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Current Research on the Immune Response to Experimental Sporotrichosis

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Abstract Sporotrichosis is often manifested as a chronic granulomatous infection and the monocytes/macrophages play a central role in the host defense system. Surface components of *Sporothrix schenckii* have been characterized and suggestions have been made as to their possible role in pathogenicity. Ergosterol peroxide, cell-wall compounds (alkali-insoluble fraction-F1 and lipid extract-LEY), and exoantigen from the yeast form of the fungus have been characterized as virulence factors, activating both innate, by cytotoxins linked to the activation of reactive oxygen and nitrogen species (H_2O_2 and NO), and adaptive immune response to produce cytokines Th1 and Th2 profile. In this study, preliminary results have demonstrated that, in systemic sporotrichosis, TLR-4 triggers the innate immune response, activating an oxidative burst. These data represent the first report of the participation of TLR-4 in murine sporotrichosis, in the presence of lipids from the cell wall of *S. schenckii*. These results taken together may open new perspectives of study leading to an antifungal agent that could be used to benefit the entire population.

Keywords *Sporothrix schenckii* · Systemic infection · Cytokines · Nitric oxide · Hydrogen peroxide · TLR-4 · Th1/Th2 response

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Introduction

Sporotrichosis was first reported in 1896 when Schenck isolated the causative agent and included it in the genus Sporothricum. Later, in 1900, Henkton and Perkins renamed this fungus *Sporothrix schenckii* [1, 2]. *S. schenckii* is a thermally dimorphic fungus with a mycelial and a yeast phase. In the environment or in the laboratory, at temperatures ranging from 25 to 30°C, *S. schenckii* grows as a filamentous mold. The organism grows readily on standard media, such as Sabouraud dextrose agar. Within a period ranging from days to weeks, white to cream-colored colonies are seen, which become brown to black and wrinkled over subsequent weeks, producing conidia which are either dark or hyaline, arranged along the hyphae with a bouquet-like appearance [3]. In the wild, *S. schenckii* is a saprophyte. It has been isolated from straw, wheat grains, fruits, tree bark, wood, shrub thorns, rose brushes, ploughed land, spiders, flies, dead insects and larvae, dust, animal droppings, algae, and sea animals [4].

In vivo, at 37°C, *S. schenckii* grows as a yeast-like cell. In this form, the organism reproduces by budding and does not form conidia. Typically, the yeast forms are 4–6 μm in diameter and often cigar-shaped. In vitro, at 37°C, the species assumes the yeast phase on supplemented media, such as brain–heart infusion or blood-cysteine-glucose agar [3].

People of any age, race, or sex may be affected, but the disease is more common in adult males, primarily because of their occupational and recreational exposure. Sporotrichosis has been reported in many workers, including farmers, vegetable growers, gardeners, florists, forestry workers, paper manufacturers, miners, slaughterhouse workers, veterinarians, laboratory workers, and workers handling packing materials. Children are just as susceptible as adults [5, 6].

The infection usually occurs as a consequence of traumatic implantation of the fungus into the skin, often associated with lymphangitis, but inhalation of the conidia can lead to pulmonary infection which, rarely, may also spread to bones, eyes, central nervous system, and viscera [2, 7]. Another mode of transmission is through animal bites or scratches. The animals most commonly responsible are armadillos and cats, but horses, dogs, snakes, rats, and birds have also been involved [8].

The size of the initial inoculum, the host immune response status, the virulence of the fungus, the depth of traumatic inoculation, and thermal tolerance are all thought to have a role in the development of sporotrichosis [9].

