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Age-related changes in porcine humoral and cellular immune parameters

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ABSTRACT

The sequence of development of porcine systemic and local humoral and cellular immunity was analyzed by the age-dependent patterns of total serum immunoglobulin (Ig) levels, responsiveness of peripheral blood lymphocytes (PBL) to common mitogens (phytohemagglutinin, PHA; concanavalin A, ConA; poke-weed mitogen, PWM), or allogeneic PBL in a mixed lymphocyte culture (MLC), and the expression/distribution patterns of intestinal mucosal immune cell subsets, in order to establish the basic immune parameters for an assessment of immunocompetence in clinically normal pigs. Thus, we surveyed the species-related physiological values of these parameters in different age/technological categories, comprising infant (suckling and weaned pigs) and adult (gilts, sows, and boars) swine, kept in intensive rearing conditions. The highest level of total Igs was determined in neonatal pigs (43.5 ± 4.6 g/L), and this value was significantly higher ($P<0.001$) than that in weaned pigs (12.1 ± 1.5 g/L). Among adult swine, the highest level of total Igs was recorded in sows (32.0 ± 3.1 g/L), and this was much higher ($P<0.001$) than that found in gilts (25.1 ± 3.5 g/L) or boars (21.2 ± 5.0 g/L). The reactivity of PBL in neonatal pigs decreased by 60-70% compared to weaned pigs, regardless of

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the stimulator applied. In adult swine, the strongest reactivity of PBL, except to PHA, was determined in sows. Histomorphometric analyses showed that $CD45RA^+$, $CD45RC^+$ and IgA^+ cells in sections of the jejunum and ileum were significantly higher in weaned pigs ($P<0.05$) compared to neonatal pigs. The immune parameters obtained for a sample of the swine population in Croatia may serve as a basis for further research on porcine systemic and local (intestinal) immune responsiveness regarding (i) differences among breeds, (ii) the influence of paragenetic factors, (iii) the impact of microbial etiology diseases, particularly those accompanied by immunosuppression, and (iv)the validation of specific/nonspecific immunomodulation.

Key words: age-dependent, total immunoglobulin levels, lymphocyte reactivity, swine

Introduction

In recent years, the porcine immune system has been studied extensively in order to i) elucidate the role of immune cells and molecules during infectious diseases, (ii) understand basic immunological reactions and the immune function in transplantation, and (iii) generate a model for various aspects of human immunology. Consequently, experimental interest in the porcine immune system has increased remarkably (ROTHKÖTTER, 2009). The structure and functional anatomy of the lymphoid tissues/organs and the number/subset of lymphoid cells of the pig has been described earlier (BIANCHI et al., 1992; BINNS and PABST, 1994). In addition, several aspects of the porcine immune system, such as prenatal (ŠINKORA and BUTLER, 2009) and postnatal ontogeny (JUUL-MADSEN et al., 2010), including functional maturation of cellular immunity (BROWN et al., 2006), antibody repertoire development (BUTLER et al., 2009) and development of the mucosal immune system (BAILEY, 2009), have been analyzed extensively.

Health problems in suckling and weaned pigs cause significant economic losses worldwide. In postnatal pigs, changes in the activation of the immune system caused by antigenic stimuli, commensal microbiota, pathogens and environmental antigens result in the appearance of activated T and B cells in the peripheral lymphoid pool (BUTLER et al., 2006) and the influx and expansion of these cells in the mucosal immune system (BAILEY, 2009). In pigs, the mucosal immune system effectively controls the expression of active immune responses to pathogens and the tolerance of harmless antigens (BAILEY, 2009). The further development and maturation of adaptive immunity in suckling and weaned pigs, as a consequence of interaction with environmental antigens, results in their reaching adult immunocompetence at 7 to 9 weeks of age, as determined by the immune cell distribution in the small intestine (VEGA-LOPEZ et al., 1995), and by defining the role of the intestinal lamina propria as a mucosal effector site for perorally delivered vaccines (STOKES and BAILEY, 2000). However, in addition to the phenotypic characterization of porcine immune cells in developing pigs (LUNNEY and PESCOVITZ, 1987; BIANCHI et al., 1992; VALPOTIĆ and STOKES, 1994; BROWN et al., 2006), their functional maturation has also been studied during *in vitro* assays (VALPOTIĆ et al., 1989; HOSKINSON et al., 1990; BROWN et al., 2006).

