Anat Embryol (1997) 195:41–50

ORIGINAL ARTICLE

N.N. Barman · A.T.J. Bianchi · R.J. Zwart · R. Pabst
H.J. Rothkötter

Jejunal and ileal Peyer's patches in pigs differ in their postnatal development

Accepted: 31 May 1996

Abstract The postnatal development of the jejunal and ileal Peyer's patches was studied before and after weaning in 1-, 1.5- and 2-month-old pigs. The follicles of the jejunal Peyer's patches grew with age and were two times longer and wider in specified pathogen-free and conventional pigs than in germ-free animals, thus indicating an influence of the living microbial antigens from the gut lumen. In germ-free pigs the size of the ileal Peyer's patch follicles increased between the 1st and 2nd month, whereas in the specified pathogen-free and conventional animals these follicles were comparable in size in all three age groups. In 1- to 1.5-month-old pigs the interfollicular area of jejunal Peyer's patches was wider (0.1 ± 0.04 mm) than that of the ileal Peyer's patch (0.04 ± 0.03 mm). Immunohistological studies showed that in germ-free pigs preferentially surface IgM^+ but few IgA^+ B cells were present in the follicles, domes and dome epithelia. In specified pathogen-free and conventional pigs the B cells expressed different levels of surface or cytoplasmic IgM or IgA. In all groups studied, more T cells were observed in the jejunal than in the ileal Peyer's patch. Here, few T lymphocytes were found because of the small interfollicular areas. Small numbers of Null cells were distributed in the interfollicular regions of all animals. The results show that living microbial antigens have a major influence on the jejunal and ileal Peyer's patches in pigs. The morphological differences between the two types of Peyer's patches are an indication that they develop differently during postnatal life. So far it remains unclear whether these morphological differences reflect a specific function of the pig's ileal Peyer's patch, such as the expansion of the genetically determined B cell repertoire as has been reported for sheep.

Key words Lymphocyte subsets · Nutrition · Peyer's patch morphology · Postnatal development · Pig

N.N. Barman[1] · R. Pabst · H.J. Rothkötter (✉)
Centre of Anatomy 4120, Medical School of Hannover,
D-30623 Hannover, Germany
Fax: ++49 (511) 532 2948

A.T.J. Bianchi · R.J. Zwart
Agricultural Research Department,
Institute for Animal Science and Health, Lelystad,
The Netherlands

Present address:
[1] Department of Microbiology, College of Veterinary Science,
Guwahati, Assam, India

Introduction

The Peyer's patches (PP) are the organized lymphoid tissue of the gut involved in the uptake of antigens and induction of mucosal immune responses. They consist of many follicles, between which preferentially T lymphocytes are present in the interfollicular area. Towards the luminal aspect the follicles are covered by the dome area containing T and B lymphocytes and antigen presenting cells (for review see Pabst 1987). Antigens are taken up and transported into the dome by the M cells, which are specialized cells of the dome epithelium (for reviews see Neutra and Kraehenbuhl 1992; Gebert et al. 1996). Although the mechanisms of antigen presentation and stimulation of lymphoid cells in the dome area have not been examined in detail, it is well known that lymphoid cells stimulated or produced in the PP finally reach the intestinal mucosa and function as immunoglobulin A-producing plasma cells or as antigen-specific T cells (for review see, for example, McGhee and Kiyono 1992).

The distribution along the small intestine and the morphology of PP differ markedly between species. In humans, discrete PP up to ~10 cm in length are distributed throughout the small intestine, with increased numbers in the ileum. The follicles of these PP are round with large domes and well-developed interfollicular areas (Cornes 1965). In several mammalian species such as ruminants (e.g. sheep, cattle), omnivora (e.g. pigs) and carnivora (e.g. dogs), in addition to several discrete PP in the jejunum, there is a long continuous PP in the ileum, with a maximum length of ~2 m (Pabst et al. 1988; Landsverk et al. 1991). This ileal PP has been examined

in a number of species, but especially in sheep. In this species the ileal PP has long columnar-shaped follicles and very small domes and interfollicular areas. It consists of more than 90% B cells (Reynolds and Morris 1983; Hein et al. 1989). The morphology of the ileal PP in cattle is comparable (Landsverk et al. 1991).

There is a lot of evidence that the characteristic morphology of the ileal PP in ruminants reflects a special function (for review see Griebel and Hein 1996). In the ileal PP follicles of sheep many B cells are already produced during gestation without the influence of antigen (Reynolds and Morris 1983, 1984). Resection of the ileal PP in sheep before birth results in severe B lymphopenia in postnatal life (Gerber et al. 1986). In addition, many of the newly formed cells do not leave the ileal PP but die in situ (Reynolds 1986). In sheep it has been observed recently that somatic hypermutations occur in the ileal PP follicles independent of the influence of antigens, thus increasing the genetically determined B cell repertoire (Reynaud et al. 1991, 1995). After this highly active period, the ileal PP involutes in later life. In 18-month-old sheep few if any follicles are found in the ileum (Reynolds and Morris 1983, 1984). All these observations support the hypothesis that the ileal PP in sheep may function in a comparable fashion to a primary lymphoid organ for B cells.

In comparison to ruminants little is known about the morphology and function of the ileal PP in the pig, which is an omnivorous animal. Comparing the function of the ileal PP of sheep (ruminant) and pigs (omnivora) can provide important basic knowledge about lymphocyte production and function in the PP and the implications of the type of digestion and the luminal antigens for this process. A few reports indicate that there might be a difference between the ileal PP and the jejunal PP. In young minipigs (1.5 months) long-shaped follicles, tiny domes and small interfollicular areas have been observed in the ileal PP, but these follicles do not reach the length of those in sheep, nor is the percentage of B cells as high (Binns and Licence 1985; Rothkötter and Pabst 1989). In an immunohistological study no marked differences between the jejunal and ileal PP were observed (Bianchi et al. 1992). One-month-old pigs had larger follicles in the ileal PP than in the jejunal PP and the birth rate of lymphocytes in the two types of PP was comparable (Pabst et al. 1988).

