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Identification, localization and quantification of porcine intestinal immune cell subsets during the first seven weeks of postnatal ontogeny

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ABSTRACT

By using immunohistology and digital image analysis for histomorphometry, we investigated the influence of porcine postnatal development on distribution and quantitative patterns of naïve/memory lymphoid cell subsets ($CD45RA^+$, $CD45RC^+$, respectively), helper/cytotoxic T cell subsets ($CD4^+$, $CD8^+$, respectively) as well as of IgA^+ plasma cells in the gut-associated lymphoid tissues (GALT) of pigs reared under intensive conditions during the first 7 weeks of life. Current research on the postnatal ontogenesis/maturation of porcine small intestinal mucosal immune system have shown that newborn pigs are immunologically rather immature and, thus, totally dependent on passively acquired immunity. Their jejunal mucosa almost lacks immune cells, with the exception of a low number of $CD45RA^+$ lymphoid cells (1.25×10^{-5}) at Day 0 *post partum*. A very few $CD45RC^+$ lymphoid cells (8.79×10^{-6}) appeared at Day 7, followed by a similar number of IgA^+ plasma cells (7.65×10^{-6}) which were observed at Day 14. Such first signs of the postnatal development of both innate

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and adaptive immunity by identification/localization of tested cell subsets residing porcine GALT, *i e.* jejunal mucosal sites are proceeded by a lag period of three weeks until the appearance of low quantities of $CD4^+$ (3.62×10^{-5}) and $CD8^+$ (3.13×10^{-5}) T cells at Day 35 after birth. After that phase of early postnatal development of the crucial cell subsets participating in the antigen-specific immunity on the gut mucosal surfaces of young pigs, further progress was visible through their different distribution patterns within the jejunal mucosa compartments, and a gradual increase in their numbers, particularly between Day 35 and Day 49 of age. A significant increase was recorded within that period in the numbers of naïve, memory and plasma cells, whereas the number of helper and cytotoxic T cells only slightly increased between Day 42 and Day 49 of life.

Key words: postnatal development, immune cells, gut mucosa, pigs

Introduction

At birth, the porcine intestinal mucosal immune system is poorly developed and it has been suggested that this may lead to harmful allergic or damaging inflammatory conditions by inappropriate responses to feed and microbial antigens in the post-weaning period (BAILEY et al., 2005 b; BAILEY and HAVERSON, 2006; LALLES et al., 2007; GALLOIS et al., 2009). The kinetics of development of the gut immune system may therefore play a critical role in determining the outcome of the response to both harmless and harmful intraluminal antigens. This system develops gradually during the first 6 weeks of life in several stages, and in immediate dependence on environmental influences (PABST et al., 1988; PABST and ROTHKÖTTER 1999; BAILEY and HAVERSON, 2006; LEWIS et al., 2012). In neonates, the porcine small intestinal mucosa almost lack lymphoid cells, except the small number of antigen presenting cells and T cells, and therefore they have very poor or no reaction to the same dose of antigen that triggers an adequate immune reaction in adults (BAILEY et al., 2005a; BAILEY and HAVERSON, 2006). Their small intestinal wall contains rudimentary Peyer's patches (PP), which nonspecifically expand due to the rapid intrafollicular proliferation of B cells 2 weeks following birth (ROTHKÖTTER and PABST, 1989). Simultaneously with the dramatic increase in B cells, the production of T cells within the small intestinal lamina propria (LP) and interfollicular areas (IFA) of the PP was found to be less intensive and rather slower (PABST et al., 1988). The gradual appearance of some conventional, activated, T cells is proceeded by the influx of antigen presenting dendritic cells (DC), expressing major histocompatibility complex class II (MHC II^+) molecules and co-expressing CD45 and CD16 membrane markers (HAVERSON et al., 2000). Between the second and fourth weeks of life, increasing numbers of mature $CD4^+$ cells appear within the villous LP, whereas $CD8^+$ T cells only appear in significant numbers as late as 4 to 6 weeks of life (HAVERSON et al., 1999). Similarly, a significant number of IgA^+ plasma cells have been reported after the fourth week of life (ROTHKÖTTER et al., 1999), although the appearance of IgA-producing B cells within the LP has been demonstrated on day six of life (PABST and ROTHKÖTTER 1999). The IgM^+ immunoblasts appear earlier, but only exceed the number of IgA^+ B cells after 3

