Abstract
Transcutaneous delivery of vaccines to specific skin dendritic cells (DC) subsets is foreseen as a promising strategy to induce strong and specific types of immune responses such as tolerance, cytotoxicity or humoral immunity. Because of striking histological similarities between human and pig skin, pig is recognized as the most suitable model to study the cutaneous delivery of medicine. Therefore improving the knowledge on swine skin DC subsets would be highly valuable to the skin vaccine field. In this study, we showed that pig skin DC comprise the classical epidermal langerhans cells (LC) and dermal DC (DDC) that could be divided in 3 subsets according to their phenotypes: (1) the CD163neg/CD172aneg, (2) the CD163neg/CD172apos (DDC) that could be divided in 3 subsets according to their phenotypes: (1) the CD163neg/CD172aneg, (2) the CD163neg/CD172apos, and (3) the CD163apos/CD172apos DDC. These subtypes have the capacity to migrate from skin to lymph nodes since we detected them in pso-afluent lymph. Extensive phenotyping with a set of markers suggested that the CD163neg DDC resemble the antibody response-inducing human skin DC/macrophages whereas the CD163neg/CD172apos DDC share properties with the CD8+ T cell response-inducing murine skin CD103apos DC. This work, by showing similarities between human, mouse and swine skin DC, establishes pig as a model of choice for the development of transcutaneous immunisation strategies targeting DC.

Introduction
Vaccines targeting skin, through intradermal or epicutaneous delivery, present several advantages compared to the vaccine given intramuscularly, such as dose sparing [1] and better activation of cytotoxic and mucosal responses [2,3]. Current knowledge supports that optimal induction of immune responses depends on the dendritic cell (DC) subtypes that are targeted by vaccines. Thus the identification of the DC subtypes in the skin and the analysis of their specialization function in immunity are key steps in the development of cutaneous delivered vaccines. Most information on skin DC has been obtained in the mouse model (for review see [4]). Mouse epidermis contains a unique DC subset, the Langherans cells (LC), that can be identified in the mouse by their high expression of Langerin (CD207). Some migrating LC, en route for the lymph nodes (LN), are also found in the dermis [5]. The role of LC is still unclear in the mouse, but converging results suggest that they behave as immunoregulatory cells [6,7,8]. Mouse dermal DC (DDC) comprise four distinct subsets, the discrete CD207apos/CD172apos/CD103apos, the CD207apos/CD172apos/CD103apos, the CD207apos/CD172apos/CD11bahigh and CD207apos/CD172apos/CD11bapos DDC subsets [5]. The CD207apos/CD172apos/CD103apos DDC have received much attention recently as they play a key role in cross-presentation for tolerance induction and in mounting a CD8+ T cell immune response [5,8,9]. Although playing a major role in specific immune responses, they represent less than 5% of the DDC [5,10], but 13% of the skin draining LN DC [5].

Much less data are available for the human skin DC. Human CD207bahigh LC have been identified in epidermis, but contrary to mouse, they were described as the most efficient skin DC subset to expand antigen specific CD8+ T cells by antigen cross-presentation [11]. Besides, two DC subsets (CD14apos and CD1aapos DDC) were distinguished in the human dermis [11,12], but they do not share cell surface phenotype with the mouse DDC. For instance, the CD207 marker was not found expressed by human DDC. The minor CD14apos DDC subset expresses several macrophagic markers such as CD163, DC-Sign/CD209 and the mannose-receptor/CD206, and was found to prime CD4apos T cells into cells that induce isotype switching in B cells. The largely represented CD1apos DDC subset was revealed to activate CD8+ T cells better than CD14apos DC but less efficiently than LC.

Results on human skin DC functions were generated in vitro, whereas the mouse DC functional data were obtained in vivo. These different experimental approaches may explain the discordant functional results obtained from the two species. Alternatively, intrinsic differences in the mouse and human skin structures such as the hairiness and the stratum corneum thickness might be associated to evolutionary divergences in DC subpopulation roles. Evaluation of novel vaccine strategies in vivo the skin would thus greatly benefit from a more relevant animal model that would permit in vivo investigation. Pig skin shares strong histological similarities with human skin, such as low hairiness, thick stratum corneum with similar lipid composition [13], and dermis structure [14]. CD207bahigh LC have been identified in pig
epidermis [15,16,17]. In addition, pig DC that had migrated from skin explants expressed CD1, CD172a, MHC-II and CD80/CD86 [15]. Finally, pig is a large mammal which permitted us to adapt for the first time pseudo afferent catheterism to swine skin lymph collection [18].