In the present study an attempt was made to elucidate how the jejunal and ileal PP grow in pigs after birth and which factors influence the morphology and development of both types of PP. Germ-free, specified pathogen-free (SPF) and conventional pigs were used to examine the morphology of the jejunal and ileal PP before weaning at 1 month and after weaning at 1.5 and 2 months after birth. The three experimental groups focus on different influences stimulating the intestinal immune system. In the germ-free pigs the influence of food components was analysed. It can be assumed that the small amount of dead microbial material present in the food of these pigs is not an important stimulus of the gut immune system (Wold et al. 1989). In SPF and conventional animals the influence of different levels of living bacteria and other microbial antigens that the PP was exposed to was examined. Although the rearing conditions of these three experimental groups represent the major forms of stimuli to which the gut immune system is exposed, the rearing conditions are not completely comparable. The germ-free animals had to be delivered by caesarean section and kept in isolators separated from their mother. Thus they had no access to colostrum and breast milk. It is not possible at present to compose a sterile artificial colostrum that contains, in addition to the macronutrients, all the substances present in normal colostrum or milk. The laborious and expensive modifications of the rearing conditions of young pigs are a basis for finding out how the jejunal and ileal PP develop in an omnivorous species like the pig, whose diet and microbial load in the digestive system is different from that of ruminants.

Materials and methods

Animals and tissues

In the present study three litters of piglets were used (crossbred: Large White × Dutch Landrace; breeding stock of the Institute for Animal Science and Health, Lelystad, The Netherlands). The animals were reared germ free (n = 8), SPF (n = 8) or conventional (n = 9). Germ-free piglets were delivered by caesarean section. They were provided with sterilized powdered milk (based on cow milk; Sloten, Deventer, The Netherlands) up to day 31. After weaning on day 32, these animals were given sterilized pelleted food (Hope Farms, Woerden, The Netherlands; irradiated 5 Mrad). The sterility of germ-free pigs was checked in faeces and tonsillar swabs every week. Normally delivered SPF pigs were kept with their mother, and thus received sow milk (including colostrum) until weaning (day 31). Then they were given pelleted food (irradiated 1 Mrad). Conventional pigs were kept under standard farming conditions, and their diet was supplemented from day 10 onwards with non-irradiated pelleted food, which was also their diet after weaning on day 31. Each litter of piglets was subdivided into three age groups: 1 month (n = 3, before weaning) and after weaning 1.5 months (n = 3) and 2 months (n = 2). In the conventional animal group three animals were used at 2 months of age. As the rearing of germ-free and SPF pigs is difficult and expensive, the study was restricted to these low numbers. It is obvious that there were certain differences in the rearing conditions of the germ-free animals in comparison to SPF and conventional pigs. However, under the available experimental facilities it was not possible to provide the germ-free animals with colostrum and sow milk.

Tissue sampling

Samples of the jejunal PP and ileal PP were taken from the animals at necropsy. Jejunal PP were always excised within the first 1.5 m of gut length aboral to the duodenojejunal flexure. The ileal PP samples were excised in the middle of the continuous ileal PP. Cross-sections of the whole gut circumference were taken in each case. For routine histological examination the tissue samples were fixed in Schaffer's solution, while parallel samples were snap-frozen in liquid nitrogen and stored at –70°C until cryostat sections were made.

Table 1 Monoclonal antibodies to pig lymphocyte surface molecules used in this study

Antibodies	Surface molecules	Preferential cell type	Dilution	Reference
MAC 80	CD2	T cells	1:300	Lunney and Pescovitz (1988)
74–12–4	CD4	T helper cells	1:1000	Lunney and Pescovitz (1988)
76-2–11	CD8	T cytotoxic cells	1:40	Lunney and Pescovitz (1988)
8/1		T cell subset	1:300	Lunney and Pescovitz (1988)
CVI-SWIgA-27.9	IgA	IgA^+ B cells	1:50 000	Van Zaane and Hulst (1987)
CVI-SWIgM-28.4	IgM	IgM^+ B cells	1:50 000	Van Zaane and Hulst (1987)
MAC 320		Pig Null cells	1:40	Binns et al. (1992)
MAC 319		Pig Null cell subset	1:500	Binns et al. (1992)

Histology and morphometry of PP

The jejunal and ileal PP fixed in Schaffer's solution were embedded in glycolmethacrylate. As the blocks contained the whole gut circumference it was possible to obtain cross-sections. For each sample four histological sections 4 μm thick were stained with Giemsa and haematoxylin and eosin (H&E) and screened with a Zeiss microscope to locate jejunal PP and ileal PP. The morphometry of the PP was carried out as follows. At least ten longitudinally sectioned follicles per sample were measured at a magnification of ×100 using the divisions of an ocular grid (100 rectangles, 1 mm^2 total area). To determine the size of the follicles their width and height were recorded. The distance between neighbouring follicles was determined where the follicles were closest to each other. In this way the interfollicular area was measured. The results were recorded per sample and the average value ± SD was expressed in millimetres.

Immunohistology

Cryostat sections 5 μm thick were prepared for immunohistology. Sections were allowed to air dry for at least 1 h and finally stored at –20°C until use. Monoclonal antibodies against B cells (IgA^+, IgM^+), T cells ($CD2^+$, $CD4^+$, $CD8^+$, $8/1^+$) and Null cell subsets (Mac319, Mac320) of pigs were used (Table 1). A standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique served as detection system (Rothkötter et al. 1991). In brief, frozen tissue sections were thawed and dried at room temperature for 30 min and fixed in methanol acetone (1:1) solution for 90 s. TRIS-buffered saline (TBS) of pH 7.2 containing 0.05% Tween 20 (Serva, Heidelberg, Germany) was used as washing solution. Thoroughly washed sections were incubated with 50 μl of appropriately diluted primary antibodies (Table 1) for 30 min at room temperature in a humid chamber. After washing with TBS the sections were incubated with the secondary antibody (rabbit anti-mouse immunoglobulin 1:50; Dako, Hamburg, Germany) for 30 min. In the third step, TBS-washed slides were incubated with 50 μl of APAAP complex (1:50; Dako) for 30 min. The incubations with the last two antibodies were repeated for 15 min each to increase the staining intensity. Slides were washed in TBS and the phosphatase reaction was performed with fast red solution (10 mg fast red salt in 9.8 ml 0.1 M TBS containing 2 mg naphthol AS-MX phosphate, 200 μl *N*,*N*,-dimethyl formamide and 10 μl 1 M levamisole (Sigma, Munich, Germany)) for 25 min. The sections were briefly counterstained with haematoxylin for 45 s and mounted in glycergel (Dako).

