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Review

The immunology of the porcine skin and its value as a model for human skin☆

Artur Summerfield[a,*], François Meurens[b], Meret E. Ricklin[a]

[a] Institute of Virology and Immunology, Sensemattstrasse 293, 3147 Mittelhäusern, Switzerland
[b] Vaccine and Infectious Disease Organization-International Vaccine Centre (VIDO-InterVac), University of Saskatchewan, 120 Veterinary Road, S7N 5E3 Saskatoon, Saskatchewan, Canada

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ABSTRACT

The porcine skin has striking similarities to the human skin in terms of general structure, thickness, hair follicle content, pigmentation, collagen and lipid composition. This has been the basis for numerous studies using the pig as a model for wound healing, transdermal delivery, dermal toxicology, radiation and UVB effects. Considering that the skin also represents an immune organ of utmost importance for health, immune cells present in the skin of the pig will be reviewed. The focus of this review is on dendritic cells, which play a central role in the skin immune system as they serve as sentinels in the skin, which offers a large surface area exposed to the environment. Based on a literature review and original data we propose a classification of porcine dendritic cell subsets in the skin corresponding to the subsets described in the human skin. The equivalent of the human CD141$^+$ DC subset is CD1a$^-$CD4$^-$CD172a$^-$CADM1^{high}, that of the CD1c$^+$ subset is CD1a$^+$CD4$^-$CD172a$^+$CADM1$^{+/low}$, and porcine plasmacytoid dendritic cells are CD1a$^-$CD4$^+$CD172a$^+$CADM1$^-$. CD209 and CD14 could represent markers of inflammatory monocyte-derived cells, either dendritic cells or macrophages. Future studies for example using transriptomic analysis of sorted populations are required to confirm the identity of these cells.

1. Skin structure and advantages of the pig model

The skin is the largest organ of the mammalian body with an estimated total weight of 5 kg and a surface around 2 m^2 for adult humans (Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005). Being most exposed to the environment, it represents a major physical and immunological protection against injury and infection. Accordingly, similar to the mucosal immune system, a skin immune system (SIS) has been described representing a coordinated system in which epithelial cells, resident immune cells, and a local microenvironment including locally produced vitamins control immunity and tolerance to self and foreign antigens (Di Meglio et al., 2011; Heath and Carbone, 2013; Nestle et al., 2009). In addition, recent work indicates a major role for the skin microbiome, which is composed of up to 10^{12} microorganism/m^2, mostly localized in the intercorneocytic spaces (Grice and Segre, 2011). In addition to physical and immunological protection, the skin plays an important role in thermoregulation, transmission of stimuli, storage/synthesis, and absorption (Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005).

The skin comprises three main layers: On the top the epidermis (ectodermic origin), the dermis (mesodermic origin), composed of collagen and elastin fibers in an amorphous matrix of mucopolysaccharides and the subcutis, also called hypodermis, a fatty subdermal layer (Debeer et al., 2013). The skin is also associated to various structures such as hair follicles, sweat glands, sebaceous glands, nerves, blood vessels, and lymphatics (Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005). The epidermis is the avascular superficial layer. It consists of several layers of cornifying squamous keratinocytes that make up more than 95%. Within around 3 weeks of age keratinocytes go through different layers, namely the *stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum lucidum* and the outermost *stratum corneum* (Debeer et al., 2013; Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005). The other cells (<5%) are the Merkel receptor cells, the melanocytes, and the Langerhans cells, a type of dendritic cell (DC) typically associated with the skin (Nestle et al., 2009). Because of the absence of blood vessels in the epidermis, the tissue receives nutrients and oxygen supply by diffusion from dermal blood vessels. The dermis can be divided into a papillary

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* Corresponding author. Tel.: +41 31 8489377.
E-mail address: Artur.Summerfield@ivi.admin.ch (A. Summerfield).

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layer, the *pars papillaris*, and a reticular layer, the *pars reticularis* (Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005). Some sections of the epidermis, the rete ridges can extend downward between the dermal papilla. The dermis consisting of many cells types, mainly fibroblasts, mast cells, and dermal DC, fibrous proteins and an extracellular matrix is firmly attached to the epidermis by a basement membrane. The epidermis represents the most important component of the skin barrier function protecting the body, while the dermis provides strength and elasticity to that barrier with collagen and elastic fibers embedded in proteoglycans. Typically the thickness of the human epidermis varies between 60 and 100 μm in most areas (up to 600 μm in the plantar and palmar regions) (Debeer et al., 2013; Elias, 2007; Proksch et al., 2008; Rushmer et al., 1966; Wang and Sanders, 2005). The dermis accounts for most of the skin thickness with 0.6–3 mm. Over the skin there is a diversified microflora also involved in the protection of this tissue through multiple interactions with host keratinocytes and immune cells.