In this report, we thoroughly described and analyzed 4 swine DC subsets in epidermis, dermis and lymph, and we suggested possible correspondences with mouse and human skin DC. This provides a first analytical and dynamic picture on the swine skin DC establishing pig as a relevant model to study skin DC subsets in immune responses and to develop novel vaccine strategies.

**Results**

**Selection of the markers used for the discrimination of DC subtypes**

For FACS gating of DC from skin and lymph, we used the most widely recognized phenotypic definition of DC, as being large, MHC-IIhigh cells. We first checked, using MHC-II/CD14 staining, that CD14high dermal macrophages were not present in the MHC-IIhigh gate; similarly MHC-II/CD21 staining assured us that CD21pos dermal B lymphocytes were absent from the DC gate (Figure 1). We then tested a panel of antibodies available in pig allowing the detection of several proteins previously described on mouse and human DC (Figure 1). We thus chose to phenotypically characterize DC subpopulations distinguished by the expression of CD163 and CD172a (Figure 2).

**Definition and phenotype of four DC subtypes**

The complex marker expression patterns found on lymph and dermal DC suggested the existence of DC subpopulations. In human skin, CD163 has been described on a macrophage-like DDC subpopulation [11,12,24] whereas CD172a+ DC have been described in several species as endowed with cross-presentation capacities [5,8,9,21]. We thus chose to phenotypically characterize DC subpopulations distinguished by the expression of CD163 and CD172a (Figure 2).

Using CD163 and CD172a, 4 subpopulations could be segregated in the dermis: the quantitatively minor population (1) CD163negCD172a+ (9+/− 2%), the population (2) CD163highCD172apos (25+/− 3%), the population (3) CD163lowCD172apos, constituting the majority of the DDC (47+/− 7%) and the population (4) CD163negCD172a+ DDC (11+/− 5%). Population (1) was negative for the majority of the markers tested, with the exception of a bimodal expression of CD11R3 (CD11R3neg and CD11R3pos) and CadM1 (CadM1pos and CadM1neg) and a minor CD209neg subpopulation. The expression of CadM1 in this subpopulation (1) is in agreement with the described CadM1pos/CD172apos phenotype of cross-priming DC in mouse spleen [20] and sheep afferent lymph [21]. The CD163high DDC population (2) presented a strikingly high expression of CD16 and was positive for CD206 and CD209, in agreement with a 'macrophage like' phenotype [11,12,24]. The DDC population (3) was CD16low and presented a heterogeneous expression of CD207/Langerin. Finally, in the dermis the last population (4) highly expressed more complex expression of CD163, which discriminate 3 different DDC populations (negative, low and high CD163 expressing DCs), a homogenous expression of CD11R3 and almost no CadM1 detection. Epidermal DC were homogenous in the expression of all the markers tested, at the exception of CD209, which discriminated about 5% of positive cells from the main CD209neg population. We observed no expression of CD163 and CD206; low to null expression of CD16, depending of the animal; and a strikingly high expression of CD207.

In order to analyze the skin DC that migrate in lymph, lymph duct catheterism was conducted. FSC/SSChigh and MHC-IIhigh cells, represented around 1% of the total lymph cells. We next characterized lymph DC, they presented similar complex expression patterns as DDC for all the tested antigens, at the exception of CD209 which was not expressed on lymph DC.

**Figure 1. Phenotyping of swine DC in skin and afferent lymph.** FACS analysis of DC from dermis, epidermis and pseudo-afferent iliac lymph. Skin biopsies were first split in epidermis and dermis by dispase digestion of the basal layer, before overnight collagenase digestion of the dermis. Lymph DC were enriched by density gradient before staining. Dot-plots represent the gating strategy (FSChigh/MHC-IIhigh cells) used to electronically isolate DC from the different tissues. Histograms depict isotype control (plain histograms) versus indicated marker expression (bold, open histograms) and are representative of at least 3 independent experiments on 3 different animals.

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CD207. The epidermis presented one unique DC population, namely Langerhans cells (LC), constituting the vast majority of the epidermal DC (95\% \pm 3\%). They expressed the same levels of CD163 and CD172a than the dermal DC population (4). Moreover, with the exception of CD16 which is lower, and of CadM1 which is not expressed in the DDC population (4), LC and DDC population (4) expressed the same levels of the other markers tested, pleading for a common epidermal origin of these two DC populations.

