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The Role of Phagocytes and NETs in Dermatophytosis

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Abstract Innate immunity is the host first line of defense against pathogens. However, only in recent years, we are beginning to better understand the ways it operates. A key player is this branch of the immune response that are the phagocytes, as macrophages, dendritic cells and neutrophils. These cells act as sentinels, employing specialized receptors in the sensing of invaders and host injury, and readily responding to them by production of inflammatory mediators. They afford protection not only by ingesting and destroying pathogens, but also by providing a suitable biochemical environment that shapes the adaptive response. In this review, we aim to present a broad perspective about the role of phagocytes in dermatophytosis, focusing on the mechanisms possibly involved in protective and non-protective responses. A full understanding of how phagocytes fit in the pathogenesis of these infections may open the venue for the development of new and more effective therapeutic approaches.

Keywords Dermatophytosis · Phagocytes · Macrophages · Dendritic cells · Neutrophils · Innate immunity

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Introduction

Dermatophytosis is among the most common fungal infections worldwide [1], but little is known about the immunological mechanism involved in pathogenesis of these infections. Although the interest of immunologists in fungal infections was guided by their increased incidence in immunosuppressed patients and focused on the importance of adaptive mechanisms, innate immunity has been recognized as an equal player in antifungal defense.

Phagocytes are a very heterogeneous population of cells known to perform phagocytosis. Based on how efficiently they perform this action, they can be divided into non-professional and professional phagocytes [2]. The first one includes lymphocytes, epithelial cells, endothelial cells and fibroblasts. Neutrophils, monocytes, macrophages and dendritic cells (DC) represent the second group. Since our focus here is this last group, we refer to "professional phagocytes" as simply "phagocytes" along this review.

Even this classification, however, is grossly simple, since each of these cellular populations shows high degree of diversity. They can be resident cells, as the Kupffer cells in the liver or the Langerhans cells in the skin, or circulating cells with shorter half-lives, as neutrophils in the blood. Their phenotypes are also subjected to high variation as observed in the conversion of monocytes into inflammatory macrophages and DC, or even in the maturation process that DC shows along the antigen processing program [3–5].

In the context of the immune response to pathogens, phagocytes can be viewed as both initiators and effectors. On the one hand, by the uptake of pathogens, digesting them and performing antigen presentation, along with the cytokine production, they can initiate and shape the adaptive response. On the other hand, when they are recruited and activated in response to T helper-derived cytokines, they can potentiate their antimicrobial abilities, promoting infection clearance [6]. They can even prevent excessive damage and help in tissue renewal, exerting key homeostatic functions [7].

In this review, we aimed to present a broad view of the current state of the art in the study of phagocytes in dermatophytosis. We started with a broad view about the role of these cells in dermatophytosis pathogenesis, moving to recent findings about immune recognition and then to works about neutrophils and neutrophils extracellular traps (NETs).

Phagocytes in Dermatophytosis Pathogenesis: An Old (Unfinished) Story

The importance of phagocytes in the immunity to dermatophytosis was recognized more than 30 years ago. Cell-mediated immunity was considered critical for disease control based on clinical and experimental findings associating development of inflammation and delayed hypersensitivity reaction to infection restriction [8].

While antibodies were not considered protective due to the high titers developed by chronically infected patients [9], humoral immunity in the form of complement proteins was considered helpful in dermatophyte elimination by enhancing the recruitment and killing function of polymorphonuclear leukocytes (PMNLs) [10, 11]. In addition, Suite et al. [12] observed that secreted components of *Trichophyton violaceum* and *Trichophyton rubrum* acted as potent chemotactic factors and this enhanced recruitment of inflammatory cells promoted the intense inflammatory reaction observed in dermatophyte infections—interestingly, the authors also realized that the antifungal drug griseofulvin inhibited the PMNLs chemotaxis and proposed that successful therapeutic results obtained with this drug were not only due to its fungicidal activity but also by avoiding exacerbated inflammation [12].

Along with neutrophils, monocytes were also recognized as important effector cells in fungal elimination, able to inactivate *T. rubrum* and *Trichophyton quinckeanum* employing phagocytosis and reactive oxygen species (ROS) generation as the main tools for fungal destruction [13]. These activities were greatly enhanced when the phagocytes were activated by treatment with phorbol myristate acetate (PMA) or concanavalin A, suggesting that in in vivo conditions, these phagocytes may require further activation for optimal performance.