The pig is considered as an excellent animal model in many fields of biomedical research (Meurens et al., 2012). Indeed its anatomy, physiology, and immune system share numerous similarities with human. Regarding the skin, the pig is also very similar to its human counterpart, as opposed to many "loose-skinned" animals such as mouse and rat (Table 1) (Debeer et al., 2013; Kong and Bhargava, 2011; Marquet et al., 2011; Montagna and Yun, 1964; Sullivan et al., 2001). The skin of rodents differs significantly from humans as it is loosely connected to the subcutaneous connective tissue (Kawamata et al., 2003). In contrast pig and human skin are tightly attached to it (Sullivan et al., 2001).

Several studies assessed the porcine skin using various approaches such as histology (Debeer et al., 2013; Montagna and Yun, 1964), confocal Raman microspectroscopy (Tfaili et al., 2012), and infrared spectroscopy (Kong and Bhargava, 2011). They showed that in pigs the *stratum corneum* (SC) thickness is 20–26 μm, comparable to what is observed in humans. Complete epidermis varies from 30 to 140 μm in thickness in pigs compared to 50–120 μm in humans (Hammond et al., 2000; Mahl et al., 2006; Meyer et al., 1978). Furthermore, when a measure less dependent of body site such as the dermal–epidermal thickness ratio is considered (10:1–13:1) the pig is again very similar to human (Meyer et al., 1978). Pig as well as human also shows developed rete-ridges and *pars papillaris*, and abundant subdermal fatty tissue (Debeer et al., 2013; Kong and Bhargava, 2011; Marquet et al., 2011; Montagna and Yun, 1964; Sullivan et al., 2001). In fact, fat and not fur/hair are the main insulation components of porcine and human skin. Nevertheless, the subcutaneous fat layer is generally thicker in pigs compared to man.

Regarding blood supply in the dermis, pigs are also comparable to humans (Forbes, 1969; Montagna and Yun, 1964; Vardaxis et al., 1997). Also with respect to adnexal structures in pigs and humans similarities are evident (especially for hair follicles) even if some differences can be identified (Meyer et al., 1978). Amongst them there is the absence of eccrine glands. Moreover, in pigs apocrine glands are distributed through the skin surface. Regarding histology and the protein and lipid compositions of the different layers, obvious similarities between both species have also been identified (Debeer et al., 2013). The lack of skin pigments in many breeds of pigs is also advantageous for dermal studies.

Porcine skin has been used in many occasions as a model for human skin. This includes studies on wound healing (Ansell et al., 2012; Jung et al., 2013; Sullivan et al., 2001), burns (Abdullahi et al., 2014; Sheu et al., 2014), transdermal penetration, delivery and toxicology (Barbero and Frasch, 2009; Godin and Touitou, 2007; Mahl et al., 2006; Simon and Maibach, 2000; Yu et al., 2013), infectious diseases (Mounsey et al., 2010; Rampton et al., 2013; San Mateo et al., 1999), radiation and UVB impact (Agay et al., 2010; Brozyna et al., 2009; Smirnova et al., 2014), snake venom (Imkhieo et al., 2009) and taser (Jenkins et al., 2013) assessments, as well as stem cell research (Hao et al., 2009; Zhao et al., 2012). The pig was also employed as a model for experimentally induced allergic contact dermatitis, revealing similarities to human with respect to clinical, histological and immunohistological features (Vana and Meingassner, 2000). Recently a comprehensive study has shown that a panel of 93 monoclonal or polyclonal antibodies recognizing various human and porcine cell types and structures were cross-reacting between the two species (Debeer et al., 2013).

In the current review, we focus especially on the interest of pig in the study of skin immunology and particularly on the description and the comparison of the main immune cells such as DCs in this tissue.

2. The immunological components of the epidermis

2.1. Keratinocytes

Keratinocytes (KC) represent the first cellular line of defence in the skin and have been shown to express a wide range of TLR including TLR-1, TLR-2, TLR-4, TLR-5, and TLR-6 as well as endosomal TLR-3 and TLR-9. In addition, they are prone to activation via the inflammasome pathway (Di Meglio et al., 2011). As a result KC can secrete a wide range of pro-inflammatory cytokines, and of particular importance cationic antimicrobial peptides (AMP), such as the cathelicidins. These are believed to have an important protective role against bacterial skin infections. In humans the only cathelicidin known is LL37, while a total of 11 distinct cathelicidins have been described for pigs (Sang and Blecha, 2009). It is unknown which members of the porcine cathelicidins are produced by KC.

There are numerous publications in which porcine keratinocytes have been isolated and employed for wound healing studies, and we are referencing just a few (Eldardiri et al., 2012; Kiwanuka et al., 2011; Sullivan et al., 2001; Vardaxis et al., 1997; Yan et al., 2011). This includes also studies involving gene therapy (Pfutzner et al., 2006).

