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Recent Advances on the Role of Toll-like Receptors in Sporotrichosis – An Overview

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Authors' contributions

This mini-review was designed by author TCN and it was carried out in collaboration between all authors. Authors TCN, RAA and IZC managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Sporotrichosis is a chronic granulomatous subcutaneous mycotic infection caused by dimorphic fungus *Sporothrix schenckii* species complex which is clinically and/or epidemiologically important. The fungus is present in soil and contaminated decaying vegetation and their usual mode of infection is by traumatic inoculation into the skin and subcutaneous tissue. The immunological mechanisms involved in the prevention and control of infections caused by *S. schenckii* are not yet well understood but it has been discussed they should include both humoral and cellular immune responses. Immune status of the host and the inherent heterogeneity found within the same species might interfere with the expression of their virulence factors, leading to distinct clinical manifestations of disease. Interactions between innate and adaptive systems play an essential role on the immune response against microbial infection. Toll-like receptors (TLRs) are important in this process since they bind to pathogen surface antigens and initiate the immune response. In this

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review, we will explore and discuss recent advances in the involvement of toll-like receptors in the recognition of the etiological agent of sporotrichosis and how this process interferes with the production of mediators in response to the infection.

Keywords: Sporothrix schenckii; sporotrichosis; pattern recognition receptors; Toll-like receptors; fungus.

1. INTRODUCTION

Sporotrichosis is a universal cutaneous mycosis affecting man and a variety of animals [1]. *Sporothrix schenckii* complex fungi, which inhabits contaminated decaying vegetation in the soil, has been considered the causative agent of this disease. Currently, in certain regions of Brazil, the disease has been regarded as a public health problem due to the increase in the number of cases. Although the immunological mechanisms involved in the prevention and control of infections caused by *S. schenckii* are not yet well understood, it has been discussed they should include both humoral and cellular immune responses. It is known that interactions between innate and adaptive systems play an essential role on the immune response against microbial infection. Toll-like receptors (TLRs) are important in this process since they bind to pathogen surface antigens and initiate the immune response, however little is known about the role of these receptors in sporotrichosis. This review is aimed at demonstrating the studies published to date emphasizing the recognition of *Sporothrix* by TLRs.

2. SPOROTRICHOSIS

Sporotrichosis is a chronic granulomatous subcutaneous mycotic infection caused by dimorphic fungus *Sporothrix schenckii* which belongs to the class of Hyphomycetes [1]. This fungus is morphologically constituted by septate hyphae, conidia with hyphae laterally arranged or in groups at the end of conidiophores. When developing at 37℃, it grows in a parasitic yeast-like form. In many cases, it may grow as round-, oval- or cigar-shaped and its reproduction occurs by budding [2,3]. A few years ago, *S. schenckii* was considered the unique species responsible for sporotrichosis. However, recent molecular studies have demonstrated that *S. schenckii* species complex is clinically and/or epidemiologically important and comprised by *Sporothrix brasiliensis*, *Sporothrix mexicana*, *Sporothrix globosa*, *S. schenckii* sensu strictu, *Sporothrix luriei*, and *Sporothrix albicans* (formerly *Sporothrix pallida*) [4-7]. These species exhibit differences in their geographical distribution, biochemical properties (such as carbohydrate assimilation), diverse degree of virulence and response to antifungal therapies [7]. Sporotrichosis has a worldwide distribution but it is more prevalent in tropical and subtropical zones. Its usual mode of infection is by traumatic inoculation of the fungus, present in soil and contaminated decaying vegetation, into the skin. Most cases of sporotrichosis occur during leisure and occupational activities, such as floriculture, agriculture, mining, and wood exploitation [8].

In addition to the above mentioned causes, sporotrichosis may also be transmitted to human beings or other animals by infected feline which acts as carriers of this fungus. Generally, cats become infected by contact with soil contaminated by *S. schenckii* [9]. This is an endemic condition in Rio de Janeiro, Brazil [10]. Sporotrichosis in cats has been reported in several countries, but none of them has presented a disease outbreak as large as that seen in Brazil. Actually, more than 4,000 cases of the disease were diagnosed in Brazil between 1998 and 2012 [10].

