were collected from both the jejunum and ileum. The jejunum was dissected from the duodenum at the duodenocolic fold and from the ileum at the beginning of the continuous ilPP. The beginning of the continuous Peyer's patch was considered the start of the ileum although a few discrete Peyer's patches are typically found in the ileum prior to the continuous patch.[26] Proximal jejPP were obtained 10 cm distal to the duodenocolic fold. Distal jejPP were obtained 40 cm proximal to the continuous Peyer's patch in the ileum. The mid-jejPP were obtained from the centre of the jejunum. At least three contiguous patches from each section were minced together for a single cell suspension. Eight centimetre sections of the continuous Peyer's patch were obtained from the proximal (prox. ilPP), middle (mid-ilPP) and distal (dist. ilPP) portions of the ileum with the distal section being within 5 cm from the ileocecal junction.

Monoclonal antibodies

Monoclonal antibodies (mAb) specific for porcine CD4 (74-12-4, mouse IgG2bκ), CD8 (76-2-11, mouse IgG2aκ), and CD45 (74-9-3, mouse IgMκ),[27,28] were obtained from the American Type Culture Collection (ATCC; Rockville , MD) and purified from ascites fluid by passage through a protein A–Sepharose column using standard procedures according to the mAb isotype. The eluates were dialysed against phosphate-buffered saline (PBS) and adjusted to a concentration of 1 mg/ml. Biotin conjugated antibodies were utilized for flow cytofluorometric analysis (FCM). The conjugation of these antibodies to biotin-*n*-hydroxysuccinimide ester (Calbiochem, San Diego, CA) was carried out as described.[29]

Cell separation and surface staining

Ater removing the serosa, the jejPP and ilPP were rinsed in Hank's balanced salt solution (HBSS) and the mucosal surface gently blotted against a dry paper towel to remove the layer of mucus. Tissues were then minced gently in HBSS and the cells were washed once by centrifugation. The cell suspension was resuspended in 20 ml of a 40% Percoll (Sigma, St Louis, MO) solution, underlayed with an equal volume of 70% Percoll, and centrifuged for 30 min at 400 ***g*** as described.[30] The band at the 40–70% interface was collected, washed twice, and suspended in Dulbecco's buffered saline (Mediatech, Herndon, VA) supplemented with 0·5% bovine serum albumin (Intergen, Purchase, NY) and 0·1% NaN_3 (Flow PBS). Only cells with a light scatter characteristic of lymphocytes were analysed. More than 95% of the cells were CD45 positive, indicating the absence of contaminating epithelial cells. Fat and excess tissue were trimmed from tonsils and lymph nodes and the tissue was gently minced with sharp scissors. If the viability was less than 90%, the cell suspension was centrifuged (400 ***g*** for 30 min) through a cushion of Histopaque 1077. Peripheral blood mononuclear cells were also isolated by Histopaque gradient centrifugation. Intestinal intraepithelial lymphocytes (iIEL) were isolated from sections of the jejunum that were free of Peyer's patches as described previously.[30]

For the CD4/CD8 dual staining, 20 μl of lymphocyte cell suspensions in Eppendorf tubes (10^6 cells/tube) were reacted with 50 μl of anti-CD4 mAb (200 μg/ml). After a 30 min incubation on ice, cells were washed with flow PBS and reacted with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse immunoglobulin $F(ab')_2$ antibody (Zymed, San Francisco, CA) for an additional 30 min on ice. After washing, free binding sites were blocked by incubating the cell pellet with 30 μl of purified mouse immunoglobulin (100 μg/ml, Zymed). Cells in a 50 μl volume were then reacted with 10 μg of biotin-conjugated anti-CD8 mAb followed, after washing, by 10 μg of

streptavidin–phycoerythrin (Zymed). After a final wash, cell pellets were suspended in 300 μl of flow PBS and analysed in a Epics 752 Flow Cyotometer (Coulter, Hialeah, FL) equipped with an Argon ion laser. Data were collected for 20 000 cells using the MDADS Coulter data acquisition software in list mode. Data were analysed with ELITE software from Coulter. In the forward versus right angle light scatter histogram, electronic gates were set to include small lymphocytes only. This was confirmed by CD45 staining, which demonstrated that >95% of the cells were positive for this lymphoid cell marker. Fluorescence intensities in the red (phycoerythrin) and green (FITC) fluorescence axes were expressed on a 3 log scale. For each tissue, the mean and standard error of at least three animals were calculated. The total proportion of T cells was calculated from the two-colour analysis by the addition of CD4 SP + CD8 SP + CD4/CD8 DP lymphocytes. The proportion of CD4/CD8 DP cells as a percentage of the total CD4$^+$ subset was calculated with the following formula: (% CD4/CD8 DP × 100)/(% CD4/CD8 DP + % CD4 SP).