weeks (BROWN et al., 2006). Functional *in vitro* studies have shown lower responsiveness of circulating lymphocytes in 1-day-old suckling pigs (for 70%) as compared to that in 28-day-old weaned pigs (JANJATOVIĆ et al., 2008; KOVŠCA-JANJATOVIĆ et al*.,* 2009; KOVŠCA-JANJATOVIĆ et al*.,* 2010; POTOČNJAK et al., 2012). Further development and maturation of adaptive immunity in weaned pigs, as a consequence of interaction with environmental antigens, results in reaching adult immunocompetence at 7 to 9 weeks of age, as determined either by the immune cell distribution in the small intestine (VEGA-LOPEZ et al., 1995) or by functional *in vitro* assays (BROWN et al., 2006), and by defining the role of their intestinal LP as a mucosal effector site for perorally delivered vaccines (STOKES and BAILEY, 2000).

This study focuses on the kinetics of the development/maturation of the porcine intestinal mucosal immune system during the early (from Day 0 to Day 21 of life) and late (from Day 28 to Day 49 of life) postnatal period. By immunohistologic and histomorphometric analyses we investigated the influence of postnatal ontogenesis on the appearance, distribution and frequency patterns of naïve/memory lymphoid cell subsets ($CD45RA^+$, $CD45RC^+$, respectively), helper/cytotoxic T cell subsets ($CD4^+$, $CD8^+$, respectively) as well as of IgA^+ plasma cells in the jejunal mucosa of pigs reared under intensive conditions, during the first 7 weeks of life.

Materials and methods

Experimental animals. This study was performed on 40 pigs (crossbreeds of Swedish Landrace, Yorkshire and Pietren, from litters of 3rd parity sows) aging from 0 to 49 days, reared under intensive conditions on a commercial farm eastern Croatia. The pigs were assigned to 8 experimental groups depending on their age, starting from neonates (0 Day of life) and further to sucklings aged either 7, 14 or 21 days and weaners aging 28, 35, 42 or 49 days. The groups comprised 5 pigs each. The pigs within the groups were of both sexes and approximately of the same body weight. They were marked 1 to 5 by ear-numbers, and housed, managed and fed in accordance with the rearing technology of the farm. The pigs from the first four age groups (aging either 0, 7, 14 or 21 days) were kept with their sows in the sections of the pens with underfloor heating, while the pigs from the remaining four groups (aging either 28, 35, 42 or 49 days), which had been weaned at the age of 26 days, were kept in separate pens with concrete floors and fed with a mixture of feed for growing pigs in portions of approximately 200 g per pig per day. Experimental and animal management procedures were conducted in accordance with the EU "Directive for the Protection of Vertebrate Animals used for Experimental and other Purposes" (86/609/EEC).

Sampling. Five pigs from each of the 8 age groups were euthanatized by intracardial injection of 0.3 mL/kg of T61 (Hoechst, Munich, Germany) in accordance with the Law

on Animal Ethics and Welfare of Croatia and the aforementioned EU Directive 86/609/EEC. Immediately following euthanasia (at Days 0 to 49) 5 specimens (1 cm) of the mid jejunum from suckling and weaned pigs (either 3 to 4 cm or 9 to 10 cm distal from duodenum, respectively) were fixed in 10% phosphate-buffered paraformaldehyde (pH 7.2) for 24 hours until used for histology and immunohistology analyses.

*Monoclonal and polyclonal antibodies (mAbs/pAbs)***.** The Murine mAbs reactive with porcine leukocyte surface molecules, *i.e.* cluster of differentiation (CD) antigens or immunoglobulin A (IgA) molecule on plasma cells, that we used as the primary Abs to study *in situ* identification, distribution and quantification patterns of respective lymphoid cell subsets residing jejunal mucosa of experimental pigs are listed in Table 1. The pAbs against murine IgG (originated from rabbits or goats) conjugated with HRP were used as the secondary Abs (Table 1).