Under this idea, even though $CD4^+$ and $CD8^+$ lymphocytes are known to exert cytotoxic activity toward dermatophytes in vitro [14], their main contribution in infection control may rely more on their ability to activate phagocytes through cytokine production. Indeed, skin biopsies from patients with tinea faciei, e.g., showed enhanced levels of IFN-γ and macrophage migration inhibitor factor [15] and, in a recent model of experimental dermatophytosis, Baltazar et al. [16] demonstrated the importance of IFN-γ for infection resolution, required for the recruitment of neutrophils and macrophages and promotion of their oxidative burst response. Besides lymphocytes, many of these activating cytokines could also be provided by other cell types, as keratinocytes [17], indicating that the antidermatophyte response is already deflagrated before the induction of an adaptive response.

Nevertheless, while monocytes seem to restrict the fungal development, macrophages, on the other hand, are not equally efficient cells. Previous work from our group showed that murine peritoneal resident macrophages were able to phagocytose *T. rubrum* conidia and secrete TNF-α, but were unable to eliminate the pathogen. Instead, the fungus developed into hyphae inside the phagocytes, leading to their destruction [18]. Although this phenomenon may be associated with infection persistence, it is also possible that resident cells, even though susceptible to dermatophyte growth, may produce inflammatory and chemotactic factor that help the recruitment and activation of more efficient cells. Then after an appropriate stimulatory environment is established, these phagocytes may be able to eliminate the fungus.

Although most of these previous works studying the role of phagocytes employed in vitro or ex vivo systems, data from histopathological analysis confirmed that these cells are indeed recruited to the sites of infection in in vivo settings. The population of

Langerhans cells, e.g., were shown to be increased in the mycotic area of the epidermal/dermal layer [19], and neutrophils could also be observed in samples of the stratum corneum collected from patients, though this feature is not exclusive to dermatophytosis [20]. Further mouse models for the study of infection with *Arthroderma* confirmed the presence of neutrophils, macrophages and DC in the lesions [21].

The critical contribution of phagocytes in dermatophytosis was further corroborated with the work from de Sousa et al. [22] where they observed a correlation between defective phagocyte function and chronic infection. In chronic widespread dermatophytosis (CWD), patient-derived macrophages and neutrophils showed defective phagocytosis, reduced production of ROS and diminished secretion of inflammatory cytokines. Interestingly, none of the patients had concurrent immune deficiencies and the observed defects were limited to *T. rubrum* challenge (the dermatophyte identified in these patients), but not to zymosan, PMA or lipopolysaccharide (LPS) stimulation. Therefore, the dysfunctional response of phagocytes in these patients may turn them prone to CWD development.

Hence, while the involvement of phagocytes is an established point in dermatophytosis pathogenesis, exactly how these cells interact with and respond to dermatophytes, shaping the immune response at the tissue and organism levels, is still the area of intense research.

Advances in Innate Immune Recognition

A great revolution in the field of innate immunity has started with the introduction of the concepts of pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) by Charles Janeway [23], and these topics frequently overlay with the study of phagocyte biology in infectious diseases.

PRRs are germline-encoded receptors that recognized PAMPs—defined structures or molecular determinants that are not expressed by the host—but are essential for the microbes and show low structural diversity. PRR activation triggers signaling cascades that promotes transcriptional responses, as cytokine production, and non-transcriptional processes, as phagocytosis or cell death [24].

Establishing the dogma of self- and non-self-recognition, a framework was proposed, then, for the way the innate immunity works: a constant survey of the organism compartments for detection of microbial elements as a way to quickly identify and react to infectious process and host injury.

Currently, five main classes of PRRs are recognized: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs), RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs) [25]. Since RLRs and ALRs are associated with RNA/DNA recognition, mainly in viral infections, and no association between them and dermatophytes was described up to date, they will not be covered on this review.

