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Tailored immunity by skin antigen-presenting cells

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Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DDC, dermal dendritic cell; DLN, draining lymph node; IL, interleukin; IM, intramuscular; InfDC, inflammatory DC; LC, Langerhans cell; moDC, monocyte-derivated DC; SC, subcutaneous; TC, transcutaneous

Skin vaccination aims at targeting epidermal and dermal antigen-presenting cells (APCs), indeed many subsets of different origin endowed with various functions populate the skin. The idea that the skin could represent a particularly potent site to induce adaptive and protective immune response emerged after the success of vaccinia virus vaccination by skin scarification. Recent advances have shown that multiple subsets of APCs coexist in the skin and participate in immunity to infectious diseases. Induction of an adaptive immune response depends on the initial recognition and capture of antigens by skin APCs and their transport to lymphoid organs. Innovative strategies of vaccination have thus been developed to target skin APCs for tailored immunity, hence this review will discuss recent insights into skin APC subsets characterization and how they can shape adaptive immune responses.

Introduction

Each year, vaccination programs across the world prevent between 2 and 3 million needless deaths, protecting children against deadly diseases such as measles, polio, diphtheria, tetanus and pertussis with the ultimate aim to induce immune memory for life-time protection and in all individuals.[1] Vaccine leaders from around the world discuss critical issues surrounding the development of effective—and affordable/acceptable—vaccines. Intramuscular (IM) or subcutaneous (SC) routes, widely used for vaccination, have proven to be successful in inducing systemic humoral immunity toward several pathogens but generally failed to induce efficient and long-term cellular protection. Vaccine development faces difficulties to manipulate the appropriate arms of the immune system in predictable ways for vaccine efficacy. The nature and intensity of the acquired immune protection are variable depending of targeted pathogens and host responses. After a period of abandon that have followed the successful era of vaccinia virus vaccination and eradication of smallpox, the skin routes of vaccination have regained interest during the past two decades.

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Our increased understanding of the high immune potential of skin resident and inflammatory cells, as well as the urgent need to improve the immunogenicity of vaccines against infectious diseases (HIV, HCV, influenza…) and cancers motivates the development of innovative strategies targeting the cutaneous tissue. Numerous concepts for vaccine delivery to the skin layers have been developed in the past decade. However, some key mechanisms of vaccination remain to be elucidated in more details: (1) the nature and function of antigen-presenting cells (APCs) that can favor the initiation of immune response; (2) the molecular and cellular networks involved in skin immunization. These parameters are important to ensure optimal initiation of both innate and adaptive immunity. The challenge today is to understand how to manipulate skin APCs to increase vaccine efficacy. We propose here an overview of our recent knowledge on skin APC and inflammatory cells and their potential to modulate adaptive immunity for optimal development of vaccination strategies.

Characterization and Functions of Epidermal Antigen-Presenting Cells

Langerhans cells

The epidermis of mice and human is composed of stratified layers of squamous and keratinized cells—keratinocytes—that protect the integrity of the skin.[2,3] As the epidermis is not vascularized, nutrient supply is dependent on the dermis. The solely professional APCs that populates the epidermis, namely Langerhans cells (LCs), represent 1–5% of all epidermal cells. LCs are located mainly at the suprabasal layer of the epidermis and form a network around keratinocytes.[4] They can be identified by the constitutive expression of Langerin (CD207), and the presence of unique intracytoplasmic organelles known as Birbeck granules, which have a role in antigen uptake.[5] CD207 is a type II C-type lectin receptor homogeneously expressed at the surface of human and murine LCs that, when triggered by its ligands, is internalized in Birbeck granules, a hallmark of maturation.[6,7] CD207 expression is not exclusive to LCs as other dermal DC populations have been found to express it, as described below. Human and mouse LCs also express E-cadherin, a molecule that mediate adhesion to keratinocytes,[8] epithelial cell adhesion

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molecule (EpCAM), CD205 (DC-SIGN), and class-II major histocompatibility complex (MHCII) molecules (**Table 1**). In addition, human, but not mouse, LCs also express high levels of the CD1a molecule which is able to present non-peptidic antigens to T cells.[9]

LCs key features include radioresistance, longevity and immunosurveillance of the skin.[10] In sharp contrast with other DC subsets, LC repopulation did not depend on transplanted bone-marrow cells after lethal irradiation of mice but rather on skin local precursors.[11] Similarly, LCs from parabiotic mice, which share a common blood circulation, failed to mix, thus supporting the idea that they maintain themselves independently of circulating precursors during steady-state.[11] Conversely, it was shown that in inflammatory conditions, another type of LCs, named short-term LCs, arise from Gr-1^{hi} monocytes soon after UV light-induced depletion.[12,13] This subset is progressively replaced by long-term LCs that arise from bone marrow precursors in steady-state. A study by Nagao et al. further described this mechanism and revealed that hair follicles act as entry portals for monocyte-derivated LCs in a C-motif chemokine receptor (CCR) 2 and CCR6-dependent manner.[14]