Table 1. Primary and secondary (conjugated with horseradish peroxidase; HRP) Abs used for immunohistological identification/localization and morphometric quantification of porcine lymphoid cell subsets residing jejunal mucosa

Ab		Isotype	Ab specificity	Targeted cells/molecules	Origin
Primary	Secondary: conjugate				
MIL 13	/	IgG1	CD45RA	Leukocytes -naïve T lymphocytes	AbD Serotec, Kidlington, Oxford, UK
MIL 5	/	IgG1	CD45RC	Leukocytes -memory T lymphocytes	
MIL 17	/	IgG2b	CD4a	Helper T lymphocytes	
MIL 12	/	IgG2a	CD8a	Cytotoxic T lymphocytes	
K61 1B4	/	IgG1	IgA	IgA^+ plasma cells	
/	Rabbit anti-mouse IgG : HRP	IgG	Mouse IgG	Mouse IgG	
/	Goat anti-rabbit IgG : HRP	IgG	Rabbit IgG	Rabbit IgG	Abcam, Cambridge, UK

*Histology and immunohistology analyses***.** After fixation, the specimens of jejunum were dehydrated, embedded in the paraplast (Sigma, Sherwood Medical Industries, USA), cut into 5 μm thick serial sections and then processed for standard hemalaun (Meyer's solution; Kemika, Zagreb, Croatia) and eosin staining. These sections were examined by a light microscope (Leitz, Orthoplan, Germany) in order to select tissue areas of the jejunal mucosa suitable for immunohistological identification/quantification of the

lymphoid cell subsets tested. For immunohistology the paraplast-embedded sections were processed for the indirect immunoperoxidase (IP) method, using primary and secondary Abs (Table 1), as detailed earlier (LACKOVIĆ et al., 1997). After drying, the sections were examined by a light microscope (Eclipse E600, Nikon, Japan) and the areas selected for histomorphometry were photographed by a digital camera (DMX1200, Nikon, Japan).

Histomorphometry by digital image analysis (DIA). Histomorphometric analysis of targeted lymphoid cells within the jejunal mucosa sites was performed using the commercial software imaging program, Lucia G (version 4.11) for DIA. The quantification of these cells was performed by DIA in 12 randomly selected tissue section fields (of an average area of 695 821 μm^2) at ×200 on screen magnification from each of the 5 sampled pigs per group. Such counting included villous LP, Lieberkühn crypts (Lc) and submucosa for $CD4^+$, $CD8^+$ T cells, villous epithelium/LP and Lc for $CD45RA^+$ naïve lymphoid cells, Lc and the IFA of jejunal PP for $CD45RC^+$ memory lymphoid cells as well as villous LP and Lc for IgA^+ plasma cells The numerical data were expressed as the mean values of the number of targeted cells per μm^2 of an average tissue section field as previously described (KOVŠCA-JANJATOVIĆ et al., 2009).

Statistics. The obtained differences in the mean values of tested cells between the number of cells in the pigs at either Day 0 of life or on the day of their first observation, and the number of cells in the pigs of the corresponding age group on the consecutive days of the experiment were evaluated by statistical analysis using the Student's t test for dependent samples on the StatisticaSixSigma software (StatSoft, Inc.). The significance of differences between the number of cells in the control pigs (euthanized at either Day 0 or on the day the cell subsets tested were first found) were considered as significant at $P<0.05$ and lower values. We also calculated the difference between the index of increase or decrease in the number/percent of these cells in relation to the values obtained at either Day 0 of life or on the day they were first found (no. of the cells = 1.00 or 100%).

Results

The identification, localization and distribution patterns of $CD4^+$, $CD8^+$, $CD45RA^+$, $CD45RC^+$ i IgA^+ immune cells residing sites within the jejunal mucosa of pigs belonging to the 8 experimental groups differing in age by intervals of a week (starting from Day 0 to Day 49 of life) as well as in the first appearance and frequency of tested cell subsets during the postnatal development/maturation of the small intestinal mucosal immune system in swine, are shown in Figs 1 to 5.

Up to Day 35 of age, $CD4^+$ T lymphocytes were not observed. Then these cells were visible and rather frequent within the LP of the jejunal villi and around the Lc (Fig. 1 a, b). Their number was the highest (3.62×10^{-5}), compared with the numbers of the other cell subsets tested as recorded on the Day (age of the pigs) of their first appearance in the porcine jejunal mucosa (Table 2).