TLRs are membrane-associated receptors that are usually associated with the recognition of bacterial components, e.g., LPS (TLR4), flagellin (TLR5) and RNA (TLR3), and their signaling pathways require adapter molecules, as MyD88 and TRIF, to transduce the receptor activation into effector responses [26]. In fungal infections, TLR2 and TLR4 show prominent roles, associated with the recognition of carbohydrates, usually mannans [27]. They promote control to *Candida albicans* and *Aspergillus fumigatus* infections not only through their contribution in the production of inflammatory mediators, but also by preventing excessive damage by induction of IL-10 and regulatory T cells [28–30]. In addition, *A. fumigatus* RNA recognition by TLR3 has also been identified as another protective element by establishing robust $CD8^+$ T cell responses [31].

In dermatophytosis, there is still no direct link between TLRs and infection outcome; however, some initial works indicate a possible contribution. Recently, Cambier et al. [32] evaluate the expression of TLR2, TLR4 and dectin-1 mRNA in feline PMNs exposed to different components from *M. canis*. The authors showed an increase of TLR2 and TLR4 mRNA levels in feline PMNs stimulated with live and heat-killed arthroconidia, but not in those stimulated with the secreted components from *M. canis*. These results suggest that TLR2 and TLR4 are involved in the host immune response through the recognition of *M. canis* PAMPs.

TLR2 and TLR4 were shown to be positively expressed in response to *T. rubrum* both in vitro, employing the human keratinocyte cell line HaCaT

[33, 34], and in vivo, where immunostaining of TLR2 and TLR4 was higher in infected skin biopsies than in healthy controls [35]. Recently, Oliveira et al. [36] reported, through a similar immunohistochemistry approach, that, in patients with disseminated dermatophytosis, TLR4 expression, but not TLR2, is depressed, representing a possible mechanism behind the increased severity and persistence of this clinical form.

Curiously, an important contribution of TLR2 in response to *C. albicans* was described in mast cells for phagocytosis and nitric oxide production [37]. Since mast cells occur in the skin, where they regulate the tissue homeostasis and the immune response, especially the promotion of type 2 responses [38], they may also be a active player in dermatophyte immunity.

Another class of PRRs deeply involved in fungal recognition is CLRs. They are transmembrane receptors (similar to TLRs) and are associated with the recognition of sugars and carbohydrate-associated molecules, which are abundantly expressed in the surface of fungi, as β-glucans and α-mannans [39], through the use of C-type lectin domains. The intracellular signals that are triggered can be activating or inhibitory and, for that, they employ, respectively, integral immunoreceptor tyrosine-based activation (ITAM) or immunoreceptor tyrosine-based inhibitory (ITIM)-like cytoplasmic motifs. ITAM systems, like in dectin-1, involve the activation of the kinase Syk and the formation of the caspase recruitment domain 9-B cell lymphoma/leukemia 10-mucosa-associated lymphoid tissue (CARD9-Bcl-10-MALT) complex, leading to gene transcription. Some CLRs, as dectin-2 and mincle, do not possess intracellular domains, and they function by recruiting FcγR adaptor molecules that contain ITAM domains. Finally, ITIM motifs, as in myeloid inhibitory C-type lectin (MICL), recruit phosphates as Src homology 2 domain-containing phosphatase (SHP)-1 and SHP-2 that can both limit the response provided by the activating signals and potentiate those ones that are submitted to repression in steady-state conditions [40, 41].

CLRs are essential in the protection against fungal pathogens, as observed for *C. albicans* [42], *Pneumocystis jirovecii* [43] and *Paracoccidioides brasiliensis* [44], being an interest target for vaccine development [45]. They may also be a target for immune evasion, as observed for *Fonsecaea pedrosoi*, the main etiological agent for chromoblastomycosis, that produces chitin-like molecules to avoid dectin-1 recognition and prevent the induction of a protective response [46]. In most of these works, protection is afforded mainly due to the induction of a T_H17 response. Dectin-1 and dectin-2 activation, e.g., lead to the secretion of key cytokines in T_H17 activation—IL-1β, IL-6 and IL-23—while repressing IL-12 production [47] and even converting Treg cells into IL-17 secreting ones [48]. IL-17 would drive fungal clearance by inducing production of antimicrobial peptides and recruiting neutrophils [49].