Only a few studies concerning the development of porcine immune cell subsets in the lymphoid organs and immune molecules in the serum, monitored over several weeks or months, have been published (BIANCHI et al., 1992; WHRAY et al., 1995; SOLANO-AGUILAR et al., 2001; MARTIN et al., 2005). The age-related characteristics of mitogen-stimulated porcine lymphocytes have also been little studied (YANG and SCHULTZ, 1986; VALPOTIĆ et al., 1989; HOSKINSON et al., 1990; DORN et al., 2002). The aim of our study was to characterize age-dependent changes in (i) the levels of total serum immunoglobulins (Igs), (ii) the responsiveness of peripheral blood lymphocytes (PBL) to common mitogens and allogeneic cells, and (iii) the expression and distribution patterns of intestinal immune cell subsets, in order to establish basic immune parameters for an assessment of immunocompetence in clinically normal swine in different age/technological categories, kept under intensive rearing conditions.

Materials and methods

Pigs and experimental design. A total of seventy-four clinically normal pigs of both sexes, aged between 1 day (neonates) and 18 months (sows) from a commercial swine farm in Croatia were used (Table 1).

Table 1. Experimental groups of pigs used for analyses of systemic and local (intestinal) humoral and cellular immune parameters

Group of pigs	No.	Age	Sex	No. of pigs euthanized/ sampled ****
Neonates	16	1 day	♀ and ♂ ***	2
Weaners	16	28 days	Castrates and ♀***	2
Gilts*	16	6 months	♀	None
Sows**	16	18 months	♀	None
Boars	10	6 months	♂	None

*Non-pregnant gilts were used. **Multiparous sows were used before artificial insemination. ***Half the neonates/weaners were of either sex or females, respectively. ****Pigs were killed and sampled for immunohistology.

The animals were randomly selected and assigned to the appropriate age groups, housed separately and fed/managed according to the technology of intensive swine production. The suckling and weaned pigs, gilts and sows were crossbreds (Swedish Landrace × Yorkshire × Dutch Landrace), whereas the boars were purebreds of these breeds. Experimental and animal management procedures were conducted in accordance with the "Directive for the Protection of Vertebrate Animals used for Experimental and other Purposes" (86/609/EEC).

Sampling. Blood samples (ranging from 4 to 20 mL) were taken by *v. cava cranialis* puncture in syringes with or without heparine (10 U/mL). The samples with heparine

were intended for the isolation of PBL, while those without heparine were centrifuged and sera were further processed to determine total proteins and Igs. After blood sampling, two neonatal (1-day-old) and two weaned (28-day-old) pigs were euthanized by intracardial injections of T61 (Merck, Darmstadt, Germany) following anaesthesia with sodium pantabarbiturate. Immediately following euthanasia, samples of the jejunum and ileum were taken and fixed in 10% neutral-buffered formalin (pH 7.0-7.6) containing 4% formaldehyde, for 24 hours. The samples were then processed for immunohistology.

Determination of total serum proteins and albumin levels. The concentration of total serum proteins was determined by a standard "N" method, slightly modified biuret reaction, using a multichannel automated analyzer SMA 12/60 (Technicon, Tarrytown, New York, USA) for colorimetric measurement at a wavelength of 550 nm. Serum albumin was determined using the bromocrezol-green method (DOUMAS et al., 1971). The colour of the complex formed by the albumin and bromocrezol-green was measured at a wavelength of 630 nm using a colorimetric autoanalyser. The results were recorded as the concentrations of total proteins and albumin in serum and expressed as absolute values (g/L).

Determination of total Ig levels. In order to separate albumin from Igs and determine their absolute and relative concentrations in serum, we used strips of gelatinized cellulose acetate (cellogel), as detailed earlier (STATO and KASAI, 1965a,b), in the method of semimicroelectrophoresis (KOHN, 1968). Quantitative patterns of serum protein fractions were recorded by the densitometer Supercellomatic (Chemetron, Milan, Italy) at the wavelength of 520 nm and the results were expressed as absolute and relative percentages of concentrations of albumin, as well as of alpha, beta and gamma globulins.

Isolation of PBL and lymphocyte stimulation test (LST). LST was performed on isolated PBL from 10 to 16 pigs, depending on the group tested, in triplicate microcultures of each sample, as described earlier (VALPOTIĆ et al., 1989). The data obtained on counts per minute (cpm) values were expressed as the stimulation index (SI). The SI was calculated as mean cpm in stimulated cultures/mean cpm in control cultures.