Consistent with their role in skin immunosurveillance, LCs appear to be very motile even at steady-state. Indeed Kissenpfennig et al. demonstrated that 2 to 3% of LCs circulate naturally from the skin to the draining lymph nodes (DLNs), passing through the dermis where they can be identified as "en route" LCs.[15] This process involves constitutive expression of chemotractants such as C-motif chemokine ligand (CCL) 20 by lymphatic vessel endothelial cells, hence providing constant source of antigen from the cutaneous environment for the induction of tolerance. Accordingly, migratory LCs have also been found in healthy human skin DLNs.[16]

The differential expression of PRRs between APC subsets probably account for the diversity of the immune responses they can induce.[17] Following TLR engagement, reception of appropriated signals, and capture of antigen, maturated, and differentiated APCs migrate to the DLNs where they will promote clonal expansion of antigen-specific naïve T cells (**Fig. 1**). To do so, they exhibit unique physical properties that allow them to migrate through confined space such as lymphatics.[18] Upon inflammation, resident LCs drastically change in morphology and motility and increase their expression of CCR7, which ligands CCL19 and CCL21 are constitutively expressed by endothelial cells of the lymphatic vessels.[19] This phenomenon, largely mediated by cytokines of the IL-1 family and TNF-α produced by keratinocytes, has to overcome the autocrine effect of tumor growth factor (TGF)-β, which by upregulating E-cadherin and down-regulating CCR7 promotes LC retention in the skin.[20]

The extensive use of Langerin-Diphteria Toxin Receptor (Lang-DTR) transgenic mice, which can be conditionally depleted of Langerin$^+$ cells, has unravelled multiple functions of LCs in vivo.[15] Consistent with their role of "gatekeeper" of the skin, LCs appear to have dual functions. First, and because the skin in continuously challenged with non-pathogenic microorganisms such as commensals and auto-antigens, LCs have unique immunosuppressive and tolerogenic properties.[21] D Kaplan's group demonstrated that targeted depletion of LCs increased antigen-specific T cell counts in a mice model of contact hypersensitivity, showing that LCs act as regulator of immune responses.[22,23] Accordingly, LCs have been shown to promote and activate antigen-specific T regulatory cells (Treg) in the course of Leishmania infection,[24] allergy contact dermatitis,[25] and after targeting with myelin oligodendrocyte glycoprotein (MOG).[26] In humans, Seneschal et al. demonstrated that in absence of exogenous antigen, LCs, but not dermal DCs, constitutively promoted local proliferation and activation of skin resident memory CD4$^+$ Treg.[27] However, after infection with *C. albicans*, the study also demonstrated that LCs were capable to induce effector memory non-regulatory T cells in situ.

The spatial localization of LCs within the skin makes them the first APCs to encounter environmental antigens, and as so, they must be able to induce potent and broad responses to foreign antigens. Indeed several studies suggest that LCs can efficiently prime naïve T cells and induce their helper or cytotoxic functions. Igyarto et al. elegantly demonstrated that LCs are necessary and sufficient to induce immunity to yeast (*C. albicans*) and

Table 1. Mouse and human APC subsets and their associated markers in healthy skin

Mouse subsets	Phenotype	Human subsets	Phenotype
Langerhans cell	CD11b^{int}, CD207$^+$, CD205$^+$, CD103-, CD172a$^+$	Langerhans cell	CD1a^{hi}, CD1c$^+$, EpCAM$^+$
XCR1$^+$ DDC	CD11b^{low}, XCR1$^+$, CD207$^+$, CD103$^{+/-}$, CLEC9A$^+$, CD172a$^-$	CD141$^+$ DDC	CD1a$^{+/-}$, CD14$^{+/-}$, CD1c$^{low/int}$
CD11b$^+$ DDC	CD11b$^+$, XCR1$^-$, CD172a$^+$	CD1a$^+$ DDC	CD1a^{int}, CD1b$^+$, CD1c$^+$, CD14$^-$, CD208$^+$
CD11b^{low} DDC	CD11b^{low}, XCR1$^-$, CD172a$^+$	-	-
Monocyte-derived DC	CD11b$^+$, Ly6C$^+$, CD64$^{low/+}$, MERTK$^{-/low}$	CD14$^+$ DDC	CD1a$^-$, CD14$^+$, CD1c$^+$, CD163$^+$, DC-SIGN$^+$
Dermal Macrophage	CD64$^+$, MERTK$^+$	Dermal Macrophage	CD14$^+$, CD1a$^-$, CD1c$^-$, FXIIIa$^+$
Plasmacytoid DDC	CD11b$^-$, B220$^+$, PDCA1$^+$	Plasmacytoid DDC	CD123$^+$, CD303$^+$, CD304$^+$