The skin and the subcutaneous tissues are the most frequent human body tissues affected by sporotrichosis. Its cutaneous form is characterized by infiltrated nodular, ulcerated or erythematosquamous lesions located on exposed skin areas where fungal inoculation occurred [11]. However, the host might be systemically exposed to the infection since it may spread into the subjacent tissues and viscera especially in immunocompromised patients, such as HIV-infected subjects [12-14]. The increasing number of reports of *Sporothrix* infection in immunocompromised patients, mainly in the HIV-infected population, suggests that sporotrichosis is an emerging global health problem [4,13].

Potassium iodide has been successfully used over the years as a prophylactic agent for the treatment of uncomplicated cutaneous manifestations [7,15]. Additionally, itraconazole has also been used for the treatment of all

forms of sporotrichosis. Terbinafine also appears to be effective for non severe forms of this infection. However, amphotericin B followed by itraconazole has been used as a first choice for the treatment of subjects systemically affected by this infection, as well as for pregnant or immunosuppressed patients [7].

3. VIRULENCE FACTORS OF *S. schenckii*

It is a premise that the successful colonization and infection of the host by the pathogen is needed to induce disease [16]. In this way, microorganisms have developed specific strategies and effective battery of putative instruments, called virulence factors, in order to overcome host defenses [17,18] and to assist them in the colonization of host tissues, dissemination into the infected area and survival in hostile environments. Both the virulence factors presented by different fungi and the defense mechanisms provided by the host require action and interaction of complex processes whose knowledge allows a better understanding of the pathogenesis of systemic mycoses [17].

One of the first steps in the pathogenesis of sporotrichosis is the fungal adhesion to host cells [19]. In this context, the fungal cell wall has been considered one of the most important structures responsible for the interaction with the host. Several fungal surface molecules, such as glycoproteins, polysaccharides, lipids and pigments [20] are able to recognize adhesins present on the surface of host cells, specifically fibronectin, laminin, and type II collagen. The linking among these molecules has been responsible for a well-stablished infection and fungal dissemination throughout the body [21].

The mechanisms responsible for the pathogenesis of *Sporothrix* spp. are still poorly understood [22]. However, there is an agreement that adaptive specific molecular strategies in addition to those putative/acquired virulence factors are essential to make *S. schenckii* more prone for tissue colonization and infection [18]. In this context, the dimorphism and thermo-tolerance of *S. schenckii* have been considered as important virulence factors which provide protection against the host immune response [17], although the mechanisms involved in the morphologic conversion from filamentous molds to the yeast form remain unknown for *S. schenckii* [6].

The dimorphism is also found in other human pathogenic fungi such as *Paracoccidioides brasiliensis*, *Penicillium marneffei*, *Histoplasma capsulatum*, *Blastomyces dermatitidis* and other fungi [23,24]. As demonstrated by Medoff et al. [25], two virulent strains of *H. capsulatum* were not able to cause infection in mice under the conditions of a lethal experimental model infection once they were exposed to sulfhydryl blocking agent p-chloromercuriphenylsulfonic acid (PCMS), an agent which irreversibly inhibits the mycelium-to-yeast transitions at 37℃. It can be concluded that the conversion from mycelia to yeast is necessary for pathogenicity of this fungus because PCMS-treated mycelia failed to cause infection and consequently no fungal cells were found in tissues [25]. In addition to this data, Drutz & Frey [26] demonstrated that polymorphonuclear neutrophils (PMNs), peripheral blood monocytes and monocyte-derived macrophages were able to phagocytize conidia of *Blastomyces dermatitidis* more efficiently than its yeast form These results indicate that the immune response is more effective against conidia than against yeast cells [26].

Additionally, some exoantigens mainly formed by peptide-polysaccharide compounds found in the cell wall of the yeast-like forms of *S. schenckii* are partially released to the culture medium under in vitro conditions [27,28] and may act as virulence factors. Animal studies have shown a depression of the immune response in rats between the 4^{th} and 6^{th} week of infection with the exoantigens [29]. Additionally, these compounds showed mitogenic activity when challenged with normal lymphocytes and they were also found to be involved in the inflammatory response [29].

