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Tonsil conventional dendritic cells are not infected by porcine reproductive and respiratory syndrome virus

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) infects monocyte-derived DCs, and previous reports have shown that PRRSV does not infect conventional DCs (cDCs) *in vitro*, but the effects on cDCs from lymphoid tissues are unknown. This study analyzed the response and susceptibility of tonsil DEC205$^+$ cDCs from infected pigs. We confirmed the phenotype and lineage of *bona fide* tonsil cDCs with the mRNA expression of FLT3$^+$ and the phenotype MHCII$^+$CADM1^{high}DEC205$^+$ (DEC205$^+$cDCs). These cells were not infected by PRRSV, whereas CD163$^+$ tonsil cells were infected. The numbers of tonsil cDCs and CD163$^+$ cells were not affected by PRRSV, in contrast to the reduction in alveolar macrophage numbers. DEC205$^+$cDCs exhibited an increase in the expression of IL-12 at 5 days postinfection, suggesting a proinflammatory response by these cells to the virus. In summary, this study confirms that, *in vitro* and *in vivo*, cDCs are not susceptible to PRRSV but can respond against it.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of the greatest losses in the porcine industry. This virus belongs to the *Arteriviridae* family and is classified in the *Porartevirus* genus and divided into PRRSV-1 (European) and PRRSV-2 (American) species (Adams et al., 2017). PRRSV affects pigs of different ages, and the infection can be classified into three stages: an acute stage with viral loads in the serum and virus replication in the lymphoid tissues, a persistent stage with lower replication in the lymph nodes (LNs) and the virus clearance stage (Lunney et al., 2016). After infection by the oronasal route, PRRSV replicates in monocyte/macrophages of the nasal and tracheal mucosa, tonsils, lungs and other lymphoid tissues (Li et al., 2012; Rossow et al., 1994). However, the main replication sites of PRRSV are tissue-resident and alveolar macrophages. A characteristic of PRRSV its long-term persistence in the lungs and especially in the LNs; in the tonsils, viral RNA has been found until 251 days postinfection (dpi) (Allende et al., 2000).

DCs are the most effective antigen presenting cells (APCs) and are essential to providing innate resistance and activating the adaptive response thanks to their role as the sentinels of the immune system (Banchereau and Steinman, 1998; Schlitzer and Ginhoux, 2014; Steinman and Hemmi, 2006). DCs are classified as classical/conventional DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs are located in the LNs and nonlymphoid tissues, and the resident cDCs of the LNs can remain there for their entire lifespan, whereas tissue cDCs constantly migrate throughout the afferent lymphatic vessels into the LNs to present antigens (Förster et al., 2012; Merad et al., 2013). In human tonsils there seems to be no presence of CD141^{hi} cross-presenting migratory DCs (cDC1) (Haniffa et al., 2012); however, a transcriptional analysis of human tonsil cDC1 and cDC2, indicates that these cells express the lymphoid migration receptor CCR7, along with other chemokines and chemokines receptors (Lindstedt et al., 2005). Mice lack of tonsils but their nasal-associated lymphoid tissue (NALT) functions in a very similar way (Velin et al., 1997), DCs from porcine tonsil, and other lymphoid tissues, have been previously characterized by us (Parra-Sanchez et al., 2018) (MHCII^{high}CADM1^{high}CD3$^-$CD21$^-$CD163$^-$), and the expression levels of FLT3 and XCR1 and FCeR1α can be used to discriminate between cDC1 (MHCII^{high}CADM1^{high}CD172a$^{-/lo}$) and cDC2 (MHCII^{high}CADM1^{high}CD172a$^+$) cells in the lineage. We also evaluated the expression of DEC205, a C-type lectin receptor also expressed by blood cDCs (Parra-Sanchez et al., 2018). Recently, Soldevila et al. (2018) defined different myeloid cell populations from porcine tonsil, including cDCs (Soldevila et al., 2018).

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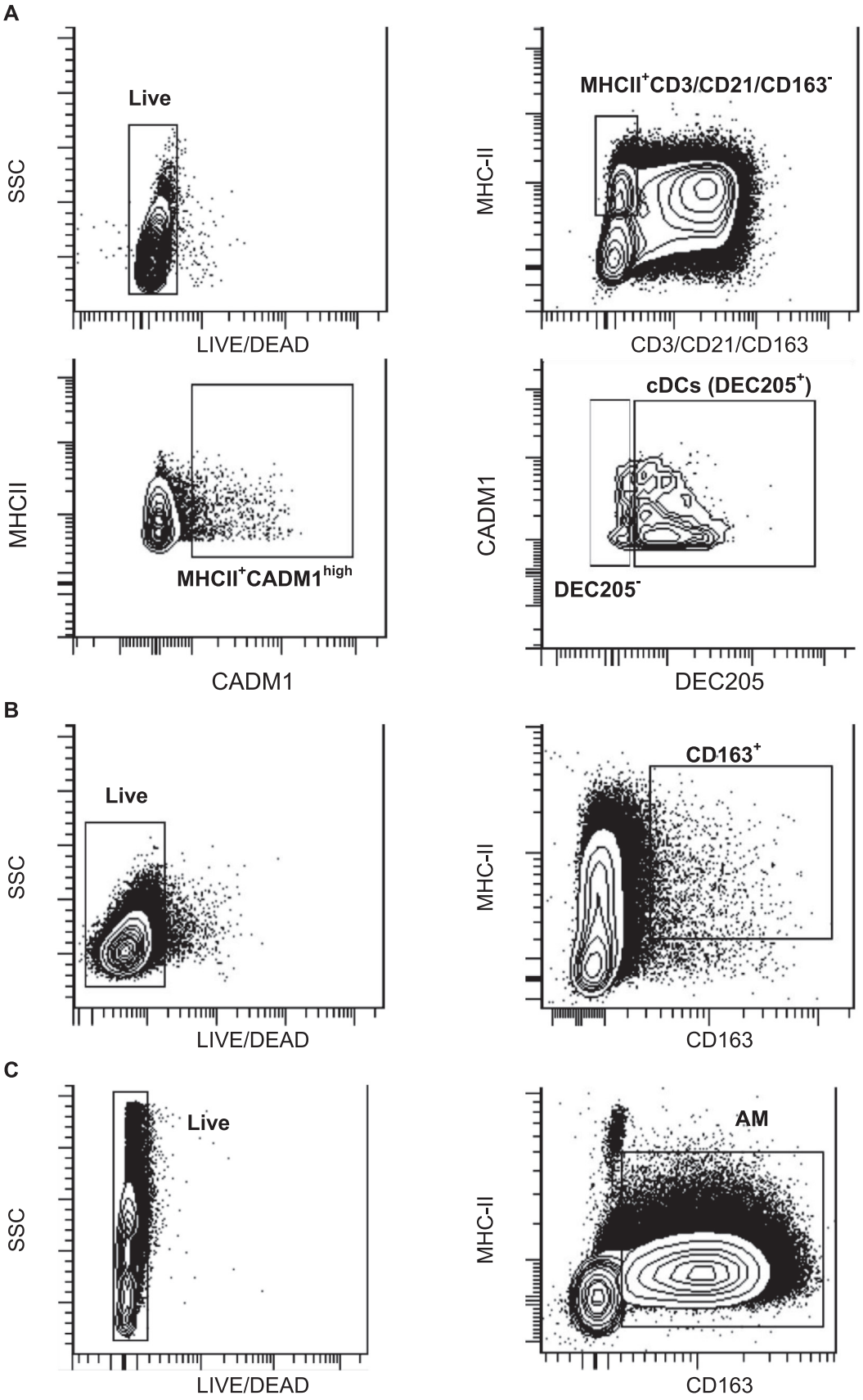