Four conventional DC subsets have been described in mice, which can be identified by their level of expression of a group of markers. In addition, murine skin contains myeloid monocyte-derived DCs and dermal macrophages. Plasmacytoid DCs are almost absent in healthy skin. Human counterpart DCs are identified based on the expression of CD1a, CD1c, CD14, and CD141. High expression of the cellular marker is denoted by $^+$, while intermediate, low, and lack of expression are denoted by^{int,low}, respectively. DDC, dermal dendritic cell.

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extracellular bacteria (*S. aureus*) by promoting induction of Th17 cells.[28] Evidences also suggest that LCs can mediate Th2-like cellular responses after epicutaneous sensitization with protein antigens.[29] Similarly, freshly isolated human LCs are endowed with the capacity to stimulate allogeneic CD4$^+$ T cells toward a Th2 profile in vitro.[16]

The ability of LCs to cross-prime foreign antigens and initiate cytotoxic CD8$^+$ T cells responses has long been controversial. Early studies either suggested that LCs were efficient in cross-presentation[30,31] or that they were not involved.[32] However, these studies did not take into account the existence of the CD207$^+$ CD103$^+$ dermal subset, as is was not identified at the time. Later on, Henri et al. showed that CD207$^+$ CD103$^+$ were the solely skin DC subset able to cross-present self-antigens using a mouse model in which keratinocytes constitutively expressed membrane-bound ovalbumin.[33] However, another study demonstrated that LCs are involved in the acquisition of effector functions by CD8$^+$ T cells that infiltrate the skin during TLR-induced inflammation.[34] Thus, rather than being responsible for cross-presentation of self-antigens during steady-state, LCs could be able to interact closely with CD8$^+$ T cells during an inflammatory response. Accordingly, Liard et al. showed that after intradermal immunization of mice with a particulate antigen, LCs were the main subset responsible for the generation of IFN-γ-secreting CD8$^+$ T cells in DLNs.[35] Interestingly, after intradermal delivery of plasmid DNA, a second wave of LCs from epidermal precursors—reminiscent of long-term LCs described in[13]—was present in DLNs two weeks after immunization and was essential for CD8$^+$ T cell priming.[36] Conversely, primary LCs residing in the epidermis at the time of vaccination could not prime CD8$^+$ T