In addition to those peptide-polysaccharide compounds, other cell wall components have been suggested as virulence factor of *S. schenckii* [29-31]. Castillo et al. [32] demonstrated that cell surface antigens of the fungus *S. schenckii*, present in both the mycelial phase and the yeast-like form, can impair the antibody production by the host. Moreover, studies have shown that fungal cell surface lipids can inhibit the phagocytosis of yeast cells by peritoneal macrophages under *in vitro* conditions, suggesting a possible mechanism of escape by this pathogen during fungal infection [33,34]. Yet, in another study, TLR-4 deficient (C3H/HeJ) and control mice (C3H/HePas) were infected with *S. schenckii* yeast cells and the immune response was assessed by production of NO and

TNF-α). The authors found that both pro-inflammatory mediators are reduced in TLR-4 deficient mice, suggesting that TLR4 has an important role in the recognition of surface lipids and in the activation of this pathway of immune response [35]. Additionally, Negrini et al. [36] evaluated the involvement of TLR-2 and fungal surface soluble (SolAg) and lipidic (LipAg) antigens in phagocytosis of *S. schenckii* and production of immune mediators by macrophages obtained from TLR-2 knockout (TLR-$2^{-/-}$) and control mice (C57BL/6). The results showed that TLR-2 knockout animals had statistical lower percentage of macrophages with internalized yeasts compared to control mice. In addition, macrophages were also pre-exposed to SolAg and LipAg for further evaluation of the percent of phagocytosis. The results showed that SolAg and LipAg impaired phagocytosis and immunological mediator production (TNF-α, IL-1β, IL-12 and IL-10) for both control and knockout mice. The authors suggested that these fungal surface antigens might block the binding of the fungus to macrophages which explains the reduced percentage of cells with internalized yeasts in macrophages pre-exposed to antigens in comparison to those only exposed to fungus. It is unclear at this point which receptor is blocked by the antigens, but the results suggest that the absence of TLR-2 enhanced this effect, because the percentage of cells with internalized yeasts was lower in TLR-$2^{-/-}$ animals compared to WT [36].

Yeast forms of *S. schenckii* are also able to produce ergosterol peroxide by a H_2O_2-dependent enzymatic oxidation of ergosterol. This peroxide compound may be considered as an escape mechanism from reactive oxygen species released during the phagocytosis process [30]. Additionally, sialic acid residues present on the cell surface of *S.schenckii* yeast cells are important to protect the cells from phagocytosis by peritoneal macrophages in mice. *S. schenckii* yeast cells treated with neuraminidase, responsible for the enzymatic removal of sialic acid residues, were taken up by activated macrophages much more efficiently than by untreated cells, which suggests that sialic acid has a protective action against phagocytosis of the fungus [33].

In addition to the above mentioned strategies, production of melanin, dark brown or black pigments, also seems to be related to the infectivity of the fungus. Although not essential for survival of the fungal cell, these pigments contribute to the growth and development of the species, allowing the survival and competition of fungi in the environment. These pigments contribute to the protection of conidia against oxidative damage by free radical and against UV radiation. By providing resistance to phagocytosis by macrophages, these pigments contribute to the pathogenicity of the fungus [37], regardless of the immune status of the host [38], but genotypic variations among strains can contribute to different clinical manifestations of the disease [38]. It is important to mention that dimorphic and pathogenic fungi other than *S. schenckii* are able to produce melanin, as the *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Blastomyces dermatitides* and *Coccidioides posadasii* [39].

In addition, many efforts have been made to isolate and identify antigenic substances from fungi and to clarify the relationship between the chemical nature and immunological activity of fungal antigens upon infection [27,28,40].

Interestingly, recent studies have demonstrated that adverse conditions found in *S. schenckii* natural habitats are responsible for the differential expression of virulence factors which could confer survival advantages [6]. Fernandes et al. [41] observed quantitative and qualitative differences in the expression of proteins of 13 different strains of *S. schenckii* from 5 different geographic regions (1,000 to 2,000 km far from each other) and of 10 strains from geographic close regions (200 to 400 km far from each other). The results of that study suggest that inherent heterogeneity within the same species might interfere with the expression of their virulence factors and, consequently, with the manifestations of disease.

4. HOST DEFENSE AGAINST *S. schenckii*

In order to survive, animals must maintain contact with the environment to exchange air, ingest food and eliminate body wastes. In the mean time, the microorganisms are universal and inhabit these environments. These microorganisms have evolved mechanisms to capitalize on these sites of environmental contact as points of entry [16]. For this reason, hosts present various protective mechanisms to prevent microbial entry.

It is also known that there is a need of a wide variety of mechanisms to control each type of microorganism. The skin is the predominant host

barrier which excludes most microorganisms. Moreover, this organ system can be damaged by trauma of various types, allowing the invasion of organisms with pathogenic potential, as in the case of sporotrichosis [42].

