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## Immunophenotyping of dendritic cells of the residing porcine gut-associated lymphoid tissues

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### ABSTRACT

The aim of the study was to analyze the distribution patterns of porcine dendritic cells (DCs) of the residing mucosa of the ileum, caecum and colon, as well as mesenteric/colonic lymph nodes (MLN/CLN) of adult pigs. Three-color immunofluorescence (IF) was applied using monoclonal antibodies specific for porcine cell surface molecules (CD172, CD11R1 and CD16) on DCs. DCs were strategically localized within the lamina propria (LP) of the ileal villi, adjacent to the epithelial layer and around the crypts of the caecum/colon. Within the ileal LP, CD16<sup>+</sup> and CD11R1<sup>+</sup> cells were prevalent, whereas CD172<sup>+</sup> cells were much rarer. Conversely, in the caecum the latter cells were more prominent, the CD16<sup>+</sup> cells were also numerous, while CD11R1<sup>+</sup> cells were extremely rare. In the colonic mucosa the most frequent were CD16<sup>+</sup>. CD11R1<sup>+</sup> cells were very rare, whereas CD172<sup>+</sup> cells were almost absent. In the MLN, CD172<sup>+</sup> cells were prevalent within the follicular area, CD16<sup>+</sup> cells were rare and none of the CD11R1<sup>+</sup> cells were examined. Within the perifollicular area the most numerous were CD16<sup>+</sup> cells, CD11R1<sup>+</sup> cells were less prevalent, while CD172<sup>+</sup> cells were sporadic. In the CLN, CD16<sup>+</sup> cells were the most frequent in the perifollicular area, CD11R1<sup>+</sup> cells were less frequent and none of the CD172<sup>+</sup> cells were observed. Within the follicular area the most numerous were CD11R1<sup>+</sup> cells, CD172<sup>+</sup> cells were very rare and CD16<sup>+</sup> cells were absent.

**Key words:** dendritic cells, gut-associated lymphoid tissues, pig

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## Introduction

Populations of dendritic cells (DCs) can be found in all tissues, except the central nervous system, and they are particularly numerous at mucosal sites that are in contact with the external environment. Dendritic cells (DCs) are the most efficient antigen-presenting cells in the immune system. Upon recognition of pathogens, DCs become activated and migrate to lymphoid structures where they present relevant antigens to lymphocytes. The uptake of pathogens and the mechanisms leading to proper processing and presentation of relevant antigens are crucial to an immune response. Namely, in adult animals, the common characteristics of these cells are pleomorphic morphology and high expression of MHC class II molecules. Their number grows rapidly after birth, probably due to response to stimuli from upcoming antigens (BRANDTZAEG et al., 1992). It is believed that the DCs originating from precursor cells in the bone marrow (HOLT et al., 1994) multiply very quickly, especially in the intestinal mucosa (FOSSUM, 1989). This multiplying may further accelerate the acute inflammatory response to bacterial and viral infections or protein antigens (MacPHERSON et al., 1995; McWILLIAM et al., 1996). Changes in the expression of their functional surface markers then occur, which leads to changes in their activities accompanied by antigen entry and presentation of antigens. Today it is known that their functionality is associated with the stages of differentiation and maturation (STUMBLES et al., 1999). For example, immature or freshly isolated DCs consume large insoluble antigens (such as bacterial cells) by phagocytosis, and they are then processed and displayed to immune cells. On the sites of mucosa, endocytosis through pinocytosis is the main mechanism for intake of soluble and small insoluble antigens. These antigens are quickly degraded, processed and transported to the cell membrane, where they are displayed within MHC class II molecules. DCs are also very successful in presenting antigens to the MHC class I molecules and subsequent promotion of cytolytic T lymphocyte responses.

