The porcine dendritic cell family

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ABSTRACT

Considering the pivotal roles played by dendritic cells (DCs) in both innate and adaptive immune responses, advances in the field of porcine immunology DC biology have recently progressed rapidly. As with the more extensively studied murine and human DCs, porcine DC can be generated from bone marrow haematopoietic cells or monocytes, and have been analysed in various immunological and non-immunological tissues. Both conventional DC (cDC) and plasmacytoid DC (pDC) have been characterized. The function of porcine monocyte-derived DC has not only been characterized in terms of antigen presentation and lymphocyte activation, but also their response to various ligands of pattern recognition receptors. These have been characterized in terms of the induction of DC maturation and pro-inflammatory, Th1-like or Th2-like cytokines secretion. Porcine pDC most effectively sense virus infections and are characterized by their capacity to produce large quantities of IFN-α and the pro-inflammatory cytokines TNF-α, IL-6 and IL-12. As such, the DC family as a whole is a powerful ally in the host battle against pathogen attack. Nevertheless, DC in particular tissue environments or under particular stimuli can down-regulate immune response development. This is not only important for preventing over-activation of the immune system and also for ensuring tolerance against self or "friendly" substances including food components, but may also be used as a mechanism of pathogens to evade immune responses.

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1. Introduction

Dendritic cells (DCs) are a heterogeneous group of potent antigen-presenting cells (APCs) with the unique capacity to prime naive T-cell responses [1]. In order to fulfil their role as sentinels of the immune system, they express several families of specialized pattern recognition receptors (PRRs) for particular pathogen-associated molecular patterns (PAMPs). These include Toll-like receptors (TLRs), nucleotide- binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and C-type lectin receptors (CLRs) all reacting directly with pathogen components [2–4]. In addition, many other receptors exist such as Fc receptors and activated complement component receptors, reacting with complexes of pathogen antigen with antibody or complement. Another element essential to DC biology is their migratory behaviour in response to chemokine gradients. DCs are the main cellular element controlling T lymphocyte activation and regulation. Furthermore, they are involved in B-cell responses, possibly through the delivery of native antigen to B lymphocytes [5–7], but certainly through the

production of B-cell stimulatory factors important for B-cell proliferation, differentiation and isotype switching [8,9]. DCs also play a functional role for NK cell activation [10,11], and are in a two-way communication with neutrophils [12].

Understanding DC biology requires the consideration of the heterogeneity and independence of DC functional plasticity. An important element therein is the functional and phenotypic differentiation of "conventional DC" (cDC) from "plasmacytoid DC" (pDC). The term cDC summarizes all DC subsets with "professional antigen presenting" function, while pDC represent the "professional interferon-α producers" [13]. Indeed, pDC are also referred to as "natural interferon producing cells" (NIPC), a functional entity first described 25 years ago [14]. However, more recent studies have demonstrated that also pDC have important antigen-presenting functions and that the two DC subsets complement each others by having a distinct regulation of MHC class I- and II-dependent antigen presentation [15–17]. Furthermore, DCs show a high level of heterogeneity, particularly in the specialized roles of various DC subsets dependent on their tissue localization and local immunological environment, which guides their function (Table 1).

One general functional consideration of all DC subsets is their critical roles as immunological sentinels. Being strategically located at sites of pathogen entry, such as mucosal surfaces and

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Table 1
Comparative phenotype and tissue localisation of porcine DC subsets and related antigen presenting cells

	CD172a	CD1	CD4	CD11R1	CD14	CD16	MHCII	CD80/86	References
MoDC	+	+	−	−	+/low	+	+	+	[23,24]
GM-CSF BMDC	+	+	−	−	+	+	+	+	[23]
Flt3L BMDC	+	+	−	−	+/−[a]	+	+	+	unpublished
blood cDC	low	+/−[b]	−	−	−	+/−[b]	high	+	[40]
pDC	low/−[c]	+/−[b]	+	−	−	+/−[b]	+	low	[40]
thyroid gland DC	+	n.d.[d]	n.d.	n.d.	n.d.	n.d.	+	n.d.	[78]
thymus DC	+	+	n.d.	n.d.	n.d.	n.d.	+	n.d.	[77]
skin DC	+	+	n.d.	n.d.	±	n.d.	+	+	[62]
mucosal tissue DC	+/−[e]	−[f]	n.d.	+/−[e]	−[f]	+	+	+	[46,47,61]
mucosal lymph DC	+/−[g]	+/−	n.d.	+	n.d.	+/−[g]	+	+	[47]
monocytes	+	−	−	−	+	+	+/−	low	[40,44]
macrophages	+	+/−	−	−	+/−	+	low	low	[118,119]
fibrocytes	+	+	n.d.	n.d.	+	+	+	+	[25]

[a] Two subsets.
[b] Variable, two subsets with some animals (see also Fig. 1).
[c] Can be negative in mucosal tissue [55].
[d] Not determined.
[e] Four subsets based on CD172a/CD11RI expression and dependent on localization in LP, PP and MLN [47].
[f] Negative on LP DC [46].
[g] Two subsets: CD172a⁺CD1⁺CD16⁺ and CD172a⁻CD1⁻CD16⁻.

dermal layers, DC can rapidly interact with pathogen PAMPs resulting in cell activation, pathogen uptake and degradative processing of the pathogen (antigen). A critical element therein is the concomitant uptake of antigen with PAMP recognition. The latter represents the "danger" signalling which the DC requires to be activated. In the absence of the "danger" signal, the DCs tend to function more as tolerogenic DC, inducing lymphocyte anergy. Such processes are involved in responses to self-antigens and tolerance of food antigens.

Following interaction with a pathogen (and its PAMPs), cDC endocytic activity—particularly macropinocytosis—is enhanced during the first 2 h after stimulation, followed by down-regulation as the DC mature. The latter relates to a prolonged capacity for efficient presentation of the endocytosed and processed antigen [18]. With completion of the DC maturation process, important biological changes occur to the DC. Chemokine receptor expression is modified, enabling migration to the inductive sites of the adaptive immune system. For example, the CCR1 and CCR5 receptors for inflammatory chemokines such as CCL3 and CCL5 are down-regulated, while the CCR7 receptor for CCL19 and CCL21 is up-regulated [8]. The latter provides DC with the signal for entry into the inductive sites of the adaptive immune system such as the lymph node. DC activation also results in an increase of cell surface MHC and co-stimulatory molecule expression—such as CD80 and CD86—as well as the production of immunoregulatory and/or inflammatory cytokines. MHC class II antigenic peptide complexes are stabilized on the cell surface to ensure efficient stimulation of T-cell responses. Overall, the induced functional changes are usually associated with increased T-lymphocyte stimulatory capacity, although this depends on the stimulus received [19].

