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Characterization and expression of DEC205 in the cDC1 and cDC2 subsets of porcine dendritic cells from spleen, tonsil, and submaxillary and mesenteric lymph nodes

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ABSTRACT

Conventional dendritic cells (cDCs) are divided into the following different subtypes: cDC1, which promotes a Th1 response, and cDC2, which stimulates a Th2 and Th17 response. These cells have not been characterized in porcine lymphoid tissues. DEC205 is a receptor that increases antigen presentation and allows DCs to cross-present antigens. The objectives of this work were to characterize cDCs subsets in the tonsil, submaxillary and mesenteric lymph nodes and spleen lymphoid tissues and to determine their expression of DEC205 by flow cytometry. The cDC1 (MHCII^{high}CADM1^{high}CD172a$^{-/low}$) and cDC2 (MHCII^{high}CADM1^{high}CD172a^{+}) phenotypes were confirmed by the expression of characteristic cDC1 and cDC2 transcripts (FLT3, XCR1 and FCER1α). Among all lymphoid tissues, the spleen had the highest frequency of total cDCs. The cDC1:cDC2 ratio showed that all lymph tissues had higher levels of cDC1 than levels of cDC2. DEC205^{+} cDCs were found in all analyzed tissues, albeit with different frequencies. Our research will facilitate the study on the function of these cells and the investigation of the strategies for DEC205 targeting and functional studies.

1. Introduction

Dendritic cells (DCs) are distinguished from other antigen-presenting cells by their sentinel role in both non-lymphoid and lymphoid tissues as well as their ability to migrate to the T cell-rich areas of lymph nodes (Steinman, 1991). DCs serve as the link between the innate and adaptive immune response by driving naïve lymphocytes into distinct classes of effector cells to initiate T cell-mediated immunity (Steinman, 1991; Mellman and Steinman, 2001; Vega-Ramos et al., 2014). There are two major DC populations: plasmacytoid DCs (pDCs) and conventional or classical DCs (cDCs). cDCs are divided into two different subtypes or subsets: cDC1 and cDC2 (Schlitzer et al., 2015a). Cross-presentation of antigens to CD8 T cells and the Th1 differentiation of these cells characterize the cDC1 cells, whereas the cDC2 subtype is specialized to activate CD4 T cells and promote Th2 and Th17 differentiation (Schlitzer and Ginhoux, 2014; Schlitzer et al., 2015b).

The cDC1 and cDC2 subtypes have been characterized in various species, and several studies have evaluated markers that facilitate the study of these cells, especially in the context of research that uses animal models for human diseases (Summerfield et al., 2015; Dutertre et al., 2014; Guilliams et al., 2016). Guilliams et al. (2016) proposed various cell markers that are common between mice, human and macaque to identify the cDC1 and cDC2 subtypes in different tissues. Specifically, the cDC1 subtype is identified as XCR1^{hi}CADM1^{hi}CD172a^{-}IRF8^{hi}IRF4^{lo}, and the cDC2 subtype as XCR1^{-}CD172a^{hi}IRF8^{lo}IRF4^{hi} (Guilliams et al., 2016). Swine are an important animal model for human research due to their physiologic, anatomic and immunologic similarity. In the field of DCs, Marquet et al. (2011, 2014) characterized the skin cDCs and found that the CD172a^{-}XCR1^{+} (which is homologous to human BDCA3^{+}) and CD172a^{+}XCR1^{-} (homologous to human BDCA1^{+}) subsets correspond to cDC1 and cDC2, respectively (Marquet et al., 2011; Marquet et al., 2014). Maisonnasse et al. (2016a,b) described similar results in lung and bronchoalveolar lavage (Maisonnasse et al., 2016a; Maisonnasse et al., 2016b). Auray et al. (2016) recently characterized porcine blood cDCs, and classified cDC1 as CD135^{+}CD172a^{lo}CADM1^{+} and cDC2 as CD135^{+}CD172a^{+}CADM1^{+} (Auray et al., 2016); meanwhile, Edwards et al. (2017) classified cDCs as CD1^{-} (Lin^{-}CD172a^{+}CD1^{-} CD4^{-}), and

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CD1$^+$ cDC (Lin$^-$CD172a$^+$CD1$^+$CD4,) (Edwards et al., 2017). These previous characterizations are fundamental for further studies that use DCs as models for human health research.