Accordingly, DCs as powerful antigen presenting cells (APC) in the mucosal and other tissues, are capable of activating and expanding naive, Ag-specific CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes (ALVAREZ et al., 2013). However, in most cases their further maturation leads to a fall in these capabilities and migration of DCs to afferent lymph nodes or the bloodstream. In fact, after exposure to antigens in the mucosa, DCs migrate to T-dependent areas of secondary lymph nodes to stimulate the primary immune response. In the intestinal mucosa DCs are close to M cells (specialized epithelial cells that are responsible for sampling of intraluminal antigens), where they intake antigens and migrate to the mesenteric lymph nodes (MLN) or PP (RUEDL and HUBELE, 1997). Although the promoters of the DCs migration (proinflammatory cytokines IL-1 and TNF or bacterial LPS) are well characterized, the molecular events associated with the turnover of these cells and their homing to regional lymph nodes or tissues are less well known. During

their maturation and migration to secondary lymph nodes, DCs are able to stimulate virgin T cell responses regulated with molecules of MHC class I and II, and are therefore called “natural” adjuvants of the immune system.

Given that the DCs and their functions have been well studied in laboratory rodents and humans, and somewhat less in the domestic pig (HAVERSON et al., 2000; BIMCZOK et al., 2005 and 2006), in this paper we will try to determine the presence and distribution of these cells in the mucosa of the distal small intestine (ileum), cecum and colon, as well as in MLN and colonic lymph nodes (CLN) of adult pigs, in order to prove their strategic distribution as APC.

### Materials and methods

*Experimental animals.* Archival samples stored by the Division of Veterinary Pathology, Infection and Immunity, Department of Clinical Veterinary Sciences, University of Bristol, UK, were used in this study. The archival samples were obtained from four conventionally farmed pigs of the New Hampshire breed, of both sexes, at the age of 6 months, weighing from 80 to 120 kg.

*Samples.* For preparation of archival samples, pigs were processed in a commercial slaughterhouse and samples were taken from distal part of the ileum, caecum and colon, as well as mesenteric/colonic lymph nodes (MLN/CLN). The samples were immediately imbedded in OCT fixation medium (Tissue-Tek; BDH, Lutterworth, UK), immersed in pantothenic acid and liquid nitrogen and stored at -85 °C.

*Monoclonal antibodies (mAbs) and conjugates.* Identification and localization of DCs were performed using mAbs specific for pig DCs (kindly donated for research purposes and testing by J. Lunney, K. Haverson and Y. Kim) and with conjugates for three-color immunofluorescence, as listed in Table 1.

Table 1. mAbs and conjugates

Clone	Specificity	Isotype	Conjugat*	Dilution	Source
SWC3	CD172	IgG2b	TRITC	1/5	J. Lunney
MIL4	CD11R1	IgG1	BIOT/AMCA	1/10	K. Haverson
G7	CD16	IgG1	FITC	5 µg/mL	Y. Kim

\*TRITC = tetrametil rodamin-5-izotiocijanat., BIOT/AMCA = biotin/streptavidin complex, IR = infra red, FITC = fluorescein izotiocijanat

*Three-color immunofluorescence histochemistry.* Frozen tissue samples were cut in cryostat at -25 °C into 5 µm thick sections, dried at room temperature for 24 hours, fixed in acetone for 15 minutes and stored at -85 °C. The following protocol for three-color

immunofluorescence histochemistry were applied: blocking of background staining was performed by incubating the tissue sections with 5% rabbit serum and 5% pig serum for 1 hour; the primary mAbs specific for CD172 and CD11R1 were applied to cover the sections and incubated for 4 hours; the slides were gently rinsed in 10% solution of PBS and the secondary antibodies IgG2b (TRITC), IgG1 (BIOT/AMCA), and IgG2a (IR) were added and incubated for 2 hours; the primary mAb specific for CD16 conjugated with FITC was added and incubated overnight in a humid chamber. Mouse serum was used for this step for blocking nonspecific reactions; the slides were gently rinsed with PBS solution and AMCA conjugate was applied and incubated for 1 hour; tissue sections were fixed with a coverslip and stored without access to light.

Tissue specimens were analysed using a fluorescence microscope with four filters (green, red, blue and IR) and a digital camera in the dark chamber. The intensity of expression to CD 172, CD11R1 and CD16 was measured in 4 randomly selected areas on two samples of the ileum, caecum and colon, as well as the mesenteric/colonic lymph nodes (MLN/CLN) from each pig. The graduation of antibody expression in specific tissue was determined as follows: - no expression; + weak expression; ++ medium expression; +++ strong expression.