Fig. 1. Phenotypes of tonsil DEC205$^+$ cDCs, DEC205$^-$ cells, CD163$^+$ cells, and AM. Representative analysis strategy for sorting DEC205$^+$ cDCs: after the selection of live cells and the exclusion of lymphocytes and macrophages/monocytes, the DEC205$^+$ cDCs and DEC205$^-$ cells were sorted (A). Representative analysis strategy for sorting tonsil CD163$^+$, cells: after the selection of live cells, MHCII$^+$CD163$^+$ cells were sorted (B). Representative analysis strategy for the identification of AM after selecting live cells (C). Tonsil total cell number is available in the Supplementary Fig. 2.

It has been previously reported that PRRSV can infect, replicate and modulate the response of monocyte-derived dendritic cells (moDCs) (Flores-Mendoza et al., 2008; Wang et al., 2007) and bone marrow-derived dendritic cells (bmDCs) (Chang et al., 2008; Silva-Campa et al., 2010). Recently, we demonstrated that subtypes of tracheal cDCs, cDC1 and cDC2 cells, are refractory to PRRSV infection but can differentially express cytokines in response to PRRSV (Resendiz et al., 2018). In this study, we investigated the early response of tonsil DEC205$^+$cDCs after infection with PRRSV.

2. Results and discussion

2.1. Analysis of tonsil cDCs expressing DEC205

As previously reported by others (Auray et al., 2016; Parra-Sanchez et al., 2018), porcine cDCs express DEC205. In this work, we sought to evaluate this marker to improve the characterization of porcine cDCs. Tonsil cDCs (CD3$^-$CD21$^-$CD163$^-$MHCII$^+$CADM1^{high}) from healthy and infected pigs were analyzed as previously described (Parra-Sanchez et al., 2018), with the difference that in this work, the expression of DEC205 was used to sort DEC205$^+$cDCs and DEC205$^-$cDCs (Fig. 1A and supplementary figure 1). Tonsil CD163$^+$ cells (Fig. 1B) corresponding to macrophages were also sorted (Soldevila et al., 2018). CD163$^+$ cells from the bronchoalveolar lavage (BAL) (Fig. 1C) corresponding to alveolar macrophages (AM) were also evaluated. To confirm the identity of cDCs, we evaluated the expression of FLT3 on both the DEC205$^+$ and DEC205$^-$ cells as well as on the CD163$^+$ and MHC-II$^+$CADM1$^-$ cells as controls (Fig. 2). We, along with others, have demonstrated the importance of FLT3 for distinguishing *bona fide* cDCs (Auray et al., 2016; Parra-Sanchez et al., 2018; Resendiz et al., 2018). Our results showed that the cell population with the higher expression of FLT3 was the DEC205$^+$cDCs, in contrast with the DEC205$^-$ population, which expresses lower FLT3 transcripts along with the tonsil macrophages (CD163$^+$ cells) and MHC-II$^+$CADM1$^-$ cells. AM were not included in this analysis. Our results confirmed the *bona fide* lineage of DEC205$^+$cDCs and showed that the expression of DEC205 in combination with the expression of the rest of the markers used improves the characterization and analysis of cDCs. Herein, the population of CD3$^-$CD21$^-$CD163$^-$MHCII$^+$CADM1^{high}DEC205$^+$ cells will be referred to as "cDCs". The expression of DEC205 on cDCs is in accordance with previous reports that evaluated the subtypes of cDCs in the blood, cDC1 and cDC2 cells, which were DEC205$^+$ (Auray et al., 2016) and previous reports from our laboratory that evaluated the distribution of DEC205$^+$ cDC1 and cDC2 cells in different lymphoid tissues (Parra-Sanchez et al., 2018). These results contribute to a better understanding of cDCs from the tonsils and can be used to evaluate cDCs from other lymphoid tissues.

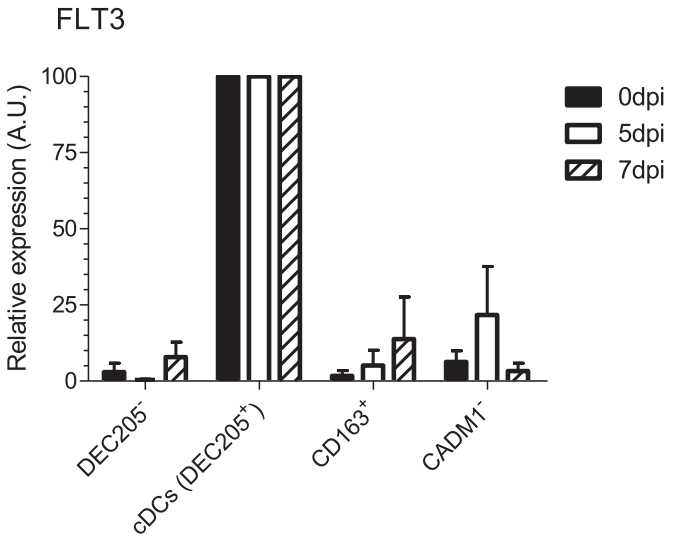

Fig. 2. Expression of FLT3 on tonsil DEC205$^+$cDCs, DEC205$^-$ cells, CD163$^+$ cells, and CADM1$^-$ cells from healthy and infected pigs. The results are expressed in arbitrary units relative to the cell population with the highest expression of FLT3, which was set as 100. The expression of the other populations were normalized to the highest FLT3-expressing population in each individual animal. Each bar represents three animals, and the standard error is indicated.