DCs express various groups of receptors, including the Toll-like receptors (TLR), which are involved in the activation and maturation of the DCs (Zanoni and Granucci, 2010), and C-type lectin receptors (CLRs), which are involved in the antigen recognition (Figdor et al., 2002). A member of the CLRs family, DEC205, is a transmembranal type I receptor with an extracellular domain, a fibronectin type II domain and multiple lectin type C domains. DEC205 has been determined to increase antigen presentation and allow DCs to perform cross-presentation (Witmer-Pack et al., 1995; Trumpfheller et al., 2006). In DCs, DEC205 has the capacity to induce and increase various types of immune responses (Macri et al., 2016). In cattle, DEC205 is highly expressed in various DC subpopulations in the afferent lymphatic vessels (Gliddon et al., 2004). This receptor is also highly expressed on human myeloid CD11c$^+$ DCs as well as in monocytes and on T and B lymphocytes (Kato et al., 2006). DEC205 has been characterized in swine (Flores-Mendoza et al., 2010), and a recent study showed that the porcine blood cDC1, cDC2 and pDCs are DEC205$^+$ (Auray et al., 2016). However, there are no studies on the expression of this important receptor on the porcine cDCs subtypes from lymph tissues.

DCs migrate continuously from tissues to the draining lymph nodes, and resident DCs spend their entire life span in lymph nodes. Many important reports have described the cDCs in the lymph nodes of humans and mice. Recently, Granot et al. (2017) reported a detailed description of the cDC subset distribution in human mucosal tissues, the associated lymph nodes and other lymphoid tissues. The study concluded that the distribution of the cDCs subsets basically depends of tissue site and that this distribution maintained throughout life (Granot et al., 2017). Little information regarding the DCs (and subsets) in swine is available; only one report has described the cDCs in the lymph tissues of swine (Jamin et al., 2006). In this study, the cDCs were described as CD172$^+$CD11R1$^+$CD1$^{+/-}$CD80/CD86$^{+/-}$, and the spleen was the tissue with the highest frequency of cDCs. Other study evaluated the phenotypic and functional properties of antigen presenting cells (MHC-II^{high}CD172a$^+$) from the mediastinal lymph node (Lopez-Robles et al., 2015). Unfortunately, no additional studies on the classification of cDCs in lymph nodes, much less the characterization of the cDC1 and cDC2 in these tissues, have been reported. The aim of this paper is to contribute to a full characterization of the cDC1 and cDC2, as well as the expression of DEC205 in these subsets, in the porcine lymph nodes and the spleen.

2. Materials and methods

2.1. Animals

Conventional pigs (2–4 months old) were obtained from a farm that was free of porcine reproductive and respiratory syndrome virus (PRRSV), influenza virus and swine enteric coronavirus diseases. The animals were then housed at the animal facility of the Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD) with *ad libitum* access to water and food. The animals were euthanized according to the ethical standards of the Mexican Official Norm NOM-033-ZOO-1995.

2.2. Tissue collection and cell harvesting

After euthanasia, the tonsil, submaxillary and mesenteric lymph nodes, and spleen were collected and placed in 50 mL Falcon tubes with 10 mL of cold sterile phosphate-buffered saline (PBS) supplemented with 50 µg/mL gentamicin (Gibco, USA). In a sterile environment, the lymphoid tissues were washed three times with PBS with gentamicin, and all the debris was removed. Each tissue was macerated in its entirety with a 100 µm nylon cell strainer and a syringe plunger; the cells were collected in 50 mL RPMI 1640 medium (ThermoFisher, USA) with 2 mM EDTA, 50 µg/mL gentamicin, penicillin-streptomycin (100 units/mL and 100 µg/mL respectively) (Sigma, USA) and amphotericin B (1.25 µg/mL) (Sigma, USA). Finally, the cells were centrifuged at 328 x g for 10 min at 25 °C, and the viability was evaluated with trypan blue exclusion stain. Erythrocytes, if present, were lysed with a lysis buffer (10 mM $NaHCO_3$, 155 mM NH_4Cl, and 10 mM EDTA) and washed with RPMI medium.