In the lymph, the same 4 DC subpopulations were observed, however their distribution was quite different from skin (Table 1) since the minor dermal DC population (1) CD163neg/CD172aneg represented 26\% \pm 3\% of the lymph DC, whereas the subtype (2) CD163high/CD172apos, well represented in the dermis, represented only 24\% \pm 2\% of the lymph DC. The lymphatic distribution of these two subpopulations is significantly different from the distribution of their skin counterparts (p<0.001). The lymphatic distributions of populations (3) and (4) were not different from skin (respectively 52\% \pm 0\% and 13\% \pm 0\%).

Lymph DC subpopulations expressed mostly the same markers than their skin counterparts. The most striking similarities were: i) the CadM1 expression of the CD172aunm DDC population (1), ii) the CD16high profile of the CD163unm DDC population (2), iii) the CD207high expression of the DDC (4)/LC populations. The main differences between lymph and skin DC were the higher expression of CD16 on CD163low lymph DC (populations (3) and (4)) compared to their skin counterparts, a higher general expression of CD206 and a more homogenous weak to null expression of CD209 in all the lymph DC subpopulations (Figure 2).

In order to demonstrate that lymph DC subpopulations came from skin territories, we proceeded to skin FITC painting and we observed, after two days, lymph DC having incorporated FITC. The same lymph DC subpopulations (1), (2), (3) and (4) were observed in FITCpos DC than in FITC neg DC, although not exactly in the same proportions (Figure 3).

Thus, as emphasized in Table 1, we described here 4 different skin DC subtypes and followed their in vitro migration from skin to LN, through the afferent lymphatic.

Figure 2. Characterization of the 4 DC subsets present in skin and lymph. FACS analysis of DC from dermis, epidermis and pseudo-afferent iliac lymph. DC were gated using the same criterions as in Figure 1 (FSC\textsuperscript{high}/MHC-II\textsuperscript{high}). The expressions of the markers identified in Figure 1 were differentially reexamined on lymph and dermal DC subpopulations characterized as CD163\textsuperscript{neg}/CD172a\textsuperscript{neg} (1), CD163\textsuperscript{high}/CD172a\textsuperscript{pos} (2), CD163\textsuperscript{pos}/CD172apos (3), and CD163\textsuperscript{neg}/CD172apos (4). In epidermis, Langerhans cells (LC) were retrieved in the same gate than population (4) from dermis and lymph. Histograms depict isotype control (plain histograms) versus indicated marker expression (bold, open histograms) and are representative of at least 3 independent experiments on 3 different animals.

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In the lymph, the same 4 DC subpopulations were observed, however their distribution was quite different from skin (Table 1) since the minor dermal DC population (1) CD163\textsuperscript{neg}/CD172a\textsuperscript{neg} represented 26\% \pm 3\% of the lymph DC, whereas the subtype (2) CD163\textsuperscript{high}/CD172a\textsuperscript{pos}, well represented in the dermis, represented only 24\% \pm 2\% of the lymph DC. The lymphatic distribution of these two subpopulations is significantly different from the distribution of their skin counterparts (p<0.001). The lymphatic distributions of populations (3) and (4) were not different from skin (respectively 52\% \pm 0\% and 13\% \pm 0\%).

Lymph DC subpopulations expressed mostly the same markers than their skin counterparts. The most striking similarities were: i) the CadM1 expression of the CD172aunm DDC population (1), ii) the CD16\textsuperscript{high} profile of the CD163\textsuperscript{high} DDC population (2), iii) the CD207\textsuperscript{high} expression of the DDC (4)/LC populations. The main differences between lymph and skin DC were the higher expression of CD16 on CD163\textsuperscript{low} lymph DC (populations (3) and (4)) compared to their skin counterparts, a higher general expression of CD206 and a more homogenous weak to null expression of CD209 in all the lymph DC subpopulations (Figure 2).

In order to demonstrate that lymph DC subpopulations came from skin territories, we proceeded to skin FITC painting and we observed, after two days, lymph DC having incorporated FITC. The same lymph DC subpopulations (1), (2), (3) and (4) were observed in FITC\textsuperscript{pos} DC than in FITC\textsuperscript{neg} DC, although not exactly in the same proportions (Figure 3).