Immunohistological analysis

The in situ distributions of B and T lymphocyte subsets as well as the Null cells were examined in a semi-quantitative way at a magnification of ×100 using a Zeiss microscope. One representative section per organ per animal was evaluated. The frequency of distinctly stained positive cells in different compartments (interfollicular area, follicle, dome) of the PP was expressed using the following terms: more frequent = most lymphoid cells of the compartment were stained positively; frequent = stained cells were less dense but covered the whole compartment; less frequent = positively stained cells were concentrated in the middle of the compartment or distributed thinly; occasional = countable number of positive cells scattered through the compartment; absent = no positively stained cells. The intraepithelial lymphocytes of the follicle-associated epithelium (FAE) were counted per dome epithelium.

Results

Histology and morphometry of PP

In the samples of either jejunal or ileal PP the size of the various follicles was similar, as was the size of the interfollicular areas. Thus, the morphometrical technique used in this study and the numbers of follicles examined were adequate to analyse the postnatal development of the PP. The histological changes in the follicles, interfollicular areas and domes of the jejunal and ileal PP were examined in germ-free (Fig. 1a–d), SPF (Fig. 1e–h) and conventional pigs at 1, 1.5 and 2 months. The follicles of jejunal and ileal PP were distinct, with the exception of 1-month-old germ-free pigs (Fig. 1a,c). The follicles of the jejunal PP were ovoid in pre-weaning life and became elliptical thereafter. The follicles of ileal PP were ovoid. In comparison to germ-free pigs the height of the follicles of the ileal PP increased dramatically in the pre-weaning life of SPF and conventional pigs (Fig. 1c,g). In the jejunal PP the interfollicular area of all animal groups was wider (Fig. 1a,b,e,f) and in SPF and conventional animals it was densely populated with lymphoid cells. In contrast, the interfollicular area of the ileal PP was narrow and wedge-shaped in all animals (Fig. 1d,g,h). The dome of the jejunal and ileal PP was cone-shaped. In pre-weaning life it was elongated and with age it became short and wide. The histological changes and morphometry of PP were comparable in conventional and SPF pigs. Therefore all figures present PP in germ-free and SPF pigs only. A schematic summary of the morphological configurations of jejunal and ileal PP is given in Fig. 2, including follicular and interfollicular area sizes at different ages in germ-free and SPF pigs.

Lymphocyte subset distribution

During postnatal life there was an increase in the subset of positive lymphocytes with age and with the higher

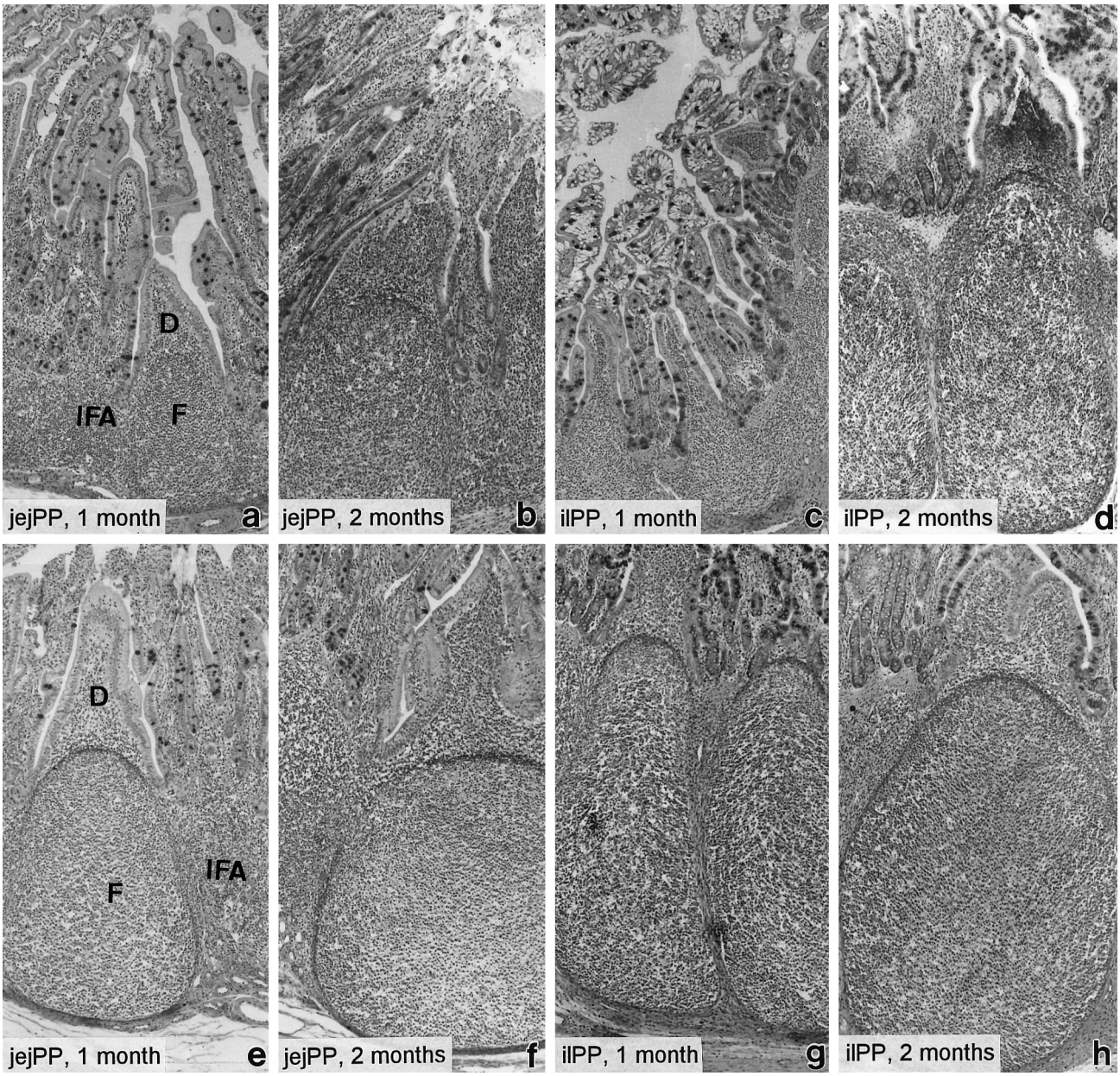

Fig. 1 Histology of jejunal and ileal Peyer's patches (PP) in germ-free (**a–d**) and specified pathogen-free (SPF) pigs (**e–h**). The jejunal (**a, b, e, f**) and ileal (**c, d, g, h**) PP are shown at 1 month (**a, c, e, g**) and 2 months (**b, d, f, h**) of age. In germ-free and in SPF pigs the size of the follicles (*F*) and interfollicular area (IFA) increase with age in the jejunal PP. In germ-free animals there is a marked increase in the length of the ileal PP follicles with age, whereas the follicles in the ileal PP of SPF pigs have a comparable length in both age groups. In germ-free animals the domes (*D*) and follicles (*F*) of the ileal PP (**c**) at 1 month of age are not distinct, but in SPF animals these compartments are distinct in both age groups. ×43