2.2. Tonsil cDCs from PRRSV-infected pigs are not infected

By *in vitro* analysis, it has been previously reported that cDCs are not infected with PRRSV (Resendiz et al., 2018), in contrast to moDCs (Flores-Mendoza et al., 2008) and bmDCs (Chang et al., 2008). In this work, we performed intracellular staining with an anti-N protein antibody to determine whether tonsil cDCs from experimentally infected pigs were positive for PRRSV. In this experiment, it was not possible to distinguish between DEC205$^+$ cDCs and DEC205$^-$ cells. The results showed PRRSV$^-$ DCs in the tonsils (Fig. 3), in agreement with previous results with the tracheal cDC1 and cDC2 subtypes (Resendiz et al., 2018). In contrast, macrophages from the tonsils and AM were infected with PRRSV (Fig. 3). We observed infected tonsil macrophages at 5 dpi (2 of 3 pigs) and at 7 dpi (1 or 3 pigs); in both cases, there was a low frequency of positive cells. In contrast, AM from all pigs were positive. The tonsils are an important site for PRRSV replication and latency (Allende et al., 2000; Molina et al., 2008; Xiao et al., 2004). Our results corroborate that tonsil macrophages are targets of viral infection, as reported for other CD163$^+$ cells from other lymphoid tissues (Yuste et al., 2017). The high percentage of PRRSV$^+$ AM in the BAL are in agreement with other reports (Patton et al., 2009) and confirm these cells as the main target of PRRSV infection. These results support previous observations from our laboratory, which showed that *bona fide* cDCs are refractory to PRRSV infection both *in vitro* (Resendiz et al., 2018) and *in vivo*. cDCs from other tissues, such as the lungs, are also refractory to PRRSV infection (Bordet et al., 2018).

2.3. PRRSV does not disturb the tonsil cDC number

It has been reported that PRRSV infects macrophages and induces apoptosis in the lungs and in different lymphoid tissues, including the tonsils (Duan et al., 1997; Morgan et al., 2016; Rodriguez-Gomez et al., 2013). Immunohistochemical analysis showed that macrophage-like cells are the main targets, and the numbers of these cells can be diminished at early times in the infection (3 dpi) (Duan et al., 1997). Given these reports, we evaluated the percentage of tonsil cDCs in uninfected control and PRRSV-infected pigs. First, we evaluated the proportion of macrophages from the tonsils and AM. As expected, a significant reduction in the percentage of AM was observed at 5 and 7 dpi. In contrast, the frequency of macrophages from the tonsils was unaffected at 5 and 7 dpi (Fig. 4), suggesting that at early times post-infection, the cells in the tonsils remained unaffected (Supplementary Fig. 2), as previously reported (Rodriguez-Gomez et al., 2013), or a rapid replacement of tonsil macrophages may occur during PRRSV infection (Xiao et al., 2004). To evaluate the number of DCs after infection, we first analyzed the proportion of MHC-II$^+$CADM1$^+$ cells, which ranged from 2% to 4% of the total MHC-II$^+$ cells in control and infected pigs (3 and 5 dpi). Within this population, cDCs ranged from 85% to 95% and remained unaffected (Fig. 4). These results suggest that after PRRSV infection (at least at 3 and 5 dpi), the proportion of tonsil cDCs was not affected by PRRSV infection, in contrast to that of AM, which showed a significant reduction. The tonsils have been considered a privileged site during PRRSV infection; in the acute (19 dpi) and persistent (67 dpi) infection stages, the tonsils have a high amount of virus/g of tissue and a low number of IFN-γ-secreting cells/g of tissue compared with the spleen. The noninfection of cDCs and the lack of a change in the frequency of cDCs could imply that these cells would have