2.3. Flow cytometry and cell sorting

Before labeling, the cells were blocked with 10% porcine serum in PBS for 10 min and centrifuged at 328 x g for 10 min at 25 °C after the addition of 10 mL of PBS with 2 mM EDTA and 5% bovine fetal serum (PBS/EDTA). In some experiments, cells from the submaxillary lymph nodes were depleted of CD3$^+$ (IgG1, clone 145-2C11; Southern Biotech, USA) and CD21$^+$ (IgG1, clone BB6-11C9.6; Southern Biotech, USA) cells using MACS anti-mouse IgG microbeads (Miltenyi Biotec, Germany) and a MS column according to the supplier recommendations. The cells were incubated with the antibodies anti-CD172a (IgG2b, clone 742215A; Monoclonal Antibody Center, USA), anti-MHCII (IgG2a, clone H42A; Monoclonal Antibody Center, USA), anti-CADM1 (IgY, clone 3E1; MBL, Japan), anti-CD3 (IgG1, clone 145-2C11; Southern Biotech, USA), anti-CD21 (IgG1, clone BB6-11C9.6; Southern Biotech, USA) and anti-CD163 (IgG1, clone MCA2311; Bio-Rad, USA). Subsequently, secondary antibodies were added: anti-IgG2b Alexa Fluor 647 (Cat No 1090-31; Southern Biotech, USA), anti-IgG1 FITC (Cat No 1070-02; BioLegend, USA) for anti-CD3, CD21 and CD163, anti-IgG2a PerCP-Cy 5.5 (Cat No 407111; BioLegend, USA), and anti-IgY Biotin (Cat No 610008; Southern Biotech, USA). Finally, streptavidin BV421 (Cat No 405226; BioLegend, USA) was added. All incubations were performed for 15 min at room temperature after which the cells were washed twice with PBS/EDTA at 328 x g for 10 min at 25 °C after each incubation. For some experiments, anti-DEC205 (clone 9HZF7 produced in our laboratory) was conjugated with the PE conjugation KIT (Bio-Rad, USA). FMO (fluorescence minus one) controls were prepared for the analysis, as well as matched isotype controls (all from BioLegend, USA): anti-mouse IgG2a (Cat No 401501), IgG1 (Cat No 400101), IgG2b (Cat No 402201), and anti-chicken IgG (Cat No 402101). The acquisitions and analyses were carried out on a FACSARIA III ™ (BD Biosciences, USA) using the FACSDiva program. Histograms were analyzed with the FlowJo software. To perform the sorting of cDC1 and cDC2, we excluded the CD3, CD21 and CD163 expressing cells; after which we selected the MHCII^{high}CADM1^{high}CD172a$^{-/low}$ cells as potential cDC1 cells and the MHCII^{high}CADM1^{high}CD172a$^+$ as potential cDC2 cells and sorted them on a FACSARIA III ™ (BD Biosciences, USA) using the FACSDiva program.

2.4. RNA extraction and quantitative reverse transcriptase PCR (qRT-PCR)

RNA from the sorted cells was extracted with the Arcturus PicoPure RNA Isolation Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer recommendations. The RNA was quantified in a Nanodrop spectrophotometer and 10 ng of total RNA was used to amplify the mRNA transcripts using real-time qPCR with the SYBR Green RT-PCR one-step kit (Agilent, USA). The amplification protocol was 50 °C for 30 min and 35 cycles of 94 °C for 30 min and 55 °C for 1 min. The amplification was carried out using the primers previously described (Maisonnasse et al., 2016a). The quantification was performed using the Ct values and the formula $2^{-\Delta Ct}$ and for each animal, the expression is presented as the relative expression to the gene with the highest expression as previously described (Maisonnasse et al., 2016a; Silva-Campa et al., 2010).