Monoclonal antibodies. The monoclonal antibodies (mAbs) reactive with porcine leukocyte surface molecules *i.e.* cluster of differentiation (CD) antigens used to study *in situ* identification, distribution and quantification patterns of respective cell subsets are listed in Table 2.

Immunohistological staining and histomorphometry. Immunophenotypes of lymphoid and myeloid cell subsets within jejunal/ileal mucosa were demonstrated by the immunohistological peroxidase-antiperoxidase (PAP) method and quantitatively analyzed using the software programme Lucia G for digital image analysis, as detailed previously (JANJATOVIĆ et al., 2008; KOVŠCA-JANJATOVIĆ et al., 2010).

Table 2. mAbs specific for swine leukocyte CD antigens used in immunohistological demonstration of porcine intestinal immune cell subsets

Marker antigens	mAbs	Cells	Donor*
CD45RA	MIL13	Leukocytes, naive lymphoid cells	Haverson
CD45RC	MIL5	Leukocytes, memory lymphoid cells	Stokes
IgA	K61.1B4	Activated B cells, IgA^+ plasma cells	Haverson

*Kindly donated for research purposes and testing for Swine CD Workshops held in Davis, CA, USA (1995), Ludhiana, India (1998), and Amsterdam, Netherlands (1999)

Statistics. Data on systemic immune parameters (levels of serum Igs and PBL reactivity for age groups of pigs) and local (intestinal) histomorphometric findings (distribution/numbers of lymphoid/myeloid cell subsets for neonatal and weaned pigs) were processed using the programme Microsoft Excel Ver.5.0 (Microsoft Corporation, USA). The significance of differences in these parameters/findings between the age groups of the pigs tested was determined by the Student *t* test. Values of $P<0.05$ and lower were considered significant.

Results

Serum protein levels and humoral immune status of pigs. Age-related concentrations of porcine serum proteins and Igs (β- + γ-globulins) are shown in Fig. 1.

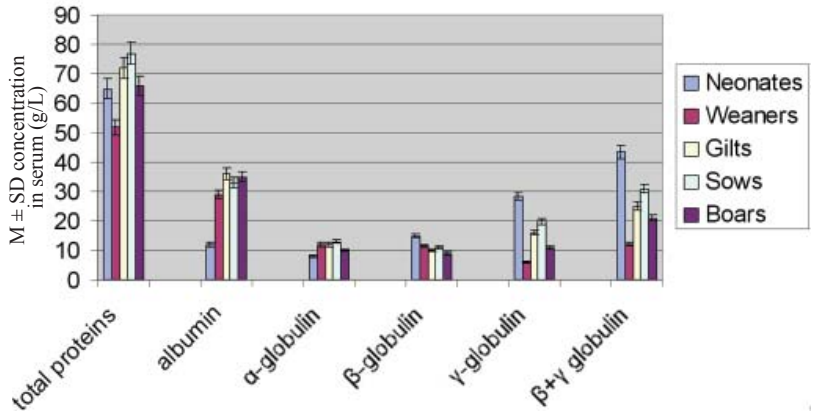

Fig. 1. Serum concentrations (M ± SD g/L) of total proteins, albumin, globulins (α- , β- and γ-globulins) and Igs (β- + γ-globulins) in different age/technological groups of pigs kept in intensive rearing conditions, for each group comprising either 10 boars or 16 animals in other categories

Since there is an intensive, massive intake of colostrum and milk proteins by neonatal pigs during the first hours of life, we recorded significantly higher levels of total serum proteins ($P<0.001$) in neonates (about 12 hours old) than in weaned pigs (28 days old)

(Fig. 1). The level of total proteins was also significantly higher ($P < 0.05$ or $P < 0.001$) in the sera of 18-month-old multiparous sows than in either 6- month-old gilts or boars, respectively.

Interestingly, the albumin level was the lowest in neonatal pigs and significantly differed ($P < 0.001$) from the values recorded in weaned pigs (Fig. 1). The gilts had a much higher concentration of albumin ($P < 0.01$) compared to sows, whereas levels in gilts *vs.* boars and sows *vs.* boars did not differ significantly. While the level of α-globulin was significantly lower ($P < 0.001$) in neonates compared to weaners, gilts and sows had significantly higher concentrations of this globulin fraction ($P < 0.5$ and $P < 0.01$, respectively) than boars (Fig. 1). Sows and gilts had similar values of α-globulin. The neonatal piglets had significantly higher levels of β-globulin ($P < 0.001$) compared to the values obtained for weaned pigs (Fig. 1). The level of β-globulin was found to be much higher ($P < 0.01$) in the serum of sows than in gilts or boars. There was no difference between the serum levels of β-globulin in gilts and boars. A significantly higher level of γ-globulin ($P < 0.001$) was detected in neonates *vs.* weaners (Fig. 1). The serum of sows had significantly higher values of γ-globulin ($P < 0.001$) compared to the sera of gilts and boars. Interestingly, gilts had much higher concentration of serum γ-globulin ($P < 0.01$) than boars of the same age.