Data analysis

Statistical analyses were performed with Epi Info, Version 6 (Center for Disease Control, Atlanta, GA). Differences in the proportion of lymphocyte subsets in the different tissues were considered significant when they reached $P < 0.05$ as determined by analysis of variance.

RESULTS

Distribution of CD4- and CD8-expressing lymphocytes in the blood, lymph nodes and mucosa-associated lymphoid tissues

CD4 and/or CD8 expressing T cells (CD4 SP + CD8 SP + CD4/CD8 DP) comprised 59 ± 2% of total lymphocytes in blood and ranged from 64 ± 4% in the retropharyngeal lymph node to 72 ± 5% in the bronchial lymph node (Fig. 1). There were no significant differences in the proportion of CD4 and/or CD8-positive cells between the lymph nodes examined. Therefore, their values were pooled and used as a group to compare to mucosa-associated lymphoid tissues. Lymph nodes as a group had a higher ($P < 0.005$) proportion of CD4 and/or CD8 expressing T cells than any of the mucosa-associated lymphoid tissues examined (Fig. 1). Of the latter, the mid-continuous ilPP contained a significantly lower ($P < 0.002$) percentage of these T cells (22 ± 2%), than the proximal and distal sections of the continuous ilPP in which CD4 and/or CD8 expressing T cells accounted for 33 ± 7% and 38 ± 4% of total lymphocytes, respectively (Fig. 1). Among Peyer's patches, the total CD4 and/or CD8 expressing lymphocyte population was significantly higher ($P < 0.005$) in the jejPP (50 ± 4%) than any of the other Peyer's patches (Fig. 1). In our analysis the CD4$^-$/CD8$^-$ DN population includes both null and surface immunoglobulin-positive cells. The CD4$^-$/CD8$^-$ DN population in the tonsil, lymph nodes, and Peyer's patches had a large proportion of surface immunoglobulin-positive cells ($n = 2$; data not shown). Thus, the middle of the continuous ilPP appears to be comprised predominantly of surface immunoglobulin-positive cells, while the jejPP and the distal continuous ilPP contain one-third fewer B lymphocytes and a correspondingly higher proportion of CD4 and/or CD8-expressing T cells.

Of the iIEL isolated from jejunum or ileum, 84 ± 8% of the cells were T cells with >90% being CD8 SP cells (data not shown). Very few, if any, CD4/CD8 DP cells were detected and a small proportion of lymphocytes were CD4 SP (<6%; not shown).

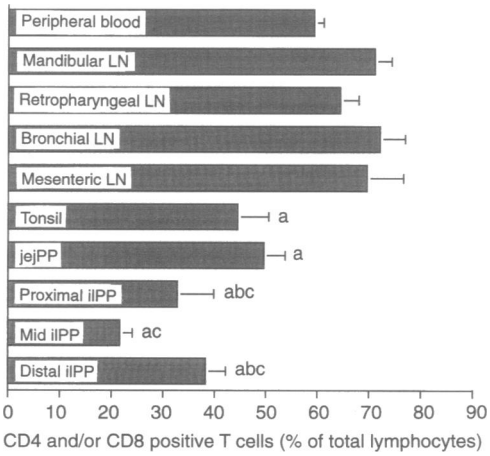

Figure 1. Percentages of CD4$^+$ and/or CD8$^+$ T lymphocytes in blood, lymph nodes, tonsils and Peyer's patches of 6–7-month-old swine. Proportions represent the sum of CD4 SP + CD8 SP + CD4/CD8 DP lymphocytes as determined by two-colour cytofluorometric analysis (mean ± SD). [a]$P < 0.005$ versus lymph nodes; [b]$P < 0.002$ versus mid.ilPP; [c]$P < 0.005$ versus jejPP.

CD4$^+$ and/or CD8$^+$ lymphocyte subsets in blood, lymph nodes and tonsils

Of the total lymphocytes in peripheral blood, 17 ± 2% were CD4 SP, 30 ± 4% were CD8 SP, and 12 ± 1% were CD4/CD8 DP cells (Fig. 2). In lymph nodes, 23–28% of total lymphocytes were CD4 SP and 27–32% were CD8 SP cells (Fig. 2) with no significant differences between peripheral (mandibular, retropharyngeal), mesenteric and bronchial lymph nodes (Fig. 2). CD4/CD8 DP lymphocytes comprised, on average, 10–13% of total lymphocytes in all of the lymph nodes, independent of location. In tonsils, the proportion of CD4 SP and CD8 SP cells was 60% or less of that in lymph nodes ($P < 0.01$), while the proportion of CD4/CD8 DP cells ranged from 1·4 to 1·7-fold higher than in the lymph nodes ($P < 0.01$; Fig. 2).