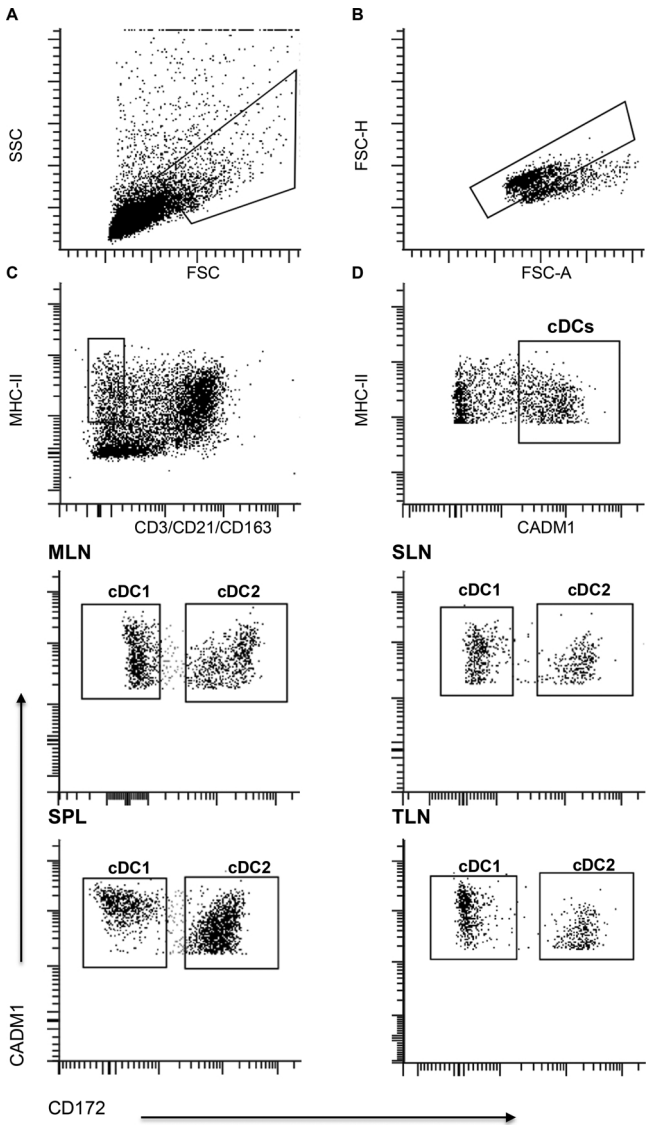

Fig. 1. Phenotype of cDCs, and cDC1 and cDC2 cells in porcine lymphoid tissues. Representative analysis of the cell selection (A) and doublet removal (B). Exclusion of $CD3^+$, $CD21^+$, and $CD163^+$ cells (C) and selection of $MHCII^{high}CADM1^{high}$ as cDCs (D). Selection of the cDC1 ($MHCII^{high}CADM^{high}CD172a^{-/low}$) and cDC2 ($MHCII^{high}CADM1^{high}CD172a^+$) cells in tonsil (TLN), submaxillary (SLN) and mesenteric (MLN) lymph nodes and spleen (SPL). For phenotypical analysis 12×10^6 cells were stained; 36×10^6 cells were stained when sorting.

2.5. Statistical analysis

All data were analyzed using GraphPad Prims v6.0 statistical software package. The comparisons of the percentages of total cDCs and the expression of DEC205 in the subsets of cDCs was performed by a one-way ANOVA and a Tukey's test, the comparison of the frequency of the cDC1 and cDC2 subtype, and the expression of DEC205 in each tissue separately, were performed using Student's *t*-tests.

3. Results

3.1. Characterization of cDCs and subtypes cDC1 and cDC2

To characterize swine cDCs and cDC1 and cDC2 subtypes, cell suspensions were prepared from the various lymphoid tissues from conventional healthy pigs and analyzed by multicolor cytometry. After gating the large cells on the basis of the forward and side scatter and removing any doublet cells (Fig. 1A-B), the $CD3^+CD21^+CD163^+$ cells were excluded and the $MHC\text{-}II^{high}$ cells were selected (Fig. 1C). The cDCs were identified as $MHCII^{high}CADM1^{high}$ and in this compartment (Fig. 1D), the cDC1 and cDC2 subtypes were classified as $MHCII^{high}CADM1^{high}CD172a^{-/low}$ and $MHCII^{high}CADM1^{high}CD172a^+$, respectively. The subsets cDC1 and cDC2 were evaluated in the tonsil, submaxillary and mesenteric lymph nodes and the spleen (Fig. 1).

To confirm the *bona fide* lineage of cDCs we evaluated the mRNA expression of FLT3, and for cDC1 and cDC2 subsets, XCR1 and FCeR1α, respectively. Cells from tonsil (n = 3) and submaxillary lymph nodes (n = 3) were sorted, and the mRNA expression of FLT3, XCR1 and FCeR1α was analyzed by qRT-PCR (Fig. 2). Both the cDC1 and cDC2 subsets in the submaxillary and tonsil tissues were positive to FLT3 (Fig. 2A and 2B, respectively), which confirmed the *bona fide* lineage of DCs. The $MHCII^+CADM1^{-/low}$ cells were also sorted. These cells were negative for FLT3 and were therefore excluded from the subsequent further analysis. To confirm the phenotypes for the cDC1 and cDC2 subtypes, the expression of XCR1 and FCeR1α was evaluated. As previously described (Maisonnasse et al., 2016a; Auray et al., 2016), the cDC1 subtype expressed transcripts for XCR1 and was negative for FCeR1α; in contrast, the cDC2 was XCR1 negative and FCeR1α positive (Fig. 2). These results confirm the identity of the cDC1 and cDC2 subsets.

3.2. Distribution of cDCs and cDC1 and cDC2 subtypes in porcine lymph nodes

The distribution of cDCs ($MHCII^{high}CADM1^{high}CD3^-CD21^-CD163^-$) was evaluated in the four lymphoid tissues (Fig. 3A): tonsil (n = 4; 10.65 ± 8.9), submaxillary (n = 5; 12.28 ± 9.1) and mesenteric (n = 3; 11.43 ± 11.8) lymph nodes and spleen (n = 3; 39.43 ± 12.08). The comparison of cDCs among the tissues evaluated in this study showed that the spleen had the highest frequency of cDCs (p = 0.006) (Fig. 3A).

Next, we evaluated the distribution of the cDC1 and cDC2 subtypes and observed that the cDC1 subtype tended to be more frequent than the cDC2 subtype in all tissues. In the submaxillary lymph node, we found the greatest difference on the cDC1:cDC2 ratio (p = 0.0003), followed by the spleen (p = 0.03). Meanwhile, the mesenteric lymph nodes and tonsil shared the same ratio (Fig. 3B).