amount of antigens the animals' gut immune system was exposed to. These larger numbers of lymphocytes were due to a certain increase in the density of the cells in the PP compartments, but more particularly to the increase in the overall size of the compartments (Fig. 2). The distribution of T and Null cell subsets in germ-free, SPF and conventional pigs did not vary greatly between 1-, 1.5- and 2-month-old pigs; therefore the T and Null cell results described below are valid for all three age groups.

B cells

Follicles

In the follicles two populations of cells were observed which expressed surface IgM on a high or a low level ($sIgM^{high+}$ and $sIgM^{low+}$). In germ-free animals 1 month of age the follicles of the jejunal and ileal PP were covered with $sIgM^{high+}$ cells (Fig. 3a,b). In addition, in the ileal PP of germ-free animals there were a few $sIgM^{low+}$

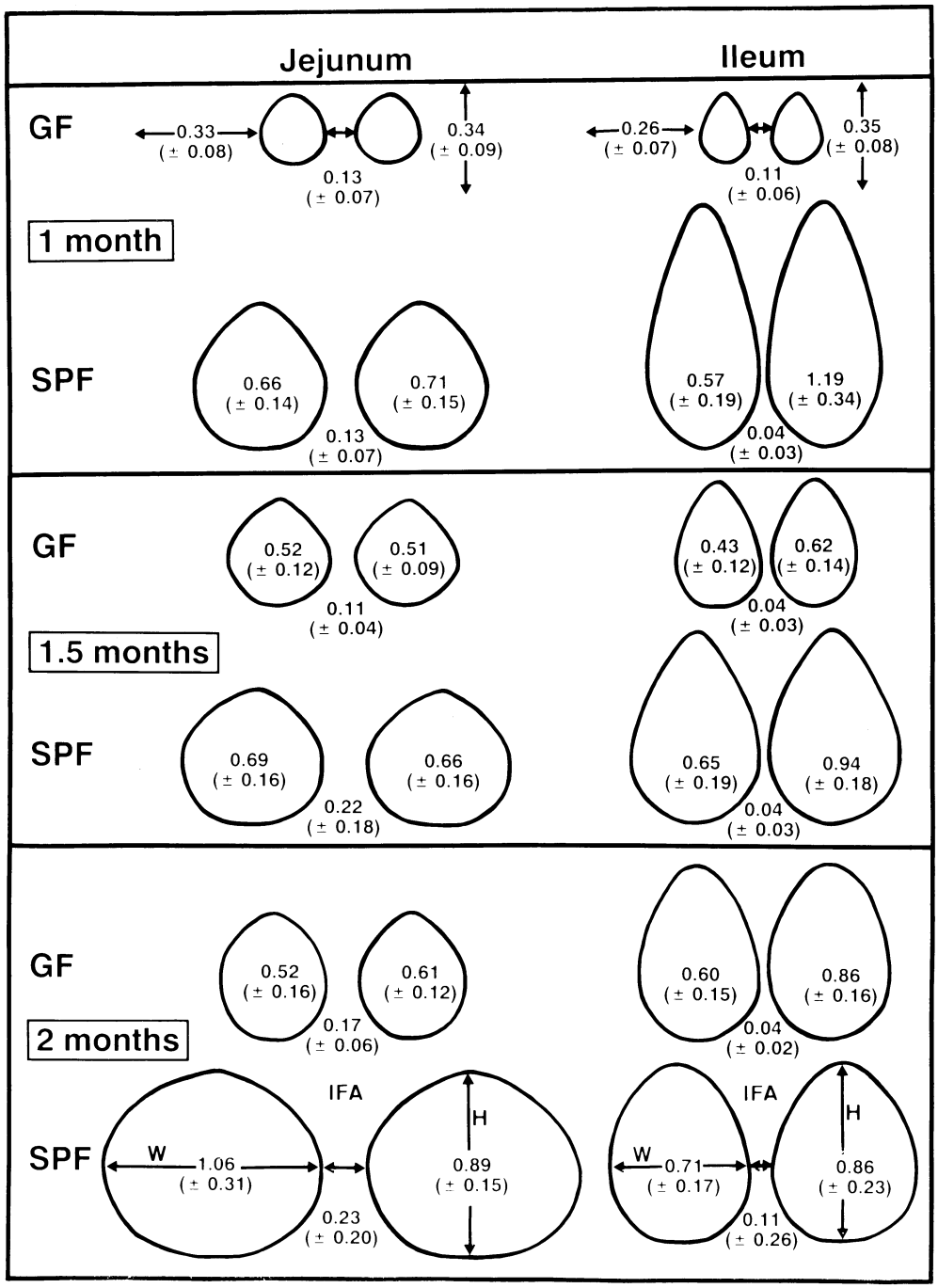

Fig. 2 Schematic summary of the morphological changes of the follicle and interfollicular area (*IFA*) of jejunal and ileal Peyer's patches at 1, 1.5, 2 months of age in germ-free (*GF*) and specified pathogen-free (*SPF*) pigs. Measurements of the width (*W*) and height (*H*) of the follicle and interfollicular region in millimetres are presented in the same scale

cells in the basolateral aspect of the follicles (Fig. 3b). Such cells were not observed in the jejunal PP of 1-month-old germ-free pigs. This distribution of $sIgM^{high+}$ and $^{low+}$ cells had changed in 2-month-old germ-free animals. Their jejunal and ileal PP follicles were more comparable to those of SPF and conventional animals. In the follicles of all age groups of SPF and conventional pigs the regions containing $sIgM^{high+}$ and $^{low+}$ cells were obvious (Fig. 3e,f). The $sIgM^{low+}$ cells were arranged like a horse-shoe in the basal and lateral area of the follicles, whereas $sIgM^{high+}$ cells were present preferentially in the centre of the follicle and towards the dome area. The jejunal and ileal PP follicles of SPF pigs contained a reticular immunoreactivity. This immunoreactivity was not present in sections incubated with the second and third antibodies alone. Therefore, the reactivity may be due to immunoglobulin on reticular cells of the follicle or to immune complexes. No surface IgA^+ ($sIgA^+$) cells were present, but a few cytoplasmic IgA^+ ($cIgA^+$) cells were identified in the follicles of jejunal PP of all animal groups.