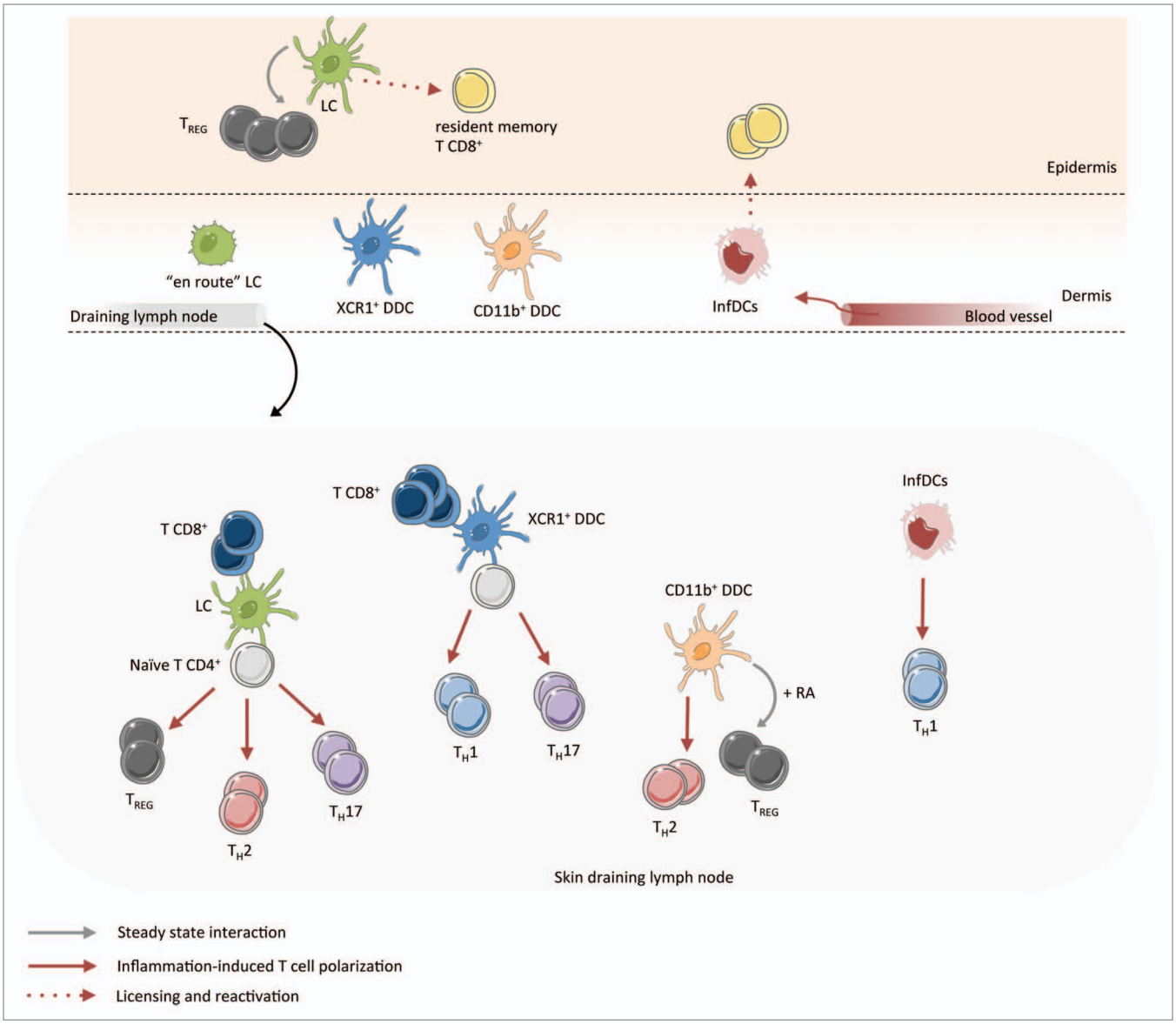

Figure 1. Mouse skin APCs shape adaptive immune responses. APC populations of the epidermis and dermis have high propensity for modulation of adaptive immune responses. This figure summarizes published works describing interaction between skin APC and T cell in skin and draining lymph node at steady-state or during inflammatory responses. InfDC, inflammatory dendritic cell; LC, Langerhans cell; T_H, T helper; T_{REG}, regulatory T cell.

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cells. Of note, bone marrow-derived CD207$^+$ dermal DCs also contributed to CD8$^+$ T cell priming.[36] These findings fits nicely with the view that steady-state, non-inflammatory LCs that are used to encounter skin antigens are tolerogenic and thus enable to efficiently prime naïve CD8$^+$ T cells, whereas LCs that are newly generated upon inflammation and entry of foreign antigen could prime naïve CD4$^+$ and CD8$^+$ T cells. However, a recent study on skin scarification with Vaccinia Virus showed that LCs are not involved in CD8-mediated immune responses.[37]

The contribution of LCs to humoral immunity has not been much studied. For instance, LCs were shown to induce protective IgG1 in serum after patch immunization with *S. aureus*-derived toxin, which revealed LCs ability to project their dendrites through tight junctions and sample antigens that have not yet breached the epidermal barrier.[38]

In humans, most studies have focused on the ability of LCs to activate and induce differentiation of naïve T cells in vitro. Human LCs preferentially induce Th2,[39,40] Th17,[41] Th22[42] and are able to cross-present antigens to CD8$^+$ T cells.[40,43]

Overall, these findings suggest a high plasticity of LCs according to the context. That is, the combination of signals they receive from the environment during steady-state or when encountering the antigen, as well as the nature of the antigen itself and its location, would shape their functions toward immunogenicity or tolerance.

Keratinocytes

Keratinocytes account for 80% of epidermal cells and although acting as structural cells, they exert key innate immune functions. Keratinocytes express various pattern recognition receptors (PRRs) such as Nod-like receptors (NLRs) 1 and 2, Toll-like receptors (TLRs) 3, 4, 5, and 9, and C-type lectins that recognize pathogen-associated molecular patterns (PAMPs), which in turn trigger activation of pro-inflammatory pathways. Recognition of PAMPs by keratinocytes lead to production of various pro-inflammatory signals such as CXC chemokine ligand (CXCL) 8, 9, 10, 11, CCL2, CCL20, tumor necrosis factor α (TNF-α), interleukin (IL) 1, 6, 10, 18, and 33.[44-46] Sugita et al. demonstrated that in mice, those innate signals where able to improve Langerhans cell (LCs) presenting functions.[47] Furthermore, the expression of MHC class II molecules on their surface make them non-professional APCs able to present antigen to CD4$^+$ T cells and stimulate their proliferation in certain conditions such as skin disorders.[48,49]