Clearly, the interaction of microbial pathogens with DC can provide insight into the pathogenesis of and defence against infectious diseases. Moreover, the central role of DC in immune defence development makes them a prime target for vaccines and immunotherapies. Recent advances in porcine immunology have allowed the characterization of the porcine DC system in this direction (as witnessed by the identified cell types shown in Table 1). This has also been possible through the availability of antibodies against cell surface markers classified in three international swine CD workshops [20–22] and studies using crossreactive antibodies—summarized in Table 2. By such means, rapid advancement in the current knowledge on porcine APC and the diversity of porcine DC function has been forthcoming. The

subsequent sections of this review will present these advancements.

2. Porcine DC subsets

2.1. Porcine DC generated in vitro

2.1.1. Monocyte-derived DC (MoDC)

Similar to other species, porcine DC can be generated by stimulating blood monocytes with interleukin-4 (IL-4) and granulocyte macrophage-colony-stimulating factor (GM-CSF). After 3–7 days of culture, non-adherent or loosely adherent cells with dendritic morphology can be harvested [23,24]. Generation of monocyte-derived DC (MoDC) in vitro can also employ a GM-CSF/ IFN-α combination. This can prove more potent than the GM-CSF/ IL4 method when seeking DC for restimulating virus-specific cytotoxic T-cells [25]. Addition of IFN-α to the GM-CSF/IL-4 cocktail also influences the DC, resulting in an enhanced T-cell

Table 2
Reagents used for labelling of surface molecules on porcine APC

CD name	Functional name	Detection	References
CD1		mAb 76-7-4	[23,24,77,119]
CD4		mAbs 74-12-4, PT90A	[40]
CD2		MAb MSA-2	[77]
CD11R1	CR3	mAb MIL4, TMG.6-5	[47]
CD11R2	CD11c (human)	anti-human mAb S-Hcl3	[40]
CD14	LPS R	mAbs CAM36A, MIL2	[23,24]
CD16	FcγRIII	mAb G7	[40,46]
CD32	FcγRII	mAb AT-10 (anti-human)	[57]
CD40		anti-human mAb G28-5	[34]
CD80/86	B7-1/B7-2	rh CTLA-4-Ig (anti-human)	[23]
CD86	B7-2	anti-human mAb HA5.2B7	unpublished[b]
CD116	GM-CSF R*	rp GM-CSF-his-tagged	[40]
CD123	IL3 R	rp IL-3-his-tagged	[40]
CD163	Scavenger R	mAb 2A10	[45]
CD172a	SIRP-α	various	[32]
CD184	CXCR4	anti-human, clone 44708	[120]
CD191	CCR1	anti-human, clone 145	[34,120]
CD206	mannose R	anti-human, clone 3.29B1.10	unpublished[b]
CD208	DC lamp	anti-human clone 104.G4	[49]
	MHC class II	various clones	[24,46,47,60,77]

[a] Detected using anti-His-Tag mAb (Roche, Basel, Switzerland).
[b] Summerfield et al.

stimulatory capacity in mixed leukocyte cultures [26]. In fact, cytokine modulation is important for manipulating the type of DC generated. TGF-β permits the generation of cells with Langerhans cell characteristics [24], while PAMPs modulate mRNA expression levels for particular TLRs [27]. Bautista et al. [28] have also demonstrated that IL-4 can be replaced by IL-13 for the generation of MoDC. Phenotypically, porcine MoDC are characterized as CD1+CD14+CD16+CD80/86+CD172a+ and MHC class II+ [23,24,28–30] (Table 1). From a comparative immunological point of view, it was unexpected to find CD14, because human CD14 is considered to be a typical monocyte/macrophage rather than a DC marker [8]. Nevertheless, MoDC from other species such as cat, cattle and dog have also been shown to express CD14 [31]. Of the other markers, the CD172a was expected due to its classification as the porcine swine workshop cluster 3 (SWC3) antigen expressed on cells of the myelomonocytic lineage [32]. It is expressed on many monocytic and granulocytic cells quite early during their differentiation [33]. Functionally, this marker represents the signal regulatory protein alpha (SIRP-α). Altogether, the co-expression of CD172a and CD1 along with relatively high levels of both CD80/86 and MHC class II represent phenotypic characteristics of porcine MoDC but no marker clearly differentiating them from monocyte-derived macrophages has been identified.

Porcine MoDC generated with GM-CSF and IL-4 relate to human MoDC in that they are in an immature state, and represent a convenient cell culture model to study the DC maturation process. Akin to their human counterparts, porcine MoDC up-regulate CD80/86, MHC classes I and II, and T-cell stimulatory activity upon maturation, while inflammatory chemokine receptors such as CCR1 and macropinocytic activity are down-regulated [23,24,27,34–36].

2.1.2. Bone marrow-derived DC (BMDC)

Porcine DC, which phenotypically and functionally resemble MoDC, can also be generated from bone marrow haematopoietic cells (BMHC), stimulated with GM-CSF plus TNF-α, or GM-CSF alone, for 7–10 days—BMDC [23]. Addition of stem cell factor to the GM-CSF/TNF-α cocktail increases the yield of DC obtained [37]. In contrast to GM-CSF, Flt3 ligand (Flt3L) stimulation induces the differentiation of both cDC and pDC, similar to the situation with human and mouse BMHC [13] (Guzylack-Piriou and Summerfield, unpublished data). Furthermore, Flt3L-induced BMHC-derived cDC phenotypically and functionally differ from GM-CSF-derived DC either generated with monocytes or BMHC. Flt3L induces the differentiation of CD14− cDC which are more sensitive to stimulation by TLR2/TLR6, TR3, TLR4, TLR5 and TLR7 ligands in terms of cytokine responses and maturation [38] (Guzylack-Piriou and Summerfield, unpublished data). This may relate to the central role of Flt3L in the generation of DC from a clonogenic BMHC DC precursor [39].

2.2. Blood DC

Porcine blood is similar to human blood in representing an important source of APC. Several populations have been identified, including monocytes, DC precursors and fibrocytes. All of these express CD172a, and in a model of antigen presentation of inactivated foot-and-mouth disease virus (FMDV) to T lymphocytes the activation was dependent on the presence of CD172a+ cells [40,41]. Similar results were also obtained with classical swine fever virus (CSFV) and protein antigens derived from this virus [25,42], as well as with tetanus toxoid [43]. Separating the CD172a+ PBMC into a major population of CD14+ monocytes and a minor population of CD14− cells showed that the latter contained the CD4− blood cDC along with the CD4+ pDC (Table 1; [40]). It is notable that although

the blood cDC and pDC differ phenotypically (CD4 expression), they are similar in their lack of CD14 expression, which distinguishes them from in vitro generated MoDC. However, this simple discrimination is not absolute. The composition of blood APC is more complex, as summarized in Fig. 1. Accordingly, it is necessary to appreciate each of the blood APC in turn, to obtain a better understanding of their roles and function in immune defences.