Although the immunological mechanisms involved in the prevention and control of infections caused by *S. schenckii* are not yet well understood, it has been discussed that they should include both humoral and cellular immune responses [28,43]. Additionally, it seems that both the site of infection and the type of pathogen are determining factors of the type of immune response to be developed. Authors have discussed that the existence of different virulence profiles in *S. schenckii* strains may play differential roles in the activation of the various arms of the immune response [44,45].

5. ROLE OF TOLL-LIKE RECEPTORS IN THE SPOROTHRICOSIS

Interactions between innate and adaptive systems play an essential role on the immune response against microbial infection. This host response involves interplay among a variety of cell surface receptors, secreted cytokines and chemokines [46].

In response to microbial infections, the innate immune system constitutes the first line of defense against invading microbial pathogens. This first defense is played by several immune pattern recognition receptors (PRRs) which are able to detect specific and evolutionarily-conserved structures on pathogens surfaces, known as pathogen-associated molecular patterns (PAMPs) [47,48]. These interactions are complex and need to be translated into a clear understanding of the roles of the respective PAMPs and PRRs under clinical conditions [49].

The Toll-like (TLRs), NOD-like (NLRs), RIG-I-like (RLRs) and C-type lectin-like receptors (CLR) contribute to the recognition of a vast range of species of microorganisms [50]. In this context, TLRs play an important role in this process being the most outstanding [47,48]. There are 13 different TLRs in mice, and 10 (TLR1-10) are expressed on humans cells. It has been suggested that TLR1, 2, 4, 5, 6 and 10 are transmembrane receptors expressed at the cell surface, while TLR3, 7, 8 and 9 are usually found on intracellular membranes [50].

Although most TLRs are found as homodimers, some TLRs can also form heterodimers, such as TLR2 in association with TLR1 or TLR6 [51]. These heterodimers can respond upon interaction with a large variety of PAMPs allowing an induction of the effective immune response to different classes of microorganisms [52].

In relation to recognition of fungi, several studies have demonstrated a crucial involvement of TLRs in this process [35,36,50-70]. Among all TLRs, TLR-2, TLR-4 and TLR-9 are particularly considered as the main ones responsible for the recognition of these pathogens [63]. Specifically for *S. schenckii*, studies have shown the involvement of receptors TLR-2 and TLR-4 in the immune response induced by this pathogen.

Studies looking at the role of TLR in the immune response induced by the fungus *S. schenckii* began in 2009 when Sassá et al. [35] demonstrated the involvement of TLR-4 in the production of proinflammatory (NO and TNF-α) and anti-inflammatory (IL-10) mediators during infection by *S. schenckii*. TLR4-deficient (C3H/HeJ) and control mice (C3H/HePas) were infected with *S. schenckii* yeast cells and immune response was assessed over 10 weeks by assaying production of cytokines by peritoneal macrophages and their correlation with apoptosis in peritoneal exudate cells. They found that both pro-inflammatory and anti-inflammatory mediators are reduced in TLR4-deficient mice, suggesting the involvement of this receptor in the recognition of this infectious agent. Translocation into the nucleus of nuclear transcription factor (NF-kB), was also higher in TLR-4 normal mice, which is consistent with the results found for cytokine production [35].

A few years later, Sassá et al. [70] investigated how TLR-4 signaling could affect inflammatory response development through evaluation of peroxide production and IL-1β, IL-6 and TGF-β release during the course of *S. schenckii* infection on TLR-4-deficient mice (C3H/HeJ). The results showed that macrophages are largely dependent on TLR-4 for inflammatory activation and that in the absence of TLR-4 signaling, an increased TGF-β release may be one of the contributing factors for the abrogated inflammatory activation of peritoneal exudate cells during mice sporotrichosis [70].

In 2012, Li et al. [65] investigated whether TLR-2, TLR-4 and NF-kB were involved in production of IL-6 and IL-8 by human keratinocytes pretreated

with anti-TLR-2, anti-TLR-4 or a NF-kB inhibitors prior to challenge with the fungus *S. schenckii* in both conidia and yeast forms. It was observed that production of those mediators was blocked using anti-TLR-2 and anti-TLR-4 and neutralized by the use of the NF-kB inhibitor, suggesting that IL-6 and IL-8 are triggered by the activation of TLR-2 and TLR-4.