2.2. Langerhans cells

Langerhans cells (LC) represent the dendritic cell (DC) subset of the epidermis. In contrast to other DC, LC originates mostly from fetal liver precursors recruited to the epidermis during embryonic life. Thereafter, LC undergoes self-renewal throughout life, at least in steady-state conditions in mice. Nevertheless, under severe inflammation leading to local LC depletion, LC can be generated from circulating monocytes (Merad et al., 2013). Numerous studies have addressed the role of LC in immunity versus tolerance and its ability to prime naïve T-cell responses with conflicting results. It appears that LC are not required for T cell priming but are necessary to license effective cytotoxic responses. Interestingly, direct antigen presentation by LCs is required for Th17 cell differentiation and is promoted by LC-derived IL-6, IL-1β, and IL-23 (Bennett et al., 2011; Igyarto et al., 2011). This may represent an important antimicrobial amplification cycle as IL-17 members and IL-22 represent potent inducers of AMPs (Sonnenberg et al., 2011).

LC in the porcine epidermis have been described as $CD172a^{+}CD1^{+}CD16^{-}CD163^{-}CADM1^{+}CD207^{+}MHCII^{+}$ cells with a typical DC morphology (Marquet et al., 2011; Nfon et al., 2008).

2.3. Lymphocytes

The epidermis also contains a small number of T cells, which can be dominated by γδ TCR expressing T cells depending on the species. While human skin is dominated by αβ T cell, in murine skin γδ T cells are more prominent (Heath and Carbone, 2013). In

Table 1
Comparison between the skin of different species.

Criteria	Guinea pig	Human	Mouse	Pig	Rat
Skin attachment	Loose-attached	Firmly attached	Loose	Firmly attached	Loose
Hair coat	Sparse or dense	Sparse	Dense (except some breed)	Sparse	Dense (except some breed)
Epidermis	Thick	Thick	Thin	Thick	Thin
Dermis	Thick	Thick	Thin	Thick	Thin
Panniculus carnosus	Present	Absent	Present	Absent	Present
Healing mechanism	Contraction	Re-epithelialization	Contraction	Re-epithelialization	Contraction

the pig, WC1$^+$ γδ T cells were described to be occasionally present in the lower levels of the epidermis (Carr et al., 1994), but to the best of our knowledge the presence of αβ T cells is not described.

3. The immnunological components of the dermis

3.1. Dendritic cells

In the dermis, DC represents the dominating and most important immune cell type and multiple subsets of DC have been described. When addressing the complexity of DC subsets in the dermis, it is necessary to take the current DC classification into account, as the different DC subsets do possess important functional specializations. This classification, based on DC ontogeny and confirmed by transcriptomic analyses, differentiates bona fide DC, which are descendants of a common DC precursor, from DC with monocytic origin (monocyte-derived DC) (Malissen et al., 2014; Merad et al., 2013). Bona fide DC can be divided into conventional DC (cDC) and the plasmacytoid DC (pDC). cDC are specialized in antigen presentation and induction of primary T-cell responses, whereas pDC represent the "natural interferon producing cells" specialized in the production of very large quantities of interferon type I in response to stimulation by nucleic acids (Summerfield and McCullough, 2009). Both pDC and cDC also play an important role in the induction of both central and peripheral tolerance. cDC consist of at least two distinct subsets with functional specialization, which have been described in detail in the mouse. The murine "CD8α/CD103 DC" corresponds to the human "CD141 DC", and the murine "CD11b DC" are similar to the human "CD1c DC" (Haniffa et al., 2013; Malissen et al., 2014). An overview of the DC family is represented in Fig. 1. The "CD8α/CD141 DC" are specialized in cross-presentation of antigens to CD8 T cells and generally promote mucosal Th1 responses, while the "CD11b/CD1c DC" subset interacts with both CD4 and CD8 T cells and are more pro-inflammatory, for example by promoting Th17 cells (Schlitzer and Ginhoux, 2014). Nevertheless, although the transcriptomic profiles of these different DC subsets appear to be conserved across several mammals, it is important to note that these functional characteristics have been mostly defined for the murine immune system only.

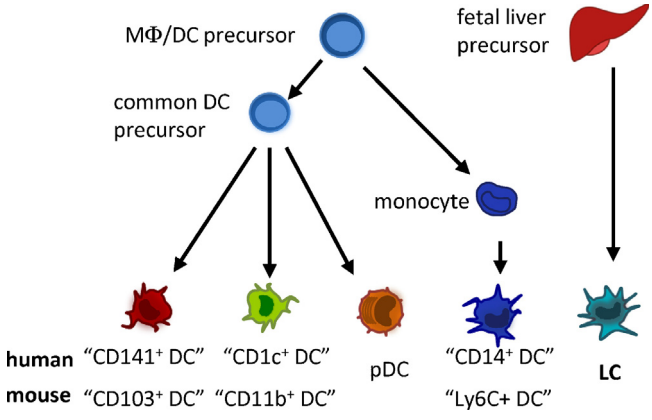

Fig. 1. Human and murine DC classification based on ontogenic development.