3.3. Expression of DEC205 in cDCs, cDC1 and cDC2 subsets

Finally, the expression of DEC205 was evaluated in the total cDCs and in the cDC1 and cDC2 subsets (Fig. 4). In all of the analyzed tissues, we found $DEC205^+$ cDCs, although the percentages of cells expressing this receptor varied within tissues and between animals (Fig. 4B). In the submaxillary and mesenteric lymph nodes and the spleen, DEC205 was expressed at low level on the cDC2 and cDC1, however, a higher percentage of cDC2 cells were positive to DEC205 (not significant), whereas in the tonsil it was present in higher level in the cDC1 subset (not significant) (Fig. 4C).

4. Discussion

DCs specialize in capturing antigens from peripheral tissues and migrating to the lymph nodes to prime naïve T cells. Today, we know that the subsets of cDCs, cDC1 and cDC2, can modulate different immune responses. For this reason, a detailed characterization of cDCs is essential to understand the immunopathology of many infectious diseases and to develop new DC-based treatments. In swine, cDC1 and cDC2 cells have been described in skin (Marquet et al., 2011; Marquet et al., 2014), blood (Auray et al., 2016; Edwards et al., 2017), lung (Maisonnasse et al., 2016a) and bronchoalveolar lavage (Maisonnasse et al., 2016b), but the characterization of the cDC subsets in lymph tissues has been lacking. In the present study, we characterized the cDC1 and cDC2 subsets of cDCs in porcine tonsil, spleen, and submaxillary and mesenteric lymph node tissues.

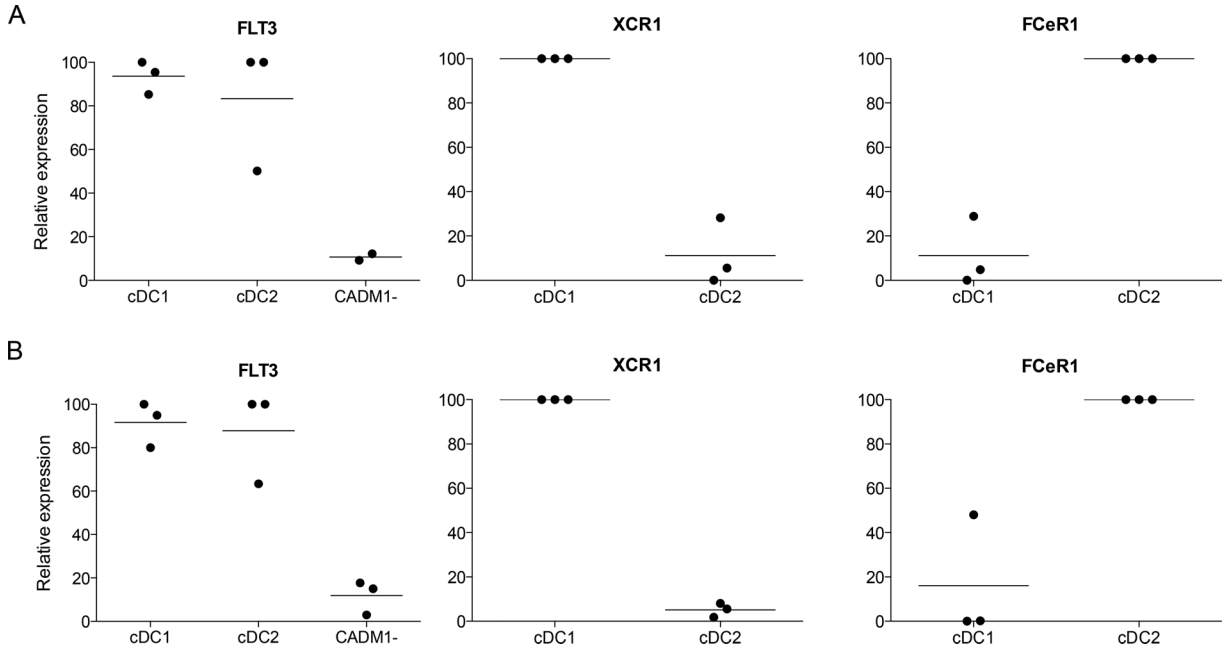

Fig. 2. Expression of cDCs-characteristic transcripts. Expression of FLT3 in the selected cDC1 and cDC2 populations, as well as the MHCII^{high}CD3$^-$CD21$^-$CD163$^-$CADM1$^-$ population. Expression of XCR1 and FCeR1a in cDC1 and cDC2 populations in submaxillary lymph nodes (A) and tonsil (B). The mean of the sorted cells was: 328, 252, and 1982 cells for cDC1, cDC2 and CADM1$^-$, respectively. The results represent the relative expression to the gene with the highest expression considered as 100, and the other genes normalized to it. This procedure was done in each animal. Figure represents the mean ± standard deviation of three different animals.