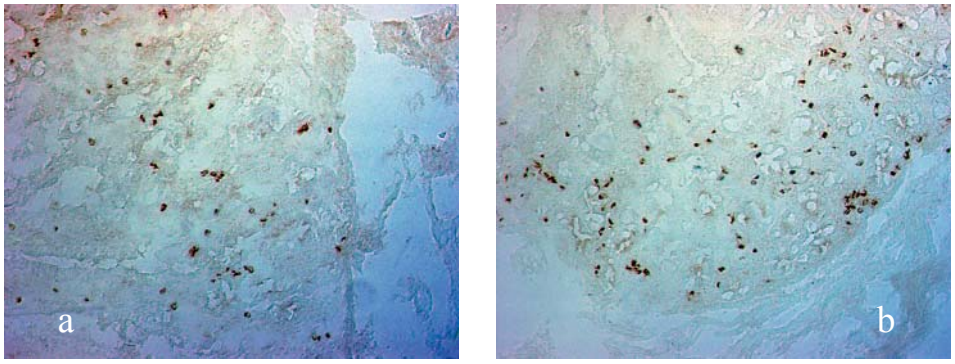

Fig. 1. Localization of $CD4^+$ T cells in villous LP, between Lc and in the submucosa of the jejunum of 35-day-old pig (a) and 49-day-old pig (b) as demonstrated by the indirect IP method; ×200.

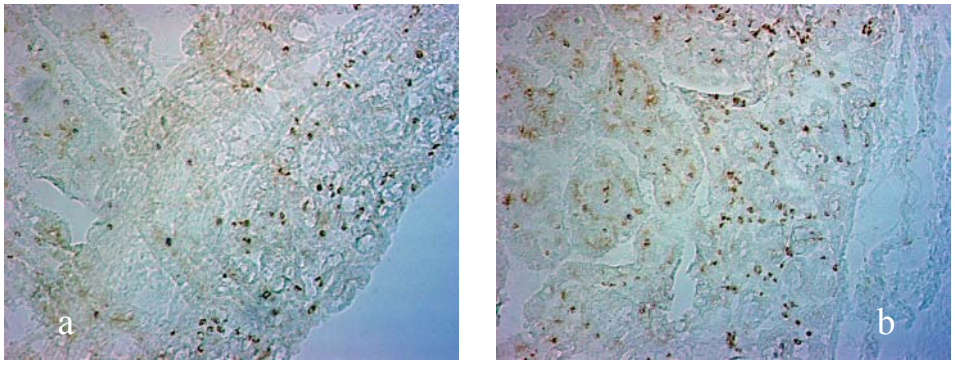

Fig. 2. Localization of $CD8^+$ T cells in villous LP, between Lc and in the submucosa of the jejunum of a 35-day-old pig (a) and a 49-day-old pig (b) as demonstrated by the indirect IP method; ×200.

On Day 42 and Day 49 of age these cells had similar distribution patterns but there were considerably more of them (+ 0.90 and + 0.92) compared to their numbers at Day 35, *i.e.* the age when they were first found in the tissue sections examined. $CD8^+$ T lymphocytes also appeared at Day 35 of age in similar, but slightly smaller numbers (3.13×10^{-5}) and had similar tissue distribution in the pigs at either 35 or 49 days of age (Fig. 2a,b). A smaller number of these cells was observed in the intestinal epithelium and under the basal membrane. Between days 42 and 49 of age these cells showed a substantial but not a continuing increase (+ 0.87 and + 0.75, respectively) compared to their numbers recorded at Day 35 (Table 2).