As mentioned above, many subsets of dermal DC have been described in man and mouse, which are reviewed in detail elsewhere (Boltjes and van Wijk, 2014; Heath and Carbone, 2013; Klechevsky, 2013). In human, the major subsets represent the CD1c$^+$CD141$^-$, the CD1c^{low}CD141$^+$ and the CD14$^+$CD209$^+$ dermal DC, the latter probably being of monocytic origin. It is likely that the human CD141$^+$ DC represent the equivalent of the murine CD8α/CD103$^+$ "cross-presenting" DC which is underlined by the expression of CLEC9A and XCR1 typically found on this subset. Accordingly, the CD1c$^+$CD141$^-$ would be equivalent to the murine CD11b$^+$ subset (Boltjes and van Wijk, 2014; Haniffa et al., 2013; Malissen et al., 2014) (Fig. 1). Under steady-state conditions plasmacytoid DC are difficult to find in the skin but they can significantly increase during inflammation (Boltjes and van Wijk, 2014).

A recent study has phenotyped porcine dermal DC and compared their phenotype to DC circulating in the skin-draining pseudoafferent lymph (Marquet et al., 2011). In the dermis as well as in the pseudoafferent lymph, a population of CD172a$^-$ and CD172a$^+$ DC were identified. Within the CD172a$^+$ subset the authors further proposed DC subsets based on the expression levels of CD163. All subsets defined differed in their expression of CD16, CD206, CD207, CD209 and CADM1 expression. The CD172a$^-$ subset in the dermis was negative for CD16, CD163, CD207 but expressed variable levels of CADM1. The CD172a$^+$CD163$^{-/low}$ were CD16$^+$, CD207$^+$ but CADM1$^-$. The further subset of CD172a$^+$ cells expressed high levels of CD16 and CD163 and was also CD206$^+$CD209$^+$. Considering that CD206 and CD209 are typically expressed on inflammatory monocyte-derived DC (MoDC) and Macrophages (MΦ) of mouse and man (Haniffa et al., 2013), this phenotype indicates a monocytic origin. Nevertheless, the CD163^{high} subset in the draining lymph differed significantly in the expression of CD206 and CD209, possibly indicating that this subset definition may not be ideal. CD14^{high} cells were proposed to represent skin MΦ (Marquet et al., 2011).

In our hands CD14 and CD209 expressing cells were rare in the dermis under steady state conditions but their number increased under inflammatory conditions. Chemically induced inflammation resulted in accumulation of a major CD209$^+$MHCII$^-$ and a minor CD209$^+$MHCII$^-$ subset in the dermis (Fig. 2). In addition, the frequency CD14$^+$ cells which were rare under steady state condition, increased. This population was partially CD209$^+$ (Fig. 2).

With the aim to propose a working hypothesis for the identification of these subsets we measured the expression of these molecules on monocyte-derived MΦ (MDM) and MoDC. In fact, MDM express higher levels of CD14 compared to MoDC (Summerfield and McCullough, 2009), but the opposite was observed for CD209 and MHCII (Fig. 3). This would indicate that CD209 could be associated with MoDC differentiation, although a previous report found comparable levels of CD209 on MDM