2.2.1. Conventional blood DC

Porcine blood carries a subpopulation of PBMC, which are CD172a+CD4−CD14−, with characteristics of cDC-high levels of MHC class II and CD80/86 (Fig. 1 and Table 1), nonadherence and potent T-cell stimulatory capacity [40]. After in vitro culture, these cells strongly up-regulate MHC and co-stimulatory molecules, and their dendritic morphology becomes clear. Based on these characteristics, we have proposed that this population contains the precursors of blood cDC. These cells have variable expression of CD1 and CD16 and can be differentiated from monocytes by lower levels of CD172a (Fig. 1 and [40]). Although the majority of porcine blood monocytes are CD1−CD4−CD14+CD16+ CD172a+ (Table 1 and Fig. 1), a detailed analysis of the monocytic population has identified at least four subsets based on CD14 and CD163 [44]. It is currently not clear how the blood cDC population phenotypically defined as CD172a+CD4−CD14− cell relates the CD14−CD163+ monocytic cell. CD163 is proposed as a macrophage marker, which is up-regulated during the differentiation of monocytes to macrophages [45] but down-regulated if monocytes are induced to differentiate into DC by GM-CSF/IL-4 [30]. This would argue for two distinct subsets of cells, a point which is not surprising considering the diversity of the DC family, but still requires further clarification (see also Dominguez et al., this volume).

Based on the frequent expression of the CD11R1 marker on DC in the mucosa [46,47], this marker has also been proposed for identification of cDC in the blood and other organs as a CD172a+CD11R1+ population [48,49]. However, this definition may not be sufficient as no functional studies have been described, and it does not consider that a subset of monocytes also express CD11R1 [50].

2.2.2. Natural interferon producing cells/plasmacytoid DC (pDC)

Although several cell types can produce type I IFN upon viral infection, pDC are particularly adept at secreting very high levels of type I IFNs [13]. Representing less of 0.5% of the PBMC [40], porcine pDC were identified through their IFN-α responses to transmissible gastroenteritis virus (TGEV) [51]. Charley and Lavenant [51] originally described them as being non-adherent, non-T, non-B, CD4+, MHC-class-II positive cells. Subsequently, they went on to describe their ontogeny [52,53] and migration into lymphoid tissue after viral challenge in vivo [54,55]. Recently, using the ovine and porcine models, it has been demonstrated that pDC also migrate in the afferent lymph of cannulated animals [56].

Phenotypically, porcine pDC can be clearly identified as CD172a^low CD4^high CD14−CD163− within the peripheral blood population (Table 1). They express no or low levels of the T-cell markers CD3, CD5 and CD6, as well as no CD21 [40], but can carry low levels of CD1 and moderate levels of CD2 (Fig. 1). Related to their interaction with immune complexes porcine pDC express Fc receptors including CD16 (Fig. 1, [40]) and CD32 [57]. In contrast to porcine pDC, ovine pDC do not express CD4 and CD172a but are characterized by high expression of CD45RB [56].

Porcine pDC appear to be the major DC population producing high quantities of IFN-α and TNF-(in response to CpG motifs [58]. This clearly relates to the human DC system, placing the porcine DC system in line with that of humans and therefore distinct from the murine model [59]. Certainly related to their human and mouse
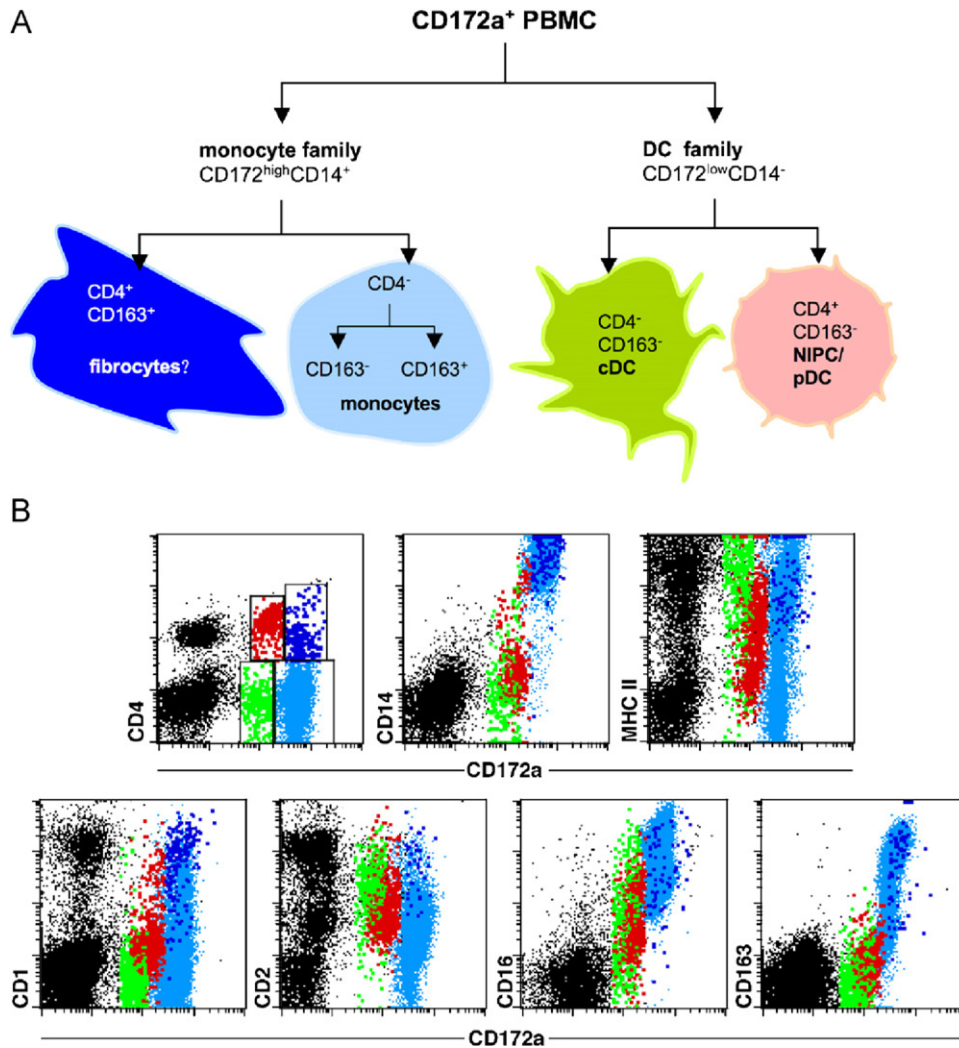
Fig. 1. Phenotype of porcine blood DC. (A) Porcine CD172a$^+$ blood APC can be differentiated into CD14$^+$ monocytic cells and CD14$^-$ DC. Two major subpopulations of CD4$^-$ monocytic cells can be defined based on CD163 expression (depicted light blue; see also Dominguez et al., this issue). Furthermore, a small CD4$^+$CD14$^+$CD163$^+$ subset with unknown function can be identified (dark blue). Porcine blood DC express relatively low levels of CD172a and lack CD14 and CD163. While cDC are CD4$^-$ (green), pDC express high levels of CD4 (red). (B) Expression of CD14, MHC class II, CD1, CD2, CD16 and CD163 on CD172a^{high}CD4$^-$ monocytes (light blue dots), CD172a^{high}CD4$^+$ monocytic cells (dark blue dots), CD172a^{low}CD4$^-$ cDC (green dots) and CD172a^{low}CD4$^+$ pDC (red dots). A representative animal is shown. Comparison of five different SPF pigs of the similar age (6–12 month old) revealed high variability in the expression levels of MHC class II, CD1, CD2 and CD16 on the DC subpopulations.