T-lymphocyte subsets in jejPP and continuous ilPP

The proportion of T-cell subsets in the discrete Peyer's patches in the proximal, middle and distal sections of the jejunum did not differ, and thus, T-cell subsets in these patches were averaged and are reported as a single mean value (Fig. 3). The proportion of CD8 SP (28%) and CD4/CD8 DP (10%) cells in the jejPP was similar to that in the lymph nodes, while the percentage of CD4 SP cells (12%) was significantly less ($P < 0.002$; jejPP versus any lymph node). In the proximal continuous ilPP the proportion of CD4 SP (8%) and CD8 SP (14%) cells was less ($P < 0.01$) than the proportion of these subsets in any of the lymph nodes (Fig. 3). In contrast, the percentage of CD4/CD8 DP cells did not differ from that of lymph nodes (Fig. 3). The mid-section of the continuous ilPP contained 8% CD4 SP cells, 8% CD8 SP cells and 4%

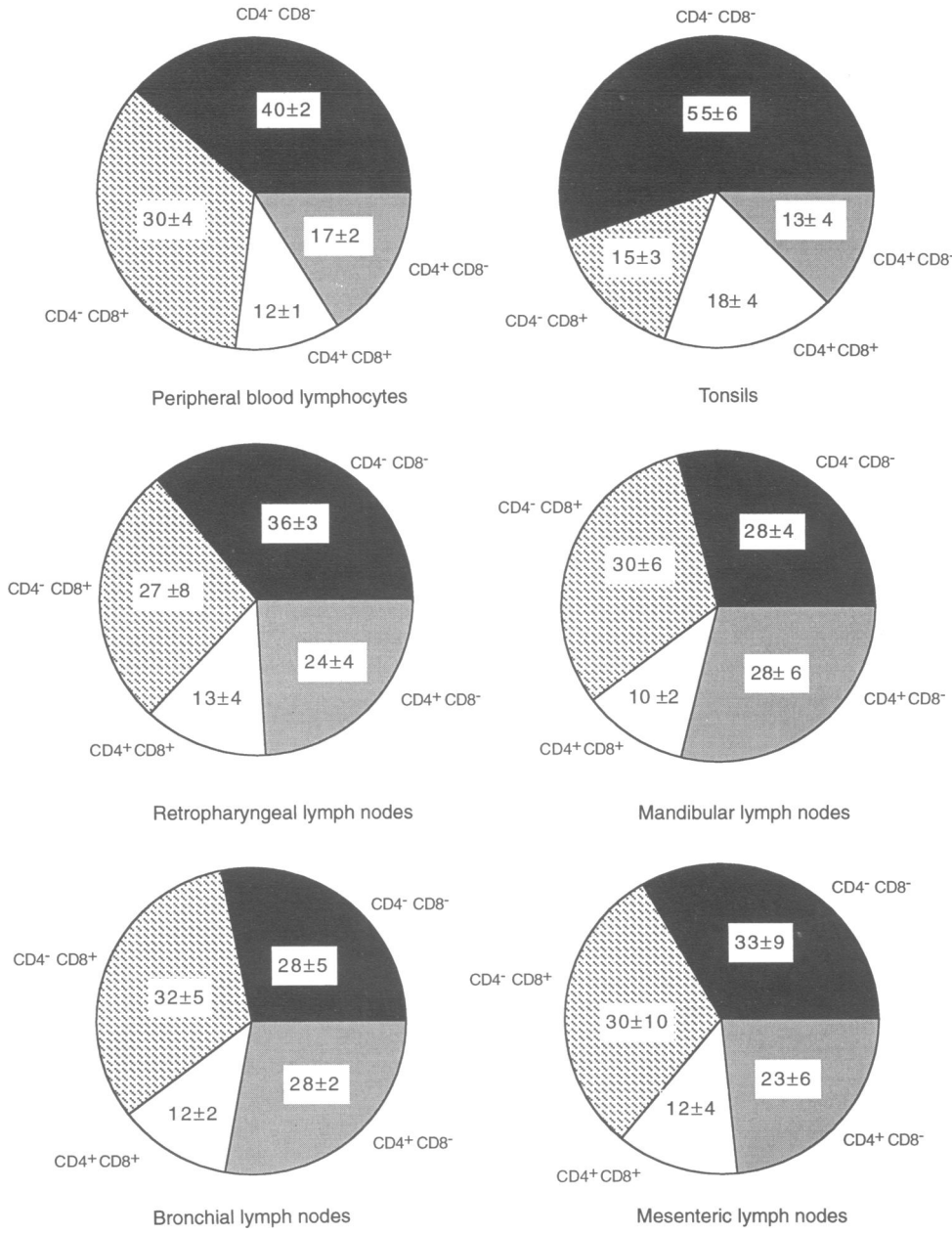

Figure 2. Lymphocyte subsets in blood, tonsils, and lymph nodes of 6–7-month-old swine. Proportions were determined by two-colour cytofluorometric analysis (mean ± SD).