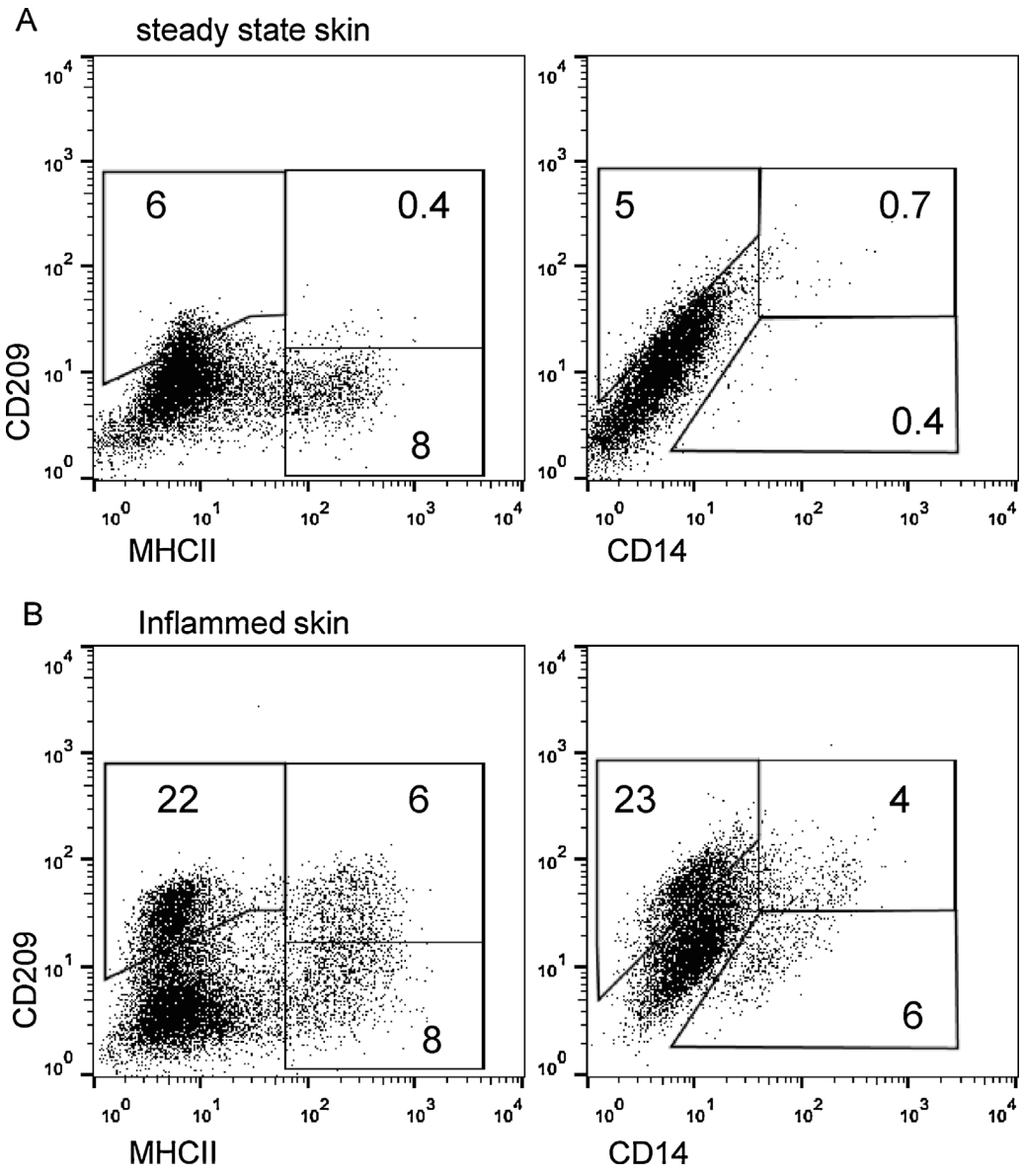

Fig. 2. CD209 and CD14 expression on porcine dermal DC/MΦ under steady state and inflamed conditions. Skin inflammation was induced by shaving, followed by topical application of 100 μl of 2% 1-chloro-2,4-dinitrobenzene (DNCB) in acetone and olive oil (1:3). After 24 h, the epidermis was removed and the dermis cut into small pieces, cultured for 24 h and dermal single cells suspensions prepared as previously described (Ricklin et al., 2010). The cells were immunophenotyped using three-color flow cytometry using MHCII (clone MSA3), CD14 (clone CAM36A) and CD209 (polyclonal antibody kindly provided by Prof. Dr. Meng, Virginia Polytechnic Insititute, Blacksburg, VA, USA (Huang et al., 2009)). The data is representative of three independent experiments.

Table 2
Comparative phenotype of human and porcine skin DC subsets.[a]

	"CD141$^+$ DC"		"CD1c$^+$DC"		pDC[b]		Inflamm. DC/MoDC		LC	
	hu[d]	po	hu	po	hu	po	hu	po[c]	hu	po
CD1a	–	–	+	+	–	–	+	+/–[c]	+	+
CD4[a]	–	–	+	–	+	+	–	–	–	–
CD14	–	–	–	–	–	–	+	+/–	–	–
CD123[b]	–	–	–	–	+	+	–	–[c]	nd	nd
CD135[b]	+	+	+	+	+	+	–	–[c]	nd	nd
CD163	–	–	–	low	–	–	+/–	+/low	–	–
CD172a	–	–/low	+	+	+	+	+	+	+	+
CADM1	+	+	–	–/low	–	–	–	–	+	+
CD206	–	–	–	–	–	–	+	+	–	–
CD207	–	–	–	–	–	–	–	–	+	+
CD209	–	–	–	–	–	–	+	+	nd	+/–

[a] Proposed porcine equivalent based on literature (Marquet et al., 2011), comparative phenotype and data presented in this manuscript.
[b] Data are from blood (Guzylack-Piriou et al., 2010; Summerfield and McCullough, 2009).
[c] In vitro data (Summerfield and McCullough, 2009).
[d] Human phenotype as reviewed (Boltjes and van Wijk, 2014; Haniffa et al., 2013; Merad et al., 2013).