Characterization and Functions of Dermal Antigen-Presenting Cells

The dermis, which is mainly composed of fibroblasts, represents the skin connective tissue layer. As blood and lymphatic vessels are present throughout the dermis, it hosts multiple immune cell populations that vary dramatically from steady-state to inflammatory conditions. In mice, four conventional dermal DC subsets can be distinguished based on surface expression markers (**Table 1**). Analysis of mice transgenic for CD207 (Langerin-EGFP) revealed that in addition to "en route" LC that transit through the dermis, at least two other dermal DC subsets express CD207 and differ from LCs in origin and function.[15,50-52] These subsets express low amounts of CD11b and can be further characterized by expression of the marker CD103 (**Table 1**). They develop from a blood-borne pre-DC progenitor and acquire their phenotype and functions through the action of granulocyte/macrophage colony-stimulating factor (GM-CSF).[10,53] It has been recently proposed that these populations should be referred to as a so-called XCR1$^+$ DC subset, based on the unique expression of this marker.[54]

Other DC subsets include CD11b$^+$ DCs, lacking CD207, CD103, and XCR1 expression and representing the majority of dermal DCs,[33,55] as well as a minor population of CD11b^{low} DCs.[56] The CD11b$^+$ population was long considered as an heterogeneous population that overlapped with other subpopulations that had not been - or poorly - characterized, including conventional dermal DCs (cDCs), dermal macrophages, monocytes, and monocyte-derived DCs (moDCs).[33,57,58] The recent benchmarking study by Tamoutounour et al. further subdivided steady-state CD11b$^+$ dermal cells into distinct subsets of cDCs, moDCs, macrophages, and monocytes, using c-mer proto-oncogene tyrosine kinase (MerTK), CD64, and CCR2 markers and gene expression comparison.[59] In this study, blood Ly-6C^{hi} monocytes were found to constitute a pool that continuously generated skin resident monocytes as well as moDCs at steady-state, while cDCs developed on a Fms-like tyrosine kinase 3 ligand (Flt3L)-dependent and CCR2-independent manner.[59] The origin of dermal macrophages appears less clear. While some resident dermal macrophages derive from monocytes, another pool seems to seed the dermis before birth from yolk-sac progenitors.[60] Whether these differential origins can be linked to differential functions has not yet been elucidated. At steady-state, resident macrophage function is primarily to survey the skin and react quickly after detection of foreign antigen. Upon inflammation, blood monocytes can further differentiate into specialized macrophage subset such as pro-inflammatory "M1", regulatory "M2" or wound-healing macrophages.[61] Healthy human dermal DC subsets include "en route" LCs, CD14$^+$ DCs, CD1a$^+$ DCs, and CD141$^+$ DCs, which resemble murine LCs, mo-DCs, CD11b$^+$ DCs, and XCR1$^+$ DCs respectively.[62] Their associated markers can be found in **Table 1**. The overall complexity to segregate dermal APCs by surface markers in line with the development of systems biology approaches fostered the identification of transcriptional signatures of cell subsets.[63-65] The use of transcription factor-deficient mice unravelled developmental relationships and functions of certain subsets.[66] However, the phenotyping of cells based on intracellular markers requires permeabilization, which unable cell sorting by flow cytometry for further uses.

Plasmacytoid DCs (pDCs) are absent from non-inflamed skin and essentially act as contributors of skin wound healing and TLR7-mediated skin inflammation.[67] As members of the innate immune system, their functions will not be further discussed here.

During pathogen- or vaccine-driven inflammation, immune populations of the dermis vary dramatically, with rapid infiltration of inflammatory cells from blood capillaries under the

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influence of pro-inflammatory cytokines and chemokines, while skin resident APCs sample foreign antigens and migrate to cutaneous DLNs where they prime naïve T cells (**Fig. 1**).[68]

Inflammatory DCs (infDCs) are a hallmark of inflammation induced cell recruitment. InfDCs arise from blood Ly-6C^{hi} CCR2^{+} monocytes and are thought to differentiate under influence of Macrophage-Colony Stimulating Factor (M-CSF).[69] It is however not fully understood what are the cellular source for M-CSF neither if other factors contribute to InfDCs differentiation. One feature that differentiates InfDCs from steady-state moDCs is the fact that they can upregulated CCR7 and thus migrate to DLN and present antigens, thought in relatively smaller number than CD11b^{+} cDCs.[59] InfDCs have been sometimes referred to as Tip-DCs (for TNF-α/inducible NO synthase (iNOS)-producing-DCs), however monocytes and activated macrophages can also express iNOS and TNF-α. In addition, Tip-DCs were actually found not to express the DC-specific zbtb46 transcription factor in *Listeria*-infected mice while InfDCs expressed it.[70,71] During inflammation, the massive recruitment of monocytes also helps to repopulate the skin with newly generated LCs[12] as well as dermal DCs,[72] which left the skin to migrate to DLNs. In addition, neutrophils can be found within an hour after skin injury and participate in the production of cytokines and chemokine that help recruitment and activation of monocytes.[73]

