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Towards a Better Understanding of *Staphylococcus aureus* Skin Infections-The Interactions with Dendritic Cells

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Abstract

Staphylococcus aureus (*S. aureus*) is the leading cause of skin and soft tissue infections in humans. Additionally, local infections further lead to dissemination and colonization of secondary infections sites including the lungs, heart valves and even medical prostheses. It is well known that this bacterial species is capable of altering host immune responses and that long-term protection against *S. aureus* is not completely effective. Antigen presenting cells represent key players in the implementation of these responses. Among these, dendritic cells (DCs) represent a wide variety of cell subsets that are heterogeneously distributed throughout the skin. As sentinels of the epithelial barrier, they are often the first cells in contact with pathogens and play a crucial role in the activation of specific T cell responses. In its planktonic form, the interactions between *S. aureus* and various DC cell subsets have been extensively studied in *in vitro* and *ex vivo* models. However, the tendency of bacteria to transition towards the biofilm lifestyle in the host suggest a necessity to study these interactions under *in vivo* conditions. Of note, mouse skin infection models provide a cheap and easy support to study the longitudinal responses of DCs through the use of fluorescence and intravital imaging. The development of models capable of comparing *S. aureus* planktonic and biofilm *in vivo* DC responses could prove essential in explaining the chronic nature of biofilms or the absence of an effective protective response. In this review, we highlight the different DC subsets found in the skin and their roles during *S. aureus* skin infections. We also address how this bacterium is capable of subverting these functions and review the known literature of existing mouse skin infection models that have and could potentially aid in the study of *S. aureus* planktonic or biofilm immune responses.

Keywords: Dendritic cells, *Staphylococcus aureus*, Skin, Immunity, Biofilm, *In vivo* models

Introduction

In healthy individuals, *Staphylococcus aureus (S. aureus)* can be found commensally in sebaceous sites in the skin microbiome [1]. Dysbiosis in the skin flora, abnormal skin barrier functions and immune abnormalities, such as in patients suffering from immunodeficiency or immunosuppressive treatments, predispose to the development of staphylococcal skin infections [2-4]. Indeed, these cocci are often isolated from patients suffering from chronic inflammatory skin diseases such as atopic dermatitis (AD) and recurring furunculosis, diseases that can be further exacerbated by the presence of *S. aureus* biofilms [3,4]. However, little is known about how this bacterial lifestyle interacts with the immune system in favor of chronicity [1,5].

The skin represents a specialized niche of immunity, as keratinocytes and Langerhans cells (LC) also participate in immune responses by the recognition of pathogen associated molecular patterns (PAMP), the antigen presentation and the secretion of antimicrobial peptides and cytokines [3,4]. The latter participate in the recruitment of professional phagocytes such as monocytes (MO), macrophages (MΦ) and polymorphonuclear neutrophils (PMN). These effector cells are implicated in the clearance of bacterial infections *via* phagocytosis and the formation of neutrophil or

macrophage extracellular traps [6-8]. In addition, dendritic cells (DC) play the role of antigen presenting cells (APC) where they induce adaptive immune responses, that can be recalled in subsequent infections [9].

The adaptive cellular responses to *S. aureus* skin infections have mainly implicated T helper (Th)17 cells. *Via* the secretion of interleukin (IL)-17A and IL-17F, these cells enhance the barrier function and antimicrobial properties of epithelial cells, while also increasing recruitment of PMNs [9]. In a murine immunization model, Th1/Th17 populations were upregulated in skin lesions of immunized mice, accompanied by an increase in PMN recruitment, resulting in a decrease of skin lesion area and bacterial burden in comparison to naïve mice [10]. Supporting this concept, IL-17A from γδ T cells has also been described in the control of *S. aureus* cutaneous infections [11,12]. Similarly, in patients with Hyper IgE Syndrome or HIV, characterized by impaired Th17 formation, an increased susceptibility to *S. aureus* skin infections is observed. However, the beneficial actions of these cells are not observed in the case of AD patients colonized by AD specific *S. aureus* strains where a Th2 immune environment is induced in lesional skin [3]. Outside of AD, a protective role of Th2 responses have been demonstrated in animal models [9,13].