Moreover, by evaluating the role of TLR-2 during the process of phagocytosis of *S. schenckii*, Negrini et al. [36] verified that the absence of this receptor resulted in a lower rate of phagocytosis of this pathogen along with reduced production of TNF-α, IL-1β, IL-12 and IL-10 compared to TLR-2-expressing controls. The findings suggested that the recognition of the fungus by TLR-2 is critical for the release of pro-inflammatory and anti-inflammatory mediators [36]. Additionally, TLR-2 knockout and control mice (C57BL/6) were used to evaluate the influence of TLR-2 over macrophages production of IL-1β, IL-12 and TNF-α, their stimulation level by NO release and the production of IFN -γ, IL-6, IL-17 and TGF-β by spleen cells. On the course of 10-weeks of sporotrichotic infection, it was demonstrated that the production of pro-inflammatory mediators and NO were strikingly dependent on TLR2 recognition. Besides, IL-17 production was not dependent on the TLR-2 expression and the absence of Th1 response in TLR-2 knockout animals was concomitant with IL-17 production. The authors concluded that the absence of TLR-2 interferes with the course of the infection induced by the fungus *S. schenckii* [67].

Despite numerous investigations on the role of TLRs in the production of the inflammatory mediators under laboratorial experimental conditions, little is known about the role of these receptors in clinical infection. However, the regulation of inflammatory process is fundamental to the protection against fungal diseases without inducing damage to the host [68,69].

This review showed recent advances in studies that, altogether, demonstrate the involvement of TLR-2 and TLR-4 in the recognition of the sporotrichosis etiological agent and how this recognition process interferes in the production of mediators in response to the infection. Besides that, it seems that a number of other receptors are also involved. The presence of PRRs interactions suggests that many aspects of innate immunity are more sophisticated and complex and, after a pathogen infection, the host can utilize these receptors differentially to mount robust immune responses. It is clear that the knowledge about the mechanisms of host-pathogen interactions are crucial for understanding the immune response against pathogens and it may also provide specific information for the design of new therapeutic options for the treatment of a variety of pathological conditions, including fungal infections.

Further investigations are needed to verify the possible involvement of other receptors of innate immune system in the recognition of the fungus *S. schenckii* and to detail the impact of these receptors on the course of the infection. As subject of future researches, it would be important to evaluate the role of TLR1 and TLR-6 (heterodimers with TLR2) for a better understanding of the innate immune response mechanisms involved in the infection induced by this microorganism.

6. CONCLUSION

The results of this review suggest a new insight in relation to how the immune system, through TLR-2 and TLR-4, recognizes and induces the production of mediators in response to the fungus *S. schenckii*. Further investigations are needed to detail the impact of other TLRs in the infection itself. A better understanding of recognition of *Sporothrix* by TLRs may provide new therapeutic strategies to combat sporotrichosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Donadel KW, Reinoso YD, Oliveira JC, Azulay RD. Sporotrichosis: Review. An Bras Dermatol. 1993;68(1):45-52. Portuguese.
2. Gori S, Luppeti A, Moscato G, Parenti M, Lofaro A. Disseminated sporotrichosis in patients with AIDS: Case report and review of the literature. Acta Cytol. 1997;41(2): 519-21.
3. Méndez-Tovar LJ, Anides-Fonseca AE, Peña-González G, Manzano-Gayosso P, López-Martínez R, Hernández-Hernández F, et al. Unknown fixed cutaneous

sporotrichosis. Rev Iberoam Micol. 2004; 21(3):150-52.