CD4/CD8 DP cells (Fig. 3). All of these values were also significantly lower than corresponding subset proportions in any of the lymph nodes ($P < 0{\cdot}01$). The distribution of CD4 SP and CD4/CD8 DP cells in the distal section of the continuous ilPP was similar to that of jejPP, while CD8 SP cells were represented in lower proportions ($P < 0{\cdot}05$, distal ilPP versus jejPP).

Relative proportion of CD4/CD8 DP lymphocytes in the CD4$^+$ subset of blood, lymph nodes and mucosa-associated lymphoid tissues

Because of a possible lineage relationship between CD4 SP and CD4/CD8 DP cells,[5] the proportion of CD4/CD8 DP cells as a percentage of the total CD4$^+$ lymphocytes was calculated. The individual means for each tissue examined are shown in Fig. 4. There were no significant differences in the proportion of CD4/CD8 DP cells as a percentage of the total CD4$^+$ lyphocytes between the lymph nodes examined (Fig. 4). Therefore, their values were pooled and used as a group to compare to mucosa-associated lymphoid tissues and blood. Similarly, there were no significant differences in this value between the different Peyer's patches, and thus, these results were also pooled for comparison with other tissues. The CD4/CD8 DP population as a fraction of the CD4 lymphocytes in the tonsil (60%) was higher ($P < 0{\cdot}01$) than the lymph nodes (range 27% to 41%) or the peripheral blood (41%). As a group the Peyer's patches had significantly higher proportion of CD4/CD8 DP cells (range 32% to 69%) than the lymph nodes ($P < 0{\cdot}01$).