## Results

*The distribution patterns and intensity expression of porcine DCs residing mucosa of the porcine small and large intestine.* Identification and localization of porcine DCs residing mucosa of ileum, caecum and colon of adult pigs are shown in Fig. 1-3. The differences in expression of particular markers in tested intestinal tissue samples with regard to the different tints of DCs is also noticeable. Thus, for example in the lamina propria of the ileal villi cells prevail that have a strong expression of CD16 (green) and medium expression of CD11R1 (blue) and CD172 (red) membrane markers (Fig. 1, Table 2). In contrast, around the crypts of the caecum DCs prevail that strongly express CD172 (red) membrane markers, while the expression of CD16<sup>+</sup> (green) and CD11R1 (blue) cells was weak (Fig. 2, Table 2). In the mucosa of the colon the most numerous were DCs that strongly express the CD16 membrane marker (green), while the expression of CD11R1<sup>+</sup> cells (blue) and CD172<sup>+</sup> cells (red) was weak (Fig. 3, Table 2).

Table 2. The intensity of expression to CD 172, CD11R1 and CD16 measured in samples of pig ileum, caecum, colon and mesenteric/colonic lymph nodes (MLN/CLN)

Tissue sample	Graduation of antibody expression in specific pig tissue*		
	anti-CD172 conjugated with TRITC	anti-CD16 conjugated with FITC	anti-CD11R1 conjugated with BIOT/AMCA
ileum	++	+++	++
caecum	+++	+	+
colon	+	+++	+
folicullar area of MLN	+++	+	-
perifolicullar area of MLN	++	+++	+
folicullar area of CLN	+	+	++
perifolicullar area of CLN	-	++	+

\* - no expression; + weak expression; ++ medium expression; +++ strong expression.

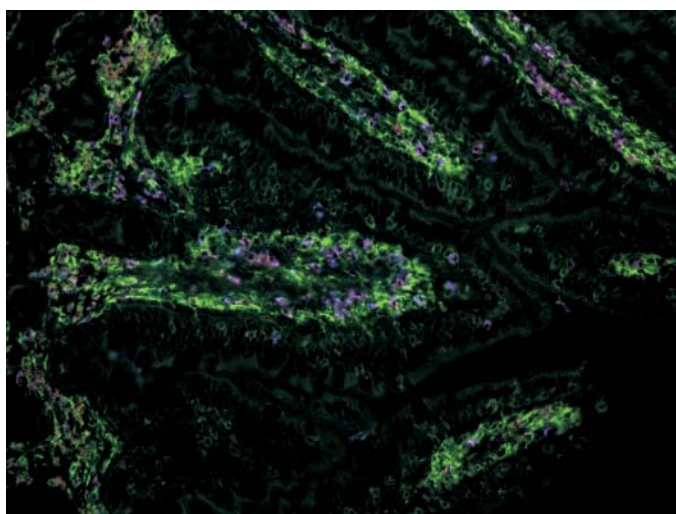


Fig. 1. The distribution patterns of DCs in mucosa of porcine ileum marked with mAbs and conjugates; (a) anti-CD172 conjugated with TRITC (red), (b) anti-CD16 conjugated with FITC (green) and (c) anti-CD11R1 conjugated with BIOT/AMCA (blue);  $\times 20$

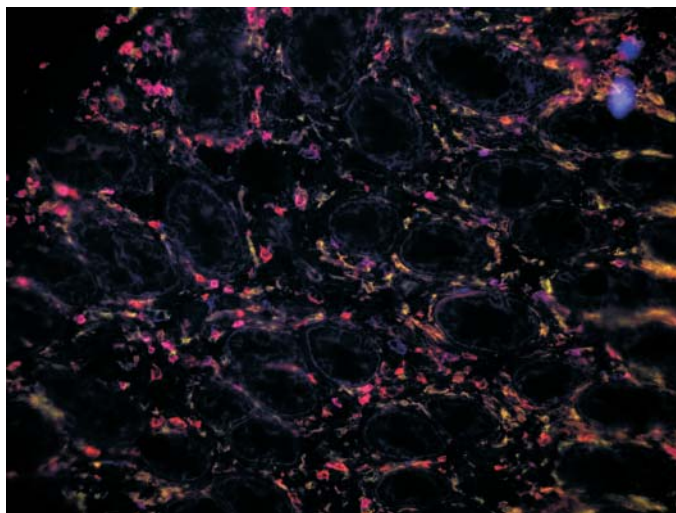


Fig. 2. The distribution patterns of DCs in mucosa of porcine caecum marked with mAbs and conjugates; (a) anti-CD172 conjugated with TRITC (red), (b) anti-CD16 conjugated with FITC (green) and (c) anti-CD11R1 conjugated with BIOT/AMCA (blue);  $\times 20$