counterparts is their ability to respond to many viruses by the production of large quantities of IFN-α (see below).

2.3. Mucosal DC

2.3.1. cDC

With the major site for pathogen entry being through the mucosa of the respiratory and digestive tract, it is not surprising that most lymphocytes are in the mucosalassociated lymphoid tissues (MALT). Consequently, it is essential to regulate the mucosal immune responses against harmless microorganisms and food antigens in contact with the mucosae. This is a major role played by mucosal DC and is reflected by their anatomical localization in the mucosal tissues. They can mediate tolerance to self-antigens and harmless entities, while acting as sentinels sensing the danger posed by invading pathogens and deleterious entities. The first report on porcine mucosal DC described a putative DC in Peyer's patches (PP) as MHCII$^+$ cell lacking T- and B-cell markers [60]. In the lamina propria (LP) of the small intestine the presence of an MHCII+CD172a$^+$CD11R1$^+$ CD16$^+$ DC subset was demonstrated [46]. Bimczok and colleagues [47] further char-

acterized DC in other immunological sites of the gut and proposed four phenotypically distinct subsets of DC based on the expression of CD11R1 and CD172a. LP DC are mainly CD172a$^+$ CD11R1+, PP DC are mainly CD172a$^+$CD11R1$^-$ in subepithelial domes and CD172a$^-$CD11R1$^-$ in interfollicular regions, and mesenteric lymph node (MLN) DC are mostly CD172a$^-$ CD11R1$^+$. Interestingly, only the CD11R1$^+$ DC subsets were present in lymph, suggesting that DC migration to MLN originates largely from the LP. With respect to antigen sampling a rare population of LP DC extending cytoplasmic processes between enterocytes have been described [61]. In the PP, it appears that antigen transfer from M cells to DC is an important process, as many DC in the subepithelial dome have been demonstrated to be adjacent to M cells [61].

In contrast to the lack of CD172a on many DC in the inductive interfollicular areas of the MLN and PP, CD172a is expressed on porcine DC is at peripheral sites of pathogen entry and antigen contact such as the skin, LP and PP subepithelial dome [46,47,62]. This would indicate a possible down-regulation of this molecule during the maturation and migration process, and would be supported by the observed expression of CD172a on MoDC, BMDC generated using either GM-CSF or Flt3L, as well as circulating blood

DC [23,37,38]. Nevertheless, with *in vitro* maturation studies using MoDC, BMDC as well as blood DC [23,57,58,63,64] no loss of CD172a was observed indicating that additional factors would be required for this process. An alternative explanation would be that CD172a⁻ DC represent lymph node tissue resident DC which are phenotypically distinct from *in vitro* generated DC.

In humans, rat, cattle and sheep, the expression of CD172a differentiates functionally distinct DC subsets. While CD172a⁺ DC are more stimulatory for T cells, it is possible that the CD172a⁻ subsets is specialized in the phagocytosis of apoptotic cells [65–69]. Future studies are required to clarify such functional differences in the pig.

DCs from the porcine upper respiratory tract have also been described. In the tracheal mucosa many DC are located above the basal membrane and inside the epithelial layer where they form a dense network with many cytoplasmic processes probably related to their important role as immunological sentinels in this organ [61]. The majority of these cells co-express CD16 and MHC class II but not CD11R1. Jamin et al. [49] recently described putative DC in the tonsils by co-expression of CD11R1 and CD208 (DC lamp) or co-expression of CD11R1 and CD172a. Nevertheless, from this study it is unclear whether a CD172a⁻ DC would exist in this organ. It is also not yet clear how CD172a is expressed in DC of non-mucosal lymphoid tissue.

Several functional properties have been assigned to mucosal DC originating from the gut including the capacity to imprint the mucosal homing receptors a4b7 integrin and CCR9 on T and B lymphocytes, the secretion of cytokines of the mucosal micro-environment such as IL-10 and TGF-β, the promotion of T regulatory and Th2 rather than Th1 responses, and the induction of IgA secretion [70–74]. Considering that the capacity of gut DC to produce retinoic acid (RA) is a requirement for many of these functions and that gut DC are likely to be themselves under the influence of RA derived from gut epithelial cells, we have tested whether porcine MoDC can acquire the function of gut mucosal DC. After treatment of porcine MoDC with RA the DC acquired the capacity to promote a4b7 integrin and CCR9 on T lymphocytes, to secrete TGF-β and to promote IgA responses [75]. We have extended these studies and also demonstrated that RA induces the expression of retinaldehydrogenase, a rate-limiting enzyme in the synthesis of RA. The drug-mediated inhibition of this enzyme in RA-treated DC abrogated their capacity to promote mucosal homing receptors expression on lymphocytes (Saurer and Summerfield, unpublished data). This underlines the important role of tissue-specific factors in governing DC function.

2.3.2. pDC

NIPC/pDC have been identified as IFN-α-positive cells by immunohistochemistry in the intestinal epithelial layer, the LP, near the PP and in the MLN early after infection with transmissible gastroenteritis virus (TGEV) [55]. Since this was associated with high levels of serum IFN-α and only few IFN-α producing cells were identified in other organs it appears that during an enteropathic virus infection IFN-α would almost exclusively originate from gut pDC triggered locally. The rapid IFN-α response of pDC as early as 6 h after infection would imply that pDC are present in mucosal tissue fulfilling their role as sentinels. This relates to the recently identified CCR9 expression and migration of mouse pDC to the small intestine under steady-state conditions [76] and also to the presence of pDC identified as CD172a⁺CD4⁺ cells in tonsils and MLN of healthy pigs [49].