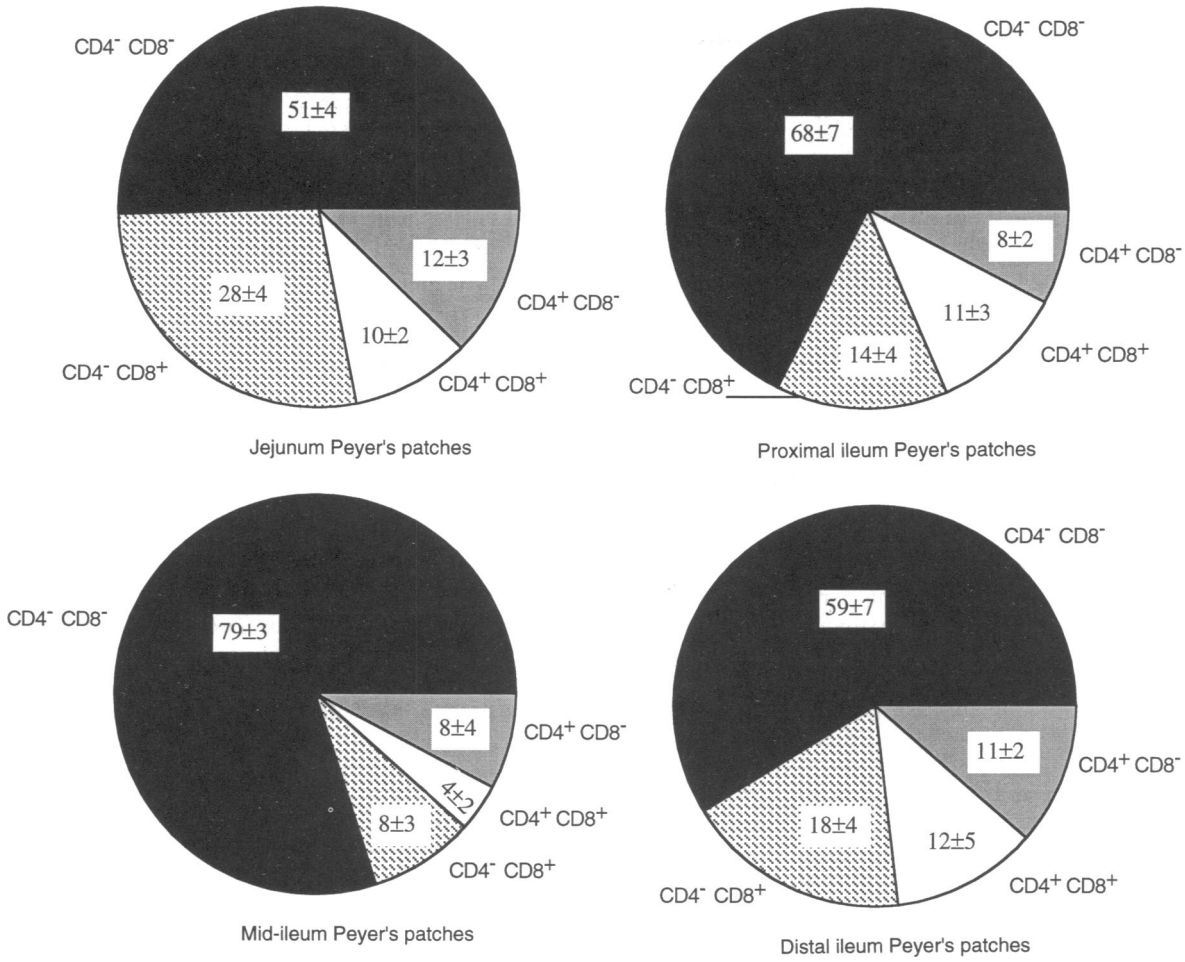

Figure 3. Lymphocyte subsets in the jejPP, proximal, mid and distal continuous ilPP of 6–7-month-old swine. Proportions were determined by two-colour cytofluorometric analysis (mean ± SD).

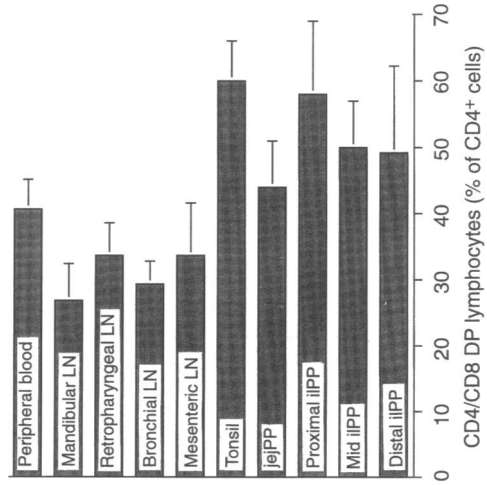

Figure 4. Relative proportion of CD4/CD8 DP lymphocytes in the CD4$^+$ subset of blood, lymph nodes and mucosa-associated lymphoid tissues. The proportion of CD4/CD8 DP cells within the CD4$^+$ lymphocyte subset was calculated as described in the Materials and Methods (mean ± SD). Statistical differences between lymph nodes, Peyer's patches, tonsils and blood are described in the text.

DISCUSSION

Substantial evidence indicates that circulating CD4/CD8 DP lymphocytes in the pig are functionally mature T cells,[5] and differ significantly in their phenotype from the immature CD4/CD8 DP cells found in the pig thymus.[31,32] It has been shown previously that the number of circulating CD4/CD8 DP T cells in the pig increases gradually with age,[5] and even more markedly in thymectomized pigs[33] (R. J. Husmann, R. M. Binns & F. A. Zuckermann, in preparation). Furthermore, CD4/CD8 DP lymphocytes are able to respond to recall viral antigen and, akin to human memory cells, express high levels of the β1 integrin.[34] All of these properties are typical of a memory cell population. In this study, the distribution of CD4/CD8 DP lymphocytes between mucosa-associated lymphoid tissues and selected peripheral and visceral lymph nodes was compared. The data demonstrate directly, for the first time, the presence of CD4/CD8 DP lymphocytes in organized lymphoid tissues of the small intestine. The presence of this lymphocyte population in Peyer's patches had been inferred previously from one-colour immunofluorescence analyses.[24] The data also showed that, among the tissues examined, CD4/CD8 DP lymphocytes were found in the greatest proportion in the tonsils. The relation between the peripheral blood CD4/CD8 DP lymphocytes and the cells of the same phenotype in the lymph nodes,

Peyer's patches and tonsils has not been established. However, because all of these tissues are secondary lymphoid organs, it is likely that the CD4/CD8 DP cells present in them belong to the same pool of lymphocytes present in the blood, which are either trafficking through these organs or are being generated in these inductive sites. These two possibilities are not mutually exclusive, and both remain to be examined.