Thus, as emphasized in Table 1, we described here 4 different skin DC subtypes and followed their in vitro migration from skin to LN, through the afferent lymphatic.
Table 1. Skin and lymph DC sub populations.

|   | % (+/- SD) CD11R3 | CD16/FcγR3 | CD206/MR | CD207/Lang | CD209/DCSign | CadM1/SYNCAM |
|---|------------------|-------------|-----------|-------------|--------------|--------------|
| 1 | CD163<sup>neg</sup>/CD172a<sup>neg</sup> | Dermis 9% (+/- 2%) * | +/± - | - | - | +/± - |
|   | Lymph 26% (+/- 3%) * | + | +/± - | - | - | +/± - |
| 2 | CD163<sup>pos</sup>/CD172a<sup>pos</sup> | Dermis 25% (+/- 3%) * | +++ ++ - | - | + | ++ +/- |
|   | Lymph 2% (+/- 2%) * | +++ | + | - | +/± - | |
| 3 | CD163<sup>pos</sup>/CD172a<sup>pos</sup> | Dermis 47% (+/- 7%) | +++ + - | + and - | - | - |
|   | Lymph 52% (+/- 8%) | ++ | ++ | + and - | +/± | |
| 4/LC | CD163<sup>neg</sup>/CD172a<sup>neg</sup> | Epidermis 95% (+/- 3%) | +++ ++ - | +++ + and - | ++ |
|   | Dermis 11% (+/- 5%) | +++ | + | - | +++ - | |
|   | Lymph 13% (+/- 8%) | ++ | ++ | + | +++ +/- | ++ |

Four DC subpopulations have been characterized in lymph and skin. Percents +/- standard deviation (SD) represent the mean of at least 4 experiments on different animals.

*Represent significant differences (student T test p < 0.001) in the percentage of DC subpopulations between dermis and lymph. Expression intensity for each antigen is depicted from (++++): Highly expressed, to (+/-): Very low expression, to (-): No expression at all. (+ and -) means that there is a bimodal expression of the antigen. The markers allowing the best discrimination between DC subtypes are highlighted in bold.

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Figure 3. Skin FITC painting attests that all the lymph DC subtypes migrate from skin. Skin FITC painting allows the detection of DC migrating from skin to lymph. FITC at 1 mg/ml in acetone/dibutylphthalate (V/V) was applied on the leg of the animal, lymph was collected before painting and 2 days after painting, and lymph cells were then stained for MHC-II, CD172a and CD163. DC were gated using the same criterions as in Figure 1 (FSC<sup>high</sup>/MHC-II<sup>high</sup>). FITC<sup>-neg</sup> and FITC<sup>pos</sup> DC subpopulations are depicted. Data are representative of two independent experiments.

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In situ characterization of skin DC

In confocal experiments, anti-CD1 antibodies appeared to more distinctly stain DC than anti-MHC-II antibodies. Moreover we and others [15] have checked that MHC-II and CD1 molecules were co-expressed on the vast majority of skin cells. We thus used CD1 instead of MHC-II to detect skin DC in situ. CD1 staining allowed the unambiguous distinction between skin DC and macrophages, since preliminary experiments showed that skin macrophages were CD1neg/CD14high whereas skin DC were CD1pos/CD14low (data not shown). Using CD1 or MHC-II as DC markers, we were not able to distinguish CD172a neg from CD172apos DC (Figure 4A), however CadM1 expression (Figure 4H), a signature of CD172a neg DC, was observed in the dermis. CD11R3 appears highly expressed on DDC and on LC (Figure 4B). As expected from flow cytometry analysis CD163, CD206 and CD209 were observed on a fraction of DDC, but not on LC (Figures 4C, E, G). CD16 was expressed on a DDC subpopulation, but also, at a weaker level, on LC (Figure 4D). As expected, CD207 was expressed on LC, but also on some DDC (Figure 4F). Finally CADM1 expression appeared in the epidermis on cells along the basal layer, corresponding to undifferentiated keratinocytes, but also on LC and on some DDC (Figure 4H). In addition, different staining combinations confirmed that CD163apos DDC expressed CD16, CD206 and CD209, but neither CD207 nor CadM1 (data not shown). Thus we were able to detect in situ the 4 skin DC subpopulations defined by Facs analysis. Except for the LC, present in the epidermis, no preferential localization of the 3 DDC subpopulations in upper or lower dermis was observed.