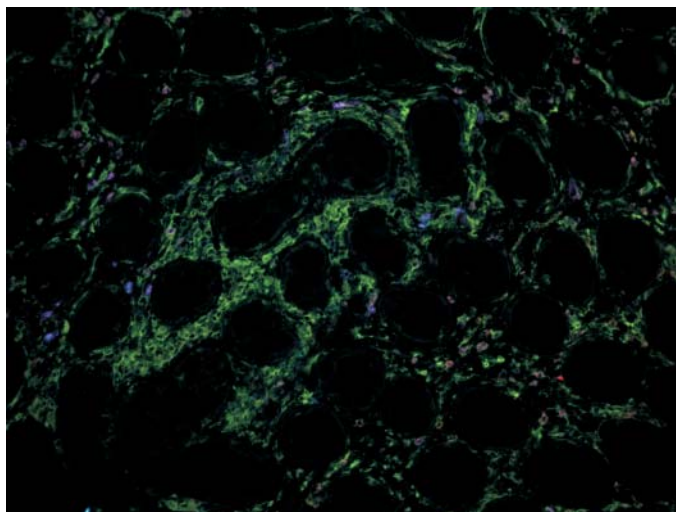


Fig. 3. The distribution patterns of DCs in mucosa of porcine colon marked with mAbs and conjugates; (a) anti-CD172 conjugated with TRITC (red), (b) anti-CD16 conjugated with FITC (green) and (c) anti-CD11R1 conjugated with BIOT/AMCA (blue);  $\times 20$

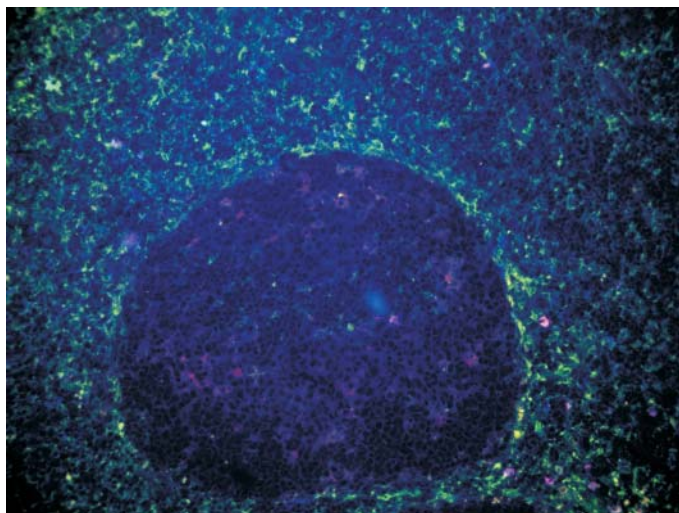


Fig. 4. The distribution patterns of DCs inside and out of the follicles of porcine MLN marked with mAbs and conjugates; (a) anti-CD172 conjugated with TRITC (red), (b) anti-CD16 conjugated with FITC (green) and (c) anti-CD11R1 conjugated with BIOT/AMCA (blue);  $\times 20$