Little is known about the tissue origin, differentiation pathway, migrating behaviour and functions of the CD4/CD8 DP T-cell population in normal animals. Nevertheless, there is mounting evidence that porcine DP T cells may represent CD4 SP cells that have acquired CD8 after antigenic exposure. Independent research groups have demonstrated that a significant proportion of porcine lymphoblasts generated during an *in vitro* response to allogeneic,[4] viral,[5] or parasite,[35] antigens are CD4/CD8 DP cells, while in the same culture CD4 SP lymphoblasts are very scarce. Furthermore, purified CD4 SP cells give rise to CD4/CD8 DP cells upon *in vitro* stimulation with recall-viral antigen.[34] Although direct proof that CD4 or CD8 SP cells can switch to a CD4/CD8 DP phenotype is not yet available, the tissue distribution of this population is consistent with its induction in sites of antigenic stimulation. For example, although as compared to lymph nodes, fewer lymphocytes bearing CD4 and/or CD8 were present in all of the mucosa-associated tissues examined, CD4/CD8 DP T cells were found in greatest proportions in the tonsils, where they accounted for 62% of the $CD4^+$ lymphocytes, as compared to 35% in the lymph nodes. Although in Peyer's patches the CD4/CD8 DP cells were equally abundant as in lymph nodes, they also accounted for a significantly larger fraction of the $CD4^+$ lymphocyte population, i.e. 44% in the jejPP, and 58% in the proximal continuous ilPP. Tonsils, having abundant numbers of T, B, and antigen-presenting cells and being positioned at the top of the respiratory and digestive tracts with unique anatomic features that trap environmental materials, are considered a major inductive site of the common mucosal immune system.[18] The presence of spontaneously interferon-γ-producing cells,[36] and significant proportions of CD45RO memory T cells in human tonsils[37] agrees with the postulate that T cells are induced to differentiate in this secondary lymphoid organ. In the pig too the tonsils are a site of major antigenic-stimulation shown by the extensive follicular lymphoid tissue and high level of lymphocyte traffic.[20,21] These findings are consistent with both a local antigen-mediated induction of CD4/CD8 DP lymphocytes and a preferential migration back to such inductive sites. These postulates are further supported by the observation that in tonsils of 3-day-old pigs, in which effector/memory cells are expected to be very scarce, only CD4 SP lymphocytes are present.[34] Further research is required to examine these possibilities.

Several possibly important differences were noted for T-cell populations in the two types of Peyer's patches in the pig. In agreement with reports from Binns & Licence,[20] and Rothkötter & Pabst,[24] but differing from data reported by Bianchi *et al.*,[22] the percentage of cells bearing CD4 and/or CD8 were higher in the jejPP than in continuous ileal PP. $CD4^+$ and/or $CD8^+$ T cells accounted for 50% of the lymphocytes in the jejPP and 20% in the middle region of the continuous ilPP. In our analyses the null cells are included in the CD4/CD8 DN population together with the B cells. A sizeable population of up to 35% null cells is present in the continuous ilPP and to a lesser extent in the discrete jejPP.[24] The null cell population in the blood is a heterogeneous population whose function is unknown.[15] The degree of heterogeneity for this subset in the PP has not been examined. B cells are also more abundant in the ilPP (63%) than in the jejPP (46%) of 1·5-month-old pigs.[24] Interestingly, the proportion of B cells decreases dramatically by more than 50% in both types of Peyer's patches by 12 months of age, with an increase in CD4 and/or CD8 T cells.[24] The proportion of B cells in the Peyer's patches from 6–7-month-old pigs detected in this study was akin to the 1·5-month-old pigs previously reported.[24] A dynamic balance between the different lymphocyte subsets present in these organs must be determining their incidence as well as the physiological role of these lymphoid organs as changes in lymphocyte subsets ensue. Of note is that all of the lymph nodes assayed drain a mucosal surface directly or indirectly, and thus, a higher level of antigenic stimulation would be expected. The degree of antigenic stimulation at any site of course could influence resident lymphocyte populations by modulating either differentiation or trafficking.