2.3.3. DC in other organs

Bautista et al. have isolated DC migrating from porcine skin explants. Phentotypically these cells resemble MoDC in terms of CD172a co-expression with CD1 and the high levels of MHC class II and CD80/86 (Table 1). Moreover, isolated skin-derived DC show variable expression of CD11R2, CD14 and CD16 [62].

Immunohistochemical analysis of porcine thymic tissue has shown DC to be large cells located in the medullary and the cortico-medullary regions, as evidenced by the presence of surrounding Hassall's corpuscles. Porcine thymic DC have also been partially purified and characterized [77]. They too relate to MoDC and skin DC in their CD1, CD172a and MHC class II expressions, but additionally express CD2 (Table 1), which can also be found on blood DC (Fig. 1).

Croizet and colleagues [78] demonstrated the value of the porcine model for characterizing DC from non-lymphoid organs, such as the thyroid DC.

2.3.4. Fibrocytes

Besides the more "classical" APC, a relatively recent addition has been described. Fibrocytes are a blood-derived cell population with fibroblastoid morphology, which is distinct from DC. Fibrocytes have been described for mice, humans and pigs [25,79,80], and represent 0.5–1% of nucleated cells in peripheral blood. They express CD13 and CD34, which would suggest a haematopoietic origin, possibly myeloid. The presence of CD14, CD16 and CD172a on porcine fibrocytes [25], and the differentiation of human fibrocytes *in vitro* from a blood-derived CD14⁺ population [81] support this hypothesis. Porcine fibrocytes originate from a CD163⁺ PBMC subpopulation [82]. Fibrocytes are important during wound healing, rapidly entering sites of injury together with inflammatory leukocytes [80]. They are also an important source of cytokines and chemokines important for T lymphocyte and DC development. These include IL-6, IL-10, macrophage-colony-stimulating factor, macrophage inflammatory protein-1a, MIP-1b and monocyte chemoattractant protein-1 [83].

It has been suggested that fibrocytes may play an early role in the induction of antigen-specific immunity [83,84], in particular the activation of T helper lymphocytes [80]. Porcine fibrocytes were also shown to be potent APC, relating to their expression of MHC class II, CD1 and CD80/CD86, as well as their endocytic activity [25]. These cells activate CD4⁺ T cells, but also efficiently stimulate virus-specific CD8⁺ CTL. In fact, fibrocytes are effective at low ratios with T lymphocytes, ratios at which MoDC are less efficient [25]. Porcine fibrocytes also respond to TLR ligands by producing large quantities of IL-6 [82]. These TLR ligands include LPS (TLR4-ligand), lipopeptide (diacylated form recognized by TLR1/TLR2 heterodimers; triacylated form recognized by TLR2/TLR6 heterodimers), and TLR7 ligands [82].

3. Porcine DC recognition of "danger"

The importance of DC within the innate immune system is due to their recognition of PAMPs through their PRRs. TLR play a central role in this concept of innate immune recognition. This results in a robust cytokine and chemokine response, along with DC activation and maturation, all essential for adaptive immune response development (Fig. 2). A simplified concept is that antigen presentation in the absence of DC activation leads to tolerance or shortlived immune responses without immunological memory development [85]. The current evidence shows that concomitant recognition of the antigen and a "danger" signal is essential for the DC to promote adaptive immune defence development [86]. With self and food antigens, the absence of the "danger" signal ensure that the DC involved are tolerogenic.

For efficacious immune defence development, the cell subset-specific recognition of PAMPs plays a critical role, resulting in the DC-derived cytokines necessary for both the innate response and
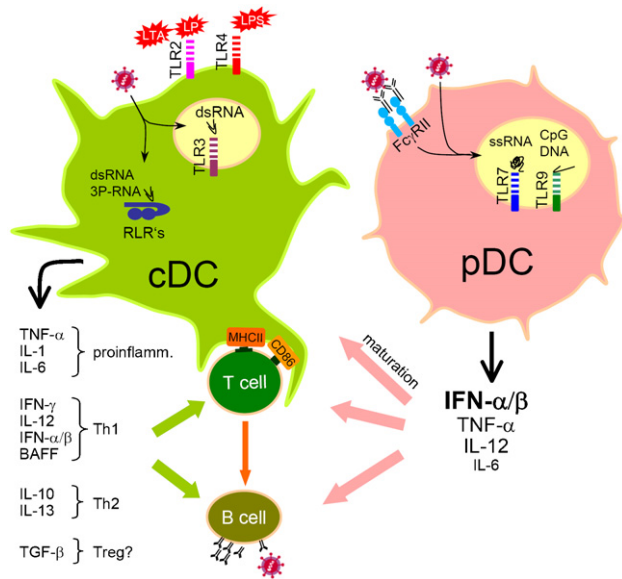
Fig. 2. Functional specialization of porcine cDC and pDC at the interface innate-adaptive immunity. The current knowledge on PAMP responsiveness and cytokine profile is schematically represented. Porcine pDC produce not only particularly large quantities of type I IFN but also TNF-α, IL-12 and IL-6 after stimulation with certain viruses, TLR7 and TLR 9 ligands. These cytokines promote cDC maturation. The latter are involved in antigen presentation and regulate lymphocyte responses. This is partially controlled by the cytokines profile, which depends on stimulation by TLR ligands, RLR ligands, viruses and bacteria as well as other factor such as the tissue environment. The cytokine profiles can be classified as proinflammatory, Th1- and Th2-like. The influence of porcine DC on differentiation of T regulatory cells (Treg) has not yet been described.

the strength and quality of the adaptive response. In addition to phenotypic differences, it is also in this area of PAMP recognition that species-dependent differences are observed. Consequently, it is important to understand how porcine APC recognition of PAMP compared with human APC, and to determine the distribution of

TLR within the porcine DC system. In Fig. 2, a schematic overview of the functional specialization and cytokine production of porcine cDC and pDC is represented. The current knowledge on the cytokine responses of MoDC is also included in the overview provided by Table 3.

3.1. Response of cDC to TLR ligands

3.1.1. Maturation and phenotypic modulation

One of the important responses induced by TLR ligation on DC is the induction of maturation. As a simple read-out of this process increased levels of surface molecules involved in antigen presentation such as MHC class II, CD40 and CD80/86 are often used. Nevertheless, the interpretations of such results alone do not permit definitive conclusions on the maturation status of a DC in terms of T-cell stimulatory activities [19].