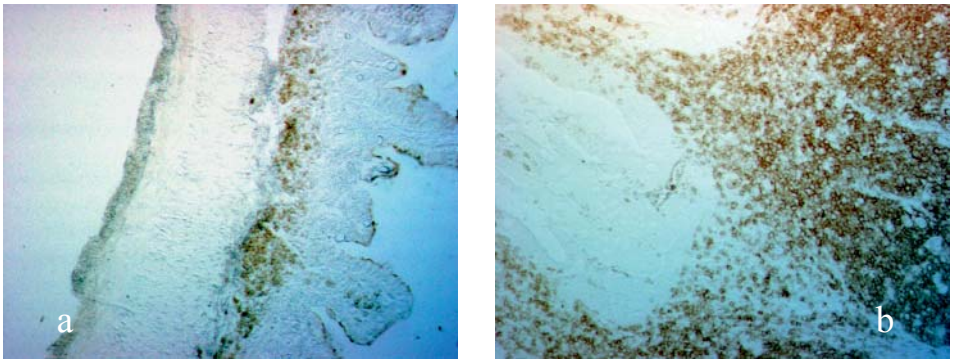

Fig. 3. Localization of jejunal CD45RA$^+$ naïve T cells either in the villous epithelium, LP and in Lc of a 0-day-old pig (a) or in the LP and Lc of a 21-day-old pig (b) as demonstrated by the indirect IP method; ×200.

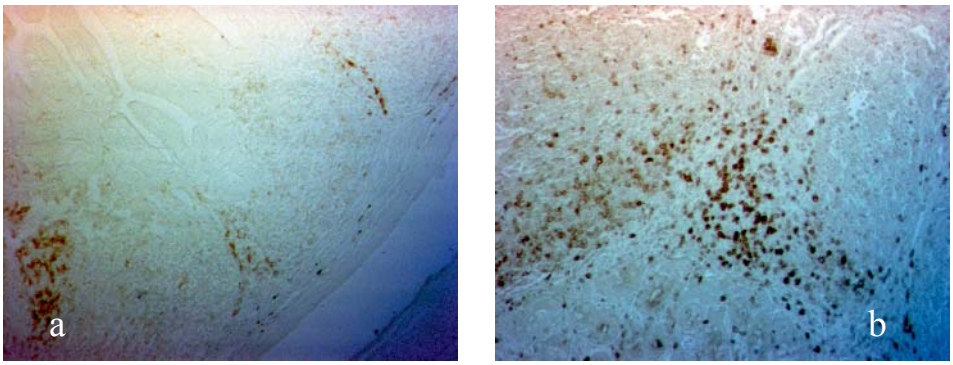

Fig. 4. Localization of jejunal CD45RC$^+$ memory T cells either in the Lc of a 7-day-old pig (a) or in the IFA of PP of a 42-day-old pig (b) as demonstrated by the indirect IP method; ×200.

Unlike the late appearance of the T cell subsets tested, numerous CD45RA$^+$ naïve lymphoid cells were observed within the villous epithelium, rarely interspersed in the LP and between the Lc of the newborn pigs at Day 0 of life (Fig. 3a). A rather large number of these cells (1.25×10^{-5}) were recorded in the jejunal mucosa of the neonates (Table 2).

Table 2. Histomorphometric values of porcine immune cell subsets residing jejunal mucosa of developing pigs; the results are expressed as the mean values of the number of cells per μm² of tissue section field as counted by DIA

Cell subset residing jejunal mucosa	Age group of pigs (days)[a]	Mean No. of the cells[b]	Index[c]	Increase/Decrease[d]
$CD45RA^+$	0	1.25×10^{-5}	1.00	-
	7	2.11×10^{-5}	1.73	+ 0.73
	14	3.36×10^{-5}	2.69	+ 1.69
	21	2.82×10^{-5}	2.26	+ 1.26
	28	6.04×10^{-5}	4.38	+ 3.38
	35	1.23×10^{-4}*	9.84	+ 8.84
	42	1.17×10^{-4}*	9.36	+ 8.36
	49	1.22×10^{-4}*	9.76	+ 8.76
$CD45RC^+$	0	/[e]		
	7	8.79×10^{-6}	1.00	-
	14	7.91×10^{-6}	0.90	- 0.10
	21	1.10×10^{-5}*	1.25	+ 0.25
	28	1.63×10^{-5}*	1.85	+ 0.85
	35	2.12×10^{-5}*	2.41	+ 1.41
	42	2.07×10^{-5}*	2.35	+ 1.35
	49	2.54×10^{-5}*	2.89	+ 1.89
$CD4^+$	0	/		
	7	/		
	14	/		
	21	/		
	28	/		
	35	3.62×10^{-5}	1.00	-
	42	6.87×10^{-5}	1.90	+ 0.90
	49	6.94×10^{-5}	1.92	+ 0.92
$CD8^+$	0	/		
	7	/		
	14	/		
	21	/		
	28	/		
	35	3.13×10^{-5}	1.00	-
	42	5.84×10^{-5}	1.87	+ 0.87
	49	5.47×10^{-5}	1.75	+ 0.75
IgA^+ plasma cells	0	/		
	7	/		
	14	7.65×10^{-6}	1.00	-
	21	1.24×10^{-5}*	1.62	+ 0.62
	28	1.18×10^{-5}*	1.54	+ 0.54
	35	2.71×10^{-5}*	3.54	+ 2.54
	42	3.02×10^{-5}*	3.95	+ 2.95
	49	2.85×10^{-5}*	3.73	+ 2.73