XCR1^{+} Dermal DCs

Dermal XCR1^{+} DCs comprise two populations of CD11b^{low} CD207^{+} cells that either express CD103 or not.[54] As this nomenclature is relatively recent, these cell subsets were often referred to as CD207^{+} dermal DCs, irrespectively of their expression of CD103. XCR1^{+} dermal DCs resemble the XCR1^{+} CD8α^{+} DCs found in lymphoid and non-lymphoid organs, which are particularly efficient in cross-presentation; therefore it was initially assumed that their unique function was to cross-present skin-derived and viral antigens.[74,75] Accordingly, XCR1^{+} dermal DCs specifically express TLR3, which recognize double-stranded RNA, and Clec9A, which bind to components of dead cells.[74,76] CD207^{+} dermal DCs reportedly induced CD8^{+} T cell responses to self-antigen[33] and after vaccinia virus,[37] and leishmaniasis infection in mice.[77] However, in contrast with CD8α^{+} DCs, CD207^{+} dermal DCs have been found to promote differentiation and function of CD4^{+} T helper effector cells. Induction of Th1 responses to bacteria[28] and experimental autoimmune encephalomyelitis (EAE),[53] and Th17 responses upon skin infection with herpes simplex virus have been noted in mice.[78] Using a systems biology approach, Haniffa et al. recently identified the human homolog of mice XCR1^{+} DCs, namely CD141^{+} dermal DCs, which also express XCR1 and Clec9A and efficiently cross-present antigens to CD8^{+}T cells in vitro.[79] In addition, vitamin D3 was described as a potent inducer for the generation of CD141^{+} DCs-like DCs in vitro, which promoted Treg differentiation and suppression of xeno-graft vs. host disease and tumor alloimmunity in mouse model.[80]

CD11b^{+} Dermal DCs

Multiple myeloid cell subsets of the dermis express CD11b. The expression of the marker CD64 differentiates conventional DCs (CD64-) from monocytes and moDCs (CD64low/$^{+}$).[59] Their function has been extensively studied in vivo in mice, however due to possible contamination with other CD11b^{+} myeloid cells, previous studies must be interpreted with care. Nevertheless, under steady-state condition, CD11b^{+} DCs found in skin and associated DLNs produce retinoic-acid, which is involved in the generation of Treg.[81] This observation was surprising, as retinoic acid production is largely mediated by CD103^{+} DCs in other organs such as the gastro-intestinal tract.[82] What mechanisms underlie this functional difference between the skin and the mucosa are not known. Polarization of Th2 cell responses is also a feature of CD11b^{+} dermal DCs. The initiation and progression of skin allergic inflammation involve a thymic stromal lymphopoietin (TSLP)-responsive DC subset expressing high levels of CD11b and able to drive differentiation of Th2 cells in mice.[83] Whether their non-responsive counterpart form a distinct subset with different functions remains to be elucidated. Accordingly, conditional depletion of dermal CD301b^{+} DCs, a population of CD11b^{+} DCs that do not express CD207, impaired Th2 cell development upon infection with *N. brasiliensis* or with others well-known Th2 cell-inducing adjuvant in mice.[84] Not all CD11b^{+} DC express CD301b, therefore this subset may represent a specific population dedicated to Th2 immunity. In humans, the corresponding CD1a^{+} dermal DCs represent the major subset of the dermis. They strongly express the CD1c (BDCA-1) marker and low levels of CD1a as compared with human LCs. Both CD1a+ dermal DCs and LCs are found in T cell-rich areas of skin DLNs and appear to have similar properties of antigen cross-presentation to CD8^{+} T cells, as well as capacity to promote differentiation of CD4+ T cells into Th2 cells in vitro.[85,86] For a comprehensive review on human dermal DC functions, see ref. 62.

Monocyte-Derivated and Inflammatory DCs

In absence of inflammation, murine and human skins contain low numbers of moDCs that develop from continuously extravasating Ly-6C^{hi} or CD14^{+} monocytes respectively.[40,59] In mice, steady-state moDCs express Il-10 transcripts, suggesting that these cells could exert immunosuppressive functions. When pulsed with OVA protein, they induced proliferation and IFN-γ production by OT-I CD8^{+} T cells and OT-II CD4^{+} T cells in vitro, though to a minor extend than CD11b^{+} dermal DCs.[59] Sorted human CD14^{+} dermal DCs produce IL-10 and TGF-β, and have been shown to inhibit cytotoxic T lymphocyte responses and preferientially polarize pre-activated CD4^{+} T cells into T follicular helper cells and stimulate B cell isotype class-switching.[40,85] Therefore, even in absence of strong inflammatory signals, moDCs are able to present the antigen and stimulate proliferation of naïve T cells in vitro. However, wether these cells exert specific function in vivo remains to be determined.