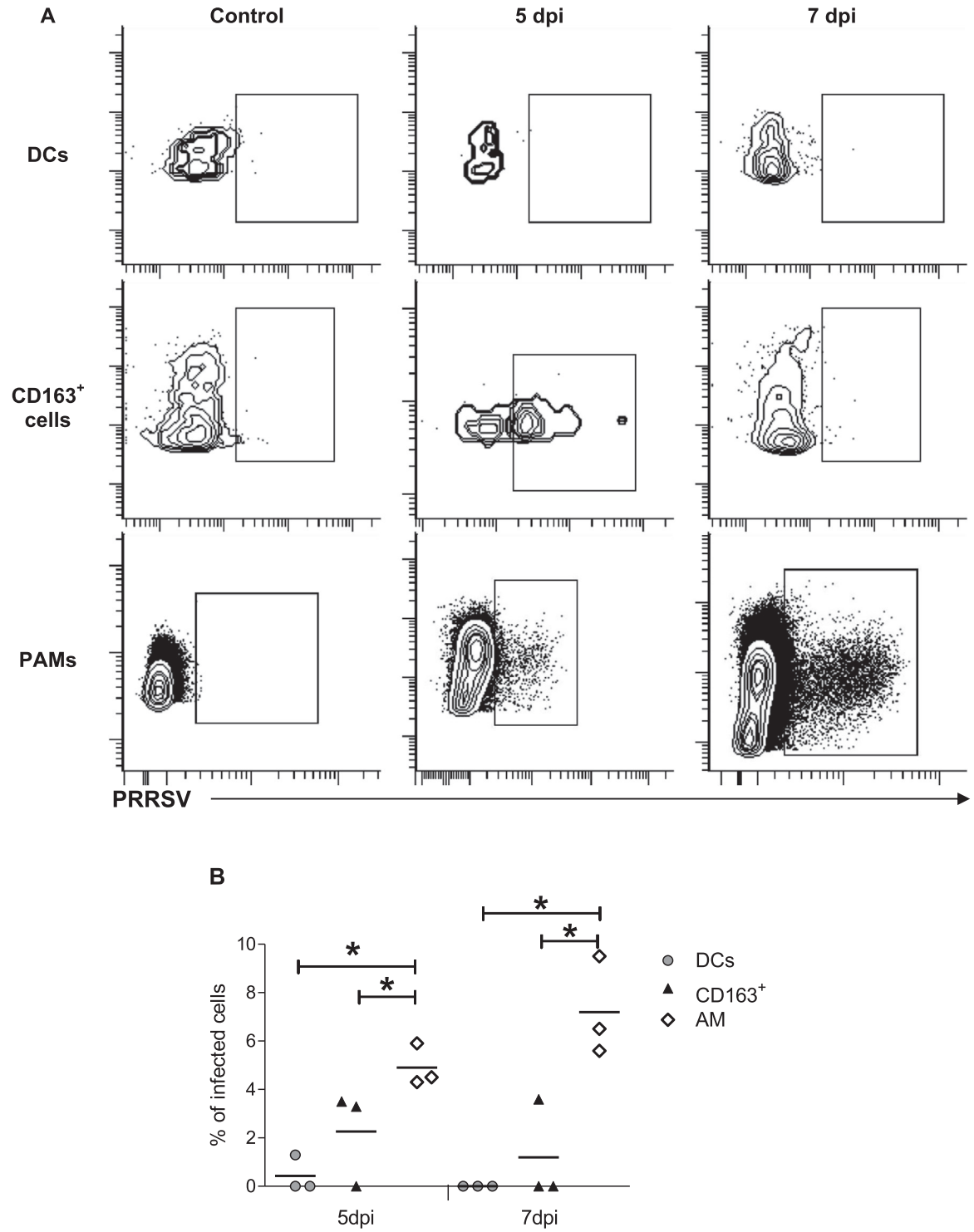

Fig. 3. Tonsil DCs, CD163$^+$ and AM from infected pigs. Representative analysis of intracellular staining of PRRSV-infected cDCs, tonsil CD163$^+$ cells and AM (A). Percentage of infected tonsil DCs, CD163$^+$ cells, and AM from infected pigs. These percentages were calculated from the intracellular labeling with the anti-N protein antibody analyzed by flow cytometry. Figure shows the mean and each symbol represents one animal. *, indicate a $p < 0.05$ (B).

the ability to prime naïve T cells during infection and suggest that the delayed/imperfect immune response to PRRSV is not due to alterations in the tonsil cDCs. However, cDCs from other regional lymphoid tissues that drain the lymph from the lungs have to be evaluated. The ability of cDCs to stimulate T cells, when these cDCs have been pre-cultured in presence of PRRSV, needs also to be evaluated.

2.4. Tonsil cDCs from PRRSV infected pigs express IL-12

From *in vitro* experiments with moDCs and bmDCs, we know that PRRSV can modulate cytokine production. If true, many of those effects may be strain-dependent. Low levels of IFN-α and high levels of IL-10, TGF-β and IL-12 have been reported. In this work, we evaluated the mRNA expression in sorted cDCs, DEC205$^-$ cells and macrophages from the tonsils of infected pigs (Fig. 5). In uninfected control pigs,

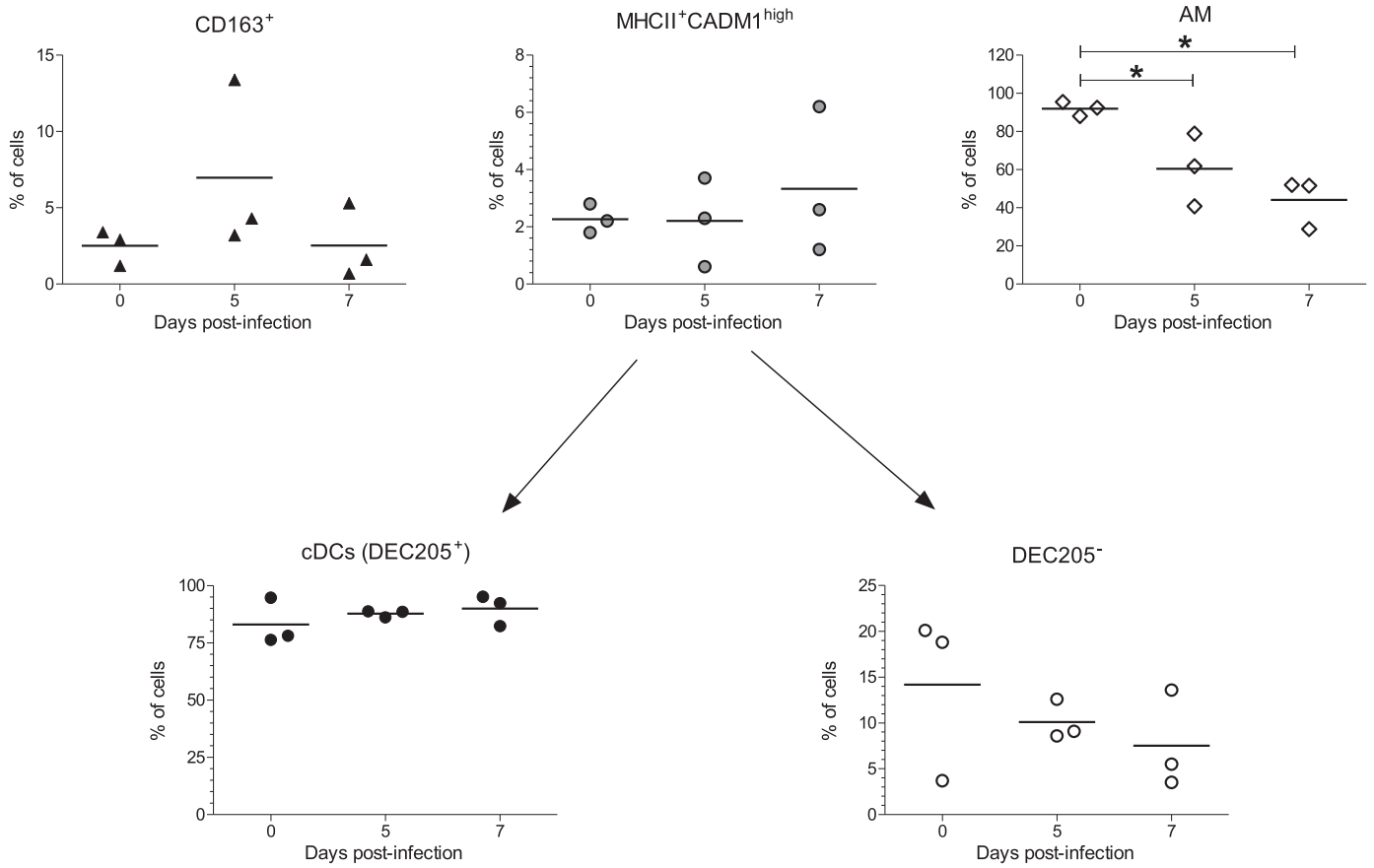

Fig. 4. Frequency of tonsil CD163$^+$, MHCII$^+$CADM1^{high}, AM and DEC205$^+$cDCs, DEC205$^-$ cells. First, the number of MHCII$^+$CADM1$^+$ cells was analyzed, and into this population, the number of cDCs (DEC205$^+$cDCs) and DEC205$^-$ cells was evaluated. The analysis included the number of CD163$^+$ and AM cells in healthy and infected pigs. Figure shows the mean and each symbol represents one animal. *, indicate a $p < 0.05$ (B).