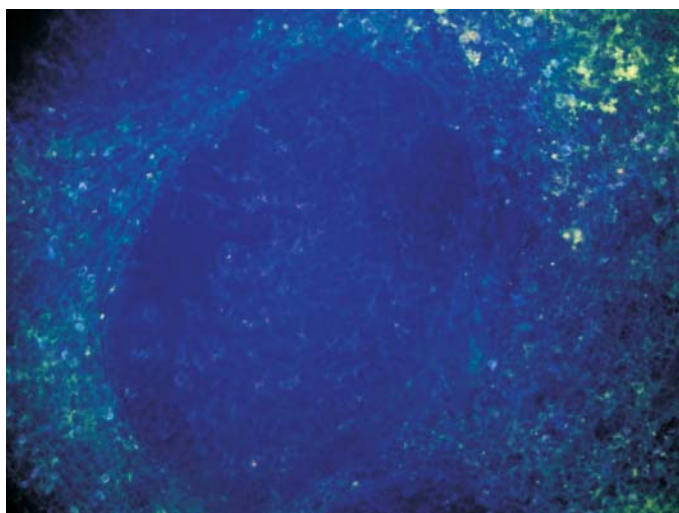


Fig. 5. The distribution patterns of DCs marked with mAbs and conjugates inside and out of the follicles of porcine CLN; (a) anti-CD172 conjugated with TRITC (red), (b) anti-CD16 conjugated with FITC (green) and (c) anti-CD11R1 conjugated with BIOT/AMCA (blue);  $\times 20$

*The distribution patterns and intensity expression of porcine DCs residing in the mesenteric/colonic lymph nodes.* Identification, localization and intensity expression of DCs residing in the regional lymph nodes of adult pigs are shown in Fig. 4 and 5 and Table 2. In follicles of the MLN, the number of DCs are smaller than in the perifollicular area (Fig. 4). Differences in expression of particular DCs membrane markers inside or outside the follicle are also noticeable. While inside the follicle CD172<sup>+</sup> cells (red) are predominant (strong expression), CD16<sup>+</sup> cells (green) are very rare (weak expression), while CD11R1<sup>+</sup> cells (blue) are absent (no expression). In contrast, in the perifollicular area of the MLN, cells that express the CD16 membrane marker (green) are the most numerous (strong expression), CD11R1<sup>+</sup> cells (blue) are less common (medium expression), while CD172<sup>+</sup> cells (red) are sporadic (weak expression). The distribution patterns and intensity expression of DCs in follicles and the perifollicular area of the CLN are shown in Fig. 5 and Table 2. The number of cells that express the CD16 membrane marker (green) is highest in the perifollicular area (medium expression) and CD11R1<sup>+</sup> cells (blue) are much less common (weak expression), while CD172<sup>+</sup> cells (red) are not found in this area (no expression). Inside the follicles the most numerous are CD11R1<sup>+</sup> cells (blue) (medium expression), while CD172<sup>+</sup> cells (red) and CD16<sup>+</sup> cells (green) are almost absent (weak expression).

### Discussion

With three-color IF applied using mAbs specific for membrane markers of porcine DCs CD172, CD11R1 and CD16, it is possible to determine phenotypic and distributional patterns of DCs in tested gut-associated lymphoid tissues of adult pigs. Our data are in accordance with previous findings of numerous APC present in LP of adult pig. Namely, DCs that strongly express MHC II<sup>+</sup>, co-expressing CD45 and CD16 markers and other myeloid markers, appear in the first week of a piglet's life (WILSON et al., 1996). At this time these cells also express CD14 marker, which is not present in older pigs, suggesting the possibility that some of these DCs originate from circulating monocytes (BIANCHI et al., 1992). Porcine intestinal DCs are located just below the epithelial cells and can be visualized so that their extensions in the epithelial layer are seen (BIMCZOK et al., 2006). The considerable heterogeneity of their phenotypes is described, but it has not yet been linked to their functional diversity. In our investigation we also noticed the differences in expression of single specific markers on DCs in tested samples of intestinal tissue and regional lymph nodes. For example, in the lamina propria of the ileal villi CD16<sup>+</sup> DCs prevail, while CD11R1<sup>+</sup> and CD172<sup>+</sup> cells are moderately distributed. Around the crypts of caecum CD172<sup>+</sup> are much more numerous, while CD16<sup>+</sup> and CD11R1<sup>+</sup> cells are very rare. In mucosa of colon the most numerous are CD16<sup>+</sup> DCs, CD11R1<sup>+</sup> cells are rare, while CD172<sup>+</sup> cells are almost absent.