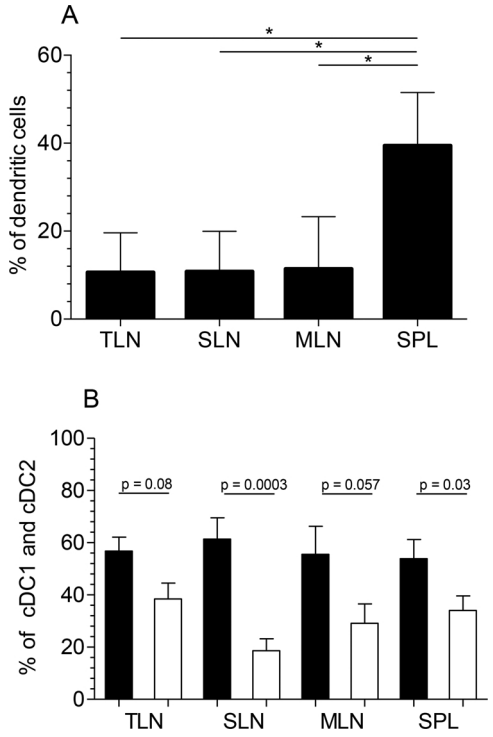

Fig. 3. Distribution of total cDCs, and cDC1 and cDC2 in various porcine lymph tissues. Percentages of cDCs (MHCII^{high}CADM1^{high}) in tonsil (TLN), submaxillary (SLN) and mesenteric (MLN) lymph nodes, and spleen (SPL) (A). Evaluation of percentages of cDC1 (black bars) and cDC2 (white bars) were defined from the compartment of cDCs in TLN, SLN, MLN, and SPL (B). Results represent the mean ± standard deviation of seven (SLN), six (TLN), three (MLN), and four (SPL) from different animals. *, indicate a $p < 0.05$.

After a lineage exclusion of CD3, CD21 and CD163 positive cells, we identified a MHCII^{high}CADM1^{high}CD3$^-$CD21$^-$CD163$^-$ population (cDCs), and within this compartment, the subsets MHCII^{high}CADM1^{high}CD172a$^{-/low}$ (cDC1) and MHCII^{high}CADM1^{high}CD172a$^+$ (cDC2) were described. This phenotype was confirmed by the expression of FLT3 and XCR1 in the cDC1 population and FLT3 and FCeR1α in the cDC2 population. We followed a similar strategy that Auray et al. (2016) to define the cDC subsets and that of Maisonnasse et al. (2016a,b) and Marquet et al. (2011) to confirm that *bona fide* cDCs are CD163 negative. Our strategy resulted in phenotypes and genotypes of the subtypes of cDCs that were similar to the previous reports.

The cDC1 cells in the lymph nodes, blood, skin and lung varied in the expression of CD172a: the lymph nodes and blood cells were CD172a$^{-/low}$ whereas the skin and lung cells were described as CD172a$^-$. In our opinion, in further studies, cDC1 must be referred as CD172a$^{-/low}$ to avoid controversy. Regarding other receptors, our results were consistent with previous reports with respect to the expression of CADM1, FLT3 and XCR1 (Maisonnasse et al., 2016a; Maisonnasse et al., 2016b; Auray et al., 2016). The cDC1 phenotype was consistently observed in all lymph tissues evaluated. Regarding cDC2, our results and the previous reports define this subtype as CADM1$^+$CD172a$^+$ and FLT3$^+$FCeR1α$^+$ (Maisonnasse et al., 2016a; Auray et al., 2016). We used CD163 as a lineage exclusion marker because in previous reports the cDC2 cells were shown to be CD163 negative: these results were consistent in various tissues. However, Auray et al. (2016) reported that the blood cDC2 were CD163 positive. These discrepancies reflect the challenges in describing these subsets (Auray et al., 2016). Although Auray et al. (2016) eliminated the monocyte-specific markers (CD14$^+$ cells) and demonstrated the expression of the cDCs receptors, the CD14$^-$CD16$^+$CD163$^+$ monocytes could represent the possible source of CD163 in the cDC2 subpopulation (Tippett et al., 2011). Another explanation is that blood cDC2 cells express CD163, but this receptor is lost after migration to lymph nodes. Our results showed that the cDCs from the trachea were CD163 negative, and the CD163$^+$ cells were FLT3$^-$ negative (manuscript in preparation). Further studies are needed to probe the implications of the expression of CD163 on cDC2 cells.