Clinical Manifestation

The sporotrichosis has several forms of clinical manifestation: (1) Lymphocutaneous, (2) fixed cutaneous, (3) multifocal (disseminated) cutaneous, and (4) extracutaneous [10, 11]. Lymphocutaneous is the most common type of sporotrichosis worldwide, beginning as a painless hardened pink papule, pustule, or nodule at the inoculation site, which rapidly enlarges, becomes violet, and ulcerates to expose a ragged necrotic base. It starts as a nodular or ulcerated lesion at the site of inoculation of the fungus and follows through regional lymphatic vessels characterized by nodular lesions that ulcerate. In the fixed cutaneous form, the fungus remains confined to the site of inoculation, which generally reflects the high immunity of the host and is more common in endemic areas. The disseminated cutaneous and extracutaneous forms have been observed mainly in immunocompromised patients with hematologic diseases, diabetes mellitus, and using immunosuppressive drugs, having infection with human immunodeficiency virus (HIV), or in individuals with a history of abuse of alcohol. Among the extracutaneous forms, the most common are the osteoarticular and lung, but there are reports of hematogenous spread with involvement of multiple organs, such as eyes and central nervous system [6, 12–15].

The sporotrichosis can occur in the visceral lesions, as well as in the skin, and impaired immunity of the hosts and/or the different virulence of individual strains could cause these different clinical manifestations. In fact, Uenotsuchi et al. [16] have suggested that *S. schenckii* of cutaneous origin are more potent to activate dendritic cells to induce subsequently stronger Th1-prone immune response than those of visceral origin. *S. schenckii* of visceral origin positively induced Th2 environment as evidenced by significantly higher IL-4 production along with the inability to induce strong Th1 immune responses. Thus, this may further explain their differential clinical manifestations in the context of the factors of the pathogen side, showing that *S. schenckii* of visceral origin may escape from the host immune defense by inducing little Th1-prone responses. On the other hand, other researchers have demonstrated that even in the same *S. schenckii* strain, and it has been reported that the conidia cultured for 7 days are more virulent than those cultured for 12 days. A different virulence is thought to be derived from a different rhamnose: mannose ratio of their surface, which has been changed during the culture [17]. Such a slight difference in the fungal surface sugar composition might contribute the differential activation of dendritic cells.

Immune Response

The immune response in the host determines the degree of invasion by the fungi. The mechanism(s) of resistance and susceptibility to *S. schenckii* infection are not known. It is not known whether specific proteins or enzymes produced by *S. schenckii* contribute to its pathogenic potential. Tsuboi and

co-workers [18, 19] have reported the production of extracellular proteinases by *S. schenckii*, but these enzymes have not yet been linked to the pathogenesis of this disease [20]. Surface components of *S. schenckii* have been characterized and suggestions made as to their possible role in pathogenicity [21, 22].

Yeast cells from virulent dimorphic fungi, in contrast to opportunistic fungi, survive in vitro phagocytosis by neutrophil granulocytes. Although virulent yeast forms ingested by polymorphonuclear cells (PMNs) trigger a respiratory burst comparable to that induced by opportunists, they are less susceptible to hydrogen peroxide and other microbicidal products of leukocytes. In an earlier study, we identified ergosterol peroxide from yeast forms of *S. schenckii* through spectroscopic methods (^{1}H and ^{13}C nuclear magnetic resonance and high resolution mass spectrometry) [23]. This substance, a presumed product of the H_2O_2-dependent enzymatic oxidation of ergosterol, has been isolated and purified, showing a molecular formula of $C_{28}H_{44}O_3$, and displays characteristic features of epidioxy sterols. It reverted to ergosterol on contact with *S. schenckii* enzyme extract. Thus it is conceivable that in *S. schenckii* ergosterol peroxide is formed as a protective mechanism to evade reactive oxygen species (ROS) during phagocytosis. The presence of ergosterol peroxide in a pathogenic fungus, reported for the first time in our study, in addition to suggesting a possible detoxification reaction, may also represent a virulence factor. Since singlet oxygen ($^{1}O_2$) may not be involved in the peroxidation of ergosterol [24] or in the oxygenation of cholesterol [25], it seems that survival of the virulent yeast forms in contact with PMNs depends on additional detoxification mechanisms besides the one leading to ergosterol peroxide synthesis.