insignificant mRNA expression of IFN-α was observed; however, after PRRSV infection, an increase in this expression was observed in some pigs. This increase was found in all the evaluated subpopulations but lacked statistical significance. Previous results from *in vitro* experiments with cDC1 cells responding to PRRSV showed an increase in the mRNA transcripts of IFN-α. This suggests that the response against PRRSV can be different between cDC1 and cDC2 cells, but is observed only when the subtypes are evaluated separately, and the *in vivo* response will depend on the cDC1:cDC2 ratio (Resendiz et al., 2018). In agreement with this hypothesis, lung cDC1 cells produced higher IFN-α levels than cDC2 cells at 10 dpi (Bordet et al., 2018). IL-12 mRNA expression was significantly increased in cDCs at 5 dpi, and the response was absent in the other cells. IL-12 production has been described in the lungs of infected pigs (Yu et al., 2016) and in infected moDCs (Park et al., 2008). IL-12 plays a central role in the response against viral infection, especially when it is produced by DCs (Nizzoli et al., 2013). cDC1 cells are the main source of this cytokine in response to PRRSV (Bordet et al., 2018), and as previously suggested, the impact of IL-12 on the induction of an immune response will depend on the ratios of IL-12 with other cytokines, mainly regulatory cytokines. The expression and production of anti-inflammatory cytokines in PRRSV infection is well documented. None of the tonsil cell populations analyzed in this study showed a significant increase in the expression of IL-10 postinfection, although cDCs from uninfected animals (2 of 3) showed elevated mRNA IL-10 transcripts, suggesting a regulatory function of these cells in the steady state (Fig. 5). There was no expression of TGF-β by cDCs; however, in DEC205$^-$ cells, at 7 dpi, an increase was observed in two of three pigs, suggesting a regulatory function for these cells. TGF-β production in response to PRRSV has been described as a way PRRSV regulates the immune response (Silva-Campa et al., 2009). DEC205$^-$ cells and cDC2 cells (Bordet et al., 2018) can be two of the sources of this cytokine with regulatory immune effects. DCs migrate to lymphoid tissues *via* CCR7 receptor binding to its ligands (Luther et al., 2002). To our knowledge, there are no studies implicating CCR7 in whether PRRSV affects cDCs migration to the lymph nodes. Using sorted cells, we analyzed the expression of the CCR7 transcript in all tonsil cell populations studied, but we did not find any differences in the expression of CCR7 between any cell populations at any time during the infection. Individual variability makes it difficult to determine significant differences; even so, the fact that there was no increase in CCR7 expression compared with healthy animals could mean one of two things: all migrating cells lower CCR7 expression when arriving at the tonsils, or PRRSV affects the migration of APCs.

3. Conclusions

To the best of our knowledge, this is the first work that studies *bona fide* cDCs from the lymphoid tissues of PRRSV-infected pigs. We used the expression of DEC205 to obtain a better characterization of cDCs and to demonstrate that these cells are not infected by PRRSV; however, in response to the virus, cDCs produce elevated mRNA transcripts of IL-12. This work only evaluated two time points postinfection, and it is possible that other effects can be demonstrated at later times or with other PRRSV strains. Further studies will evaluate the effects of cDCs on the priming of T cells and the contributions of cDCs to the immune response to PRRSV. It is known that PRRSV can modulate the immune response and, as a consequence, delay the adaptive immune response. Infection/modulation of DCs has been used to explain the immunomodulation of PRRSV; however, the lack of infection of cDCs in this and other reports (Bordet et al., 2018; Resendiz et al., 2018)