Porcine MoDC as a model of cDC relate to those from other species in their response to TLR ligands by upregulation of MHC and CD80/86 (Table 3). These include TLR2 ligands Pam3Cys lipopeptide, pseudomonas OprI lipoprotein, lipoteichoic acid (LTA) and peptidoglycan [27,35], TLR3 ligands such as synthetic double-stranded (ds) RNA polyinosinic– polycytidylic acid (polyIC) [27,28,36,87], TLR4 ligand lipopolysaccharide (LPS) [23,24,27] and the TLR7 ligands R837 and polyuridylic acid [38]. Similar to human DC, both porcine MoDC and blood cDC have been reported to be unreceptive to the TLR9 ligand CpG-ODN, when analysed for MHC class II and CD80/86 expression [58]. Nevertheless, MoDC express TLR9 mRNA [27,38], and CpG-ODN can induce the mRNA of TLR4, TLR9, IFN-g and IL12 p35, as well as increased levels MHC class II as well as CD80/86 [27]. Such discrepancies between the studies may have been caused by sequence differences in the ODN employed or may reflect differences in the responsive status of the cells employed. For example, the relatively modest increase of CD80/86 and MHC class II expression on MoDC after TLR stimulation is synergistically enhanced in the presence of IFN-α [64]. In this study it was also shown that a full phenotypic maturation only occurred during antigen presentation to T

Table 3
Response of MoDC to stimulation by TLR ligands and cytokines

	MHCII	CD80/86	endo- cytosis	T-cell activation	Cytokine secretion	References
TLR/RLR ligands						
LPS	↑	↑	↓	↑	TNF-α IL-6, IL-10[a], IL-12[a], IL-13[a], IFN-γ[a]	[23,27,35,87]
Pam-3-Cys	↑	↑	↓	n.d.[b]	IL-6	[35,75]
LTA	↑	↑	n.d.	n.d.	IL-10[a], IL-12[a], IL-13[a], IFN-γ[a]	[27]
Peptidogycan	↑	↑	n.d.	n.d.	IL-12[a]	[27]
CpG	↑	↑	n.d.	n.d.	IL-12p35[a], IFN-γ[a]	[27]
PolyIC	↑	↑	↓	↑	INF-αβIL-6, IL-12[a], IFN-γ[a]	[36,75,87]
PolyIC tf[c]	↑	↑	n.d.	n.d.	INF-αβ	[36]
mRNA tf	↑	↑↑	n.d.	↑	INF-αβ	[36]
pDNA tf	↑	n.d.	n.d.	n.d.	IL-12	[26]
Other microbial components						
CT[d]	↓	↑	↓	↓↓	TNF-α↓, IL-10	[34]
LT[e]	↓	↑	=	n.d.	n.d.	[75]
Actinobacillus	n.d.	n.d.	n.d.	n.d.	IL-6, IL-10, IL-12	[26]
Fusarium toxin	=	↓	↓	=	IL-10↓	[121]
Cytokines, cytokine/TLR ligand cocktails						
TNF-α	=	↑	n.d.	↑↑	n.d.	[64]
TNF-α/LPS	↑↑	↑↑	↓	↑		[34]
IFN-α/pIC	↑↑	↑↑	↓	n.d.	n.d.	[64]
IFN-α/TNF-α	↑↑	↑↑	↓	↑	n.d.	[64]
IFN-α/TNF-α/TLR ligand	↑↑↑	↑↑↑	↓	n.d.	n.d.	[35,64]

[a] mRNA levels analysed.
[b] Not determined.
[c] Transfection.
[d] Cholera toxin.
[e] Heat-labile enterotoxin (E. Coli).

lymphocytes. We have also observed that in contrast to MoDC, Flt3-ligand derived BMDC are clearly more responsiveness to a range of TLR ligands including TLR2, 3, 4, 7 and 9 (Guzylack-Piriou and Summerfield, unpublished data).

3.1.2. Cytokine responses to TLR ligands

TLR ligation can induce cytokine mRNA species for IL-10, IL-12p35, IL-13 and IFN-g in DC, although this depends on the TLR ligand employed [27]. While polyIC, LPS, LTA, and CpG induced the Th1 promoting cytokines IL12 and IFN-g, the Th2-like cytokines IL-10 and IL-13 were induced only by LPS and LTA (Table 3). Raymond et al. [27] also demonstrated that similar to human and mouse DC, the cytokine response of porcine DC can be modulated by cytokines. For example, IL-12p35 mRNA can be enhanced with TNF-α, IL-12 and IFN-γ and reduced with IL-10 treatment of DC. Along the same line, the Th1/Th2 cytokine profile will depend on the type of antigen encountered [88]. At the protein level MoDC have been reported to produce TNF-α and IL-6 after stimulation with poly(IC), LPS, OprI and Pam3Cys (both TLR2 ligands), while production of IL-10 and IL-12 has been difficult to detect by ELISA [34,35,75,87]. Related to the stimulation by these specific TLR ligands is the cytokine response of MoDC to heat-inactivated *Actinobacillus pleuropneumoniae* in terms of IL-6, IL-10 and IL-12 secretion [26]. MoDC also produce type I IFN after stimulation with poly(IC) and mRNA transfection [28,36,87]. The latter is dependent on the secondary structure of the mRNA molecule forming dsRNA structures [36].

It is important to note that MoDC maturation and cytokine production is highly variable and subject to immunomodulation. Another example for this is the observation that RA has a potent synergistic effect on IL-6 secretion induced by TLR ligands [75]. This relates to the fact that the DC family has a high diversity to deal with the varying environments and signals it receives *in vivo* from the local tissue environment and from pathogens. With *in vitro* analyses, it is only possible to reproduce a small fraction of this diverse scene. Therefore, what we are observing *in vitro* is true for the conditions being created, but can not be taken as a general rule for all DC subsets under all conditions *in vivo*.

3.2. Response of pDC to TLR ligands

As with cDC, the pDC can also upregulate MHC class II and co-stimulatory molecules after *in vitro* culture [40] and when stimulated with TLR ligands [58]. However, the notable trait of pDC is their ability to produce INF-α Similar to the human and mouse immune system, porcine pDC are the most potent producers of INF-α after stimulation with certain PAMPs. These PAMPs are the CpG type A motifs [58] and TLR7/8 ligands such as R837 [89]. Such characteristics show a particularly close relationship to the human DC system [13]; pDC respond to TLR7 and 9 ligands, whereas cDC such as MoDC tend to recognize more TLR2-4 ligands. In contrast, murine DC and macrophages also respond well to TLR9 ligands, although it is again the pDC which produce large quantities of IFN-([13]. Dependent on the stimulus, porcine pDC also produce large quantities of TNF-α, IL-6 and as mentioned above IL-12 [58,89]. It is this IL-12 induction, which distinguishes porcine pDC from their human counterparts [13].