It should be highlighted, however, that an adaptive independent contribution of CLRs in antifungal defense is being identified. Quintin et al. [49] showed that protection to *C. albicans* infection could be achieved in the absence of T and B lymphocytes. Through a mechanism termed "trained immunity," they showed that dectin-1 stimulation in monocytes induced stable epigenetic changes that primed the cells and increased their cytokine response, granting protection in further fungal challenges [50]. More recently, Whibley et al. [51] demonstrated that efficient resolution of *Candida tropicalis* infection relies on CARD9-dependent, but IL-17-independent, mechanisms.

Since CLRs are key receptors in fungal recognition, it is not surprising that they are also employed in dermatophyte sensing. In fact, it was recently shown that mutations in *CARD9* gene could be associated with the development of deep dermatophytosis [52, 53], a severe clinical manifestation where the infection can overcome the skin layer and reach deeper tissues. These findings reinforce the importance of these CARD9-associated molecules in dermatophyte control.

An initial study from Sato et al. [54] showed that dectin-1 and dectin-2 can recognize *T. rubrum* and *M. audouinii* and posteriors works implied a role for dectin-1 in the production of cytokines by the keratinocyte cell line HaCaT in response not only to whole *T. rubrum* stimulation but also to its secreted components [34, 55, 56]. In addition, Nakamura et al. [57] demonstrated that the contact hypersensitivity reaction in response to trichophytin is also mediated by dectin-1. Collectively, these works imply the secreted components as important triggers of the host response.

Curiously, Blake et al. [58] reported that secreted mannans exert immunoinhibitory activities and may

suppress this hypersensitivity response. Considering that mannan recognition is an important step in the interaction between *T. rubrum* and human monocytes [59], it is tempting to speculate that the balanced secretion between stimulatory and inhibitory molecules may shape the virulence potential among dermatophyte strains.

Serrano-Gómez et al. [60] showed that dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN or CD209 in humans) can recognize keratinophilic fungi as *Microsporum canis* and *Chrysosporium tropicum* through mannan moieties, and Santiago et al. [61] posteriorly confirmed that *T. rubrum* phagocytosis by human macrophages and DCs relies on this receptor. Unlike DCs, however, these human macrophages were unable to restrict *T. rubrum* growth, resembling the previous findings obtained from murine cells [18].

Another remarkable CLR in dermatophyte response is the receptor DC-HIL. Originally associated with the provision of inhibitory signals to T cells, this receptor could prevent the lymphocyte proliferation and posterior reactivation response [62]. Curiously, the same group showed after that DC-HIL could also recognize dermatophytes, as *T. rubrum* and *M. audouinii*, but not non-dermatophytes as *C. albicans*, playing a positive role in cytokine production and DC activation [63]. The authors postulated that since DC-HIL is highly expressed by epidermal Langerhans cells [64], it would be a key receptor in the immune response to dermatophytes.

Finally, a third PRR system of great interest in antifungal immunity is the NLRs. They are cytoplasmic sensors that, unlike classical PRRs, do not work by recognizing defined PAMPs, but rather they are considered sentinels for host damage. The main effector consequence of NLRs activation is the formation of inflammasomes, multimolecular platforms whose main purpose is caspase-1 activation [65].

The most studied NLR in fungal infections is the NLRP3, a protein composed of basically three portions: a N-terminal PYD domain, a central nucleotide oligomerization domain and a C-terminal leucine-rich repeats domain. Once activated, NLRP3 oligomerizes and recruits the adaptor protein ASC (apoptosis-associated speck-like protein) through its N-terminal pyrin domain (PYD). Then, ASC recruit caspase-1 through CARD–CARD interactions, leading to the protease activation. Caspase-1 performs the activation of the cytokines IL-1β and IL-18, and it is also able to induce pyroptosis, a cell death pathway that causes the leakage of the intracellular content, leading to a strong inflammatory reaction [66].

Even though the mechanisms underlying NLRP3 activation are still a matter of debate, it is accepted that NLRP3 does not work by direct recognition of PAMPs, but by responding to cell injury and alterations in homeostasis. These events could be translated in the form of potassium efflux (as ATP activation of P2X7 receptors); lysosomal disruption; altered calcium balance; increased ROS production; and dysfunctional mitochondria metabolism [67].

Due to their high inflammatory potential, inflammasomes are subject to tightly controlled mechanisms, whose the most common is at the transcriptional level. Under steady-state conditions, some components of the inflammasome, as NLRP3 and IL-1β, are not constitutively expressed and, therefore, they need to be induced before the system can be activated. These priming signals are provided by the activation of other PRRs, as TLRs, whose transcriptional programs induce the expression of the inflammasome components to significant levels [68].