Generally, levels of Igs (β- + γ-globulins) showed high variability among infant and adult swine (Fig. 1). The highest level of Igs was determined in neonatal pigs (43.5 ± 4.6 g/L), and this value was significantly higher ($P < 0.001$) than in weaned pigs (12.1 ± 1.5 g/L). The latter had the lowest level of serum Igs compared to the other age groups tested. Among adult animals, the highest level of Igs was recorded in sows (32.0 ± 3.1 g/L), and it was much higher ($P < 0.001$) than in gilts (25.1 ± 3.5 g/L) or boars (21.2 ± 5.0 g/L).

In vitro reactivity of PBL and cellular immune status of pigs. The age-related functional characteristics of porcine PBL as determined by LST are shown in Table 3.

Table 3. Age-related *in vitro* reactivity of porcine PBL to common mitogens (PHA, ConA or PWM) and allogeneic cells in MLC as validated by SI values*

	Stimulator			
Group (No. of pigs)	PHA (2 μg/mL)	ConA (10 μg/mL)	PWM (2.5 μg/mL)	MLC**
Neonates (N = 12)	25.4	11.0	29.5	5.8
Weaners (N = 14)	84.6	31.9	96.5	15.1
Gilts (N = 16)	312.3	27.5	87.7	13.0
Sows (N = 15)	240.8	38.2	90.2	22.0
Boars (N = 10)	195.1	24.5	62.1	11.9

*SI = Mean cpm in stimulated cultures/mean cpm in control cultures. **Mixing 5×10^4 responding cells with 10^5 stimulating allogeneic cells pretreated with Mitomycin C for 30 minutes at 37 ºC.

Since we wanted to assess the developmental aspects of specific and nonspecific cellular immunity, our original data expressed as cpm values were also presented as SI values. In this way, it was possible to reduce the considerable deviations obtained for nonstimulated control cultures which resulted due to differences in the ages of the pigs, their physiological and health status and experimental conditions, particularly measurements using a β-scintillation counter.

The PBL from neonatal pigs exhibited the lowest reactivity (Table 3). Such reactivity was found to be 60-70% lower than in weaned pigs, regardless of the stimulator applied (Table 3). Similarly, reactivity was much lower than that recorded in adult swine. It was 87-92% lower (when the stimulator was PHA), 55-61% (ConA), 52-67% (PWM), and 51-74% (MLC), respectively. Conversely, the reactivity of PBL in weaned pigs, with the exception of values which were 57-73% lower than in adult swine when PHA was the stimulator, was found to be slightly (10-16%) to moderately higher (27-55%) when the cells of gilts and boars were stimulated with PWM, ConA or allogeneic cells in MLC. Among adult swine, apart from when PHA was used as a stimulator, the strongest reactivity of PBL was recorded in sows. Surprisingly, cells from gilts responded 23% more to PHA stimulation than cells from sows.

However, when the data obtained by LST were expressed as cpm values, we observed a different relationship between the *in vitro* capacity of cellular immune responsiveness in the age/technological groups of the pigs tested (Fig. 2).

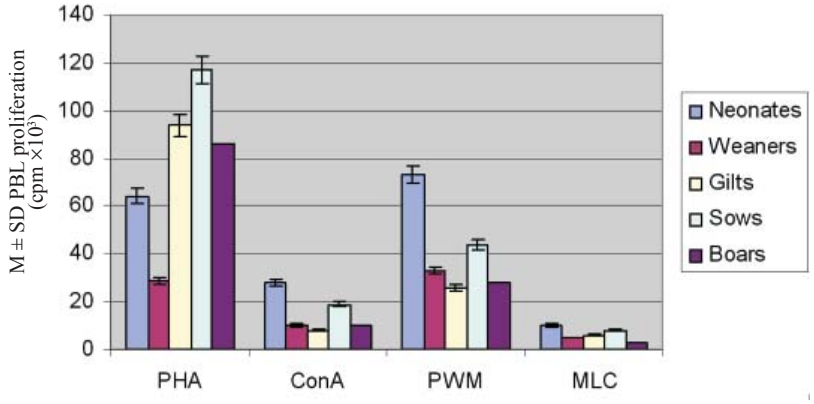

Fig. 2. *In vitro* reactivity of PBL (M ± SD cpm $\times 10^3$) from pigs of different ages/technological groups kept in intensive rearing conditions to common mitogens (PHA, ConA or PWM) and allogeneic cells in MLC; each group comprised either 10 boars or 16 animals of other categories