Clearly, the mechanisms regulating T cell activation in the context of *S. aureus* skin infections remains unclear. Further, the presence of biofilms adds a second level of complexity to the subject. Although the impact of *S. aureus* biofilms in relation to skin infections is not well documented, the chronic nature of both entities suggests an impaired adaptive immune response incapable of mediating bacterial clearance. At the center of T cell coordination, DCs play a critical role as APCs that could potentially contribute to these ineffective responses.

In this review, we discuss the different DC populations in the skin and their responses towards *S. aureus* in a cutaneous setting. We address the mechanisms employed by *S. aureus* to counter DC functions and lastly, we review the known literature on the specific interactions between biofilms and DCs in the skin.

Dendritic Cells Subsets in the Skin

Skin is composed of two anatomical distinct layers: the epidermis and the dermis. The outer epidermis is a highly stratified-epithelium mainly composed of keratinocytes, and separated from the underlying dermis by the basement membrane. The dermis is made of various stromal cells that include fibroblasts, and houses blood and lymph vessels, sweat glands and hair follicles. Skin DCs are divided into LCs in the epidermis, and dermal DCs (dDCs) in the dermis that are closely related to conventional or classical DCs (cDCs) subsets present in lymphoid tissues. They represent heterogeneous populations of highly specialized phagocytic cells. As professional APCs, their main function is to transport cutaneous antigens to skin draining lymph nodes (dLN) and to present them to naïve $CD4^+$ and $CD8^+$ T cells to initiate adaptive immune responses. They also interact with skin resident and infiltrated cells at steady state or under inflammation to modulate both innate and adaptive immune responses.

Dendritic Cells at Steady State

At steady state, the only resident APCs in the epidermis are LCs, which represent 3-5% of epidermal cells, with approximately 700 LCs/mm^2 [14]. They are related to MΦs and have unique properties. Indeed, they self-renew in the epidermis and originate, in mouse, from yolk sac- or fetal liver-derived hematopoietic precursors rather than bone marrow Hematopoietic Stem Cells [15]. Compared with dermal cDCs, LCs express lower major histocompatibility complex class II (MHC-II) levels, intermediate CD11c levels and very high levels of the C-type lectin Langerin (CD207) [16,17]. In humans, LCs are further subdivided into LC1 and LC2 subsets that differ in abundance and also transcription profile [18]. As sentinel cells, they continuously sense the external environment and maintain tolerance to skin commensals by establishing connections with the surrounding epithelium *via* protruding dendrites [19,20]. When skin inflammation occurs, they take up antigen and transport it to the dLN to initiate T cell responses. Their migratory capacity to lymph nodes T cell zone is similar to that of cDCs [20,21].

Dermal DCs originate from blood-borne precursors known as pre-cDCs. In the skin, 3 distinct subsets of migratory dDCs are found: $XCR1^+$ dDCs (or cDC1), $CD11b^+$ dDCs (or cDC2) and double negative dDCs (For reviews see Malissen 2014 [23]; Kashem 2017 [15]; Collin 2018 [21]). $CD11b^-XCR1^+$ dDCs represent a minor population of DCs in human and mice that express high levels of CD207. They include $CD103^+$ and $CD103^-$ cells. They are able to quickly migrate to the deep T cell area of the LN. $CD11b^+$ dDCs are the most abundant sub-population of DCs in the dermis at steady state. They express the CD11b and CD1c markers respectively in mice and in humans, and other numerous markers both in human and mice as $CX3CR1^+$, SIRP α^+, $CCR2^+$ and $CD11c^+$. Classically, the presence of CD14 is used to distinguish MOs and MΦs from cDCs, although a novel $CD1c^+$ $CD14^+$ DC subset that remain phenotypically distinct from MOs, has been identified in the human dermis [22]. After migration, dDCs are directed into the peripheral paracortex of the LN. Double negative dDCs represent a small population of dDCs exclusively present in the mouse dermis. Their phenotype is $XCR1^-$ $CD207^-$ $CD11b^{lo}$ [15,21,23].

Finally, $LY6C^{hi}$ MOs migrate from the blood circulation in a CC-chemokine receptor 2 (CCR2)-dependent manner to generate dermal monocyte-derived DCs (mo-DCs) that are detected in the skin at steady state. Their transcriptional

program is similar to that of CD11b$^+$ dDCs, but their capacity to migrate and activate CD4$^+$ and CD8$^+$ T lymphocytes is lower.