CD172a neg/CD163 neg DDC and lymph DC transport apoptotic bodies

In rat mesenteric lymph [25] and sheep oro-nasal and skin lymph [26], a CD172a neg DC subset has been described that contains cytoplasmic apoptotic DNA. The pig skin derived CD172a neg DC subtype (1) appeared CD11R3 low/CD163 neg/CD16-neg/CD207-neg/CD209 neg and strikingly CADM1 pos (Figure 2). It could be detected both in the dermis and the afferent lymph. We thus tested these DC for the presence of cytosolic apoptotic bodies. TUNEL pos cytoplasmic inclusions were mostly found in the CD172a neg DC from lymph and dermis (Figure 5). Moreover CD163 and TUNEL co-stainings showed the presence of apoptotic bodies uniquely into CD163 neg DC from lymph and dermis (data not shown). Finally no TUNEL staining was observed in LC (data not shown). These results indicate that transportation of apoptotic bodies from skin to lymph is a specific property of the CD172a neg/CD163 neg dermal DC subpopulation in pig.

Discussion

Using two markers selected for human and murine DC characterization, CD163 and CD172a, we described four DC subpopulations present in pig skin. Thanks to the new surgical protocol we developed, we were able to collect pseudo afferent

Figure 4. In situ skin DC phenotyping. Sections of normal swine skin were acetone/methanol fixed and stained for the indicated specificity (green) and CD1 (red). A. CD172a, B. CD11R3, C. CD163, D. CD16, E. CD206, F. CD207, G. CD209, H. CADM1. Antibodies used are described in Materials and Methods. Images are representative of at least 5 pictures from different regions of biopsies from at least 3 different animals, independently stained. Objective used 40x oil immersion. E: Epidermis, D: Dermis, Scale bars = 10 μm.

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draining swine skin, and to observe that all the skin DC subsets we described did have the capacity to migrate in the lymph. Our goal was to define specific DC subtypes that could be targeted in skin vaccination. We thus considered of great importance to ascertain that each of these subtypes had the capacity to migrate through the lymph toward the LN, where they would eventually encounter the naïve lymphocytes in order to mount the desired immune response.

We more precisely characterized the already described [17] swine epidermal LC as CD163<sup>neg</sup>CD172a<sup>pos</sup>CD207<sup>high</sup> which allowed us to identify their lymph-migrating counterpart, representing 11% of total afferent lymph DC. This subpopulation was also observed in the dermis, likely representing LC en route for the lymphatic. We defined three other DDC subpopulations: the CD163<sup>neg</sup>CD172a<sup>neg</sup> DDC subtype (1) transports apoptotic bodies and is observed in equivalent proportion (20%) in swine and sheep skin lymph [26]. Its mouse (CD172a<sup>neg</sup>/CD103<sup>pos</sup>) [5], human (CD172a<sup>neg</sup>/BDCA3<sup>pos</sup>) [20] and sheep (CD172a<sup>neg</sup>/CD26<sup>pos</sup>) [21] counterparts are endowed with unique cross-priming capacities. Of note, the human BDCA3<sup>pos</sup> DC have been observed in blood but have not yet been described in skin. Unfortunately neither CD103 nor BDCA3 nor CD26 antibodies are available in pig. CD163<sup>neg</sup>CD172a<sup>neg</sup> DDC subtype (1) does not express CD207/Lang, at the difference with mouse CD172a<sup>neg</sup>/CD103<sup>pos</sup> whereas, LC/population (4) is CD172a<sup>pos</sup>/CD163<sup>neg</sup>/CD16<sup>neg</sup>/CD207<sup>high</sup>/CadM1<sup>pos</sup>; in addition FITC painting revealed that at least one part of these lymphatic DC subpopulations originate from skin. Altogether these parameters plead for the fact that skin DC subpopulations 1, 2 and 4 might be the actual precursors of their lymphatic counterparts. We observed that the CD172a<sup>neg</sup> population (1) is overrepresented in afferent lymph compared to dermis. Interestingly, a higher migration rate has been proposed to explain the higher percentage of their mouse (CD172a<sup>neg</sup>/CD103<sup>pos</sup>) counterpart in LN (13% of the DC) than in skin (5% of the DDC) [5]. Conversely, in swine, the CD163<sup>high</sup> DDC population (2) is underrepresented in afferent lymph. These results might be interpreted by differences in their residence time in the tissue at steady state, the CD163<sup>high</sup> DDC having a low turnover whereas the CD172a<sup>neg</sup> DDC would have a more rapid total turnover in the normal skin. It is important to note that we analyzed lymphatic DC present in the lymph more than 10 days post-surgery to eliminate the consequences of inflammation due to surgery. Conversely this work was performed on healthy conventional pigs, which might imply some background inflammation. Nevertheless, we consider these conditions comparable to what can be obtained when studying human skin samples [11,12].

