Molecular Immunology 96 (2018) 1–7

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

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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ARTICLE INFO

Keywords:
Porcine
Dendritic cells
cDC1
cDC2
DEC205

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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https://doi.org/10.1016/j.molimm.2018.02.003
Received 26 September 2017; Received in revised form 8 December 2017; Accepted 1 February 2018

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).