Dendritic Cells during Inflammation

Inflammatory DCs appear transiently when inflammation occurs. Contrary to LCs and dDCs which are present at steady state and enriched in inflammatory conditions, inflammatory DCs disappear when skin returns to a steady state, once infection is cured. They represent a highly heterogeneous populations of MHC-II$^+$ CD11c$^+$ cells, with no unique surface markers. Their origin is currently not well established, with a predominant hypothesis as MO-derived cells (For reviews see Kashem 2017 [15]; Collin 2018 [21]). During skin inflammation, they are recruited in a CCR2-dependent manner, and play a major role at the site of inflammation [24].

Plasmacytoid DCs (pDCs) are interferon alpha (IFNα-producing population of cells, with a similar origin as cDCs. They are detected in the blood circulation and in lymphoid tissues at steady state [15]. In humans, pDCs located in tonsillar crypts and oro-nasopharyngeal epithelium can be exposed to extracellular bacteria such as *S. aureus* [25]. During inflammation, they are also present in the skin, playing distinct roles in inflammatory skin diseases [15,26]. In mice, they express membrane markers shared with members of the B cell family, including B220 and Ly6C. In humans, pDCs are CD11c^{lo}, CD11b$^-$, MHC-II^{low}, CD123/IL- 3R$^+$, CD303 (BDCA-2)$^+$ and CD304 (BDCA-4)$^+$ [7,15]. When activated, they secrete high concentrations of IFNα, tumor necrosis factor alpha (TNFα and IL-6 and upregulate the CD86 membrane marker [25,27]. Additionally, Chen et al. (2020) have identified a transient pDC-like subset, that infiltrate human skin wounds, expressing CD11c$^+$, HLA-DR$^+$, CD123$^+$ and CD1a$^+$ [28].

Dendritic Cell Activation after *S. aureus* Recognition in the Skin

In the skin, cutaneous DCs can recognize *S. aureus* antigens through their toll like receptors (TLR). The most documented pattern recognition receptor (PRR) in this regard is TLR2, found on the plasma membrane, which can sense a diverse range of PAMPs, namely bacterial peptidoglycan (PGN), acetylated lipoproteins and lipoteichoic acid, the latter involving heterodimerization with TLR6 along with CD36 [29,30]. CD207 and macrophage galactose-type lectin (CD301) expressed in LCs and dDCs respectively have also been shown to interact with specific carbohydrate motifs located on *S. aureus* wall teichoic acids (WTA) [31,32]. The intracellular TLRs 8 and 9 recognize ssDNA and dsDNA respectively, but have not been described in human LCs [33].

The binding of PRRs to their PAMPs leads to the internalization of bacteria by phagocytosis, inducing DC maturation, characterized by the expression of MHC and costimulatory molecules, and their migration towards skin dLNs, where they present antigens to naïve T cells. The latter has also been shown to occur directly in the skin, following infection, where DC-T cell clusters were observed at the perivascular area in the dermis of various animal models [15,34]. Recently, the expression of activity-regulated cytoskeleton associated protein/activity-regulated gene 3.1 (Arc/Arg3.1) in a proportion of mice DCs and LCs was shown to enhance migratory capacity and CD4$^+$ and CD8$^+$ T cell activation in dLNs [35]. The differentiation of naïve T cells into the different effector T cell populations is influenced by the secretion of cytokines in addition to T cell receptor and co-stimulatory molecules activation. Indeed, activation of a particular PRR influences the downstream secretion of cytokines and chemokines, thus influencing the fate of the inflammatory environment [30]. Mouse LCs expressing human langerin secreted CXCL1 (KC), IL-6 and IL-17 in response to WTA β-GlcNAc while PGN, an agonist of TLR2, induced the secretion of IL-6, IL-10 and IL-8 but not IL-12 or IL-1β in LCs isolated from human skin [32,33]. Thus, LCs have been implicated in the differentiation of Th17 cells and also the accumulation of IL-17 producing γδ T cells in the skin [36,37]. In contrast, cDC1s have been shown to drive Th1 polarization *via* the cytokine IL-12 [15,34]. Activated EpCAM$^+$CD59$^+$Ly-6D$^+$ cDC1s also mediated PMN recruitment through the secretion of vascular endothelial growth factor α following intradermal challenge with *S. aureus* [38].