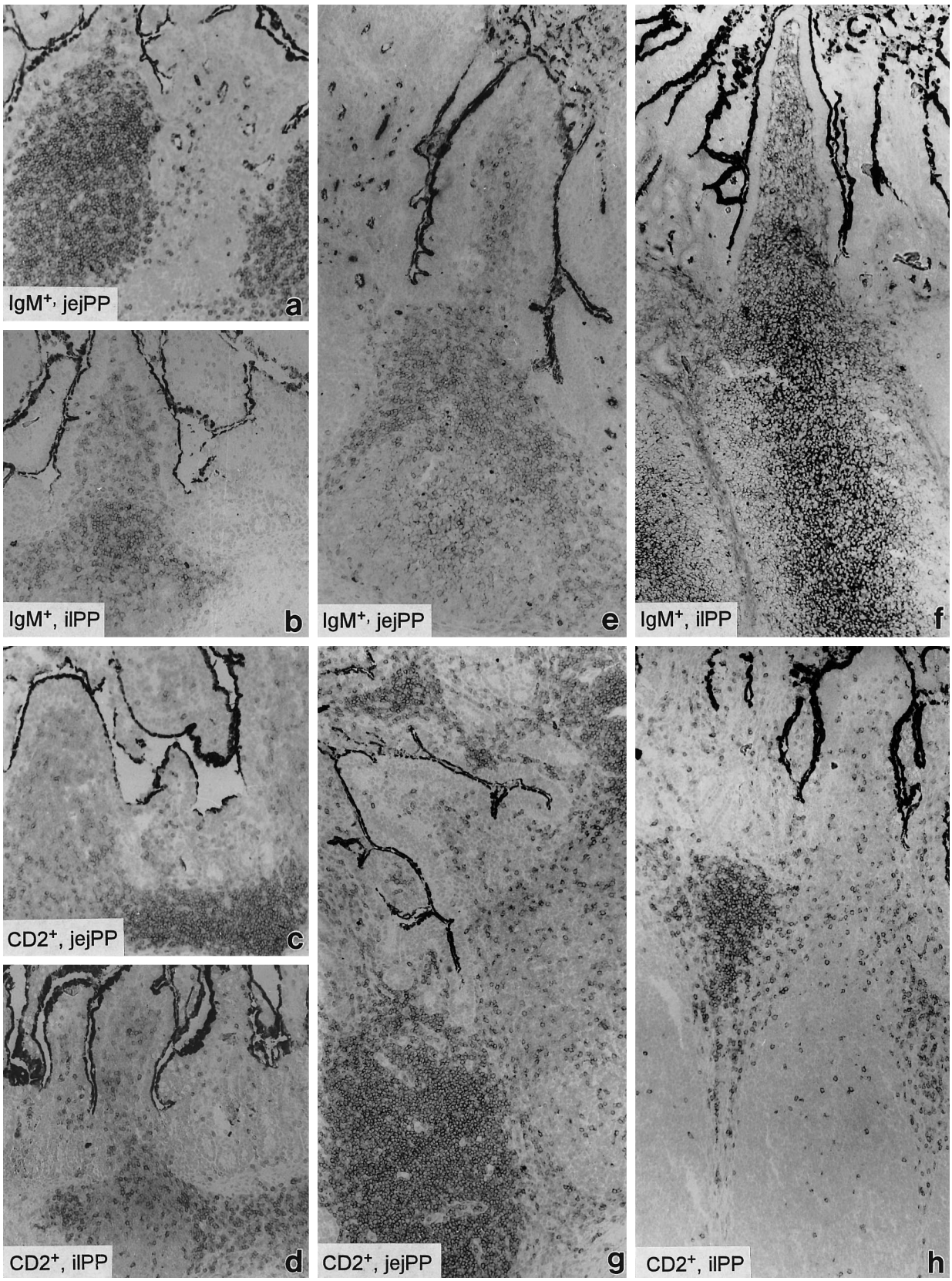

Fig. 3 Distribution of IgM$^+$ (**a, b, e, f**) and CD2$^+$ T cells (**c, d, g, h**) in the jejunal (**a, c, e, g**) and ileal (**b, d, f, h**) PP of 1-month-old germ-free (**a–d**) and SPF (**e–h**) pigs. IgM$^+$ cells are prominent in germ-free pigs (**a, b**) and in SPF pigs in the dome of jejunal PP (**e**). The interfollicular areas of the jejunal PP (**c, g**) contain many more CD2$^+$ T cells than the tiny dome areas of the ileal PP (**d, h**). ×84

Table 2 Distribution of T lymphocyte subsets in different compartments of jejunal and ileal Peyer's patches (*PP*). An increase in the size of the PP compartments was observed with age, whereas the distribution of the subsets in the various compartments was comparable. Therefore, the results obtained in all three age groups (1, 1.5 and 2 months) are summarized (*IFA* interfollicular area, *FAE* follicle associated epithelium, *SPF* specified pathogen-free; +++ very frequent, ++ frequent, + less frequent, ± occasional, – negative)

Tissue area examined	Animal group	CD2		8/1		CD4		CD8	
		Jejunal PP	Ileal PP	Jejunal PP	Ileal PP	Jejunal PP	Ileal PP	Jejunal PP	Ileal PP
Follicle	Germ-free	+	±	±	–	±	±	±	±
	SPF	+	±	–	–	±	±	±	–
IFA	Germ-free	+++	+	+++	+	++	+	+	±
	SPF	+++	++	+++	++	+++	++	+++	++
Dome	Germ-free	+	+	±	±	±	±	±	±
	SPF	+	+	+	±	+	±	±	±
FAE	Germ-free	±	±	±	±	–	–	–	–
	SPF	±	±	±	±	–	–	±	±

Interfollicular area

A small number of $sIgM^+$ cells was present in the interfollicular area of both PP of germ-free (Fig. 3a,b), SPF (Fig. 3e,f) and conventional pigs. Cells positive for cytoplasmic IgM ($cIgM^+$) were identified in germ-free pigs (1–3 per interfollicular area), whereas $cIgA^+$ cells were present in all three animal groups (1–3 per interfollicular area).