Figure 5. CD172a<sup>neg</sup> DC from lymph and dermis contain apoptotic bodies. Lymph DC were partially enriched by low density gradient. Skin biopsies were first split in epidermis and dermis by dispase digestion, before overnight collagenase digestion of the dermis. Dermal DC were then enriched by Ficoll gradient. DC were further purified using MHC-II and magnetic beads staining followed by magnetic separation. Lymph and Dermis DC were then cyto-centrifuged, fixed in PFA and reacted with anti-MHC-II (red) and anti-CD172a mAb (blue). Cells were further labelled for apoptotic DNA by the TUNEL method (green). Data are representative of 3 independent experiments.

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This work was aimed to define DC subpopulations in swine skin and to find their lymph migrating steady state counterparts. This first step accomplished, we are currently devising a transcriptomic study of the different swine skin subpopulations described here, in order to confirm their assignment to their murine, human [20] and sheep [21] counterparts. Moreover the definition of swine skin DC subsets and their assignment to their mouse counterparts will allow the design of subset targeting vaccines by the use of chimeric antibodies targeted to specific DC subsets. Antibody-based DC targeting has recently been shown to be exceptionally efficient in mouse models after a single vaccination [for review see [29]]. For instance, in mice, targeting CD11b<sup>hi</sup> DC using DCIR2 [29], Dectin-1 [30] or CIRE/DC-Sign [31] induced CD4 responses whereas targeting CD16<sup>hi</sup> spleen DC using anti-DEC-205 [29] or Clec9A [31] induced CD8 responses. Our description of swine DC subsets and the transcriptomic work in progress, will lead to the determination of target antigens specifically expressed on swine DC subtypes, allowing the development of this technology in swine which, in addition to its great interest in animal health, would be an important proof of principle for its translation in human medicine.

In conclusion, this study describes a skin DC network in swine similar to that of mouse and human. Finally the skin structure similarities between man and pig, now comforted by immunological homologies, allow us to propose the use of pig as a model of choice for the expanding field of transcutaneous vaccinations.

### Materials and Methods

**Ethics statement**

This study was carried out under licenses from the Direction of the Veterinary Services of Versailles (accreditation n°A78-93, A78-15, A78-730). This study was approved by the Regional Paris South Ethics committee (n°08-001).

**Pseudo afferent lumbar lymph duct catheterism in miniature pigs**

Pseudo-afferent lumbar lymph duct catheterization was performed in two steps. First, LN draining the flank, hindquarters, and inguinal areas were surgically resected. Two months later, after lymph vessel healing, a retro-peritoneal surgery was performed for inserting a silicone catheter (4 french, Nutricath bSQ, Vygon, Ecouen, France) in the lumbar trunk. The catheter was led out through a skin opening into a flask containing 500 units heparin. To facilitate lymph draining, pigs were left free in their cage. Lymph was collected twice a day. Cells were step-frozen in FCS containing 10% DMSO. Low-density lymph cells were obtained after centrifugation on a 1.065 density iodixanol gradient (Optiprep; Nycomed Pharma, Paris, France). The anti-CD209/DC-Sign is a kind gift from X.J. Meng [32].

**Immunochemical staining of skin samples**

Skin biopsies were snap-frozen in OCT (Sakura, Paris, France) and conserved at -80°C. Cryosections (5 µm) were cut using cryotome (Leica CM3050S, Nanterre, France). Sections were air-dried, fixed in cold methanol/acetone and stained using previously described anti-swine antibodies and isotype-specific secondary antibodies. Sections were mounted in SlowFade mounting medium (InVitrogen). Slides were examined on a LSM510 confocal microscope (Zeiss, LePecq, France), using a 40X, oil immersion objective.

**TUNEL staining of skin and lymph DC**

Magnetically enriched MHC-II<sup>pos</sup> DC were detected on slide by cytoplasmic staining. Cells were air-dried, fixed in 4% PFA before surface staining using previously described antibodies. TUNEL staining was then performed according to manufacturer instruction (R&D, Lile, France).

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Author Contributions

Conceived and designed the experiments: IS NB. Performed the experiments: FM MB FP CU CK IS NB. Analyzed the data: FM IS NB. Wrote the paper: NB IS FM.