3.3. Antiviral responses of DC

The DC family is particularly effective at sensing viruses through cytosolic and endosomal PRR, which detect viral nucleic acid. Important cytosolic receptors include the dsRNA-dependent protein kinase R (PKR) and the RLR's RIG-I and MDA-5 [90]. Generally, such receptors recognize viral replication occurring *in situ*—being cytosolic, these PRR will detect the intermediates, which are also usually cytosolic. While RIG-I can sense 5'-triphosphorylated singlestranded RNA, MDA-5 appears to be more specific for dsRNA, but the fine specifity within the helicase system is not entirely clear [91]. Although these receptors are ubiquitously expressed, their important role in the interaction of cDC with viruses has been demonstrated [92]. This is different with the endosomal PRR, all members of the TLR family including TLR3, 7, 8 and 9, which are expressed only on cells playing a specialized role in innate immune responses such as DC. TLR3 represents a receptor for dsRNA, TLR7 and 8 for single-stranded RNA and TLR9 for CpG-motif containing DNA. TLR3 will not only sense viruses with dsRNA genomes but also endocytosed dsRNA replicative intermediates produced during the replicative cycle of singlestranded RNA viruses, released from dying cells in the vicinity.

As summarized in Table 4, the studies with porcine cDC and pDC demonstrate that similar to human and mouse, pDC produce high levels of IFN type I to most viruses studied including classical swine fever virus, FMDV, influenza virus, lentiviral vectors (LV), pseudorabies virus (PRV) and TGEV, while such responses are absent or weak with cDC. The only exceptions described to date are the porcine circovirus 2, which can suppress IFN response in pDC [93], and Sendai virus which is a potent inducer of IFN type I in cDC [26].

Table 4
DC interaction with viruses and IFN-α/β induction

	cDC			pDC		References
	Infection	Maturation	INF-α/β	Infection	INF-α/β	
CSFV	++	+/−	supression	+	+++	[57,87,97]
FMDV	(+)[a]	−/+[b]	+	+	++/−[c]	[63,109,112] [110]
Influenza V	+	+	−	+	+++	unpublished
LV vector[d]	+	+	−	+	+++	[38]
PCV2	(+)[e]	−	−	n.d.[f]	supression	[89,93,122]
PRRSV	+	↓	supression	n.d.	n.d.	[101–104,115]
PRV	n.d.[b]	n.d.	n.d.	n.d.	+++	[89]
SV	n.d.	n.d.	+	n.d.	n.d.	[26]
TGEV	−	−	−	−	+++	[40,51,63]

[a] Infection with transient or abortive replication.
[b] Unpublished results (Guzylack-Piriou and Summerfield).
[c] Stimulation strain-dependent or FMDV complexed with immune immunoglobulins.
[d] HIV-derived VSV-G protein pseudotyped vector.
[e] PCV2: persisting infection.
[f] Not determined.

An important basis for the responsiveness of pDC to viruses is the expression of endosomal TLR7 and TLR9, which can be triggered by DNA and RNA viruses respectively independently of viral replication [94]. In addition, the constitutive expression of IRF7, the master regulator of type I IFN, represents a unique feature of pDC [13,95].

A good example showing the importance of pDC recognition of viruses is the response seen with CSFV. This monocytotropic, haemorrhagic RNA virus replicates efficiently in cDC without apparently inducing their activation or maturation [87]. The lack of DC activation is not due to the absence of a trigger, because the virus generates a dsRNA intermediate in infected MoDC theoretically capable of stimulating RLRs. CSFV actively prevents the cDC response to dsRNA through its non-structural N^{pro} protein targeting the IRF-3 pathway [96], on which cDC depend for induction of IFN [97]. In contrast, in pDC CSFV will induce IFN-(production [57]. As mentioned above, these cells do not rely on IRF-3 due to their high levels of constitutive IRF-7 [13]. The production of large quantities of IFN-(in the serum of CSFV-infected pigs [98,99] presumably originating from pDC [99] indicates the importance of pDC for systemic IFN-(responses, particularly when cDC activities are impaired by the pathogen. In fact, a number of RNA viruses encode proteins interfering with cellular antiviral machinery and therefore prevent activation of cDC [100].

Also with porcine reproductive and respiratory syndrome virus (PRRSV), a productive infection of cDC has been observed [101–104]. This apparently does not result in the secretion of type I IFN, although IFN mRNA is induced, indicating a block of the IFN system at the translational level [104]. Whether PRRSV can activate pDC has not yet been described.

In addition to the TLR-recognition of viral PAMPs, pDC possess other receptors to sense viruses. This has been demonstrated for TGEV, an RNA-genome coronavirus, which can activate pDC using a surface receptor interacting with the viral M glycoprotein [105]. Use of inactivated virus and subunit structures has demonstrated that this activation is independent of viral nucleic acid [106], excluding a role for TLR3 and TLR7. Moreover, mutation of the glycosylation site in the M protein yields a virus incapable of inducing pDC, but still capable of replication [107]. Also studies in our own laboratory support the conclusion of triggering through a cell surface receptor-inhibitors of endosomal acidification such as chloroquine and bafilomycin prevent CpG-induction, but not TGEV-induction of IFNa production by pDC [63]. Similar observations have been made with other viral proteins such as HIV-derived gp120 and human pDC [108].

Although porcine pDC like human pDC are highly efficient producers of IFNa and effective at sensing virus infections, not all viruses will activate pDC so efficiently. Nonenveloped viruses such as FMDV-an RNA virus of the Picornaviridae related to poliovirus and rhinoviruses—are less efficient to activate pDC. It should be noted that these viruses either fail to replicate in DC, or produce only an abortive infection [109–111]. Nevertheless, pDC can be efficiently activated by FMDV under particular conditions. In the presence of opsonizing factors such as virus-specific immunoglobulin (Ig), which mediate FcgRII-enhanced uptake of virions, pDC activation was observed-an event dependent on the presence of intact and active viral RNA [63]. Such an activity would provide important antiviral innate defences at a time early post-vaccination when the adaptive response had begun producing specific antibody, but is inadequate to protect the host from disease or virus replication. This function of pDC would have an additional advantageassisting cDC in promoting the development of an efficacious adaptive immune defence for protecting the host. FMDV can also infect cDC including skin DC and MoDC [109,112], which results in low levels if IFN-β secretion.