How *Staphylococcus aureus* Planktonic Bacteria Manipulate the Dendritic Cells

S. aureus has developed numerous strategies to avoid host immune attacks and modulate the establishment of adaptive immune responses (For a review, see Darisipudi et al., 2018 [7]; Wu et al., 2014 [25]). First, the bacteria produce numerous bicomponent pore-forming leukocidins as LukAB and LukED that target the CD11b and CCR5 receptors respectively, and lyse PMNs, MOs and MΦs, but also DCs, even after efficient phagocytosis [39-43].

Inside phagocytes, the bacteria are also able to survive and multiply. Several virulence factors are involved, as phenol-soluble modulin α (PSM α) peptides to counteract phagolysosomal acidification, the O-Acetyltransferase (OatA) to resist to lysozyme degradation and the Catalase (KatA), the Alkyl Hydroperoxide Reductase (AhpC) and the Staphylococcal Peroxidase Inhibitor (SPIN) to resist oxidative stress [44-47]. Even if DCs lack myeloperoxidase, the target of the SPIN protein, their functional capacity to prime T cells in the lymphoid tissues is impaired by the PMN-derived protein [7]. PSM peptides have additional effects on DC activity, as PSM3 produced by community-associated methicillin-resistant *S. aureus* have the capacity to induce tolerogenic DCs upon TLR2 ligand stimulation of regulatory T cells *via* the activation of the p38-CREB pathway [48,49].

The bacteria cell wall is another key component of DC

attenuated functional properties. In the skin, WTA producing *S. aureus* strains induce LC activation and maturation [50]. The lipoteichoic acid thereafter downregulates DC activation and antigen presenting activity *via* a TLR2 dependent signal pathway [51].

Another immune evasion strategy developed by *S. aureus* bacteria is DC modulation of cytokine production, by secreting several virulence factors as Esx factors, PSMs or alpha-toxin. *In vitro,* the two Esx proteins, EsxA and EsxB secreted by the type VII-like secretion system, reduce the production of Th1/Th17 pro-inflammatory cytokines by infected DCs [52]. PSMs are other key modulators of DC cytokines production [48,49,53]. In particular, they inhibit the production of IL-32, IL-6 and IL-8 by epithelial cells, and IL-32 is involved in DC maturation [53]. In AD, toxins as the alpha-toxin first contribute to the induction of a Th1 like cytokine response [54]. Bacteria further skew the immune response toward Th2, with LCs producing high amounts of IL-2 and lower amounts of IFNγ, therefore contributing to bacterial persistence in the skin, and to chronicization of the inflammatory skin disorder [3,55,56].

Finally, *S. aureus* induces an overstimulation of the host immune system by producing numerous pyrogenic superantigens [57]. They do not affect any parameters of DC function but lead to an overstimulation of T cell lymphocytes [58].

Dendritic Cells and *Staphylococcus aureus* Chronic Skin Infections- In vivo Infection Models

A contributing factor to the pathogenicity of *S. aureus* is its capacity to transition towards the biofilm lifestyle. The latter are mono- or poly-species species microbial aggregates embedded in an extracellular matrix that can be found attached to a variety of surfaces, conferring protection to environmental dangers [59]. In humans, *S. aureus* can attach to either host proteins such as collagen and fibronectin or to inert surfaces namely, implanted medical devices in order to form biofilms [60]. In non-inflammatory conditions, *S. aureus* of the human microbiota have been shown to form biofilms [5]. In pathological conditions, *S. aureus* have been linked to the severity of certain inflammatory skin diseases namely AD [1]. Moreover, *S. aureus* clinical isolates from AD patients have been shown to be strong producers of biofilms [61,62]. The capacity of biofilms to survive the treatment of antimicrobials such as antibiotics and host anti-microbial peptides, while also resisting host immune attacks makes them strong contributors to the chronic nature of skin diseases [63,64]. Thus, the study of the *in vivo* interactions between *S. aureus* biofilms and specific actors of the adaptive immune system is essential in the understanding of *S. aureus* chronic infections. Review of the current literature has shown a lack of *in vivo* information on how DCs respond to biofilms and how this differs to planktonic bacteria [7,65].