4. López-Romero E, Reyes-Montes Mdel R, Pérez-Torres A, Ruiz-Baca E, Villagómez-Castro JC, Mora-Montes HM, et al. *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. Future Microbiol. 2011;6(1):85-102.
5. Oliveira MM, Almeida-Paes R, Gutierrez-Galhardo MC, Zancope-Oliveira RM. Molecular identification of the *Sporothrix schenckii* complex. Rev Iberoam Micol. 2014;31(1):2-6.
6. Téllez MD, Batista-Duharte A, Portuondo D, Quinello C, Bonne-Hernández R, Carlos IZ. *Sporothrix schenckii* complex biology: Environment and fungal pathogenicity. Microbiology. 2014;160(11):2352-65.
7. Mahajan VK. Sporotrichosis: An overview and therapeutic options. Dermatol Res Pract. 2014;272376.
8. Barros MB, de Almeida Paes R, Schubach AO. *Sporothrix schenckii* and Sporotrichosis. Clin Microbiol Rev. 2011; 24(4):633-54.
9. Souza LL, Nascente PS, Nobre MO, Meinerz ARM, Meireles MCA. Isolation of *Sporothrix Schenkii* from the nails of healthy cats. Braz J Microbiol. 2006; 37:372-4.
10. Gremião ID, Menezes RC, Schubach TM, Figueiredo AB, Cavalcanti MC, Pereira SA. Feline sporotrichosis: Epidemiological and clinical aspects. Med Mycol. 2015;53(1): 15-21.
11. Schechtman RC. Sporotrichosis: Part I. Skinmed. 2010;8(4):216-220.
12. Hardman S, Stephenson I, Jenkins DR, Wiselka MJ, Johnson EM. Disseminated *Sporothix schenckii* in a patient with AIDS. J Infect. 2005;51(3):73-77.
13. Freitas DF, Valle AC, da Silva MB, Campos DP, Lyra MR, de Souza RV, et al. Sporotrichosis: An emerging neglected opportunistic infection in HIV-infected patients in Rio de Janeiro, Brazil. PLoS Negl Trop Dis. 2014;28(8):e3110.
14. Moreira JA, Freitas DF, Lamas CC. The impact of sporotrichosis in HIV-infected patients: A systematic review. Infection. 2015;43(3):267-276.
15. Sterling JB, Heymann WR. Potassium iodide in dermatology: A 19th century drug for the 21st century-uses, pharmacology, adverse effects, and contraindications. J Am Acad Dermatol. 2000;43(4):691-697.
16. Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited. Microbiol Mol Biol Rev. 1997;61(2):136-169.
17. Kurokawa CS, Sugizaki MF, Peraçoli MT. Virulence factors in fungi of systemic mycoses. Rev Inst Med Trop Sao Paulo. 1998;40(3):125-135.
18. Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev. 2003;67(3):400-428.
19. Hostetter MK. Adhesin and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. Clin Microbiol. 1994;7(1):29-42.
20. Pontón J, Omaetxebarría MJ, Elguezabal N, Alvarez M, Moragues MD. Immunoreactivity of the fungal cell wall. Med Mycol. 2001;39:101-110.
21. Lima OC, Bouchara JP, Renier G, Marot-Leblond A, Chabasse D, Lopes-Bezerra LM. Immunofluorescence and flow cytometry analysis of fibronectin and laminin binding to *Sporothrix schenckii* yeast cells and conidia. Microb Pathog. 2004;37(3):131-140.
22. Rodrigues AM, Kubitschek-Barreira PH, Fernandes GF, de Almeida SR, Lopes-Bezerra LM, de Camargo ZP. Immunoproteomic analysis reveals a convergent humoral response signature in the *Sporothrix schenckii* complex. J Proteomics. 2015;115:8-22.
23. Teixeira MM, de Almeida LG, Kubitschek-Barreira P, Alves FL, Kioshima ES, Abadio AK, et al. Comparative genomics of the major fungal agents of human and animal Sporotrichosis: *Sporothrix schenckii* and *Sporothrix brasiliensis.* BMC Genomics. 2014;15:943.
24. Klein BS, Tebbets B. Dimorphism and virulence in fungi. Curr Opin Microbiol. 2007;10(4):314-319.
25. Medoff G, Kobayashi GS, Painter A, Travis S. Morphogenesis and pathogenicity of *Histoplasma capsulatum*. Infect Immun. 1987;55(6):1355-1358.
26. Drutz DJ, Frey CL. Intracellular and extracellular defenses of human phagocytes against *Blastomyces dermatitidis* conidia and yeasts. J Lab Clin Med. 1985;105(6):737-750.