4. Porcine DC and lymphocyte activation

Just as in vitro analyses cover only a proportion of likely events in vivo, focusing on phenotypic modulation of DC and cytokine profiles will reveal only part of the story. During immune defence development, these modulations of the DC serve a purpose beyond the direct attack on the pathogen by the DC. That additional purpose is promoting the functional interaction with the adaptive immune system.

4.1. Interaction of DC with T lymphocytes

The interaction between T lymphocytes and DC is a bilateral process. On one side there is the central role of DC in presenting antigen and stimulating T cells. This has been shown in several models including mixed leukocyte reactions [24,26], superantigen presentation [23] as well as antigen specific T-cell restimulation [25,75,87,113,114] (see also Table 3). On the other side, T lymphocytes provide important signals to DC. With porcine MoDC cultures we have observed that after the co-culture of cytokine-matured DC with T cells in the presence of antigens, a further upregulation of MHC class II expression was obtained reaching levels clearly above those obtained with any stimuli in the absence of Tcells [64]. Interestingly, TNF-α pre-treatment of the DC was as efficient as TNF-α/IFN-α cocktails to sensitize the DC for this process. These in vitro observations indicate that MoDC maturation is a regulated multistep process. From a practical point of view this means that although TNF-α alone is not sufficient to induce MoDC maturation, the T-cell responses induced by TNF-α-treated DC can compensate to provide stimuli reaching those obtained with more potent maturation cocktails such as TNF-α/IFN-α or TLR ligands [64]. Moreover, the T-lymphocyte activity provides an additional advantage in that cocktails such as TNF-α/IFN-α, as well as those combined with TLR can have the drawback of "exhausting" the DC.

Similar to other species, it has been demonstrated that DC can modulate the type of T-cell response induced. For example, the Th1/Th2 profile will be influenced by the type of antigen presented as well as by the cytokine environment [88]. Another example of such modulation is the observation that cholera toxin-treated DC have the capacity to suppress T-cell proliferation [34]. This was associated with decreased MHC class II expression and increased IL-10 secretion of the DC and was reversible by addition of TNF-α suggesting an immunoregulatory process. PRRSV has also been described by several authors to modulate DC and monocytes towards reduced T-cell stimulatory capacity after in vitro infection [102,103]. Also here reduced expression of MHC and co-stimulatory molecules together with increased IL-10 levels have been described [101–103,115]. Such studies are valuable to understand viral pathogenesis but care must be taken to avoid any contamination of the cultures with mycoplasma as MoDC cultures apparently efficiently support mycoplasma growth, which can result in a potent antiproliferative activity [116].

DC not only determine the type of T-cell response but also their homing characteristics. As described in section "Mucosal DC", MoDC treated with RA promote $\alpha 4\beta 7$ and CCR9 expression, representing essential gut homing receptors [75].

4.2. Interaction of DC with B lymphocytes

Classically, B lymphocytes equipped with their surface Ig receptor will recognize native unprocessed antigen and would only require T-cell help for clonal expansion and differentiation into antibody producing cells. In this sense, B cells should show only an indirect requirement for APC such as cDC. Nevertheless, APC produce a number of cytokines, which have a direct stimulatory

effect on B cells. These are classical cytokines such as IL-6, IL-10 and IFN-α/β, but also more recently identified cytokines such as the B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL)-members of the tumour necrosis factor superfamily [9]. In addition, several studies also demonstrated the "delivery" of native unprocessed antigen by DC to B cells [5–7]. Our own studies with porcine DC in an *in vitro* model of FMDV-specific Ig synthesis demonstrated an important direct role of APC's during antigen-specific restimulation of immune B lymphocytes. Purified B cells produced virusspecific Ig only in the presence of Tcells and APC. Monocytes and MoDC but not pDC supported B-cell differentiation into antibody-secreting cells. While IL-2 could replace T-cells, addition of BAFF could compensate for a lack of APC. In fact, blocking of BAFF receptor abrogated the APC-derived help for B-cell responses [41]. In addition, DC also influence isotype switching. As mentioned above, RA-treated MoDC promote virus-specific IgA secretion *in vitro* [75].

Not surprisingly, similar to the interaction with T lymphocytes, the interplay of DC with B lymphocytes is also bilateral. An important role is certainly played by the "ménage à trois" of FcR expressed on DC, antibody and antigen. This permits an amplification of antigen uptake and presentation, and can also mediate efficient cross-presentation of antigen for stimulation of MHC class I-restricted T-cell responses. Furthermore, the FcR-system sensitizes DC for inflammatory and antiviral cytokine responses. One example is the response of pDC to FMDV mentioned above (Table 4). These pDC only respond by producing IFN-α to FMDV when the virus is complexed with antibodies [63]. Another example is with CSFV, where pDC sensitized with cytophilic antibodies show enhanced IFN-α production in response to lower virus quantities than when no cytophilic antibodies are present [57]. In both cases FcgRII is involved.

4.3. The advantages of the porcine model

Recent advances in the characterization of the porcine immune system, particularly in porcine DC biology, have permitted the use of the porcine model for many immunological studies. Although the library of reagents for such studies is still restricted compared to that for mouse and human studies, knowledge of porcine immunology is well advanced. With the unveiling of the sequence for the porcine genome, there will be clear advantages for using the pig. In particular, the MoDC model has a number of advantages and applicability. Large numbers of DC can be generated without killing the animal. For example, a typical figure of 30–70 million DC can be generated using monocytes isolated from 400 ml of blood. The facility to repeat blood sampling with the pig enables the use of these DC in antigen presentation assays, to monitor autologous T-cell responses in immunization experiments with outbred animals [113]. Moreover, with the MoDC being in an immature state, another advantage is the possibility to study DC maturation in response to cytokines, TLR ligands and infections.

Considering that DC are a rare cell type within the leukocyte populations, the large size of the pig, its lymphoid organs and the availability of larger volumes of blood together with repeated samplings offer an advantage allowing considerable immunological progress. In addition to this facility of recovering large volumes of blood regularly from the same animal, cannulation procedures for porcine lymph vessels at both peripheral and mucosal sites are now available. This approach allows us the much sought ability of studying DC migrating from peripheral sites over periods of several days [47,56], a procedure not possible with humans and certainly cumbersome with mice considering their size and the quantities of material obtained. Moreover, the pig is more closely related to the human—both genetically and physiologically—when compared to

mice. This is reflected by immunological similarities such as the PRR and their cellular distributions [58,59]. Finally, the advantages which porcine immunology has to offer have gained a further boost with the advent of novel technologies such as RNA interference (RNAi) [38], which can be combined with the generation of transgenic pigs [117].

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