Dome and dome epithelium

In the dome of jejunal and ileal PP of germ-free pigs (Fig. 3a,b) and of jejunal PP of SPF pigs (Fig. 3e) $sIgM^+$ cells were observed. In conventional pigs, $sIgM^+$ cells were occasionally found. Very few $sIgA^+$ cells were detected in both jejunal and ileal PP of germ-free pigs. Per dome area 2–6 $cIgM^+$ cells and 2–10 $cIgA^+$ cells were recorded. The frequency of $cIgA^+$ cells was higher in SPF and conventional pigs than in germ-free pigs. In jejunal PP of germ-free and SPF pigs and in the ileal PP of germ-free pigs, 3–8 $sIgM^+$ cells per dome epithelium were identified (Fig 3a,b,e). In the dome epithelium $sIgM^+$ cells were absent in conventional pigs and as well as IgA^+ cells in all animals.

T cells

The distribution of $CD2^+$ T cell subsets in the different compartments of the jejunal PP and ileal PP is summarized in Table 2. Only the results of the germ-free and SPF animals are presented here because the cell density and distribution pattern of T lymphocyte subsets in conventional pigs were comparable to those of the SPF pigs. In germ-free animals the T cell subset densities were lower in comparison to SPF and conventional pigs (germ-free: Fig 3c,d; SPF: Fig. 3g,h). In follicles T cells were occasionally detected; these were $CD4^+$ or $CD8^+$. In contrast, the monoclonal antibody 8/1 detected few if any cells in the follicles, whereas its staining pattern in the interfollicular area and in the domes was comparable to that of the CD2 antibody. The interfollicular areas and the domes were densely packed with T cells. In the interfollicular area the T cell subsets were not evenly distributed. In germ-free and especially in SPF pigs more $CD8^+$ than $CD4^+$ cells were observed.

Null cells

In the follicles no Null cells were detected. Countable numbers (5–10 cells/interfollicular region) of Null cells were present in the interfollicular region of jejunal and ileal PP of germ-free, SPF and conventional pigs and very occasionally positive cells were found in the domes, whereas the dome epithelium was negative for Null cells. The MAC 320^+ cells (5–10 cells/interfollicular region) outnumbered the MAC 319^+ cells (2–5 cells/interfollicular region). The Null cell subsets were not influenced at all by the rearing protocols of the three animal groups.

Discussion

In the present study the development of the jejunal and ileal PP in pigs was examined using three experimental groups (germ-free, SPF and conventional). Thus an attempt was made to differentiate between the luminal microbial and nutritional stimuli the PP are exposed to. The germ-free animals received a powdered cow-milk-based diet, whereas the SPF and conventional pigs had access to colostrum and sow milk. It is a matter of discussion whether compounds present in colostrum and sow milk are able to influence the postnatal development of PP. Both nutrient-dependent and nutrient-independent factors from colostrum are known to stimulate the protein synthesis in newborn pigs (Ulshen et al. 1991; Burrin et al. 1995). Furthermore, in sheep a colostral protein has been described that stimulates the B cell production in vitro (Julius et al. 1988). Milk contains growth factors

that are related to transforming growth factor β2. These factors induce immunosuppression in vitro (Cox and Bürk 1991; Kanda et al. 1994; Mandalapu et al. 1995). The immunoglobulins in the milk inactivate luminal antigens to such an extent that during the lactating period oral immunizations, e.g. against rotavirus, are difficult (Conner et al. 1994). Immunoglobulin A and immune complexes bind selectively to the apical membrane of M cells, thus facilitating the uptake of antigens by M cells of PP (reviewed by Gebert et al. 1996). Taken together, all these effects of colostrum and milk may have an influence on the intestinal immunity. However, in a recent study using pigs no differences in PP development and morphology were observed between animals that had received colostrum and those on a colostrum-free diet (Pabst et al. 1988). Therefore the present results may be related to food antigens and factors in the milk, but they reflect to a much greater extent the stimulation of growth of PP by living microbial antigens.

In all experimental groups the lymphoid cells became denser during the 2nd month of postnatal life. The PP follicles of 1-month-old germ-free piglets contained many $sIgM^{high+}$ cells and few $sIgM^{low+}$ cells in comparison to SPF and conventional pigs. The larger number of $sIgM^{low+}$ cells in SPF and conventional animals as well as in 2-month-old germ-free pigs indicates the different stages of B cell development in the PP follicles of these groups. The horse-shoe-shaped area of low IgM staining contains the proliferating cells of the PP follicles in pigs. One hour after flash labelling of all cells in the S-phase of the cell cycle using the thymidine analogue bromodesoxyuridine the cells that had incorporated the label were observed in this area (H. J. Rothkötter, S. Möllhoff, B. von Hörsten, R. Pabst, unpublished results). Comparable observations have been made in the cortical area of the ileal PP follicles in sheep (Griebel and Hein 1996). Using the metaphase arrest technique with vincristine it has been demonstrated that there is already a marked cell production in the jejunal and ileal PP follicles in conventional pigs at day 12 after birth (Pabst et al. 1988). The increase of $sIgM^{low+}$ cells in germ-free animals between the 1st and 2nd months of life may be an indication that in animals free of living microbial antigens, new antigens from solid food are an important stimulus inducing the cell production of the PP follicles. In pigs there are no data available about the distribution and function of follicular dendritic cells; thus the reticular immunoglobulin staining pattern observed in SPF and conventional animals within the follicles cannot yet be explained.