In this work, we used a new strategy to define *bona fide* cDCs: MHCII^{high}CADM1^{high}CD3$^-$CD21$^-$CD163$^-$. In most of the previous reports that have studied DCs, the T, B and macrophage cells were removed, but it is not clear if monocyte-derived DCs remain as well as the *bona fide* DCs under these conditions. Under our experimental

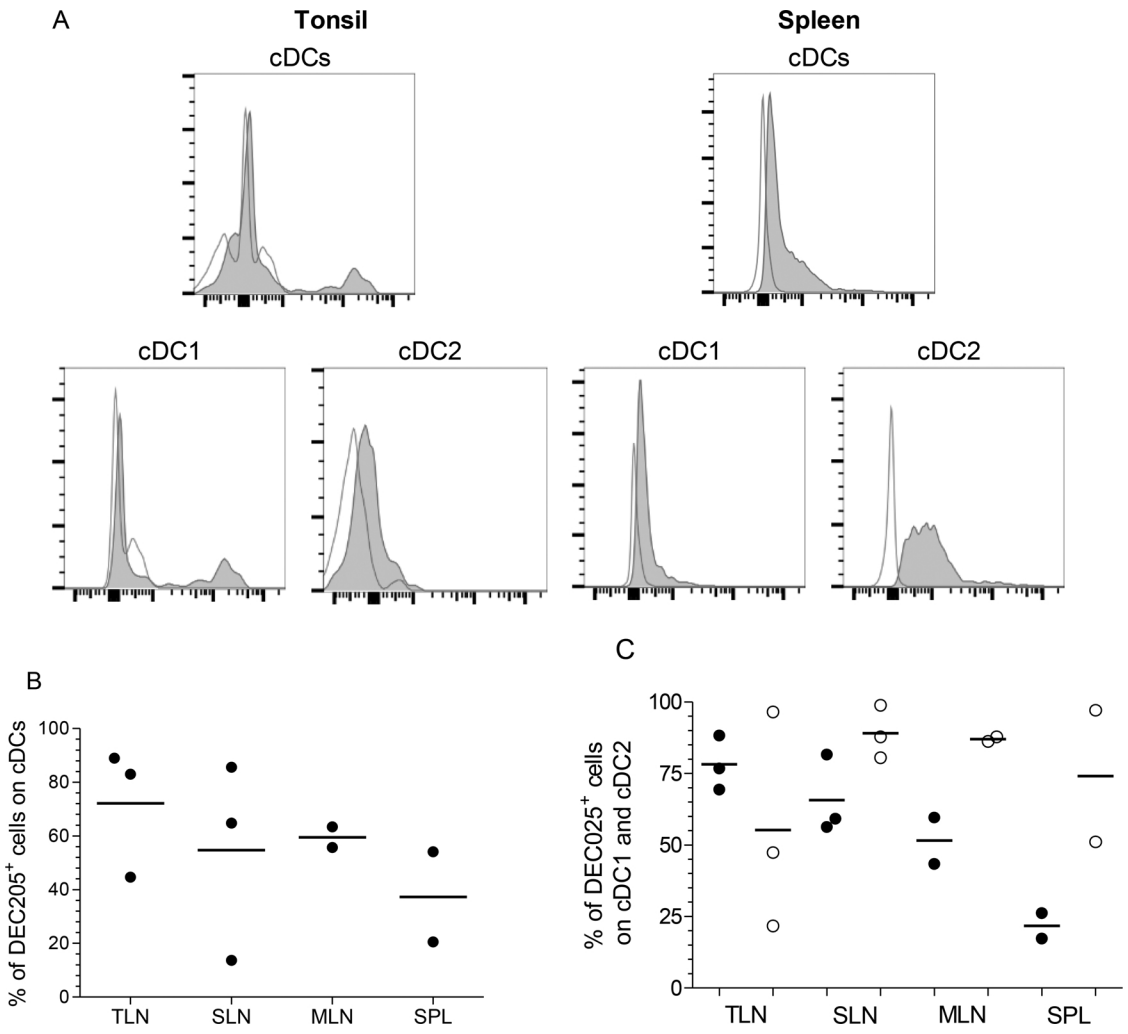

Fig. 4. Expression of DEC205 in cDCs, and the cDC1 and cDC2 populations in porcine lymphoid tissues. Representative histograms of the expression of DEC205 (gray) in tonsil and spleen. FMO was used as negative control (white) (A). Percentage of DEC205-expressing cDCs (B) and DEC205-expressing cDC1 (black dots) and cDC2 (white dots) (C) in tonsil (TLN), submaxillary (SLN) and mesenteric (MLN) lymph nodes and spleen (SPL) in all lymphoid tissues. Result are representative of three (TLN and SLN) or two different animals (MLN and SPL).