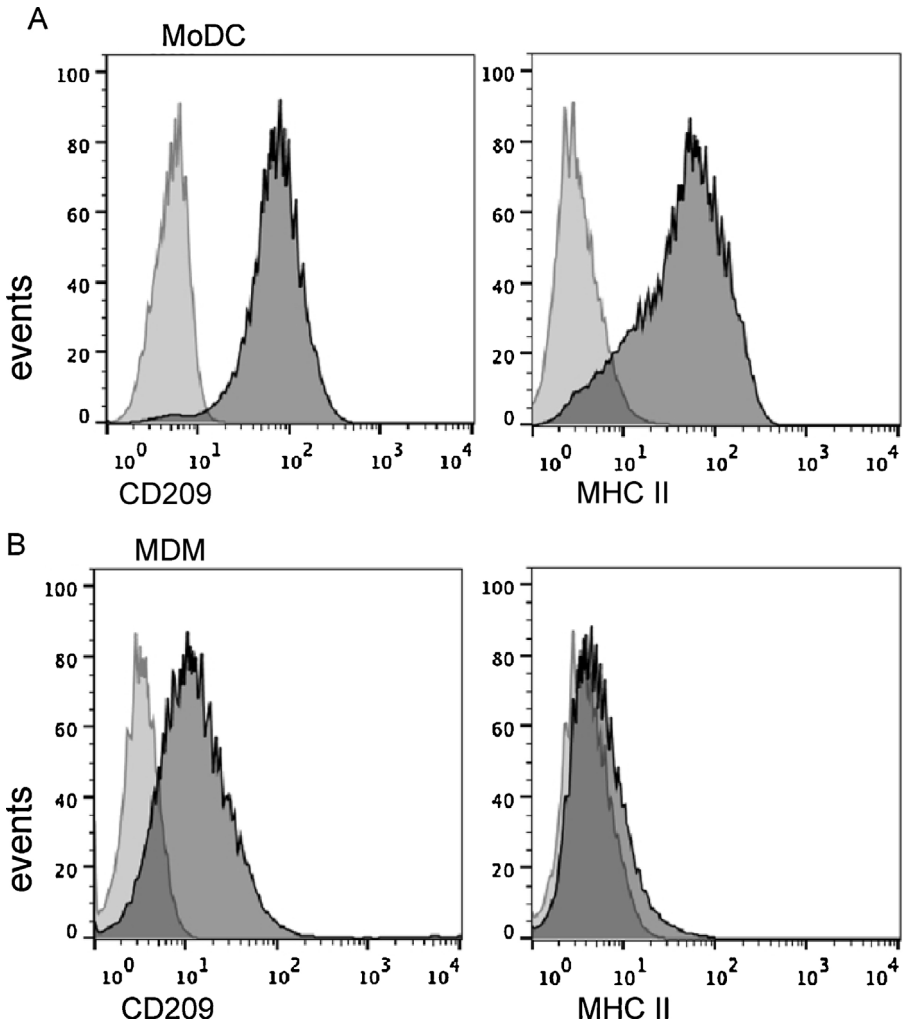

Fig. 3. Expression of CD209 and MHCII on porcine MoDC and monocyte-derived MΦ. The cells were generated as previously described (Carrasco et al., 2001).

and MoDC (Huang et al., 2009). To support this, we analyzed the expression of CD209 on blood DC and monocytes defined using six-color flow cytometry. Monocytes were identified as $CD14^+$ and blood DC as $Flt3^+$ cells (Fig. 4A (Guzylack-Piriou et al., 2010)). The $Flt3^+$ subset was further divided into a $CADM1^+$ and a $CADM1^-$ subset. Within the $CADM1^+$ cells two more subsets were defined based on CD172a expression, a $CADM1^{high}CD172a^{-/low}$ subset and a $CADM1^+CD172a^{high}$ subset. We propose that these subsets correspond to cDC1 and cDC2 as defined in Table 2 (see below). Within the $CADM1^-$, pDC were identified as $CD4^+$ cells (Fig. 4A) (Summerfield et al., 2003). Freshly isolated, all populations analyzed were $CD209^-$. After a 24 h culture period, the monocytes expressing high levels of MHCII acquired CD209. Within the $Flt3^+$ DC subsets, only a weak induction of CD209 was observed (Fig. 4B). Altogether, these data would indicate that $CD209^+$ DC in the dermis are more likely to be of monocytic origin, similar to human skin (Fig. 5).

Based on published data, the data presented in this manuscript and a comparative analysis of DC phenotypes in various species, Table 2 proposes a preliminary classification of these DC subsets according to the scheme in Fig. 1. This is based on a comparative analysis of pseudoafferent lymph DC, porcine blood DC and conserved markers of murine and human DC which we have reviewed in detail elsewhere (Summerfield et al., 2015). Although this classification requires experimental confirmation for example by a systematical transcriptomic analysis of sorted DC subsets, we have decided to summarize it in this review because we think that it will be helpful for the design of experiments by researches interested in the porcine skin or other tissues containing DC.

Plasmacytoid DC are well described in pigs in terms of phenotype and function (Summerfield and McCullough, 2009), but their presence in the skin is not directly described. Nevertheless, since that they have been found in the pseudoafferent lymph draining the skin of pigs under steady state conditions (Pascale et al., 2008), they should be present in the skin. Together with neutrophils, plasmacytoid DC are amongst the first cells to infiltrate injured and inflamed skin and probably play an important role not only in antiviral responses but also in wound healing (Gregorio et al., 2010).