Many more T cells were found in the jejunal than ileal PP. $CD4^+$ and $CD8^+$ T cells were observed not only in the dome and interfollicular area but also in the follicles of all animal groups. The monoclonal antibody 8/1 detected no lymphoid cells within the follicles. This is in accordance with the report that in vitro stimulated T cells of pigs do not express the antigen detected by the monoclonal antibody 8/1 (Saalmüller et al. 1987). In pig PP there are only few γ/δ T cells. This T cell subset was detected using antibodies against Null cells. It has been reported that in pigs a proportion of MAC 320^+ Null cells negative for the Null cell marker MAC 319 expresses the γ/δ form of the T cell receptor (Binns et al. 1992). Thus, the difference between MAC 320^+ cells and MAC 319^+ cells is approximately the percentage of γ/δ T cells. As very few MAC 320^+ or MAC 319^+ Null cells were found in this study, the numbers of γ/δ T cells in the PP must be low in pigs. Comparable results were obtained in sheep (Hein and Mackay 1991). The lymphocyte subset composition during the first 2 months of postnatal life reflects the adaptation of the gut immune system to the various influences from the intestinal lumen. The jejunal PP follicles grew continuously between the 1st and 2nd months of life in all animal groups, although the increase in size was lower in the germ-free animals. In contrast, the ileal PP follicles had a comparable size in SPF and conventional animals in 1-, 1.5- and 2-month-old animals. In germ-free pigs the ileal PP follicles were still short after 1 month, but they became longer during the 2nd month of life. The results in the ileal PP are in accordance with the shorter follicles in the isolated intestinal loops containing ileal PP in young sheep in comparison with the normal functioning ileum of the sheep (Reynolds and Morris 1984; Reynaud et al. 1995). The present observations provide evidence that jejunal and ileal PP in pigs have different growth kinetics and that both types of PP are obviously stimulated by living microbial antigens.

The morphometric analysis revealed the long shape of the ileal PP follicles and a very small interfollicular area. For this observation it was important to determine the size of the PP compartments using morphometry, because in a study concentrating on cell densities per tissue area the differences between jejunal PP and ileal PP were not that obvious (Bianchi et al. 1992). The low lymphocyte immigration into the ileal PP in comparison to the jejunal PP observed earlier in young piglets may be due to the small interfollicular areas in the ileal PP that represent the traffic areas for lymphocytes (Binns and Licence 1985; Rothkötter et al. 1990). The present results clearly demonstrate this morphological difference between the pig's jejunal and ileal PP, although the mechanisms responsible are not yet known. In addition to the luminal stimuli which are preferentially of microbial origin, there are other, probably genetically determined, mechanisms. It is obvious that during the whole life of the pig the discrete jejunal PP have a preferential function as sites for antigen sampling and induction of intestinal immune responses. They have a determined position along the small intestine (Rothkötter and Pabst 1989); they grow with increasing levels of antigens and are even present in very old animals (Pabst et al. 1988). In the large dome area of jejunal PP better antigen uptake has been detected than in the ileal PP (Gebert et al. 1994). There is an indication that the ileal PP is to a certain extent independent of the different composition of the luminal content of the upper and lower small intestine. The transposition of the ileal PP between the duodenum and the jejunum in 4- to 8-week-old pigs did not affect the ileal PP morphology and lymphocyte composi-

tion, which is also the case in sheep (Reynolds and Kirk 1989; Rothkötter et al. 1990).

Based on the morphological differences observed, the question arises whether not only the ileal PP of ruminants but also that of pigs might have a function related to B cell ontogeny. In ruminants it has been hypothesized that the intraluminal antigen load and nutritional factors produce a high unspecific mitotic activity, being an ideal stimulus to induce the high level of cell proliferation in the ileal PP follicles (Reynaud et al. 1995). The high rate of cell production is necessary to acquire somatic mutations to expand the genetically based immunoglobulin repertoire. Somatic mutations have been described not only for the ileal PP of sheep but also for PP of mice (Gonzalez-Fernandez et al. 1994; Reynaud et al. 1995). The question is whether the pig's ileal PP provides a compartment for antigen-independent B cell differentiation, because the ileal PP follicles of pigs are smaller than those of ruminants and have a lymphocyte production comparable to that of the jejunal PP follicles (Pabst et al. 1988). Studies on the development of the B cell repertoire in ileal PP in the pig are now becoming possible with the increasing knowledge of pig immunoglobulins (Butler and Brown 1994).

If the ileal PP in pigs is such a compartment for B cell differentiation the next question is whether the large amount of lymphoid tissue in the terminal ileum develops differently in pigs and in ruminants. The lamb's ileal PP follicles reach their maximum length during the suckling period, before the high levels of microbial antigens in the digestive tract appear after weaning (Reynolds and Morris 1983). Then the microorganisms are necessary in the ruminant's complex stomach to digest a herbal diet. Does the high level of luminal microbial antigens in the ruminant's post-weaning life require an effective expansion of the genetically determined immunoglobulin repertoire by somatic mutations or gene conversion occurring already in the first days of life in the ileal PP follicles? Omnivorous species do not have such large amounts of microbial antigens in their small intestine and therefore extensive lymphocyte proliferation and expansion of the B cell repertoire may not be necessary in the pig. This might explain why the ileal PP in pigs is not similar to that of ruminants and in post-weaning life develops a morphology comparable to that of the jejunal PP (Pabst et al. 1988; Rothkötter and Pabst 1989).

Acknowledgements The authors express their sincere gratitude to Dr. R.M. Binns, Cambridge, England, and Dr. A. Saalmüller, Tübingen, Germany, for their donations of monoclonal antibodies, to S. Fassbender, A. Herden and S. Schlecht for their skilful technical help, and to S. Fryk for secretarial assistance and correction of the English. The study was funded by the Deutsche Forschungsgemeinschaft, SFB 280 'Gastrointestinal Barrier', Project C1. Dr. Barman was supported by a grant from the German Academic Exchange Service.