The killing of intracellular and extracellular pathogens by phagocytes is due in part to the production of oxygenated free radicals. Under normal physiological conditions, H_2O_2 is generated in small quantities and is rapidly used or degraded, but long exposure to high concentrations of H_2O_2 can destroy biological structures and lead to irreversible cell damage [26]. ROS are utilized in the body as oxidative, cytotoxic agents, and are produced by phagocytic cells during the respiratory burst induced by infection [27]. The majority of ROS, including hydrogen peroxide (H_2O_2) and superoxide ions (O_2^-), are produced via two pathways, involving phagocyte NADPH oxidase or hypoxanthine metabolism. Both H_2O_2 and O_2^- can function independently, as cytotoxic agents, or form other toxic molecules, including the hydroxyl radical $^{\bullet}OH$, hypochlorous acid (HOCl), and peroxynitrite ($ONOO^-$) in the presence of NO [28].

We still know very little about the molecular mechanisms of innate immune recognition and the receptors involved. Innate immunity host cells recognize fungal organisms mainly through components of the fungal cell wall, acting as pathogen-associated molecular patterns (PAMPs). The PAMPs are bound and recognized by pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), on the surface of host cells. This recognition leads to activation of phagocytic effector functions and further induction of adaptive T helper (Th) responses by antigen-presenting cells [29, 30].

Over the last few years there have been significant advances in our understanding of the biology of TLRs in mammals. This discovery of mammalian homologues of the Drosophila Toll receptor protein has attracted interest in the role of these proteins in innate immunity, with various ligand specificities [31]. Several features of TLR-4 have linked this protein to innate immune responses. The overexpression of a constitutively active TLR-4 protein drives NF-kB activation, B7.1 expression, and production of cytokines (IL-1, IL-6, and IL-8) in transfected THP-1 monocytes [32]. In other studies, in which the location of TLR-2 was monitored after macrophage stimulation with zymosan, a presumed TLR-2 ligand, TLR-2 was shown to be localized first in phagocytic cups and subsequently in phagosomes, upon phagocytosis of the zymosan. This striking observation suggests that macrophages use TLRs to regulate the contents of phagosomes [33]. However, the functional consequences of the complex interplay between fungal morphogenesis and TLR signaling in vivo remain largely undefined.

A genetic defect in TLR-4 is associated with impaired generation of the superoxide anion by macrophages in response to LPS [34], suggesting that the triggering of certain TLR by PAMP results not only in the induction of gene expression, but also in the promotion of rapid processes such as the induction of an oxidative burst. The importance of reactive oxygen metabolites in macrophage

microbicidal activity is suggested by evidence that peritoneal macrophages produce reactive oxygen metabolites upon activation or exposure to pathogens [35] and that macrophage activity is inhibited by free radical scavengers, such as superoxide dismutase, an enzymatic O_2^- scavenger, and catalase, an enzymatic scavenger of H_2O_2 [36].

Research is in progress in our laboratory to check the influence of TLRs in the infection induced by *S. schenckii*. Preliminary studies have demonstrated that TLR-4 is important in this mycosis. Macrophages from the infected C3H/HeJ mice, which have a natural point mutation in the TLR-4 gene (Fig. 1a), were deficient in H_2O_2 production, generating an average of 1.48 ± 0.41 nmols/2×10^5 peritoneal exudate cells (PECs), while the C3H/HePas mice, which have competent TLR-4 (Fig. 1b), produced on average 13.62 nmols/2×10^5 PECs, over the whole 10 weeks of study, when challenged with lipid extract from the yeast form of the fungus (LEY), showing the presence of a moderate oxidative burst in the C3H/HePas infected with the fungus *S. schenckii*. This is the first report of the TLR-4 participation in murine sporotrichosis in the presence of the lipids from the cell wall of this fungus.

These results confirm the previous observation that lipid extract was found to inhibit the phagocytic process in murine experimental sporotrichosis [37]. The effect of cell-wall components and exoantigen (ExoAg) obtained from *S. schenckii* during macrophage/fungus interaction was analyzed with respect to production of hydrogen peroxide, nitric oxide, and tumor necrosis factor-α. High release of NO and TNF-α and moderate production of H_2O_2 were observed in the macrophage cultures. This was expected, since in the induction of iNOS gene expression, LPS-derived activation of a transcription factor, nuclear factor-kB (NF-kB), occurs, and this is translocated from the cytosol to the nucleus where it binds to region 1 in the promoter of the iNOS gene; also, many LPS-responsive genes, including that of TNF-α, are known to share recognition sequences that can be recognized by NF-kB for the activation of gene expression. In this light, the high levels of NO and TNF-α in response to LEY in macrophage cultures demonstrate an important role for this cytokine, among other factors, in the modulation of NO release in response to this fungal infection. Recent experiments in our laboratory have shown that