Namely, PBL from neonatal pigs exhibited the highest responsiveness to ConA, (28 ± 16 cpm $\times 10^3$), PWM (73 ± 22 cpm $\times 10^3$) or allogeneic cells in MLC (10 ± 4 cpm 10^3), whereas cells from sows exhibited the strongest response to PHA (117 ± 28 cpm10^3). The responses of cells from neonates to PWM were significantly different ($P<0.05$), with the exception of that recorded in sows (44 ± 8 cpm $\times 10^3$), than those from weaners, gilts and boars. The neonates also responded much better to allogeneic cells ($P<0.05$) than the boars. However, their response to ConA was not significantly different from the responsiveness of the other groups of pigs tested. The weaners responded to ConA, PWM or allogeneic cells similarly to the adults, but exhibited the lowest PHA-induced response (29 ± 23 cpm $\times 10^3$), which was significantly lower ($P<0.05$ or $P<0.01$) than those recorded in either gilts (94 ± 28 cpm $\times 10^3$), boars (86 ± 31 cpm 10^3) or sows (117 ± 28 cpm10^3), respectively. In adults, the strongest reactivity of PBL was observed in sows, regardless of the stimulator applied. However, these differences were not significantly different.

Identification and distribution of immune cells in the small intestinal mucosa. Age-related development of cellular ($CD45RA^+$ and $CD45RC^+$ lymphoid cells) and humoral (IgA^+ plasma cells) immunity was analyzed only in neonatal and weaned pigs (Figs 3, 4 and 5). Numerous $CD45RA^+$ naïve lymphoid cells were observed in the neonates. These cells were mostly located inside the ileal Peyer's patches and in the interfollicular areas (IFA), while fewer were found in the villous lamina proprial of the jejunum (Fig. 3a).

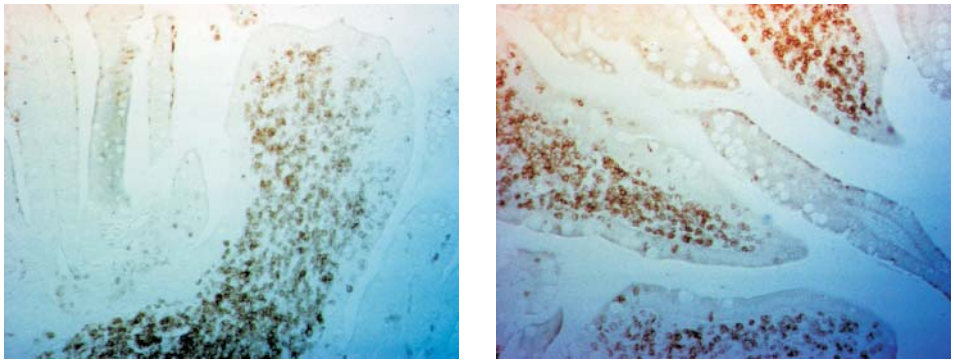

Fig. 3. Identification and distribution patterns of $CD45RA^+$ naïve lymphoid cells in the lamina propria of jejunal villi from neonatal (a) and weaned (b) pigs; PAP staining, magnification ×200

In weaners, these cells were found to be more frequent and their distribution was predominantly within the lamina propria of the jejunal villi (Fig. 3b). A small number of $CD45RC^+$ memory lymphoid cells were examined in neonates and they were mostly in the IFA of the ileal Peyer's patches (Fig. 4a). Their distribution in weaners was limited to the IFA (Fig. 4b).

The numbers of $CD45RA^+$, $CD45RC^+$ and IgA^+ cells in sections of porcine jejunum and ileum were determined by computer-assisted histomorphometry and are presented in Table 4. Quantitative immunophenotypic analyses showed that all three cell subsets studied were significantly more numerous in weaned pigs ($P<0.05$) compared to those recorded in neonatal pigs (Table 4).