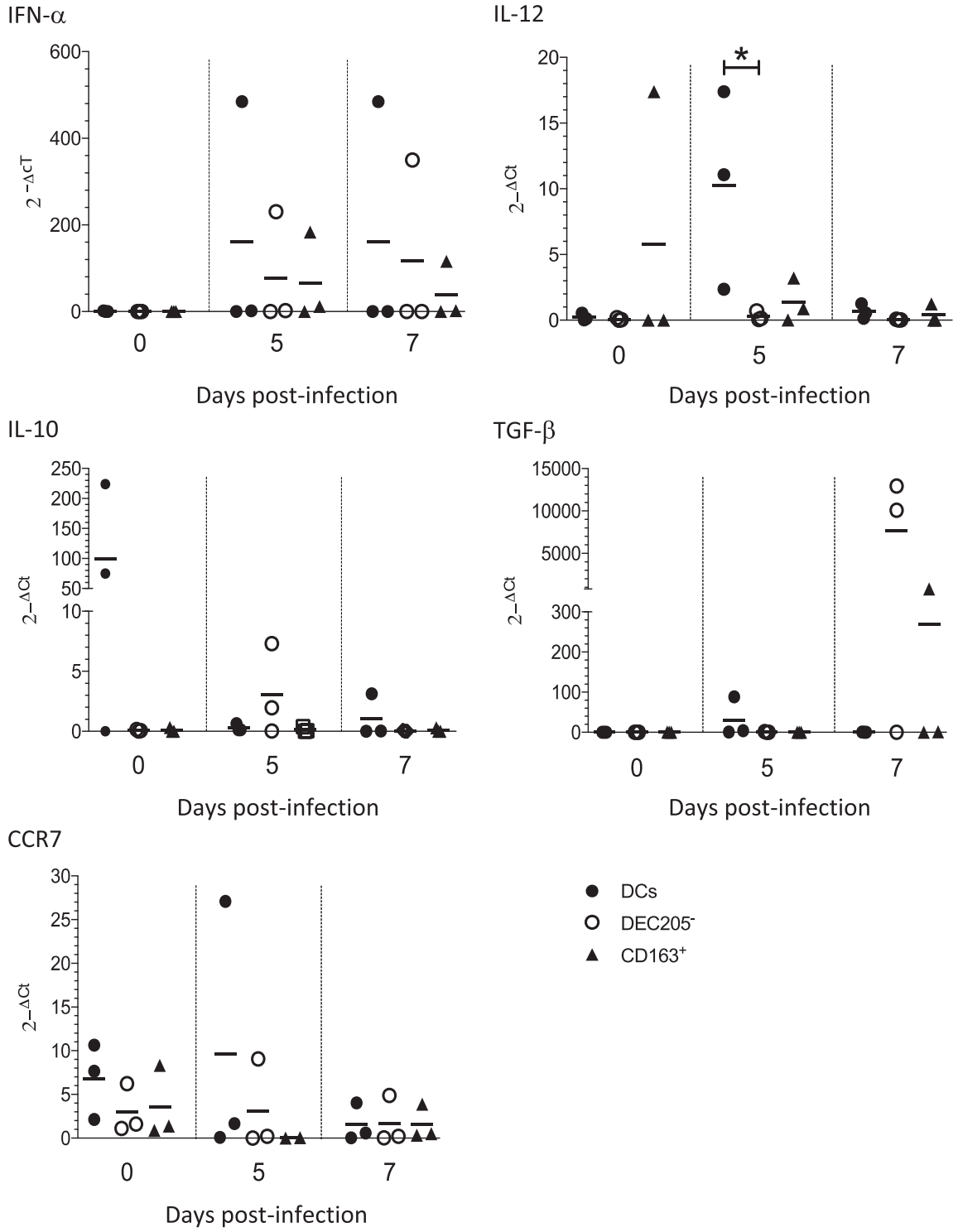

Fig. 5. Expression of cytokines in tonsil DEC205^{+} cDCs, DEC205^{-} cells, and CD163^{+} cells from PRRSV-infected and uninfected pigs. The values resulting from the formula $2^{-\Delta Ct}$ and the mean from three animals are shown for the expression levels of IFN-α, IL-12, IL-10, and TGF-β cytokines and CCR7 at day 0 (control) and 5 and 7 day post-infection. Figure shows the mean and each symbol represents one animal. *, indicate a $p < 0.05$ (B).

suggests that there are other mechanisms involved. The evaluation of other lymphoid tissues is needed to demonstrate whether the distribution of cDCs, the number of naïve T cells and the way the virus drains in these tissues can explain the delayed immune response. Additionally, it is important to evaluate how the cDC/PRRSV interaction affects T cell priming *in vitro* and *in vivo*.

4. Materials and methods

4.1. Animals and virus

Nine seven-week old pigs were obtained from a PRRSV and influenza virus-free farm. The animals were accommodated in the animal facility of the Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD) and had free access to food and water. Six animals were

infected using the PRRSV2 strain CIAD008 (GenBank accession no. DQ250071.1). The virus was propagated and titrated as previously reported (Resendiz et al., 2018). Pigs (n = 6) were infected by the intranasal route with 4 mL of 2×10^5 $TCID_{50}$ of virus per 2 mL; three pigs were euthanized at 5 dpi and three at 7 dpi. Three pigs were used as controls and remained uninfected. The euthanasia was carried out according to the Mexican Official Norm NOM-033-91 ZOO-1995 ethical standards.

4.2. Tissues and single cell suspensions

The tonsils and lungs were harvested from the euthanized pigs; the tonsils were placed in a 50 mL Falcon tube containing 15 mL of sterile and cold phosphate-buffered saline (PBS) supplemented with 50 μg/mL gentamicin (PBS/gentamicin) (Cat No. 15750-060, Gibco, Massachusetts, USA) and transported to a sterile environment. The connective tissue was removed from the tonsils, and the tonsils were washed three times with PBS/gentamicin. Once clean, the tissue was macerated with a syringe plunger on a 100 μM nylon cell strainer and transferred to a 50 mL Falcon tube with 50 mL RPMI 1640 medium (Thermo Fisher Scientific, Massachusetts, USA) supplemented with 2 mM EDTA, gentamicin (100 μg/mL), penicillin-streptomycin (Cat No. P4333 Sigma-Aldrich, Darmstadt, Germany) (100 units/mL and 100 μg/mL, respectively) and amphotericin B (1.25 μg/mL) (Cat No. A2942, Sigma-Aldrich, Darmstadt, Germany). The cells were isolated by centrifugation (328 × *g* for 10 min at 25 °C). Single cell suspensions from the tonsils were enriched with an OptiPrep™ (Cat No. D1556; Sigma, Darmstadt, Germany) gradient enrichment protocol found on application sheet C20 from the manufacturer with some modifications: we used RPMI 1640 medium as a diluent for the 11.5% iodixanol solution and Hanks' balanced salt solution with 5% fetal bovine serum and 2 mM EDTA as the suspension solution.

The lungs were collected in a 1 L beaker with 200 mL of sterile and cold PBS/gentamicin and taken to a sterile environment. A bronchoalveolar lavage (BAL) was performed with 250 mL of PBS with 2 mM EDTA to recover the pulmonary AM. The BAL was centrifuged (328 × *g* for 10 min at 25 °C) in 50 mL Falcon tubes, and the cells were used as a positive control for PRRSV infection.

4.3. Antibodies and DC sorting

Single cell suspensions from the tonsils were blocked with 10% porcine serum in PBS for 10 min; afterwards, the cells were washed with 10 mL of PBS/EDTA and centrifuged at 328 × *g* at 25 °C for 10 min. For the live/dead separation, the cells were incubated with a Zombie Aqua™ Fixable Viability Kit (Cat No 423101; BioLegend, USA) for 15 min at room temperature with PBS and washed once with PBS/EDTA at 328 × *g* at 25 °C for 10 min. For the labeling of cDCs, the single cell suspensions were incubated at room temperature for 15 min with anti-MHCII (IgG2a, clone H42A; Monoclonal Antibody Center, USA), anti-CD3 (IgG1, clone 145-2C11; Southern Biotech, USA), anti-CD21 (IgG1, clone BB6-11C9.6; Southern Biotech, USA), anti-CD163 (IgG1, clone MCA2311; Bio-Rad, USA), and anti-CADM1 (IgY, clone 3E1; MBL, Japan) primary antibodies. After the incubation, the cells were washed twice with 2 mL of PBS/EDTA and centrifuged at 328 × *g* at 25 °C for 10 min. Next, the cells were incubated with the following secondary antibodies: anti-IgG2a-PerCP-Cy5.5 (Cat No 407111; BioLegend, USA), anti-IgG1-FITC (Cat No 1070-02; BioLegend, USA) and anti-IgY-Biotin (Cat No 610008; Southern Biotech, USA). Then, the cells were incubated with Streptavidin BV421 (Cat No 405226; BioLegend, USA). To sort $DEC205^+$ cDCs and $DEC205^-$ cells, an anti-DEC205 antibody (clone 9HZF7, produce in house) conjugated using a PE Conjugation Kit (Bio-Rad, USA) was used in addition to the antibodies mentioned above. To sort tonsil macrophages, the single cell suspensions were labeled with a Zombie Aqua™ Fixable Viability Kit, anti-MHCII and anti-CD163 antibodies, and the corresponding secondary antibodies. The sorted cells were used for RNA extraction and qPCR analysis.