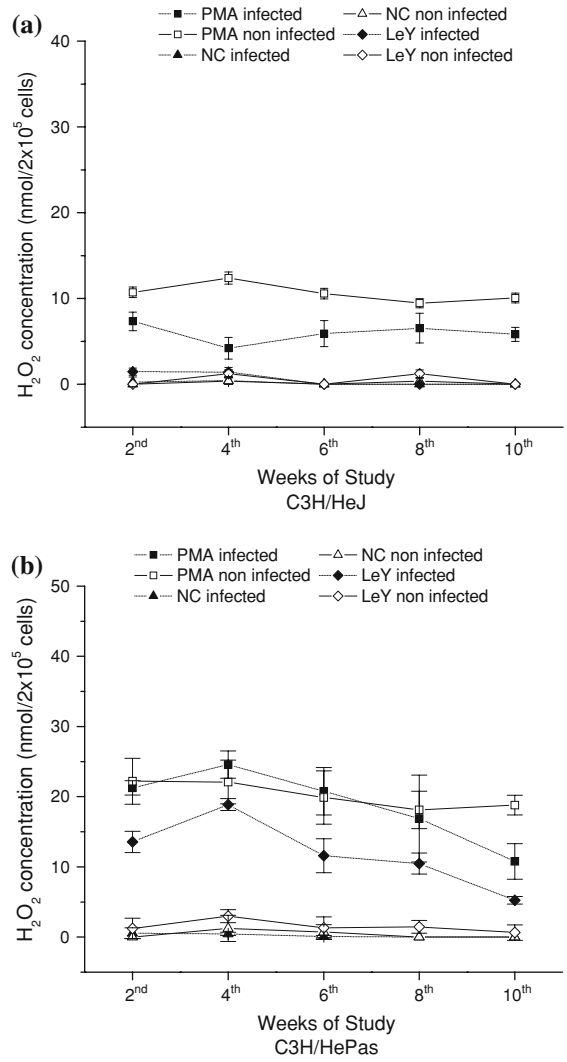

Fig. 1 Hydrogen peroxide production by PECs (peritoneal exudate cells) from TLR4-defective (C3H/HeJ-a) and competent (C3H/HePas-b) mice during *S. schenckii* infection. PECs were challenged with PMA (phorbol myristate acetate—positive control), LEY (lipid extract from yeast) or NC (negative control—PBS). After 1-h of incubation at 37°C in a mixture of 95% air and 5% CO_2, the reaction was interrupted with 50 µl of 4N NaOH, and the absorption at 620 nm was measured with an automated microplate reader. Results are shown as mean ± standard deviation of three experiments in triplicate (GrafPad Instat program—Tukey Test). The level of significance was set at $P < 0.001$ when H_2O_2 production by infected C3H/HePas cells treated with LEY was compared with infected C3H/HeJ (TLR4-defective) cells challenged with the same stimulus at the same period of infection

the F1 fraction of *S. schenckii* induces a granulomatous reaction, whereas ExoAg participates in the humoral immune response (data not published).

Granuloma formation is a critical event in the immune response against *S. schenckii*, an essential component of normal host defense. Granuloma is thought to be a result of a T-cell-dependent inflammatory response that is important in the host defense against *S. schenckii* [3, 38].

The induction and expression of cellular immunity in mycotic disease depends on a complex sequence of interactions between antigens, macrophages, and lymphocytes. The cellular response to *S. schenckii* infection involves both neutrophils and monocytes [39]. The fact that sporotrichosis is more severe and usually disseminated in nude mice and in patients with AIDS lends support to the idea that T cell-mediated immunity is important in limiting the extension of infection with *S. schenckii* [40].