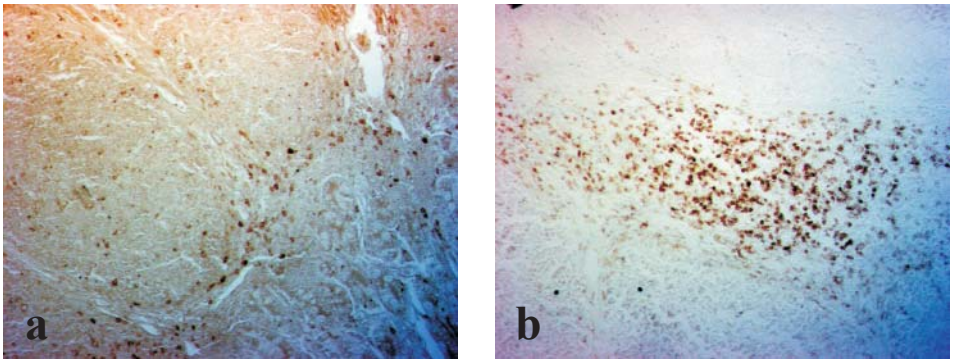

Fig. 4. Identification and distribution patterns of $CD45RC^+$ memory lymphoid cells in the IFA of the ileal Peyer's patches of neonatal (a) and weaned (b) pigs ; PAP staining, magnification ×200

IgA^+ plasma cells were rare in neonates, visible in small numbers around the crypt areas and in the villous lamina propria of the jejunum (Fig. 5a). These cells were even less abundant in the lamina propria of the jejunal villi of weaners. A strong reaction to secretory IgA was noticed in the lumen of crypts and in the apical portion of plasma cells within the crypts (Fig. 5b).

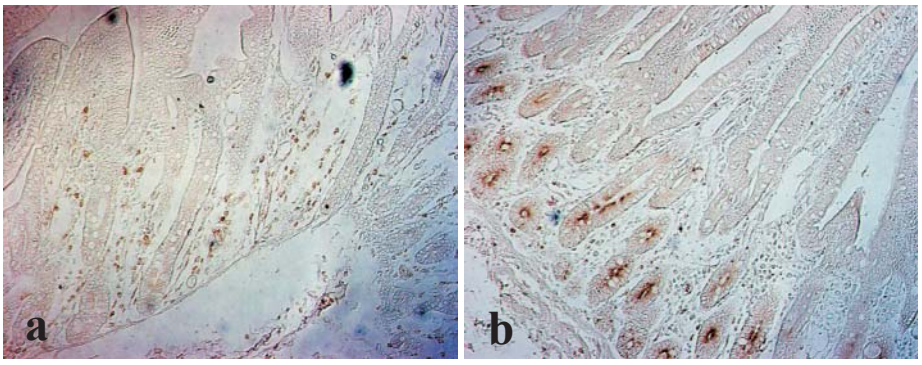

Fig. 5. Identification and distribution patterns of IgA^+ plasma cells in the lamina propria of the jejunal villi of neonatal pigs (a) and in the crypts of the jejunum in weaned (b) pigs; PAP staining, magnification ×200

Table 4. Histomorphometric values of porcine immune cell subsets in the small intestinal mucosa of young pigs; the results are expressed as mean values of the number of cells per μm^2 of tissue section field.

Group of pigs* (age)	Mean No. of immune cell subsets in the small intestinal mucosa**		
	$CD45\ RA^+$	$CD45RC^+$	IgA^+
Neonates (1 day)	1.71×10^{-5}	3.97×10^{-6}	4.05×10^{-6}
Weaners (28 days)	1.02×10^{-4}***	1.98×10^{-5}***	1.85×10^{-5}***

*Groups comprised 2 pigs each. **As counted in 12 randomly selected fields of an average area of 695821μm^2 per sample from 2 pigs per group. ***Significantly higher values at $P<0.05$ than in neonatal pigs.

Discussion

Our study on age-related changes in the humoral and cellular patterns of the porcine immune system will provide a basis for understanding how (particularly within the peripheral blood and small intestinal lamina propria) this system develops from birth to adulthood, in terms of both structural and functional maturation towards immunocompatence. Also, we examined potential differences in the molecular and cell variables of adoptive immunity in adult swine, in relation to their sex and physiological/reproductive status.