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The ability of inflammatory moDCs (InfDCs) to prime T cell responses is less clear. Following hapten-induced skin inflammation, only few numbers of newly differentiated InfDCs can upregulate CCR7 and migrate to DLNs, and their T cell stimulatory properties are very low.[40,43] In addition, InfDCs were found to overexpress type-I IFN-related transcripts as compared with steady-state moDCs.[59] Thus, under particular inflammatory conditions, InfDCs preferentially remain in the tissue, where they produce pro-inflammatory signals that stimulate the innate arm of immunity. Accordingly, dermal InfDCs have been shown to activate skin natural killer (NK) cells and memory $CD8^+$ T cells even in the absence of antigen, through secretion of IL-15 and IL-18, after microbial infection.[87] An heterogeneous group of inflammatory cells producing large amounts of TNF-α and iNOS has also been referred to as Tip-DCs. This population appears to have direct microbicide functions but poor T cell inductive properties, mirroring the phenotype of InfDCs that are generated upon sterile inflammation.[88]

López-Bravo et al. demonstrated that subcutaneous infection with *leishmania* induced efficient migration and induction of Th1-biased cellular responses by infected InfDCs.[89] If this infection model may not be representative of what occurs during natural infection, it nonetheless reveals that InfDCs could be able to migrate to DLN and initiate adaptive immunity in the context of skin vaccination. In patients with psoriasis, sorted InfDCs induced allogeneic T cell to differentiate into Th1 and Th17 cells.[90] Likewise, Segura et al. demonstrated that InfDCs isolated from patients suffering from rheumatoid arthritis or untreated inflammatory tumors were able to induce Th17 cell differentiation in vitro.[91] Thus, it seems likely that these cells can exert different functions according to the inflammatory context. In regards to what happens in other tissues, InfDCs would primarily act to stimulate antigen-experienced rather than naïve T cells.[88]

Targeting of Skin APCs by Vaccination

Intramuscular and subcutaneous vaccinations are the main routes currently used for conventional vaccines. However, the muscle and the subcutaneous tissue represent poor inductive site as they contain few, if any, numbers of APCs.[92] A tremendous body of literature points out the critical role played by APCs in initiating the adaptive immunity, that is required for protection against pathogens.[93] Recent advances in the understanding of skin APC populations and functions, in line with development of new devices make the skin particularly attractive for vaccination. Here we will briefly discuss how skin APCs can be targeted by transcutaneous and intradermal routes of vaccination.

Spatial Targeting of Skin APCs

Several methods have been developed in the past few years, which enable the targeting of skin immune actors at different depths. Antigen can be directly delivered into the dermis by conventional intradermal needles, microneedles, or pressure-injector (e.g. gene gun). Several clinical trials have compared intramuscular and intradermal routes of vaccination for a wide array of pathogens, including rabies, hepatitis B virus, and influenza, showing similar or superior immune responses by intradermal route with lower antigen dose.[92] In mice, we showed that intradermal immunization with HIV-p24 protein-loaded particles induced superior humoral and cellular responses in serum and mucosa as compared with subcutaneous and intramuscular routes.[94] This seems to be largely mediated by LCs, as their migration to the dermis and subsequent capture of the antigen was shown to be responsible for $CD8^+$ T cell responses.[35] Injection of vaccine into the dermis induces strong inflammatory responses even in absence of adjuvant, however it also induces higher local side effects and pain than conventional routes of vaccination.[95]

Passive transcutaneous vaccination consists in a topical application of vaccine compounds. As only small molecules with high lipophilicity can penetrate through intact epidermis,[96] targeting of epidermal LCs and other dermal DCs can be rendered possible by transfollicular penetration through hair follicles.[92] Hair follicle opening allows large molecules and particles (less than 300 nm in diameter) to flow and penetrate the epidermis and the dermis.[97] Recently, Vogt et al. described the penetration and uptake of HIV-p24 protein-loaded particles after cyanoacrylate stripping of human skin explants. More importantly, this process allowed maturation and activation of epidermal LCs that efficiently uptaked the particles.[98] Accordingly, we have shown that transcutaneous anti-influenza vaccination induced both $CD4^+$ and $CD8^+$ T cell responses in humans, which were superior to that obtained after intramuscular immunization.[99,100] Other methods to target epidermal cells include microneedle and needle-free patches. Standardized micron-scale needles (25 µm and 1 mm) can be grouped in microarrays and enable large molecules to enter the epidermis through microperforations.[101] The use of dissolving polymer microneedle patches has been shown to induce robust humoral responses to influenza in mice.[101] Also, antigens such as bacterial toxins can be delivered directly onto the skin[102] or deposed on needle-free patches[103] after disruption of the stratum corneum, which allow their passive transport throughout the epidermis and induce serum and mucosal humoral responses in both mice and humans.[102-104]

Thus, different methods exist that allow spatial targeting of either or both epidermal and dermal APCs, which could be used to potentiate vaccine responses.