It is believed that there are several problems related to the determination of porcine intestinal DC function. First, porcine endothelial cells probably participate in early responses because they strongly express molecules of MHC class II (WILSON et al., 1996) and are capable of secretion of anti-inflammatory cytokines. Second, anti-CD11 mAb, which is usually used in rodents and human, does not react with the same subpopulation of cells in pigs. Therefore, the mAbs specific for these marker in pigs are noted as CD11R1, CD11R2 and CD11R3, and match the mAbs CD11a, CD11b and CD11c specific for these markers in rodents and humans (BAILEY, 2009). Using these mAbs BIMCZOK et al. (2005) determined by immunohistology, a subpopulation of cells in porcine intestines that express MHC class II molecules. These cells are classified in four subpopulations based on the difference in expression and predominance of CD11R1 and CD172 markers. Thus, CD11R1<sup>+</sup> CD172<sup>+</sup> cells are predominant cells in the intestinal villi, CD11R1<sup>-</sup>CD172<sup>+</sup> cells prevail in Payers patches (PP), and CD11R1<sup>+</sup> CD172<sup>-</sup> cells in the regional lymph stream and MLN. However, our results obtained in the villi of the ileum is different, because in the ileum CD16<sup>+</sup> cells prevail, but not CD172<sup>+</sup> and CD11R1<sup>+</sup> cells. Also, in the perifollicular area of the MLN, we found many CD16<sup>+</sup> cells, while CD172<sup>+</sup> were moderately distributed and CD11R1<sup>+</sup> were sporadic. Besides the three mentioned subpopulations of DCs, a fourth was discovered (outside PP) which expresses CD172 and CD16 markers, and has a strong ability to stimulate primary T-cell immune response (HAVERSON et al., 2000), but the relationship between these populations and those expressing CD11R1 has not been determined.

Recently, it was found that the rearing environment affects the development, maturation and activities of the intestinal immune system of piglets (BAILEY et al., 2005), including its individual cell components, such as DCs (INMAN et al., 2010). DCs rapidly accumulate in the intestinal mucosa of piglets kept separately from sows in highly hygienic conditions. It is interesting that in young piglets (2-5 days old) kept in normal farm conditions with the sow, accumulation of DCs starts earlier than in older pigs (12-28 days old) kept in the same conditions (INMAN et al., 2010). Moreover, commensal strains of *E. coli* cause the on extensive multiplication of DCs in the LP (HAVERSON et al., 2007). As a result, it is likely that the differences in the expression of surface markers on DCs, as well as differences in individual subpopulations of DCs in the tested gut-associated lymphoid tissues of adult pigs with regard to this expression, indicate their diversity in the stages of maturation and/or activities, and perhaps even in patterns of migration from the mucosa of the intestine to regional lymph nodes (MLN/CLN).

In fact, our data show that the number of DCs in follicles of MLN is lower than those in the perifollicular area. In contrast, the number of DCs of CLN is lower in the follicular area. As in the intestinal mucosa, there are differences in the expression of DC membrane markers within and outside the follicle. While in the follicle of MLN

CD172<sup>+</sup> cells are prevalent, CD16<sup>+</sup> cells are very rare, and CD11R1<sup>+</sup> cells are absent. The situation in the follicle of CLN is quite different, so here the most numerous are CD11R1<sup>+</sup> cells, whereas CD172<sup>+</sup> cells are extremely rare, and CD16<sup>+</sup> cells are almost absent. In the perifollicular area of the MLN and CLN, CD16<sup>+</sup> DCs are the most numerous, CD11R1<sup>+</sup> cells are significantly reduced in number. CD172<sup>+</sup> cells are sporadic in the perifollicular area of the MLN but these cells were not found in the perifollicular area of the CLN.

Given that in the available literature we did not find any data on quantitative differences in subpopulations of DCs located within or outside the follicles of the regional lymph nodes of pigs, we could not compare our results or draw conclusions about their relevance. However, based on the above-mentioned data of other authors (BAILEY et al., 2005; HAVERSON et al., 2007; SUMMERFIELD and McCULLOUGH, 2009; INMAN et al., 2010), as well as our data, we assume that they could be the result of a dynamic cellular metabolism of DCs, that in the tested tissues of adult pigs from commercial farms they undergo different stages of maturation, exhibiting different activities, such as entry and processing of intraluminal antigen, and migration from the LP to the regional lymph nodes after contact with these antigens. A variety of substances, whether of synthetic or natural origin can restore or stimulate nonspecific and specific immunity in domestic animals, particularly in pigs, and, hence, act as immune response modifiers (IRMs) and/or adjuvants (VALPOTIĆ et al., 2013). Further experimental investigations are needed to estimate if natural non-AGP (antibiotic growth promoters) substances are able to modulate distribution patterns of porcine dendritic cells (DCs).