Using quantitative electrophoresis on cellulose acetate gels, we recovered two fractions of serum proteins, i.e. β- and γ-globulins actually comprising total serum Ig levels. The levels obtained in gilts, sows and boars ranging from 21-31 g/L (Fig. 1) were compatible with the range of values determined in many other studies of Ig levels in adult swine (18.5-39 g/L) described/compiled more recently (MARTIN et al., 2005; BUTLER et al., 2009; JUUL-MADSEN et al., 2010). Furthermore, the level of total serum Igs in gilts (25 g/L) was very similar to data obtained for adult swine (24.4 g/L or 25.4 g/L, respectively) by others (BUTLER, 1995; KORTBEEK-JAKOBS, 1981). The greatest differences were observed in the total Ig levels of neonatal (43.5 g/L) or weaned pigs (12.1 g/L) compared to values determined (neonates - 98.1 g/L and weaners - 29.1 g/L) earlier (KORTBEEK-JAKOBS, 1981). These differences may be ascribed to: (i) genetic variations in the quantitative expression of humoral immunity (*i. e.* Ig levels) among various breeds of swine (purebred Dutch Landrace *vs.* crossbreds used in our study), (ii) stress-induced immunosuppression in our pigs, which were kept in intensive rearing conditions, (iii) the small size of the sample (of 6 pigs) used by the aforementioned authors, whereby differences could be attributed to extreme individual values deviating substantially from physiological values, and (iv) due to differences in colostrum intake.

Generally, our results on mitogen-induced PBL reactivity in adult animals differed from those obtained by other authors, probably due to differences in breeds (unlike us, they used mostly purebred swine) and the age of the animals used (infant or adult), rather

than the concentrations of cells (10^5 vs 2×10^5 cells/mL)/mitogens (2 µg/mL or 10 µg/mL of PHA or ConA *vs.* 5 µg/well, respectively) applied (KORTBEEK-JAKOBS, 1981; YANG and SCHULTZ, 1986; LUNNEY and PESCOVITZ, 1987; HOSKINSON et al., 1990; BECKER and MISFELDT, 1993; BINNS and PABST, 1994; DORN et al., 2002; BROWN et al., 2006), or the variety of stimuli tested (KORTBEEK-JAKOBS, 1981; YANG and SCHULTZ, 1986; BRIM et al., 1995), but may also have been due to the different experimental procedures/conditions employed (LUNNEY and PESCOVITZ, 1987; BECKER and MISFELDT, 1993; BINNS and PABST, 1994; DORN et al., 2002). Whereas PHA as a T-cell mitogen induced a somewhat lower response in crossbred (gilts, sows) or purebred (boars) adults (ranging from 195.1-312.3), it is obvious that ConA (prevalently T-, but also B-cell mitogen) in the concentration applied stimulated PBL in the adult swine used in our study to a much lower extent (24.5-38.2) than in the purebred Dutch Landrace pigs (363.0 and 695.0, respectively) tested by KORTBEEK-JACOBS (1981). Also, much higher values were obtained for *in vitro* reactivity of PBL subsets from adult swine of the Durock breed (LUNNEY and PESCOVITZ, 1987). However, the PBL responses of crossbred neonatal and weaned pigs to PHA (25.4 and 84.6, respectively) or ConA (11.0 and 31.9, respectively) were also substantially lower than those determined in purebred (HOSKINSON et al., 1990; BECKER and MISFELDT, 1993) or crossbred pigs (VALPOTIĆ et al., 1989; VALPOTIĆ and STOKES, 1994) during the perinatal period. As expected, both groups of infant pigs had much lower T-cell responsiveness to PHA or ConA than adults, but the B-cell reactivity of weaners to PWM was slightly higher (96.5) than that of adult swine, which ranged from 62.1 (boars) to 90.2 (sows). Interestingly, purebred adult swine of the Dutch Landrace breed (KORTBEEK-JACOBS, 1981) had much lower responses to specific B-cell antigens of microbial origin (*E. coli* K88) or xenogeneic (goat) antibody to porcine IgM (2.7 or 12.0, respectively), than either the neonatal (29.5) or weaned (96.5) crossbreds used in our study. Accordingly, it seems likely that the B-cell function is established earlier in the postnatal period than the T-cell function. However, this is not altogether true if we consider that the T-cell responsiveness of weaned pigs (15.1) to allogeneic cells in MLC is not significantly different than that of gilts (13.0), sows (22.0) and boars (11.9).