Binns & Licence[20] demonstrated that the rate of lymphocyte entry was consistently low in most of the continuous ilPP, except for the proximal and distal ends (about 20 cm in young pigs), when compared with the discrete PP of the duodenum, jejunum, proximal colon and tonsils, where it is relatively high.[21] Emigration of T cells from the gut to the systemic circulation via the lymphatics has been described recently as an important migratory pathway in pigs.[38] The observation that CD4/CD8 DP lymphocytes account for a significantly larger fraction of the $CD4^+$ lymphocyte population in Peyer's patches and palatine tonsils than in lymph nodes, provides additional support for the idea that these cells acquire the CD4/CD8 DP phenotype after antigen stimulation in inductive sites of the mucosal immune system and then migrate into the circulation, perhaps via lymphatics, to seed other tissues.[34]

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REFERENCES

1. Binns R.M. & Pabst R. (1994) Lymphoid tissue structure and lymphocyte trafficking in the pig. *Vet Immunol Immunopathol* **43,** 79.
2. Pescovitz M.D., Lunney J.K & Sachs D.H. (1985) Murine anti-swine T4 and T8 monoclonal antibodies: distribution and effects on proliferative and cytotoxic T cells. *J Immunol* **134,** 37.
3. Lunney J.K. & Pescovitz M.D. (1987) Phenotypic and functional characterization of pig lymphocyte populations. *Vet Immunol Immunopathol* **17,** 135.
4. Saalmüller A., Redehasse M.J., Bühring H.-J., Jonjic S. & Koszinowski U.H. (1987) Simultaneous expression of CD4 and CD8 antigens by a susbtantial proportion of resting porcine T lymphocytes. *Eur J Immunol* **17,** 1297.
5. Pescovitz M.C., Sakopoulos A.G., Gaddy J.A., Husmann R.J. & Zuckermann F.A. (1994) Porcine peripheral blood $CD4^+/CD8^+$ dual expressing T-cells. *Vet Immunol Immunopathol* **43,** 53.
6. Blue M.L., Daley J.F., Levine H. & Scholssman S.F. (1985) Coexpression of T4 and T8 on peripheral blood T cells demon-

strated by two color fluorescence flow cytometry. *J Immunol* **134,** 2281.