27. Polonelli L, Morace G. Exoantigen studies of *Sporothrix schenckii*, *Ceratocystis minor*, and *Graphium penicilliodes* cultures. J Clin Microbiol. 1982;15(3):362-365.
28. Carlos IZ, Sassá MF, Sgarbi DBG, Placeres MC, Maia DC. Current Research on the immune response to experimental sporotrichosis. Mycopathologia. 2009; 168(1):1-10.
29. Carlos IZ, Sgarbi DBG, Placeres MC. Host organism defense by a peptide-polysaccharide extracted from the fungus *Sporothrix schenckii*. Mycopathologia. 1999;144:9-14.
30. Sgarbi DBG, Silva AJR, Carlos IZ, Silva CL, Angluster J, Alviano CS. Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii.* Mycopathologia. 1997;139:9-12.
31. Fernandes KSS, Coelho ALJ, Bezerra LML, Barja-Fidalgo C. Virulence of *Sporothrix schenckii* conidia and yeast cells, and their susceptibility to nitric oxide. Immunology. 2000;101:563-569.
32. Castillo MC, Tapia FJ, Arciniegas E. Ultrastructural localization of specific surface antigens in the dimorphic fungus *Sporothrix schenckii.* J Med Vet Mycol. 1990;28(1):91-94.
33. Oda LM, Kubelka CF, Alviano CS, Travassos LR. Ingestion of yeast forms of *Sporothrix schenckii* by mouse peritoneal macrophages. Infect Immun. 1983;39(2): 497-504.
34. Carlos IZ, Sgarbi DB, Santos GC, Placeres MC. *Sporothrix schenckii* lipid inhibits macrophage phagocytosis: Involvement of nitric oxide and tumour necrosis factor-alpha. Scand J Immunol. 2003;57(3):214-220.
35. Sassá MF, Saturi AE, Souza LF, Ribeiro LC, Sgarbi DB, Carlos IZ. Response of macrophage Toll-like receptor 4 to a *Sporothrix schenckii* lipid extract during experimental sporotrichosis. Immunology. 2009;128(2):301-310.
36. Negrini TC, Ferreira LS, Alegranci P, Arthur RA, Sundfeld PP, Maia DC, et al. Role of TLR-2 and fungal surface antigens on innate immune response against *Sporothrix schenckii.* Immunol Invest. 2013;42(1):36-48.
37. Romero-Martinez R, Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H. Biosynthesis and functions of melanin in *Sporothrix schenckii.* Infect Immun. 2000;68(6):3696-3703.
38. Zhang Z, Liu X, Lv X, Lin J. Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. Mycopathologia. 2011;172(6):439-446.
39. Taborda CP, da Silva MB, Nosanchuk JD, Travassos LR. Melanin as a virulence factor of *Paracoccidioides brasiliensis* and other dimorphic pathogenic fungi: A mini review. Mycopathologia. 2008;165(4-5): 331-339.
40. Shimonaka H, Noguchi T, Kawai K, Hasegawa I, Nozawa Y, Ito Y. Immunochemical studies on the human pathogen *Sporothrix schenckii*: Effects of chemical and enzymatic modification of the antigenic compounds upon immediate and delayed reactions. Infect Immun. 1975; 11(6):1187-1194.
41. Fernandes GF, Amaral CC, Sasaki A, Godoy PM, Camargo ZP. Heterogeneity of proteins expressed by Brazilian *Sporothrix schenckii* isolates. Medical Mycology. 2009;47:855-861.
42. Appenzeller S, Amaral TN, Amstalden EM, Bertolo MB, Neto JF, Samara AM, et al. *Sporothrix schenckii* infection presented as monoarthritis: Report of two cases and review of the literature. Clin Rheumatol. 2006;25(6):926-928.
43. Maia DC, Sassá MF, Placeres MC, Carlos IZ. Influence of Th1/Th2 cytokines and nitric oxide in murine systemic infection induced by *Sporothrix schenckii.* Mycopathologia. 2006;161(1):11-19.
44. Brito MM, Conceição-Silva F, Morgado FN, Raibolts PS, Schubach A, Schubach TP, et al. Comparison of virulence of different *Sporothrix schenckii* clinical isolates using experimental murine model. Med Mycol. 2007;45(8):721-729.
45. Fernandes GF, dos Santos PO, Rodrigues AM, Sasaki AA, Burger E, de Camargo ZP. Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii* sensu stricto isolates compared with *S. globosa* and *S. brasiliensis* species. Virulence. 2013; 4(3):241-249.