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#### References

- ALVAREZ, B., T. PODEROSO, F. ALONSO, A. EZQUERRA, J. DOMÍNGUEZ, C. REVILLA (2013): Antigen targeting to APC: from mice to veterinary species. *Dev. Comp. Immunol.* 41, 153-163.
- BAILEY, M. (2009): The mucosal immune system: Recent developments and further directions in the pig. *Develop. Comp. Immunol.* 33, 375-383.
- BAILEY, M., K. HAVERSON, C. INMAN, C. HARRIS, P. JONES, G. CORFIELD, B. MILLER, C. STOKES (2005): The influence of environment on development of the mucosal immune system. *Vet. Immunol. Immunopathol.* 108, 189-198.

- BIANCHI, A. T. J., R. J. ZWART, S. H. M. JEURISSEN, H. W. M. MOONEN-LEUSEN(1992): Development of the B-cell and T-cell compartments in porcine lymphoid organs from birth to adult life - an immunohistological approach. *Vet. Immunol. Immunopathol.* 33, 201-221.
- BIMCZOK, D., H. J. ROTHKÖTTER (2006): Lymphocyte migration studies. *Vet. Res.* 37, 325-328.
- BIMCZOK, D., E. N. SOWA, H. FABER-ZUSCHRATTER, R. PABST, H. J. ROTHKÖTTER(2005): Site specific expression of CD11b and SIRP alpha (CD172a) on dendritic cells: implications for their migration patterns in the gut immune system. *Eur. J. Immunol.* 35, 1418-1427.
- BIMCZOK, D., A. POST, T. TSCHERNIG, H. J. ROTHKÖTTER (2006): Phenotype and distribution of dendritic cells in the porcine small intestinal and tracheal mucosa and their spatial relationship to epithelial cells. *Cell Tissue Res.* 325, 461-468.
- BRANDTZAEG, P., T. S. HALSTENSEN, H. S. HUITFELDT, P. KREJCI, D. KVALE, H. SCOTT, P. S. THRANE (1992): Epithelial expression of HLA, secretory component (poly-Ig receptor), and adhesion molecules in the human alimentary tract. *Ann. N. Y. Acad. Sci.* 664, 157-179.
- FOSSUM, S. (1989): Dendritic leukocytes: features of their *in vivo* physiology. *Res. Immunol.* 140, 883-891.
- HAVERSON, K., S. SINGHA, C. R. STOKES, M. BAILEY (2000): Professional and non-professional antigen-presenting cells in the porcine small intestine. *Immunology* 101, 492-500.
- HAVERSON, K., Z. REHAKOVA, J. SINKORA, L. ŠVER, M. BAILEY (2007): Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: A study in germ-free pigs. *Vet. Immunol. Immunopathol.* 119, 243-253.
- HOLT, P. G., S. HAINING, D. J. NELSON, J. D. SEDGWICK (1994): Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airway. *J. Immunol.* 153, 256-261.
- INMAN, C., K. HAVERSON, S. R. KONSTANTINOV, P. H. JONES, C. HARRIS, H. SMIDT, B. MILLER, M. BAILEY, C. STOKES (2010): Rearing environment affects development of the immune system in neonates. *Clin. Exp. Immunol.* 160, 431-439.
- MacPHERSON, G. G., C. D. JENKINS, M. J. STAIN, C. EDWARDS (1995): Endotoxin-mediated dendritic cell release from the intestine. Characterization of released dendritic cells and TNF dependence. *J. Immunol.* 154, 1317-1322.
- McWILLIAM, A. S., S. NAPOLI, A. M. MARSH, F. L. PEMPER, D. J. NELSON, C. L. PIMM, P. A. STUMBLES, T. N. C. WELLS, P. G. HOLT (1996): Dendritic cell are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J. Exp. Med.* 184, 2429-2432.
- RUEDL, C., S. HUBELE (1997): Maturation of Peyer's patch dendritic cells *in vitro* upon stimulation via cytokines or CD40 triggering. *Eur. J. Immunol.* 27, 1325-1330.
- STUMBLES, P. A., A. S. McWILLIAM, P. G. HOLT (1999): Dendritic cells and mucosal macrophages. In: *Mucosal Immunology* (Ogra, P. L., J. Mestecky, M. C. Lamm, W. Strober, J. Bienenstock, J. R. Mcghee, Eds.), 2<sup>nd</sup> ed., Academic Press, San Diego, CA, SAD, 397- 412.