7. Blue, M.L., Daley J.F., Levine H., Craig K., & Scholssman, S.F. (1986) Biosynthesis and surface expression of T8 by peripheral blood T4$^+$ cells in vitro. *J Immunol* **137,** 1202.
8. Ortolani C., Forti E., Radin E., Cibin R. & Cossarizza A. (1993) Cytofluorometric identification of two populations of double positive (CD4$^+$, CD8$^+$) T lymphocytes in human peripheral blood. *Biochem Biophys Res Comm* **191,** 601.
9. de Maria A., Malnati M., Moretta A. *et al.* (1987) CD3$^+$4$^-$8$^-$ WT31- (T cell receptor $\gamma\delta^+$) cells and other unusual phenotypes are frequently detected among spontaneously interleukin-2 responsive T lymphocytes present in the joint fluid in juvenile rheumatoid arthtritis. A clonal analysis. *Eur J Immunol* **17,** 1815.
10. Burdick, J.F., Beschorner W.E., Smith W.J. *et al.* (1984) Characteristics of early routine renal allograft biopsies. *Transplantation* **38,** 679.
11. Gianello, P.R., Blancho G., Fishbein J.F. *et al.* (1994) Mechanisms of cyclosporin-induced tolerance to primarily vascularized allografts in miniature swine. *J Immunol* **153,** 4788.
12. Mosley R.L., Styre D. & Klein J.R. (1990) CD4$^+$CD8$^+$ murine intestinal intraepithelial lymphocytes. *Int Immunol* **2,** 361.
13. Lefrancois L. (1991) Phenotypic complexity of intraepithelial lymphocytes of the small intestine. *J Immunol* **147,** 1746.
14. Takimoto H., Takamura T., Takenchi M. *et al.* (1992) Age-associated increase in the number of CD4$^+$CD8$^+$ intestinal intraepithelial lymphocytes in rats. *Eur J Immunol* **22,** 159.
15. Binns R.M. (1994) The null/$\gamma\delta$TcR+ T cell family in the pig. *Vet Immunol Immunopathol* **43,** 69.
16. Hirt W., Saalmüller A. & Reddehase M.J. (1990) Distinct γ/δ T cell receptors define two subsets of circulating porcine CD2$^-$CD4$^-$CD8$^-$ T lymphocytes. *Eur J Immunol* **20,** 265.
17. Binns R.M., Duncan I.A., Powis S.J., Hutchins A. & Butcher G.W. (1992) Subsets of null and $\gamma\delta$ T-cell receptor$^+$ T lymphocytes in the blood of young pigs identified by specific monoclonal antibodies. *Immunology* **77,** 219.
18. Bernstein J.M., Yamanaka N. & Nadal D. (1994) Immunobiology of the tonsils and adenoids. In: *Handbook of Mucosal Immunology* (eds P.L. Ogra, M.E. Lamm, J.R. McGhee, J. Metstecky, W. Strober & J. Bienenstock), p. 625. Academic Press, San Diego.
19. Stokes C.R., Bailey M. & Wilson A.D. (1994) Immunology of the porcine gastrointestinal tract. *Vet Immunol Immunopath* **53,** 143.
20. Binns R.M. & Licence S.T. (1985) Patterns of migration of labelled blood lymphocyte subpopulations: evidence for two types of Peyer's patch in the young pig. In: *Microenvironments in the Lymphoid System* (ed. G.G.B. Klaus), p. 661, Plenum, New York.
21. Pabst R. & Binns R.M. (1989) Heterogeneity of lymphocyte homing physiology: several mechganisms operate in the control of lymphoid and non-lymphoid organs *in vivo*. *Immunol Rev* **108,** 83.
22. Bianchi A.T.J., Zwart R.J., Jeurissen S.H.M. & Moonen-Leusen H.W.M. (1992) Development of the B- and T-cell compartments in porcine lymphoid organs from birth to adult life: an immunological approach. *Vet Immunol Immunopathol* **33,** 201.
23. Jonjic N., Jonjic S., Saalmüller A., Rukavina D. & Koszinowski U.H. (1987) Distribution of T-lymphocyte subsets in porcine lymphoid tissues. *Immunology* **69,** 395.
24. Rothkötter H.J. & Pabst R. (1989) Lymphocyte subsets in jejunal and ileal Peyer's patches of normal and gnotobiotic minipigs. *Immunology* **67,** 103.
25. Nickel R., Schummer A. & Seiferle E. (1979) *The Anatomy of the Domestic Animals*, Vol. 3., p. 366. Verlag Paul Parey, Berlin.
26. Stokes C.R., Vega-Lopez M.A., Bailey, M., Telmo E. & Miller B.G. (1992) Immune development of the gastrointestinal tract of the pig. In: *Neonatal Survival and Growth* (eds M.A. Varley, P.E.V. Williams & T.L.J. Lawrence), No. 15, p. 9. British Society of Animal Production, Oxford.
27. Pescovitz M.D., Lunney J.K. & Sachs D.H. (1984) Preparation and characterization of monoclonal antibodies reactive with porcine PBL. *J Immunol* **133,** 368.
28. Lunney J.K. (1993) Characterization of swine leukocyte differentiation antigens. *Immunol Today* **14,** 147.
29. Jackson S., Sogn J. & Kindt T.J. (1982) Microdetermination of rabbit immunoglobulin allotypes by ELISA using specific antibodies conjugated to peroxidase or with biotin. *J Immunol Methods* **48,** 299.
30. Lefrancois L. (1993) Isolation of mouse small intestinal intraepthelial lymphocytes. In: *Current Protocols in Immunology* (eds J.E. Colligan, A.M. Kruisbeek, D.H. Margullies, E.M. Shevac & W. Strober), p. 3.19.1 John Wiley & Sons, Inc, New York.
31. Saalmüller A., Hirt W. & Reddehase M.J. (1989) Phenotypic discrimination between thymic and extrathymic CD4$^+$CD8$^+$ porcine T lymphocytes. *Eur J Immunol* **19,** 2011.
32. Pescovitz M.D., Hsu S.-M., Katz S.I., Lunney J.K., Shimada S. & Sachs D.H. (1990) Characterization of a porcine CD1-specific mAb that distinguishes CD4$^+$/CD8$^+$ double-positive thymic from peripheral T lymphocytes. *Tissue Antigens* **35,** 151.
33. Licence S.T & Binns R.M. (1995) Major long term changes in $\gamma\delta$TcR and CD2$^+$ T cell subsets after neonatal thymectomy in the pig: a longitudinal study lasting nearly two years. *Immunology* **85,** 276.
34. Zuckermann F.A. & Husmann R.J. (1996) Functional and Phenotypic analysis of porcine peripheral blood CD4/CD8 double positive lymphocytes. *Immunology*, in press.
35. Dillender M.J. & Lunney J.K. (1993) Characteristics of T lymphocyte cell lines established from NIH minipigs challenge inoculated with *Trichinella spiralis*. *Vet Immunol Immunopath* **35,** 301.
36. Quiding M., Granstrom G., Nordstrom I. & Ferruga B. (1993) High frequency of spontaneous interferon-gamma-producing cells in human tonsils: role of local accessory molecules and soluble factors. *Clin Exp Immunol* **91,** 157.
37. Secrist H., Egan M. & Peters M.G. (1994) Tissue specific regulation of IL-4 messenger RNA expression in human tonsil. *J Immunol* **152,** 1120.
38. Rothkötter H.J., Hriesik C. & Pabst R. (1995) More newly formed T than B lymphocytes leave the intestinal mucosa via lymphatics. *Eur J Immunol* **25,** 866.