conditions, those cDCs were consistently observed in different lymph tissues such as tonsil, submaxillary and mesenteric lymph nodes as well as the spleen. In the tonsil, submaxillary and mesenteric lymph nodes, the cDCs represent fewer than 10% of the MHC-II cells. Bimczok et al. (2005) identified DCs by the expression of MHC-II and classified four different subpopulations by their expression of CD11R1 and CD172a in the lamina propria, Peyer's patches, subepithelial domes and interfollicular regions (Bimczok et al., 2005). However, the expression of these two receptors cannot rule out the presence of other types of antigen presenting cells. Porcine CD11R1 is analogous to human CD11b, and it is expressed in some monocytes (Summerfield and McCullough, 2009). In 2006, Jamin et al. (2006) characterized porcine DCs in the tonsil, spleen and lymph nodes. The results showed two populations: $CD172a^{+}CD11R1^{+}CD1^{+/-}CD80/86^{+/-}$ DCs and $CD172a^{+}CD4^{+}CD1^{+/-}CD80/86^{+/-}$ pDCs. The CD11R1 clone used for this classification is characteristically expressed on porcine neutrophils, eosinophils, basophils and mast cells (Haverson et al., 1994). In contrast, CD1a is expressed on some thymocytes, B cells, DCs, macrophages and Langerhans cells (Piriou-Guzylack and Salmon, 2008). Due to the lack of specific cDC markers, combinations of different receptors, the elimination of the cells that are relevant including B cells, T cells and $CD163^{+}$ subsets, and the analysis of the expression of specific transcripts, such as FLT3, is necessary for the accurate classification of cDCs. For these reasons, we believe that our strategy can facilitate the work with porcine *bona fide* cDCs.

The cDC1 and cDC2 subpopulations of the tonsil, submaxillary and mesenteric lymph nodes and spleen were evaluated. In all of these tissues, the cDC1 population predominated over the cDC2 subtype, but the differences were significant only in the submaxillary lymph nodes and spleen ($p < 0.05$). In the swine respiratory system, the cDC2 subtype predominates over the cDC1 cells (Maisonnasse et al., 2016a; Maisonnasse et al., 2016b). This result implies that in this tissue, there are more cells prone to anti-inflammatory responses than pro-inflammatory ones. Guilliams et al. (2016) reported that in various organs of mice, including the spleen, there is a higher percentage of cDC2. This is similar to the results reported for human tissues (Granot et al., 2017) including some lymph nodes in which the percentage of cDC2 cells is higher than that of the cDC1 population in children and adults. On the other hand, in the mouse mesenteric lymph node, there is a higher percentage of $MHCII^{+}CD103^{+}$ cDCs, which correspond to the cDC1 phenotype, than the $MHCII^{hi}CD103^{-}$ (cDC2) (Bode et al., 2008). These discrepancies from other studies could be due to the different classification strategies, as well as the subject of study in the analysis. A study concerning the implications of the predominance of the cDC1 subtype of cells in porcine lymph tissues on the immune response may be necessary.

DEC205 is a C-type lectin receptor that is expressed in various DCs subtypes (Figdor et al., 2002; Gutierrez-Martinez et al., 2015) and has been extensively studied due to its ability to increase the immune response (Bozzacco et al., 2007; Fukaya et al., 2012; Coconi-Linares et al.,

2013; Birkholz et al., 2010; Njongmeta et al., 2012). In all tissues analyzed in our experiment, we found DEC205$^+$ and DEC205$^-$ in both, cDC1 and cDC2 subtypes. This result differs from the results for porcine blood in which all cDC1 and cDC2 are DEC205$^+$ (Auray et al., 2016). The difference could be due to the localization of the cells or the differences in the classification strategy of the subpopulations. In human tonsil (Kato et al., 2006), there are DEC205$^+$ and DEC205$^-$ DCs, similar to the porcine tonsil. However, almost all of the blood DCs are DEC205$^+$. In this study, we did not evaluate the expression of DEC205 in the pig blood cDCs; nevertheless, as mentioned above, these subpopulations have been shown to be DEC205$^+$ (Auray et al., 2016). It is possible that the differences seen in the expression of DEC205 on human cDCs could be similar to those previously found between the lymph tissues and porcine blood. The expression of DEC205 on immune cells, including DCs, in bovine afferent lymphatic vessels has been evaluated (Gliddon et al., 2004). Using DC-LAMP, DEC205 expression, and the selection of the biggest cells, the authors concluded that all DCs are DEC205$^+$. Possibly, the porcine tonsil resembles the bovine afferent lymph in demonstrating a high expression of DEC205$^+$ cDCs. This could be related to the results obtained in tonsil and human blood. In this way, the expression of DEC205 could vary as a function of the tissue in which the DCs are located. However, the low frequency of cDCs in these tissues did not allow a proper statistical analysis of the expression of DEC205 on the cDC1 and cDC2 subsets to be performed. In addition, it will be important to perform functional tests to confirm our results.

5. Conclusion

In conclusion, in this work we established a basis for the characterization of the cDCs in various lymphoid tissues. Specifically, to our knowledge, this is the first study to classify and analyze the distribution of cDC1 and cDC2 cells in various porcine lymphoid tissues. Our research will facilitate the study of the functions of these cells and their contributions to various aspects of the immunological responses. Furthermore, the observed presence of DEC205$^+$ cells, specifically cDCs, in the various porcine tissues will contribute to the investigations of strategies for DEC205-targeting and functional studies.

Conflicts of interest

State any potential conflicts of interest here or “The authors declare no conflict of interest”.

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