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Review

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

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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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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