To identify PRRSV-infected cells, cDCs, and $CD163^+$ cells from the tonsils and BAL, the cells were fixated and permeabilized using a Leucoperm™ kit (Cat No BUF09B; Bio-Rad, USA) according to the manufacturer's instructions and labeled with PRRSV anti-N protein-FITC antibody (Cat No SDOW17-A, Rural Technologies Inc., USA).

As controls, FMO (fluorescence minus one) controls and the isotype controls anti-mouse IgG2a (Cat No 401501), IgG1 (Cat No 400101), and anti-chicken IgG (Cat No 402101, all from BioLegend, USA.) were used. $DEC205^+$ and $DEC205^-$ cell sorting was carried out by selecting live cells; excluding $CD3^+$, $CD21^+$ and $CD163^+$ cells; and selecting the $MHCII^+CADM1^{high}DEC205^{+/-}$ cells as cDCs. For the sorting of $CD163^+$ cells as macrophages and/or monocytes and/or monocyte-derived DCs, the dead cells were excluded, and then the $MHCII^+CD163^+$ cells were selected. The analyses, acquisitions, and cell sorting were performed with a FACSAria™ III flow cytometer (BD Biosciences, USA) and FACSDiva™ software.

4.4. Extraction of RNA and quantitative reverse transcriptase PCR (qRT-PCR)

RNA was extracted with an Arcturus PicoPure RNA Isolation Kit (Thermo Fisher Scientific, Lithuania) following the manufacturer's instructions. After RNA quantification with a Nanodrop spectrophotometer, the amplification of the mRNA transcripts was performed with 10 ng of total RNA using qPCR with a SYBR Green RT-PCR one-step kit (Agilent, USA). The protocol for the amplification was 48 °C for 30 min and 35 cycles of 94 °C for 30 min and finally 60 °C for 1 min. The primers used for the amplification have been previously reported and evaluated by us and others: reference gene PPIA (Peptidylprolyl isomerase A), IL-10 and TGF-β (Silva-Campa et al., 2009), FLT3 (Maisonnasse et al., 2016), IFN-α (Flores-Mendoza et al., 2008) and CCR7 (Beltrán-Beck et al., 2014); the IL-12 primer sequences were obtained from the Porcine Immunology and Nutrition database of the USDA (http://www.ars.usda.gov/Services/docs.htm?docid=6065). The Ct values and the formula $2^{-\Delta Ct}$ were used for the quantification, as previously reported (Silva-Campa et al., 2010).

4.5. Statistical analysis

Comparisons of cytokine and FLT3 expression were analyzed by one-way ANOVA and Fisher's LSD Multiple-Comparison Test. The comparison of the percentage of infected cells was performed using a Student's *t*-test. All the analyses were completed using GraphPad Prims v6.0 statistical software.

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Conflicts of interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.virol.2019.01.012.

References

Adams, M.J., Lefkowitz, E.J., King, A.M.Q., Harrach, B., Harrison, R.L., Knowles, N.J.,

Kropinski, A.M., Krupovic, M., Kuhn, J.H., Mushegian, A.R., Nibert, M., Sabanadzovic, S., Sanfacon, H., Siddell, S.G., Simmonds, P., Varsani, A., Zerbini, F.M., Gorbalenya, A.E., Davison, A.J., 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). Arch. Virol. 162, 2505–2538.

Allende, R., Laegreid, W.W., Kutish, G.F., Galeota, J.A., Wills, R.W., Osorio, F.A., 2000. Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. J. Virol. 74, 10834–10837.

Auray, G., Keller, I., Python, S., Gerber, M., Bruggmann, R., Ruggli, N., Summerfield, A., 2016. Characterization and transcriptomic analysis of porcine blood conventional and plasmacytoid dendritic cells reveals striking species-specific differences. J. Immunol. 197, 4791–4806.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Beltrán-Beck, B., Romero, B., Boadella, M., Casal, C., Bezos, J., Mazariegos, M., Martín, M., Galindo, R.C., Pérez de la Lastra, J.M., Villar, M., Garrido, J.M., Sevilla, I.A., Asensio, F., Sicilia, J., Lyashchenko, K.P., Domínguez, L., Juste, R.A., de la Fuente, J., Gortázar, C., 2014. Tonsils of the soft palate do not mediate the response of pigs to oral vaccination with heat-inactivated Mycobacterium bovis. Clin. Vaccin. Immunol. 21, 1128–1136.

Bordet, E., Blanc, F., Tiret, M., Crisci, E., Bouguyon, E., Renson, P., Maisonnasse, P., Bourge, M., Leplat, J.-J., Giuffra, E., Jouneau, L., Schwartz-Cornil, I., Bourry, O., Bertho, N., 2018. Porcine reproductive and respiratory syndrome virus type 1.3 lena triggers conventional dendritic cells 1 activation and t helper 1 immune response without infecting dendritic cells. Front. Immunol. 9.

Chang, H.C., Peng, Y.T., Chang, H.L., Chaung, H.C., Chung, W.B., 2008. Phenotypic and functional modulation of bone marrow-derived dendritic cells by porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 129, 281–293.

Duan, X., Nauwynck, H.J., Pensaert, M.B., 1997. Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Microbiol. 56, 9–19.

Flores-Mendoza, L., Silva-Campa, E., Resendiz, M., Osorio, F.A., Hernandez, J., 2008. Porcine reproductive and respiratory syndrome virus infects mature porcine dendritic cells and up-regulates interleukin-10 production. Clin. Vaccin. Immunol. 15, 720–725.

Förster, R., Braun, A., Worbs, T., 2012. Lymph node homing of T cells and dendritic cells via afferent lymphatics. Trends Immunol. 33, 271–280.

Haniffa, M., Shin, A., Bigley, V., McGovern, N., Teo, P., See, P., Wasan, P.S., Wang, X.N., Malinarich, F., Malleret, B., Larbi, A., Tan, P., Zhao, H., Poidinger, M., Pagan, S., Cookson, S., Dickinson, R., Dimmick, I., Jarrett, R.F., Renia, L., Tam, J., Song, C., Connolly, J., Chan, J.K., Gehring, A., Bertoletti, A., Collin, M., Ginhoux, F., 2012. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. Immunity 37, 60–73.

Li, J., Yin, Y., Guo, B., Zhou, S., Zhang, Y., Liu, X., Sun, T., 2012. Sequence analysis of the NSP2, ORF5, and ORF7 genes of 11 PRRS virus isolates from China. Virus Genes 45, 256–264.

Lindstedt, M., Lundberg, K., Borrebaeck, C.A., 2005. Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. J. Immunol. 175, 4839–4846.