[a]Groups comprised 5 pigs each. [b]As counted in 12 randomly selected fields of the average area of 695 821 μm² per sample from 5 pigs per group. [c]Ratio between no. of the cells in the pigs at either Day 0 or on the day they were first observed (no. of the cells = 1.00 or 100%) and No. of the cells in the pigs of corresponding age group on consecutive days of the experiment. [d] Difference (+ or -) between index (no. of the cells) at either Day 0 or on the day they were first observed. [e] None of the cells tested were observed. *Significantly higher ($P<0.01$) than in the control pigs (euthanized at either Day 0 or on the day of firstat day of the first observation of the cell subsets tested); unmarked differences were not significant.

Between Day 7 and Day 49 of age their distribution patterns were similar to that observed at Day 0 of age, with the exception of their more pronounced frequency within the LP of villi at Day 21 of age (Fig. 3 b), when they were more numerous (+ 1.26) than in the newborns (Table 2). At Day 35 of age these cells reached the highest number (1.23×10^{-4}) and had increased (+ 8.84) statistically significantly ($P<0.01$) in comparison to their number at Day 0 of life (Table 2). Less frequent $CD45RC^+$ memory lymphoid cells were first observed at Day 7 of age, mainly in the Lc of the jejunum (Fig. 4 a), and their number (8.79×10^{-6}) was much lower than that first recorded for naïve lymphoid cells at Day 0 of age (Table 2). However, these cells increased earlier than the naïve lymphoid cells (at Day 21 vs. Day 35 of age), and continued to increase progressively, reaching significantly higher numbers ($P<0.01$) between Day 21 and Day 49 of age (Table 2). Between Day 14 and Day 35 of age their distribution resembled that observed at Day 7 of age, whereas at Day 42 of age these cells were visible in the jejunal LP, the epithelium of Lc , the IFA and inside the follicles of the PP (Fig. 4b). In 14-day-old pigs rarely scattered IgA^+ plasma cells were observed interspersed in the LP of the villi and localized around the Lc (Fig. 5 a), while in 21-day-old pigs (and in older age groups of pigs) a strong positive reaction to secretory IgA (sIgA) was visible in the lumen and the apical part of the Lc (Fig. 5 b). A very low number of sIgA-producing plasma cells (7.65×10^{-6}) was first recorded at Day 14 of age (Table 2). Soon (at Day 21) the number of these cells was found to have significantly increased ($P<0.01$), reaching the highest number (3.02×10^{-5}) and ratio of increase (+ 2.95) at Day 42 of age compared to their number at Day 14 of age (Table 2).

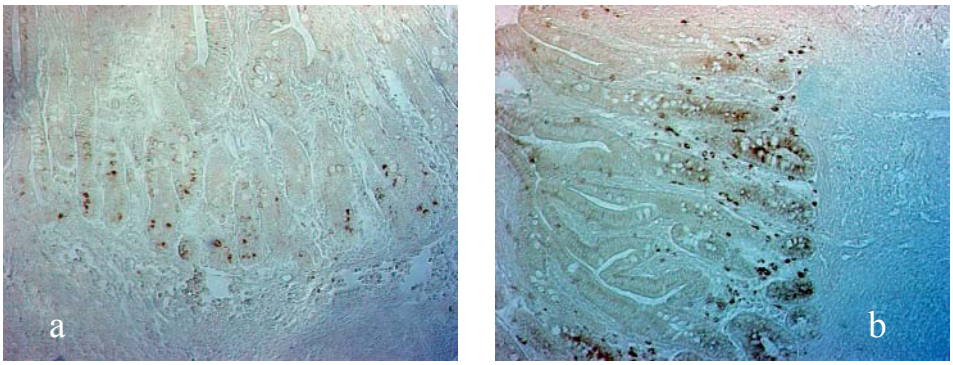

Fig. 5. Localization of IgA^+ plasma cells in villous LP and Lc of jejunum of a 14-day-old pig (a) and a 21-day-old pig (b) as demonstrated by the indirect IP method; ×200.