Animal models, namely rodents, provide the complex interactions between host cells that are absent in *in vitro* conditions, while allowing the study of a variety of infections in different contexts, such as those present at the different depths of the skin, in wounds and also in the presence of implanted materiel [65-67]. Epicutaneous colonization models have demonstrated a beneficial effect of the oral administration of *Lactococcus lactis* on the maturation of pDCs in the skin dLN [68]. The langerin receptor can be used in inducible ablation models when expressed with the diphtheria toxin receptor (Langerin-diphtheria toxin receptor knock-in mice) to study the functional roles of LCs and cDC1s during inflammation [38]. To selectively deplete a subset of DCs, transcription factors specific to certain subsets can also be targeted, such as *Batf3* in the case of cDC1s [38]. The development of humanized rodent models has led to the use of xenografts containing tissue resident LCs and T cells, while the use of transgenic mice constitutively expressing human langerin on LCs have shown an increase in inflammatory markers following the recognition of WTA [32,69]. The implication of downstream signaling pathways for both T cell polarization and innate immune activation, can further be studied in DC specific MyD88 KO mice [70]. Models where bacteria are introduced into the subcutaneous level of skin tissue have been essential in studying the effectors of innate immune memory. Indeed, after an initial subcutaneous inoculation of *S. aureus* in the flanks of mice, an increase in Langerin$^+$ DC infiltration was observed in the abscess of mice during a subsequent infection compared to mice that never received the initial dose of bacteria [71,72]. Seeing as *S. aureus* preferentially transition towards the multicellular lifestyle when under harmful conditions in the host, it is likely that bacteria in colonized tissue contain a mixture of planktonic or biofilm bacteria or an intermediate between the two phenotypes. However, this parameter is rarely verified in *in vivo* models and thus, the specificity of the observed responses to either lifestyle is not clear.

In vivo biofilm infection models involve more invasive colonization of the skin tissue at the intradermal or the subcutaneous level of the skin. Biofilms are introduced either directly by inoculation into tissues [73-75] or by inserting abiotic supports, such as tissue cages, catheters or medical sutures, through incisions or pre-formed air pouches [76-78]. Abiotic supports are either sterile upon insertion and subsequently inoculated with bacteria to allow biofilm formation or are already pre-colonized by biofilms [79-81]. The common entry of *S. aureus* through wounds can be mimicked by mouse wound infection models which involve disruption of the skin barrier through skin incisions, external wounds or by scalding followed by bacterial inoculation [82-85]. However, the use of less invasive techniques has facilitated the monitoring of the evolution of infections; for example, using bioluminescent *S. aureus* strains to follow

the bacterial charge at the infection site over time and to study their dispersion towards secondary sites in the same animal [78,82,83,86-88]. Intravital imaging of transgenic reporter mice, such as the LysM-EGFP or CX3CR1-EGFP mouse lines, allows insight into the interactions of immune cell populations, namely PMNs, MOs and DCs, with *in vivo* biofilms [75,89,90]. To visualize cDC populations specifically, CD11c-YFP reporter mice have been used in both bacterial and non-infectious models [91-93]. The application of multiphoton imaging in *in vivo* models has also improved our understanding of the interactions between DCs and T cells in the lymph node and could prove useful in highlighting differential adaptive immune responses between planktonic and biofilm *S. aureus* [94-97].

Conclusion

Biofilm specific immune evasion strategies, coupled with the rise in multidrug resistant *S. aureus* strains, have made it essential to develop alternative therapeutic strategies. Namely, anti-infectious immunotherapy has proven to be effective at controlling *S. aureus* implant associated biofilm infections in mice. For example, local administration of pre-activated inflammatory MΦs at biofilm infection sites or MO metabolic reprogramming by oligomycin containing nanoparticles, both amplify pro-inflammatory responses and promote biofilm clearance [98,99]. In the case of cancer immunotherapy, the manipulation of DCs is becoming a promising avenue of investigation [100]. The chronic nature of both biofilm infections and tumors may suggest common deficiencies in DC functionality and initiation of adaptive immunity. Further research is required to determine if DC manipulation can be applied as an alternative or adjuvant therapy to antibiotics treatments against biofilms.

Conflicts of Interest

The authors declare no conflict of interest.

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