Lunney, J.K., Fang, Y., Ladinig, A., Chen, N., Li, Y., Rowland, B., Renukaradhya, G.J., 2016. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): pathogenesis and Interaction with the Immune System. Annu Rev. Anim. Biosci. 4, 129–154.

Luther, S.A., Bidgol, A., Hargreaves, D.C., Schmidt, A., Xu, Y., Paniyadi, J., Matloubian, M., Cyster, J.G., 2002. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. J. Immunol. 169, 424–433.

Maisonnasse, P., Bouguyon, E., Piton, G., Ezquerra, A., Urien, C., Deloizy, C., Bourge, M., Leplat, J.J., Simon, G., Chevalier, C., Vincent-Naulleau, S., Crisci, E., Montoya, M., Schwartz-Cornil, I., Bertho, N., 2016. The respiratory DC/macrophage network at steady-state and upon influenza infection in the swine biomedical model. Mucosal Immunol. 9, 835–849.

Merad, M., Sathe, P., Helft, J., Miller, J., Mortha, A., 2013. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting %. J. Annu. Rev. Immunol. 31, 563–604.

Molina, R.M., Cha, S.H., Chittick, W., Lawson, S., Murtaugh, M.P., Nelson, E.A., Christopher-Hennings, J., Yoon, K.J., Evans, R., Rowland, R.R., Wu, W.H., Zimmerman, J.J., 2008. Immune response against porcine reproductive and respiratory syndrome virus during acute and chronic infection. Vet. Immunol. Immunopathol. 126, 283–292.

Morgan, S.B., Frossard, J.P., Pallares, F.J., Gough, J., Stadejek, T., Graham, S.P., Steinbach, F., Drew, T.W., Salguero, F.J., 2016. Pathology and virus distribution in the lung and lymphoid tissues of pigs experimentally inoculated with three distinct type 1 PRRS virus isolates of varying pathogenicity. Transbound. Emerg. Dis. 63, 285–295.

Nizzoli, G., Krietsch, J., Weick, A., Steinfelder, S., Facciotti, F., Gruarin, P., Bianco, A., Steckel, B., Moro, M., Crosti, M., Romagnani, C., Stolzel, K., Torretta, S., Pignataro, L., Scheibenbogen, C., Neddermann, P., De Francesco, R., Abrignani, S., Geginat, J., 2013. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. Blood 122, 932–942.

Park, J.Y., Kim, H.S., Seo, S.H., 2008. Characterization of interaction between porcine reproductive and respiratory syndrome virus and porcine dendritic cells. J. Microbiol. Biotechnol. 18, 1709–1716.

Parra-Sanchez, H., Puebla-Clark, L., Resendiz, M., Valenzuela, O., Hernandez, J., 2018. Characterization and expression of DEC205 in the cDC1 and cDC2 subsets of porcine dendritic cells from spleen, tonsil, and submaxillary and mesenteric lymph nodes. Mol. Immunol. 96, 1–7.

Patton, J.B., Rowland, R.R., Yoo, D., Chang, K.O., 2009. Modulation of CD163 receptor expression and replication of porcine reproductive and respiratory syndrome virus in porcine macrophages. Virus Res. 140, 161–171.

Resendiz, M., Valenzuela, O., Hernandez, J., 2018. Response of the cDC1 and cDC2 subtypes of tracheal dendritic cells to porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 223, 27–33.

Rodriguez-Gomez, I.M., Gomez-Laguna, J., Barranco, I., Pallares, F.J., Ramis, G., Salguero, F.J., Carrasco, L., 2013. Downregulation of antigen-presenting cells in tonsil and lymph nodes of porcine reproductive and respiratory syndrome virus-infected pigs. Transbound. Emerg. Dis. 60, 425–437.

Rossow, K.D., Bautista, E.M., Goyal, S.M., Molitor, T.W., Murtaugh, M.P., Morrison, R.B., Benfield, D.A., Collins, J.E., 1994. Experimental porcine reproductive and respiratory syndrome virus infection in one-, four-, and 10-week-old pigs. J. Vet. Diagn. Invest. 6, 3–12.

Schlitzer, A., Ginhoux, F., 2014. Organization of the mouse and human DC network. Curr. Opin. Immunol. 26, 90–99.

Silva-Campa, E., Cordoba, L., Fraile, L., Flores-Mendoza, L., Montoya, M., Hernandez, J., 2010. European genotype of porcine reproductive and respiratory syndrome (PRRSV) infects monocyte-derived dendritic cells but does not induce Treg cells. Virology 396, 264–271.

Silva-Campa, E., Flores-Mendoza, L., Resendiz, M., Pinelli-Saavedra, A., Mata-Haro, V., Mwangi, W., Hernandez, J., 2009. Induction of T helper 3 regulatory cells by dendritic cells infected with porcine reproductive and respiratory syndrome virus. Virology 387, 373–379.

Soldevila, F., Edwards, J.C., Graham, S.P., Stevens, L.M., Crudgington, B., Crooke, H.R., Werling, D., Steinbach, F., 2018. Characterization of the myeloid cell populations' resident in the porcine palatine tonsil. Front. Immunol. 9.

Steinman, R.M., Hemmi, H., 2006. Dendritic cells: translating innate to adaptive immunity %. J. Curr. Top. Microbiol. Immunol. 311, 17–58.

Velin, D., Fotopoulos, G., Luthi, F., Kraehenbuhl, J.P., 1997. The nasal-associated lymphoid tissue of adult mice acts as an entry site for the mouse mammary tumor retrovirus. J. Exp. Med. 185, 1871–1876.

Wang, X., Eaton, M., Mayer, M., Li, H., He, D., Nelson, E., Christopher-Hennings, J., 2007. Porcine reproductive and respiratory syndrome virus productively infects monocyte-derived dendritic cells and compromises their antigen-presenting ability. Arch. Virol. 152, 289–303.

Xiao, Z., Batista, L., Dee, S., Halbur, P., Murtaugh, M.P., 2004. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. J. Virol. 78, 5923–5933.

Yu, Z., Huang, C., Zhang, Q., Feng, W.H., 2016. Porcine reproductive and respiratory syndrome virus (PRRSV) induces IL-12p40 production through JNK-AP-1 and NF-kappaB signaling pathways. Virus Res. 225, 73–81.

Yuste, M., Fernandez-Caballero, T., Prieto, C., Alvarez, B., Martinez-Lobo, J., Simarro, I., Castro, J.M., Alonso, F., Ezquerra, A., Dominguez, J., Revilla, C., 2017. Splenic CD163(+) macrophages as targets of porcine reproductive and respiratory virus: role of Siglecs. Vet. Microbiol. 198, 72–80.