In a previous study, we developed a murine model of disseminated sporotrichosis [41]. We demonstrated that delayed hypersensitivity to injected soluble antigen (foot pad test) and lymphocyte proliferation in the in vitro spleen cell tests for detection of cellular immune response (to antigen or mitogen) showed depressed responses in *S. schenckii*-infected mice challenged between the 4th and 6th week after fungal inoculation.

In response to antigens or inflammatory signals generated at sites of tissue injury, macrophages undergo a process of cellular "activation", which is associated with morphological, functional, and biochemical changes in the cells. One prominent characteristic of activated macrophages is their increased capacity to release pro-inflammatory and cytotoxic mediators, which aid in antigen destruction. Cytokines are low-molecular-weight regulatory proteins or glycoproteins secreted in response to a number of stimuli by cells in the body. Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine that has a key role in the cytokine network with regard to the pathogenesis of many infectious and inflammatory diseases [42], playing a crucial part in acute and chronic inflammation. It is able to induce the expression of other pro-inflammatory cytokines, such as interleukin-1 (IL-1) [43] and several chemokines [44]. Carlos et al. [43] have demonstrated that the production of IL-1 and TNF-α by adherent PECs taken from *S. schenckii*-infected mice was severely reduced between the 4th and 6th weeks and increased after the 8th and 10th weeks of infection. Together, these results show that the cellular immune response of the host plays a significant role in the pathogeny of this infectious disease, and a depressed response frequently indicates a worsening of the disease, with greater involvement of the host.

In 1999, in an investigation of *S. schenckii* [45], our group isolated a peptide-rhamnomannan from the pathogenic yeast form of the fungus. This substance, which may play a role in fungal virulence, was tested in an animal model of systemic disease, and depression of the immune response was observed in the animals between the 4th and 6th weeks of infection. Concomitantly, this compound showed mitogenic activity when used to challenge normal lymphocytes and was also found to be involved in the inflammatory response. This report opened new perspectives on the mechanism of the implantation of the fungus in tissues, which occurs in this systemic mycosis by modulation of the cellular immune response by this antigen.

When the macrophages are activated, they show accelerated metabolic rate, motility, and phagocyte activity. These cells are capable of carrying out parallel functions: besides phagocytosis, they may secrete enzymes, components of the complement, coagulation factors, and cytokines [46]; they also act as a first line of defense of the host by releasing a great number of factors, including the ROS and reactive nitrogen species (RNS), such as nitric oxide (NO), known as a powerful mediator of the inflammatory and immune responses [47–50].

It has been reported that nitric oxide (NO) produced by activate macrophages is a primary mechanism against many pathogens [51] such as *Penicillium marneffei* [52], *Histoplasma capsulatum* [53], *Cryptococcus neoformans* [54], and *Candida albicans* [55]. In general, accumulation of nitrite correlated with macrophage fungicidal activity. Fernandes et al. [56] have demonstrated that the capability of macrophages to kill *S. schenckii* could be attributed to NO production, because when NOS was inhibited by the addition of L-NMMA, there was a complete inhibition of the killing activity against *S. schenckii* conidia and yeast cells form, showing that NO is a key cytotoxic mediator involved in the murine macrophage defense against *S. schenckii*.

In their studies, Fernandes et al. [57] have demonstrated that the inhibition of NO production in vivo enhances the resistance of mice to the infection with *S. schenckii* yeast cells, and is related mainly with increased T-cell proliferation after Con

A challenge and cytokine balance between IFN-γ and interleukin-10 (IL-10), together to decreased percentage of apoptotic cells in iNOS−/− mice—results that bring significant implications in our understanding of the role of NO in immunosuppression induced by *S. schenckii.*

Besides having a microbicidal role, NO commonly acts as a signaling molecule. NO-mediated reactions may prime the macrophage to deliver a more potent oxidative burst. ROI and RNI may act cooperatively (generating peroxynitrite) or independently, stimulating other mechanisms. It is possible that NO enhances the function of the NADPH-oxidase complex, either by direct chemical modification of component proteins or by acting as a secondary messenger to stimulate other mechanisms that potentiate the activity of ROI [58]. This versatile molecule is generated by oxidation of the terminal guanidine nitrogen atoms of L-arginine by an NADPH-dependent enzyme, NO synthase (NOS) which has constitutive and inducible isozymes. Generation of this mediator by the inducible NOS (iNOS) has now been demonstrated in human macrophages following their activation in vitro [59].