Discussion

Although much attention has been paid to swine as a model of human diseases and as a xenotransplant donor, undoubtedly the species also has great economic value as a commercially important source of food. However, health problems in swine kept under intensive conditions of rearing, particularly in young pigs, cause significant economic losses worldwide. Thus, immunological research in swine husbandry and nutrition is performed to develop safe and sustainable pork production.

In recent years, experimental interest has increased remarkably in the pig immune system, to address specific issues in veterinary and biomedical research and immunomodulation (ROTHKÖTTER et al., 2002; GALLOIS et al., 2009; ŠINKORA and BUTLER, 2009; BUTLER et al., 2009; JUUL-MADSEN et al., 2010; POTOČNJAK et al., 2012). The concept that the immune system consists of distinct subsystems is of particular importance for researchers in veterinary immunology, to focus on the porcine gut mucosal immune system due to its significance and complexity. In pigs, like in most animals, this system effectively controls the expression of active immune responses to enteric pathogens and tolerance of harmless dietary and commensal bacterial antigens (BAILEY et al., 2005a,b). Hence, the developmental aspects of the porcine intestinal mucosal system have gained increasing interest (STOKES et al., 2004; BROWN et al., 2006:; BAILEY and HAVERSON, 2006; BAILEY, 2009; INMAN et al, 2010; LEWIS et al., 2012) and indicated lines for future investigations.

This study extended existing knowledge about development and maturation of the porcine intestinal immune system by phenotypic characterization of immunologically dominant gut leukocyte subsets following their sequential appearance (at weekly intervals from Day 0 to Day 49 of life) and by numerical assessment of their age-related changes (during the first 7 weeks after birth) in the jejunal mucosa of healthy pigs reared under intensive farm conditions.

Since we did not find similar investigations, regarding the number of age groups of pigs used and numerical values obtained by DIA, we will only briefly, but critically compare our data with those of other authors that we presume to be comparable. Immediately after birth (at Day 0 of life) only a small number of $CD45RA^+$ cells is found within the jejunal LP of newborns, consistent with their immunologically naïve status. Partly these cells could be functionally immature DC, which are strongly MHC II^+ and co-express CD45 and CD16, along with other myeloid markers, and appear within the first week of life, as reported earlier (HAVERSON et al., 2000; LALLES et al., 2007), but we did not further characterize $CD45RA^+$ cells by the expression of myeloid markers. Since these cells progressively proliferated and were significantly increased at Day 35 vs. Day 0, we speculate that they may undergo functional maturation to the antigen-presenting DC.

According to our follow-up observations on a weekly basis this is the only subset of the immune cells residing the intestinal mucosa until Day 7 of age, which is in agreement with the finding of very few leukocytes in the LP in conventionally reared pigs, at birth (BIANCHI et al., 1992). It has been suggested that the porcine LP becomes populated with immune cells according to a clearly staged time frame (BAILEY et al., 2001). Consistent with that, we found a very low number of $CD45RC^+$ cells in 7-day-old pigs, suggesting that these cells may be less antigen-experienced, since the sucklings are well antigen-protected by maternally derived colostral/milk antibodies. However, consistent with their advanced memory status, a significantly higher number of these cells was recorded in 21-day-old pigs, as compared with that found at their appearance (Day 7 of age) and this trend of gradual increase continues until Day 49 of age.

During the second week of life, IgA^+ plasma cells appeared within the LP around the jejunal crypts, with significantly increasing numbers recorded from Day 21 to Day 49 of age, which is in accordance with the reported appearance of these cells in significant numbers as late as 3 to 6 weeks of life following the expansion of the B-cell repertoire between 2 to 4 weeks of life (ROTHKÖTTER et al., 1991; BAILEY et al., 2005b).