Altogether, although the proposed classification of porcine DC subsets is preliminary, Table 2 indicates important phenotypic similarities between human and porcine skin DC subsets.

3.2. Macrophages

As mentioned above under steady state conditions MΦ are rare in the dermis but monocytes, which rapidly differentiate into MΦ are efficiently recruited after injury and inflammation. Two functionally distinct monocyte populations exist which differ

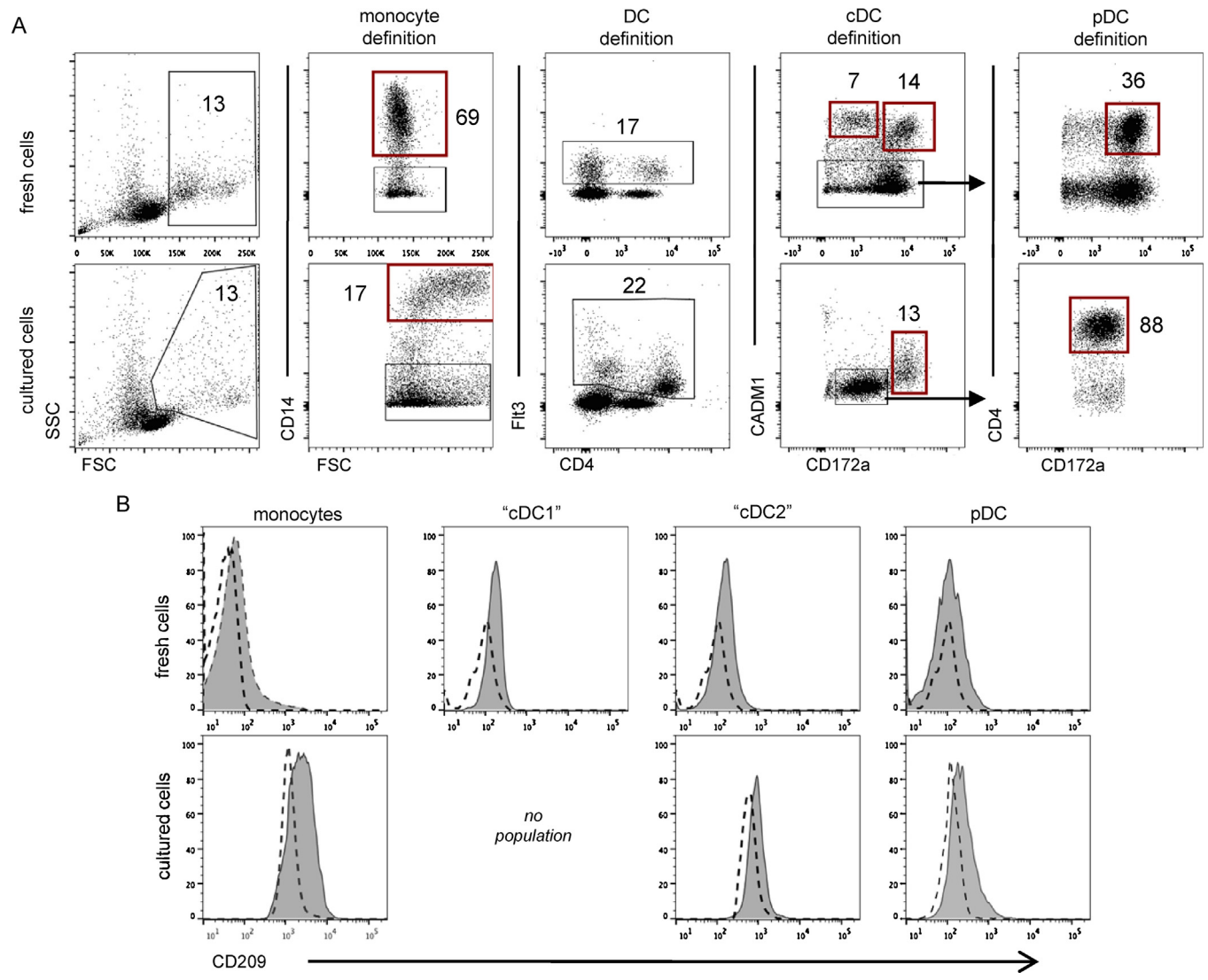

Fig. 4. Expression of CD209 on fresh and cultured blood DC subsets defined using six-color flow cytometry. PBMC were prepared and analyzed freshly or cultured for 18 h before immunophenotyping. Gates on cells with high forward and side scatter, followed by doublet discrimination gates were set. Monocytes were defined as $CD14^+$ cells and DC as $CD135^+CD14^-$ cells (Guzylack-Piriou et al., 2010). DC subsets defined by CD172a (clone 74-22-15, ATCC), CADM1 (clone 3E1, MBL, Woburn MA) and CD4 (clone PT90A, WSU, WA) were further gated as shown in A to determine the expression of the CD209 shown in B. Details of the experimental protocol can be obtained upon request.