References

Bianchi ATJ, Zwart RJ, Jeurissen SHM, Moonen-Leusen HWM (1992) Development of the B- and T-cell compartments in porcine lymphoid organs from birth to adult life: an immunohistological approach. Vet Immunol Immunopathol 33:201–221

Binns RM, Licence ST (1985) Patterns of migration of labelled blood lymphocyte subpopulations: evidence for two types of Peyer's patch in the young pig. Adv Exp Med Biol 186:661–668

Binns RM, Duncan IA, Powis SJ, Hutchings A, Butcher GW (1992) Subsets of Null and γ/δ-T cell receptor$^+$ T lymphocytes in the blood of young pigs identified by specific monoclonal antibodies. Immunology 77:219–227

Burrin DG, Davis TA, Ebner S, Schoknecht PA, Fiorotto ML, Reeds PJ, McAvoy S (1995) Nutrient-independent and nutrient-dependent factors stimulate protein synthesis in colostrum-fed newborn pigs. Pediatr Res 37:593–599

Butler JE, Brown WR (1994) The immunoglobulins and immunoglobulin genes of swine. Vet Immunol Immunopathol 43:5–12

Conner ME, Matson DO, Estes MK (1994) Rotavirus vaccines and vaccination potential. Curr Top Microbiol Immunol 185:285–337

Cornes JS (1965) Number, size and distribution of Peyer's patches in the human small intestine. I. The development of Peyer's patches. II. The effect of age on Peyer's patches. Gut 6:225–233

Cox DA, Bürk RR (1991) Isolation and characterization of milk growth factor, a transforming-growth-factor-β2-related polypeptide, from bovine milk. Eur J Biochem 197:353–358

Gebert A, Rothkötter HJ, Pabst R (1994) Cytokeratin 18 is an M-cell marker in porcine Peyer's patches. Cell Tissue Res 276: 213–221

Gebert A, Rothkötter HJ, Pabst R (1996) M cells in Peyer's patches of the intestine. Int Rev Cytol 167:91–159

Gerber HA, 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: 201–213

Gonzalez-Fernandez A, Gilmore D, Milstein C (1994) Age-related decrease in the proportion of germinal center B cells from mouse Peyer's patches is accompanied by an accumulation of somatic mutations in their immunoglobulin genes. Eur J Immunol 24:2918–2921

Griebel PJ, Hein WR (1996) Expanding the role of Peyer's patches in B-cell ontogeny. Immunol Today 17:30–39

Hein WR, Mackay CR (1991) Prominence of γ/δ T cells in the ruminant immune system. Immunol Today 12:30–34

Hein WR, Dudler L, Mackay CR (1989) Surface expression of differentiation antigens on lymphocytes in the ileal and jejunal Peyer's patches of lambs. Immunology 68:365–370

Julius MH, Janusz M, Lisowski J (1988) A colostral protein that induces the growth and differentiation of resting B lymphocytes. J Immunol 140:1366–1371

Kanda Y, Yamamoto N, Abe Y (1994) Growth factor from human milk: purification and characterization. Life Sci 55:1509–1520

Landsverk T, Halleraker M, Aleksandersen M, McClure S, Hein W, Nicander L (1991) The intestinal habitat for organized lymphoid tissues in ruminants; comparative aspects of structure, function and developments. Vet Immunol Immunopathol 28:1–16

Lunney JK, Pescovitz MD (1988) Differentiation antigens of swine lymphoid tissues. In: Miyasaka M, Trnka Z (eds) Differentiation antigens in lymphohemopoietic tissues. Dekker, New York, pp 421–454

Mandalapu P, Pabst HF, Paetkau V (1995) A novel immunosuppressive factor in human colostrum. Cell Immunol 162:178–184

McGhee JR, Kiyono H (1992) Mucosal immunity to vaccines: current concepts for vaccine development and immune response analysis. Adv Exp Med Biol 327:3–13

Neutra MR, Kraehenbuhl JP (1992) Transepithelial transport and mucosal defence. I. The role of M cells. Trends Cell Biol 2:134–138

Pabst R (1987) The anatomical basis for the immune function of the gut. Anat Embryol 176:135–144

Pabst R, Geist M, Rothkötter HJ, Fritz FJ (1988) Postnatal development and lymphocyte production of jejeunal and ileal Peyer's patches in normal and gnotobiotic pigs. Immunology 64:539–544

Reynaud CA, Mackay CR, Müller RG, Weill JC (1991) Somatic generation of diversity in a mammalian primary lymphoid organ: the sheep ileal Peyer's patches. Cell 64:995–1005

Reynaud CA, Garcia C, Hein WR, Weill JC (1995) Hypermutation generating the sheep immunoglobulin repertoire is an antigen-independent process. Cell 80:115–125

Reynolds JD (1986) Evidence of extensive lymphocyte death in sheep Peyer's patches. I. A comparison of lymphocyte production and export. J Immunol 136:2005–2010

Reynolds JD, Kirk D (1989) Two types of sheep Peyer's patches: location along gut does not influence involution. Immunology 66:308–311

Reynolds JD, Morris B (1983) The evolution and involution of Peyer's patches in fetal and postnatal sheep. Eur J Immunol 13:627–635

Reynolds JD, Morris B (1984) The effect of antigen on the development of Peyer's patches in sheep. Eur J Immunol 14:1–6

Rothkötter HJ, Pabst R (1989) Lymphocyte subsets in jejunal and ileal Peyer's patches of normal and gnotobiotic minipigs. Immunology 67:103–108

Rothkötter HJ, Zimmermann HJ, Pabst R (1990) Size of jejunal Peyer's patches and migration of lymphocyte subsets in pigs after resection or transposition of the continuous ileal Peyer's patch. Scand J Immunol 31:191–197

Rothkötter HJ, Ulbrich H, Pabst R (1991) The postnatal development of gut lamina propria lymphocytes: number, proliferation, and T and B cell subsets in conventional and germ-free pigs. Pediatr Res 29:237–242

Saalmüller A, Jonjic S, Bühring HJ, Reddehase MJ, Koszinowski UH (1987) Monoclonal antibodies reactive with swine lymphocytes. II. Detection of an antigen on resting T cells down-regulated after activation. J Immunol 938:1832–1857

Ulshen MH, Lecce JG, Stiles AD, Lyn-Cook LE (1991) Effects of nursing on growth and development of small bowel mucosa in newborn piglets. Pediatr Res 30:337–341

Van Zaane D, Hulst MM (1987) Monoclonal antibodies against porcine immunoglobulin isotypes. Vet Immunol Immunopathol 16:23–36

Wold AE, Dahlgren UIH, Hanson LÅ, Mattsby-Baltzer I, Midvetdt T (1989) Difference between bacterial and food antigens in mucosal immunogenicity. Infect Immun 57:2666–2673