46. Pasare C, Medzhitov R. Toll-like receptors: Linking innate and adaptive immunity. Adv Exp Med Biol. 2005;560:11-18.
47. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009;22(2): 240-273.
48. Stokes BA, Yadav S, Shokal U, Smith LC, Eleftherianos I. Bacterial and fungal pattern recognition receptors in homologous innate signaling pathways of insects and mammals. Front Microbiol. 2015;6:19.
49. Sorrell TC, Chen SC. Fungal-derived immune modulating molecules. Adv Exp Med Biol. 2009;666:108-120.
50. Plato A, Hardison SE, Brown GD. Pattern recognition receptors in antifungal immunity. Semin Immunopathol. 2015; 37(2):97-106.
51. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. Nat Immunol. 2010;11(5):373-384.
52. Netea MG, van de Veerdonk F, Verschueren I, van der Meer JW, Kullberg BJ. Role of TLR1 and TLR6 in the host defense against disseminated candidiasis. FEMS Immunol Med Microbiol. 2008; 52(1):118-123.
53. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature. 1999;401(6755):811-815.
54. Netea MG, van der Graaf CA, Vonk AG, Verschueren I, van der Meer JW, Kullberg BJ. The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. J Infect Dis. 2002;185:1483-1489.
55. Meier A, Kirschning CJ, Nikolaus T, Wagner H, Heesemann J, Ebel F. Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. Cell Microbiol. 2003;5(8):561-570.
56. Braedel S, Radsak M, Einsele H, Latgé JP, Michan A, Loeffler J, et al. *Aspergillus fumigatus* antigens activate innate immune cells via toll-like receptors 2 and 4. Br J Haematol. 2004;125(3):392-399.
57. Netea MG, Warris A, van der Meer JW, Fenton MJ, Verver-Janssen TJ, Jacobs LE, et al. *Aspergillus fumigatus* evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. J Infect Dis. 2003;188:320-326.
58. Netea MG, Sutmuller R, Hermann C, van der Graaf CA, van der Meer JW, van Kriken JH, et al. Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. J Immunol. 2004;172(6): 3712-3718.
59. Villamón E, Gozalbo D, Roig P, O'Connor JE, Fradelizi D, Gil ML. Toll-like receptor-2 is essential in murine defenses against *Candida albicans* infections. Microbes Infect. 2004;6:1-7.
60. Biondo C, Midiri A, Messina L, Tomasello F, Garufi G, Catania MR, et al. MyD88 and TLR2, but not TLR4, are required for host defense against *Cryptococcus neoformans.* Eur J Immunol. 2015;35:870-878.
61. van der Graaf CA, Netea MG, Verschueren I, van der Meer JW, Kullberg BJ. Differential cytokine production and Toll-like receptor signaling pathways by *Candida albicans* blastoconidia and hyphae. Infect Immun. 2005;73:7458-7746.
62. Netea MG, van der Meer JW, Kullberg BJ. Role of the dual interaction of fungal pathogens with pattern recognition receptors in the activation and modulation of host defence. Clin Microbiol Infect. 2006;12:404-409.
63. Romani L. Immunity to fungal infections. Nat Rev Immunol. 2011;11(4):275-288.
64. Bourgeois C, Kuchler K. Fungal pathogens-a sweet and sour treat for toll-like receptors. Front Cell Infect Microbiol. 2012;2:142.
65. Li M, Chen Q, Sun J, Shen Y, Liu W. Inflammatory response of human keratinocytes triggered by *Sporothrix schenckii* via Toll-like receptor 2 and 4. J Dermatol Sci. 2012;66(1):80-82.
66. Redlich S, Ribes S, Schütze S, Eiffert H, Nau R. Toll-like receptor stimulation increases phagocytosis of *Cryptococcus neoformans* by microglial cells. J Neuroinflammation. 2013;10:71.
67. Negrini TC, Ferreira LS, Arthur RA, Alegranci P, Placeres MC, Spolidorio LC, et al. Influence of TLR-2 in the immune response in the infection induced by

fungus *Sporothrix schenckii.* Immunol Invest. 2014;43(4):370-390.

68. Becker KL, Ifrim DC, Quintin J, Netea MG, van de Veerdonk FL. Antifungal innate immunity: Recognition and inflammatory networks. Semin Immunopathol. 2015; 37(2):107-116.

69. Romani L, Puccetti P. Controlling pathogenic inflammation to fungi. Exp Rev Anti Infect Ther. 2007;5:1007-1017.

70. Sassá MF, Ferreira LS, Ribeiro LC, Carlos IZ. Immune response against *Sporothrix schenckii* in TLR-4-deficient mice. Mycopathologia. 2012;174(1):21-30.

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