Physical Targeting of Skin APCs

Several classes of vaccines based on micro or nanoparticles could be used to aim vaccine compounds specifically at skin immune cells. Interesting candidates include virus-like particles (VLPs), DNA and attenuated viral vectors, as well as a family of polymeric biodegradable nanoparticles, such as poly-D L-lactide-co-glycolide and poly-D, L-co-glycolic acid (PLGA), poly-D, L-lactic acid (PLA), and others, that can carry proteins, peptides or DNA. It is clear that the nature and size of the antigen have

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an impact on its uptake by APCs. Particles of 20 to 200 nm in diameter can be internalized by professional APCs in a clathrin-dependent manner, whereas larger particles (0.5 and 5 μm) are taken up by phagocytosis (or macropinocytosis) by macrophages. Using antigen-loaded poly(lactide-co-glycolide) (PLGA) particles of different sizes ranging from 300 nm to 17 μm, Joshi et al. elegantly demonstrated that maximal uptake and activation by DCs were reached with the smallest particles in vitro.[105] This is in accordance with our observation that 40 nm particles are found in greater numbers than 200 nm or modified vaccinia Ankara (MVA) particles in DLNs of transcutaneously immunized mice.[97] Therefore, nanoparticles represent an efficient antigen delivery system for optimized targeting of skin APCs.

Specific Targeting of Skin APCs

There are several ways by which the interaction between antigen and APCs can be improved for vaccination. For example, because DCs express high levels of mannose receptors, mannosylation of the antigen-delivery system has been proposed to improve the targeting and activation of APCs.[106] Another strategy to improve the efficiency of nanoparticle-based vaccines is endocytic pathway targeting, by using C-type lectin receptors. Cruz et al. demonstrated that targeting the DC-SIGN improved antigen processing by human DCs and thus resulted in increased antigen-specific T-cell activation with reduced antigen concentrations 10 to 100-fold.[107] Similarly, several TLR and NOD ligands have been incorporated into PLGA nanoparticles, such as TLR-4 ligand (7-acyl lipid A),[108] TLR-9 ligand (CpG),[109] and NOD 1 and NOD 2 ligands (CL235 and CL365, respectively)[110] which enhances the immunogenicity of particle-based vaccines by increasing APC activation.

More specifically, vaccine-induced immunity could also be tailored by targeting selected APC populations by using antigens that are coupled with antibody specific for surface molecules.[111] For instance, targeting of the XCR1$^+$ DC-specific C-type lectin receptor Clec9A (also known as DNGR-1) with antibody coupled to antigen induced efficient cross-presentation and induction of CD8$^+$ T cells.[112,113] When anti-Clec9A antibody was coupled with a tumor-expressed peptide, development of B16 melanoma lung pseudometastases was prevented, and eradication of tumor cells enhanced.[112] Likewise, targeting of antigen to CD205, which is expressed on the surface of LCs and some dermal DCs, resulted in induction of tolerance.[114] Thus, targeting of CD205$^+$ skin DCs could induce deletion of pathogenic CD8$^+$ T cells and improve prognosis of skin allergic inflammatory diseases such as psoriasis and atopic dermatitis, as demonstrated in a murine model of type I diabetes.[115] These few examples clearly illustrate how vaccines could be designed to specifically target distinct subsets of skin APCs, thus tailoring adaptive immunity.

Conclusion

Skin routes of vaccination have proven high efficacy and efficiency. For instance, intradermal vaccination has achieved protective humoral immunity against major pathogens using lower dose of antigens as compared with conventional intramuscular and subcutaneous routes. It is now clear that this has to do with skin unique and rich immune network, which by its diversity and propensity for modulation can tailor the adaptive arms of immunity. The future of skin vaccination will benefit from our increased knowledge of APC functions and interactions, not only allowing the improvement of immune response intensity, but also its quality, polyfunctionality and persistency. By spatially, physically, and specifically targeting specialized APC subsets, future vaccines might be able to tailor immune responses against infectious diseases and tend toward a personalized medicine that is adapted to the individual.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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