The contribution of NLRP3 in fungal immunity is a well-established issue. For example, the inflammasome/IL-1β axis is essential for induction of T_H17 responses in *C. albicans* infections [69, 70] and it is suggested that proteases secreted by the fungus could directly activate NLRP3 [71]. Other classical fungal pathogens as *A. fumigatus*, *Cryptococcus neoformans* and *P. brasiliensis* were also shown to activate this system in macrophages and DC [72–74]. Considering that NLRP3 responds to glucans, it is conceivable that many other fungal pathogens may activate this system [75, 76] and, indeed, dermatophytes are NLRP3 activators.

Li et al. [77] were the first group to demonstrate the involvement of the inflammasome against a dermatophyte, *Trichophyton schoenleinii*. Employing the human monocytic cell line THP-1, they observed that IL-1β secretion requires a classical NLRP3/ASC/Caspase-1 system and three activation pathways were involved: potassium efflux, ROS production and lysosomal rupture. Curiously, it also requires viable fungi, since heat-killed cells induced less cytokine secretion.

Subsequently, Mao et al. [78] presented similar results in response to *M. canis*. In addition to THP-1

cells, they observed a requirement for this inflammasome in bone marrow-derived DC and, even more interesting, also in in vivo conditions (intraperitoneal infection model), where IL-1β levels recovered in the peritoneal lavage were reduced in animals knockout for NLRP3 and ASC. They also observed that dectin-1, Syk and CARD9 were required for optimal cytokine secretion, suggesting that IL-1β may require multiple contributions.

Indeed, a cross talk between CLRs and NLRs is well known. Besides cytokine production, CLRs also drive non-transcriptional events, as phagocytosis and ROS generation [79]. CLRs can, therefore, promote inflammasome activation not only by providing its priming signal, but also by giving the activating, second signal in the form of ROS generation [80].

Our group showed that the NLRP3 inflammasome is involved in the response to *T. rubrum* [81]. We reported that bone marrow-derived macrophages required this classical inflammasome for appropriate IL-1β production and, interestingly, cells lacking the interleukin-1 receptor (IL-1R) became highly susceptible to the fungal growth. When infected with *T. rubrum*, mice knockout for IL-1R were unable to mount an adequate IL-17 response, suggesting that IL-1 may exert autocrine/paracrine actions at the cellular level and is required for the shaping of the immune response at the whole organism level.

Besides the NLRP3 inflammasome, a more recent, non-canonical caspase-8 inflammasome has also been associated with antifungal response. This inflammasome is triggered in response to dectin-1 activation, and it did not require the pathogen internalization, but still relies on the CARD9-Bcl-10-MALT platform. Described by Gringhuis et al. [81] in DC, the CARD9-Bcl-10-MALT complex triggered after dectin-1 activation not only performs its known NF-κB-dependent transcriptional action, but can also recruit and activate ASC and caspase-8, leading to processing of IL-1β independent of caspase-1 [82, 83]. Apparently, however, this system is activated only by a subset of pathogens, as some species of *Candida* and mycobacteria. Although IL-1β production by THP-1 cells in response to *M. canis* was observed to not be reduced when caspase-8 was knocked down [78], it is still possible that this non-canonical inflammasome could be involved against other dermatophyte species. Therefore, future studies are required before we rule out its involvement in dermatophytosis.

It is worth mentioning that molecular recognition is not only a host strategy to detect possible threats. Pathogens may also employ similar systems to identify targets and establish the infection in the host. Fungal pathogens, as *Coccidioides immitis*, *A. fumigatus* and *P. brasiliensis*, may express adhesins that recognize host molecules and help the colonization process [84–86]. Similarly, *Trichophyton* species were also shown to express adhesins [87, 88]. Esquenazi et al. [88] showed that the pharmacological blockade of phagocytosis in murine peritoneal macrophages reduced, but did not prevented, *T. rubrum* internalization, suggesting the dermatophyte can actively invade phagocytes. Together with our findings that macrophages are permissive to *T. rubrum* growth [18, 61, 81], it is feasible that dermatophytes could act as facultative intracellular parasites invading the host cell as part of their life cycle.