in their expression of chemokine receptors, their inflammatory potential and their contribution to wound healing (Brancato and Albina, 2011). In humans, $CD16^+CCR2^{low}CX3CR1^{high}$ monocytes probably represent the orthologues of $Ly6C^{low}$ murine monocytes, whereas $CD16^-CCR2^{high}CX3CR1^{low}$ monocytes would be equivalent to $Ly6C^{high}$ murine monocytes (Auffray et al., 2009). Functionally equivalent subsets of monocytes have been described in the blood of pigs but not yet in the dermis. In the pig, all monocytes express CD16, but CD163 can help to differentiate the equivalents of the monocyte subsets described above. $CD163^+$ monocytes are $CCR2^{low}CX3CR1^{high}$ and $CD163^-$ monocytes are $CCR2^{high}CX3CR1^{low}$ (Ezquerra et al., 2009; Moreno et al., 2010). The phenotype of porcine MΦ has been reviewed previously (Ezquerra et al., 2009) but their clear differentiation from DC remains difficult (Summerfield and McCullough, 2009). Also in the porcine skin this issue requires clarification. Marquet et al. (2011) proposed that MΦ in contrast to DC are $CD14^+$. This is certainly possible but the observation that in vitro differentiated MoDC also remain $CD14^+$ (Carrasco et al., 2001) points on the possible existence of $CD14^+$ MoDC in the skin of pigs, similar to human.

3.3. Lymphocytes

Even under steady state conditions the dermis contains a large number of lymphocytes which has been estimated to even exceed the number of cells in the blood circulation. In fact, cutaneous leukocyte antigen (CLA), CCR4, and CCR10 determines T cell cutaneous tropism (Sheridan and Lefrancois, 2011) and the large majority of CLA^+ lymphocytes reside in the skin (Clark et al., 2006). Importantly, the skin contains many resident T memory cells crucial in immunity against infections (Gebhardt et al., 2009; Wakim et al., 2008). In addition, the majors subsets of $CD4^+$ T cells are present in particular Th1, Th2 and Th17, the latter know to play an important role against fungal and bacterial infections (Miossec et al., 2009). While porcine T cells are well described and the major subsets can be differentiated phenotypically (Mair et al., 2014), information on the presence and functions in the skin is still rare.

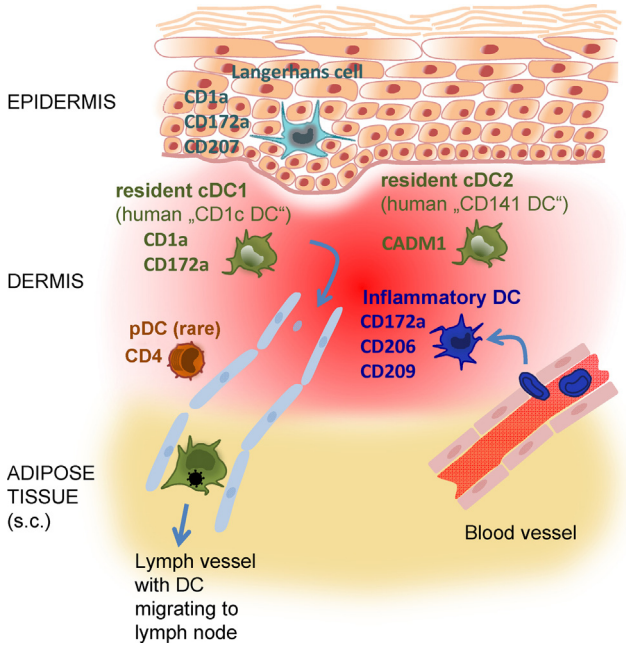

Fig. 5. Main DC subsets presenting in porcine skin. The most important markers to identify the subsets are indicated and more information can be taken from Table 1. Note that under steady state condition pDC are rare in porcine skin.

4. Conclusions

Although the porcine and human skin have remarkable similarities in structures and the pig has been extensively used as a model for research in dermatology, wound healing, drug delivery and toxicology, the knowledge of the immunological elements in the skin have not been extensively studied. Considering that the pig immune system is well described and a considerable tool box is available (Mair et al., 2014) there are great opportunities to further exploit the pig model for research in which other models do not offer the required key features described in this review, and have not provided the expected extrapolation for humans. This review also points on important knowledge gaps of the skin immune system, in particular with respect to knowledge on the immune populations present in the porcine skin. Nevertheless, we are convinced that certain fields of research such needle-free vaccine and drug delivery systems can greatly profit from the porcine model, and are only starting to evolve. Importantly, this will also be most useful for veterinary medicine. In conclusion, we believe that the pig skin model offers more possibilities than currently exploited and will further evolve with studies making use of the available information and tools, which will constantly increase.

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