Our observation of the appearance of $CD4^+$ and $CD8^+$ T cells as late as 7 weeks of life contrasts with the reported finding of their increasing numbers between either 2 to 4 or 4 to 6 weeks of life, respectively (BIANCHI et al., 1992; BAILEY et al., 2001; LALLES et al., 2007; GALLOIS et al., 2009). Moreover, we recorded rather low numbers of $CD4^+$ and $CD8^+$ T cells at Day 35 after birth, and only a slight but not significant increase in their numbers between Day 35 and Day 49 of life. Such discrepancies from the abovementioned data from others on the kinetics of development and frequency of T cell subsets crucial for antigen-specific immunity within the gut mucosa, could be explained by the lack of standardization of methods applied and the heterogeneity of pig-rearing conditions, *e. g.* exposure to pathogens. Also, they could be ascribed to differences in breed, age, sex and antigenic stimulation of the experimental pigs (conventional or specific pathogen free), as well as to usually not stated environmental conditions in all studies.

Current research on the development/maturation of the porcine small intestinal mucosal immune system during the early (from Day 0 to Day 21 of life) and late (from Day 28 to Day 49 of life) postnatal period performed by qualitative (phenotypic/distribution patterns of lymphoid cell subsets tested) and quantitative (enumeration of targeted lymphoid cell subsets by DIA) immunohistological approaches has (i) provided valuable data on the kinetics of the development of the gut (jejunal) mucosal immune system, particularly of cellular components of the adaptive immunity, (ii) provided important details on the time of the appearance of the immune cell subsets tested and their age-related numerical changes, (iii) aided our understanding of the immune maturation of the local (intestinal) immunity, and (iv) provided opportunities to use young pigs to study the effects of manipulation of the mucosal immune system for optimal efficiency under specific environmental and husbandry conditions.

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SAŽETAK

Pomoću imunohistoloških i histomorfometrijskih analiza računalnom obradom slike istraživali smo utjecaj postnatalnog razvitka u svinje na distribucijske i kvantitativne pokazatelje subpopulacija djevičanskih/memorijskih limfoidnih stanica ($CD45RA^+$ odnosno $CD45RC^+$), pomoćničkih/citotoksičnih T-stanica ($CD4^+$ odnosno $CD8^+$), kao i za IgA^+ plazma stanice u limfatičkim tkivima probavnog sustava (LTPS) prasadi uzgajane u intenzivnim uvjetima tijekom prvih 7 tjedana života. Istraživanje postnatalnog razvitka/sazrijevanja imunosnog sustava sluznice tankoga crijeva svinje pokazalo je da je neonatalna prasad imunološki prilično nezrela, pa je stoga u potpunosti ovisna o pasivno stečenoj imunosti. U sluznici njihovog jejunuma gotovo potpuno nedostaju imunosne stanice, s izuzetkom malobrojnih $CD45\ RA^+$ limfoidnih stanica ($1{,}25\times10^{-5}$) 0. dana nakon prasenja. Sedmog dana *post partum* pojavljuje se nešto malo $CD45RC^+$ limfoidnih stanica ($8{,}79\times10^{-6}$), a potom se 14. dana mogu identificirati malobrojne IgA^+ plazma stanice ($7{,}65\times0^{-6}$). Ovi prvi znakovi postnatalnog razvitka urođene i stečene imunosti, temeljem identifikacije/lokalizacije subpopulacija istraživanih stanica koje naseljavaju LTPS svinje, odnosno sluznicu jejunuma, nastavljaju se nakon razdoblja od tri tjedna do pojavljivanja malobrojnih $CD4^+$ ($3{,}62\times10^{-5}$) i $CD8^+$ ($3{,}13\times10^{-5}$) T-stanica 35. dana nakon prasenja. Nakon ove faze ranog postnatalnog razvitka ključnih subpopulacija stanica koje sudjeluju u antigenski specifičnoj imunosti na sluzničkim površinama crijeva mlade prasadi, daljnji je napredak vidljiv u različitosti obrazaca njihove raspodjele u odjeljcima sluznice jejunuma i postupnom porastu njihove brojnosti, napose između 35. i 49. dana starosti. Tijekom tog razdoblja zabilježen je značajan porast brojnosti djevičanskih, memorijskih i plazma stanica, dok je brojnost pomoćničkih i citotoksičnih T-stanica samo blago porasla između 42. i 49. dana života.

Ključne riječi: postnatalni razvitak, imunosne stanice, sluznica crijeva, prasad