As various subsets of immune cells have been demonstrated in many studies, on different sections of the intestinal mucosal immune system, originating from a number of purebred or hybrid swine of different ages/physiological status, and analyzed using a variety of monoclonal antibodies reactive with porcine CD/SWC molecules (BIANCHI et al., 1992; VALPOTIĆ and STOKES, 1994; VEGA-LOPEZ et al., 1995; WHRAY et al., 1995; SOLANO-AGUILAR et al., 2001; BROWN et al., 2006; BUTLER et al., 2009), we can only speculate on the developmental/quantitative peculiarities and distributional/migratory patterns of these cells, and on possible comparative similarities with our findings. However, we suggest that such *in situ* approaches, supplemented by numeric data obtained by morphometry, are the most relevant among nonfunctional immunological methods for

studying sequences of events in the ontogeny of intestinal immunity (as performed in the current study) and for the evaluation of innate or adoptive immunity and its exogenous modulation on the mucosal surfaces of the gut, as we described recently (JANJATOVIĆ et al., 2008; KOVŠCA-JANJATOVIĆ et al., 2010).

The immune parameters obtained for a sample of the swine population in Croatia may serve as a basis for further research on porcine systemic and local (intestinal) immune responsiveness, in relation to (i) differences among breeds or hybrids, (ii) differences in age, development and physiological status, *i. e.* technological categories of swine (iii) the influence of paragenetic factors, such as rearing technology/management, diet regimes, ambient and environmental stressors, (iv) the impact of microbial etiology diseases, particularly those accompanied by immunosuppression, and (v) the validation of specific/nonspecific immunomodulation and natural alternatives to in-feed antibiotic growth promoters.

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SAŽETAK

Slijed razvitka sustavne i lokalne humoralne i stanične imunosti u svinje analiziran je s pomoću dobno ovisnih promjena u razinama serumskih imunoglobulina (Ig), odgovorima limfocita periferne krvi (LPK) na uobičajene mitogene (fitohemaglutin, PHA; konkanavalin A, ConA; poke-weed mitogen, PWM) ili specifični antigen (alogenske LPK u miješanoj kulturi limfocita, MKL), te u obrascima ekspresije/distribucije subpopulacija imunosnih stanica u sluznici crijeva radi utvrđivanja temeljnih imunosnih pokazatelja za procjenu imunosne kompetencije u klinički normalnih svinja. Stoga smo istražili za vrstu specifične fiziološke vrijednosti

tih pokazatelja u različitih dobnih, odnosno tehnoloških kategorija, koje su uključivale mladu prasad (sisančad i odbijenike) i odrasle svinje (nazimice, krmače i nerastove) držane u uvjetima intenzivnog uzgoja. U neonatalne prasadi utvrđema je najviša razina ukupnih Ig (43,5 ± 4,6 g/L), i ta je vrijednost bila značajno viša (P<0,001) od one u odbijene prasadi (12,1 ± 1,5 g/L). U odraslih svinja, najviša razina ukupnih Ig zabilježena je u krmača (32,0 ± 3,1 g/L) i bila je mnogo viša (P<0,001) od onih utvrđenih u nazimica (25,1 ± 3,5 g/L) i nerastova (21,2 ± 5,0 g/L). Reaktivnost LPK u neonatalne prasadi bila je snižena za 60 - 70% u odnosu na onu zabilježenu u odbijene prasadi bez obzira na primijenjeni stimulator. U odraslih svinja, najjača je reaktivnost LPK, osim na PHA, utvrđena u krmača. Histomorfometrijske analize pokazale su da su CD45RA+, CD45RC+ i IgA+ stanice u dijelovima jejunuma i ileuma značajno brojnije u odbijene prasadi (P<0,05) u usporedbi s vrijednostima u neonatalne prasadi. Dobiveni imunosni pokazatelji na uzorku populacije svinja u Hrvatskoj mogu poslužiti kao temelj za dalja istraživanja njihovog sustavnog i lokalnog (crijevnog) imunosnog odgovora s obzirom na: (I) različitosti među pasminama, (II) utjecaje paragenetičkih činitelja, kao što su uvjeti uzgoja/držanja, način prehrane, te ambijentalni i okolišni stresori, (III) učinke bolesti mikrobne etiologije, posebice onih povezanih s imunosupresijom, kao i na (IV) vrednovanje specifične/nespecifične imunomodulacije.

Ključne riječi: dobno, razina imunoglobulina, reaktivnost limfocita, svinja