Neutrophils and NETs

Neutrophils do not show resident behavior as macrophages and DCs, and they have limited lifespan, being continuously renewed by the bone marrow. In steady-state conditions, they are restrained to the vascular compartment but readily mobilized in response to inflammation and tissue injury [5].

Besides their classical microbicidal activities, involving secretion of enzymes and production of ROS, neutrophils may also provide cytokines that help to shape the inflammatory response. Cambier et al. [32], for example, demonstrated that feline neutrophils could secrete IL-1β, TNF-α and IL-8 in response to *M. canis* stimulation. In addition, Taylor et al. [89] uncovered neutrophils as a source of IL-17 in *Aspergillus* infections, suggesting that other fungal pathogens, dermatophytes included, may also employ a similar mechanism.

Another remarkable effector function of neutrophils is the induction of NETs. NETs were recognized as the result of a particular pathway of cell death (termed NETosis) where neutrophils release their antimicrobial protein content along with their DNA material, creating a net of strands that entraps and eliminates microbes within [5].

It is curious to observe that NET release is triggered in response to large pathogens, as *C. albicans* hyphae and mycobacteria aggregates, but not against single

cells. Branzk et al. [90] showed that microbes small enough to be internalized do not induce NETs because the phagocytosis process impedes the neutrophil elastase translocation to the nucleus, avoiding the cell degradation into the traps. Considering that pathogenic fungi are relatively large, it is not surprising that NETs are an important component in antifungal immunity.

Indeed, aside *C. albicans* [90], the formation of NETs against fungal pathogens was already demonstrated for *A. fumigatus* [91], *P. brasiliensis* [92] and *C. neoformans* [93].

Dermatophytes can also induce the formation of NETs, and their avoidance may be an evasion strategy for disease establishment. Heddergott et al. [94] showed that the zoophilic dermatophyte *Arthroderma benhamiae* expresses a hydrophobin layer on its surface, avoiding cytokine production by human phagocytes and NET induction. Mutants unable to express the HypA protein, however, were significantly more efficient inducers of NETs and susceptible to neutrophil killing. Curiously, a similar immune evasion strategy was well established for *Aspergillus*, in which resting conidia express the hydrophobic protein RodA to escape immune recognition [95, 96]. Future works employing in vivo models may confirm the importance of this strategy in dermatophytosis pathogenesis.

Phagocytes as a Therapeutic Target?

Since phagocytes are pivotal players in the immune response to dermatophytes, strategies that target their function, enhancing their protective abilities, may represent an interesting therapeutic approach in the infection management.

In agreement with the findings observed in CWD patients [22], Gregurek-Novak [97] observed that PMNLs from patients with chronic dermatophytosis presented defects in phagocytosis and random mobility, but no alteration in the lymphocyte compartment. Interestingly, successful treatment of these patients with terbinafine normalized these parameters. Besides its direct antifungal properties, terbinafine would help control disease by restoring the phagocytes function and their antimicrobial actions, accelerating disease resolution.

In the same line, Wakabayashi et al. [98] previously reported that feeding of guinea pigs with lactoferrin, a protein from milk, potentiated the fungicidal activity of splenic mononuclear cells, although it did not change the oxidative burst or the phagocytic ability of PMNLs. Even though the authors focused on the ex vivo system and did not analyzed the supplementation effect in the infection outcome in vivo, their work suggests that immune modulation is a reasonable therapeutic strategy.

Conclusions

Phagocyte biology has attracted much attention in recent years. Their recognition as key players in the immune response against infectious agents has prompted a new area of research to investigate the details around their interaction with pathogens. Fortunately, their role in dermatophytosis is also being scrutinized, but much there is to be done.

While considerable advances have been obtained in the understanding of the immune response in dermatophytosis both in experimental systems and in human patients, we still know little about how the infection develops in the natural condition. What is the contribution of skin resident cells and the tissue matrix? Which host and pathogen determinants are involved among the different clinical presentations and how they affect the phagocyte response? Therapeutic or prophylactic vaccines could be interesting approaches and what they should target? As long we see the development of new investigational and diagnostic tools, as artificial skin systems and proteomic and genomic profiling, we may get close to these answers.

The translation of experimental findings in the clinical practice may seem distant, but it is certainly the most secure way to achieve fruitful results.

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