- SUMMERFIELD, A., K. C. McCULLOUGH (2009): The porcine dendritic cell family. *Dev. Comp. Immunol.* 33, 299-309.
- VALPOTIĆ, H., G. MRŠIĆ, B. GRŠKOVIĆ, D. ŠPOLJARIĆ, D. KEZIĆ, S. SREČEC, M. MATAUŠIĆ-PIŠL, G. LACKOVIĆ, D. CAPAK, D. MIHELIC, K. VLAHOVIĆ, I. VALPOTIĆ, A. PIRKIĆ, D. ANĐELINOVIC, M. POPOVIĆ (2013): Effect of polyoxyethylene and polyoxypropylene nonionic block copolymers on performance and recruitment of immune cell subsets in weaned pigs. *Acta Vet. Scand.* 55, 54-63.
- WILSON, A. D., K. HAVERSON, K. SOUTHGATE, P. W. BLAND, C. R. STOKES, M. BAILEY (1996): Expression of major histocompatibility complex class II antigens on normal porcine intestinal endothelium. *Immunology* 88, 98-103.

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**SAŽETAK**

Cilj istraživanja bio je odrediti razmještaj svinjskih dendritičnih stanica (DS) u sluznici ileuma, cekuma i kolona te u mezenterijskim limfnim čvorovima (MLČ) i limfnim čvorovima kolona (LČK) odraslih svinja. Trobojna imunofluorescencijska (IF) pretraga korištena je uz upotrebu monoklonskih protutijela specifičnih za svinjske površinske molekule (CD172, CD11R1, MHC II i CD16) na dendritične stanice. DS su strateški lokalizirane unutar lamine proprije (LP) crijevnih resica, neposredno uz epitelni sloj te uokolo kripte cekuma i kolona. Unutar LP ileuma prevladavale su CD16<sup>+</sup> i CD11R1<sup>+</sup> stanice, dok su CD172<sup>+</sup> stanice bile mnogo rijede. Nasuprot tome, u cekumu je bila izraženija prisutnost CD172<sup>+</sup> stanica, dok su CD16<sup>+</sup> stanice bile također brojne, a CD11R1<sup>+</sup> stanice izrazito rijetke. U mukozi kolona najčešće su bile CD16<sup>+</sup> stanice, CD11R1<sup>+</sup> stanice su bile vrlo rijetke, dok su CD172<sup>+</sup> stanice bile gotovo odsutne. U MLČ su CD172<sup>+</sup> stanice prevladavale unutar područja folikula, CD16<sup>+</sup> stanice su bile rijetke, a nije zapažena niti jedna CD11R1<sup>+</sup> stanica. Unutar perifolikularnog područja najbrojnije su bile CD16<sup>+</sup> stanice, CD11R1<sup>+</sup> stanice bile su mnogo manje zastupljene, dok su CD172<sup>+</sup> stanice bile sporadične. U LČK, CD16<sup>+</sup> stanice su bile najčešće u perifolikularnom području, CD11R1<sup>+</sup> stanice su bile mnogo manje zastupljene, a nije primjećena niti jedna CD172<sup>+</sup> stanica. Unutar područja folikula najbrojnije su bile CD11R1<sup>+</sup> stanice, CD172<sup>+</sup> stanice su bile vrlo rijetke, a CD16<sup>+</sup> stanice nisu primijećene.

**Ključne riječi:** dendritične stanice, limfno tkivo, probavni sustav, svinja

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