The expression of iNOS is regulated mainly by cytokines. The activation of the iNOS gene promoter is the main way in which that isoenzyme is regulated by cytokines in murine macrophages [60]. Depending on the type of cytokine, microbial stimulus and cell type, various signaling paths are involved in promoting or inhibiting iNOS expression [61].

Nitric oxide can inhibit the secretion of such inflammatory cytokines as interferon-gamma (IFN-γ) and IL-2 by T cells [62]. High NO levels synthesized by NOS not only destroy adjacent tissue but can also infiltrate macrophages or T cells at the site of inflammatory injury, causing apoptotic death of these inflammatory cells. This mechanism can be considered beneficial for the host because it eliminates unnecessary cells, preventing the release of potentially cytotoxic intracellular contents to the extracellular domain [62, 63]. Regulation of the transcription of iNOS is carried out by the cytokines derived from T cells. The Th1 cytokines, such as IFN-γ and IL-2, upregulate NO synthase, whereas Th2 cytokines, such as IL-4 and IL-10, are responsible for negative regulation [64].

The dichotomy of cellular and humoral immunity to pathogens has been shown to be based on differentiation of subtypes of T cells, responsible for the Th1/Th2 balance [65]. The Th clones differ in the two classes of cytokine they release. Th1 cells produce IFN-γ, IL-2, and TNF-α, and are efficient in the elimination of intracellular pathogens by activation of macrophages. Th2 cells release IL-4, IL5, IL-6, and IL-10, which activate humoral immunity and are released in markedly greater amounts in the presence of persistent antigen [66].

The production of cytokines is normally transitory and strictly controlled. They act by locking on to specific receptors in the cell membrane, initiating a cascade that leads to the induction or inhibition of countless cytokine-regulated genes in the cell nucleus [67].

The Th1 cells seem to be more susceptible to regulation by NO than by Th2 cells [68]. At the inflammatory focus, the macrophages are activated and express iNOS, producing high NO levels, which seem to have an effector function [69]. The induction of iNOS is an important effector response in defense of the host against pathogens [70, 71].

IL-12 is the main mediator of the innate immune response to intracellular microorganisms and a key inducer of the immunity mediated by cells, the adaptive response against these microorganisms. This cytokine is mainly produced by activated macrophages and dendritic cells [72]. IL-12 acts in different periods of the immune response and is involved in maintaining a long-term response [73]. IL-4 and IL-10 downregulate IL-12 being known as powerful cytokine inducers of the Th2 response [74].

The defense of the host by macrophages is mediated partially by a signal known as macrophage activating factor (MAF). IFN-γ is a powerful MAF that stimulates the expression of some membrane proteins, increasing or reducing the synthesis and secretion of multiple enzymes and thus enhancing the generating of ROS [75].

Macrophages activated by IFN-γ are capable of generating TNF-α, IL-6, IL-12, and NO, which enhance the ability of these cells to control the development of pathogens [8]. NO is in turn produced in response to such cytokines as TNF-α, IFN-γ, and IL-12 [35]. IFN-γ also has an important regulatory role in the development of the Th1 adaptive response against fungal pathogens. This role is mediated by its ability to maintain IL-12 responsiveness in CD4+ T cells [76].

In pathological states, the local production of IFN-γ by macrophages can help perpetuate chronic disease. However, the production of IFN-γ by antigen-presenting cells (APCs) should act not only to enhance innate immunity, but also to establish a link between innate immunity and the developing adaptive immune response [50].

Two independent pathways appear to control fungal infection: one is the IL-12/IFN-γ axis and the other is mediated by TNF-α. IL-12 increases NK and T-cell proliferation and cytotoxic activity and also promotes the development of a Th1-type immune response by inducing secretion of IFN-γ in T cells [77]. IL-12 and IFN-γ drive Th1 development [78], though many researchers have shown that other cofactors, including IL-1α and TNF-α, are required for the development of Th1 cells to produce maximal levels of IFN-γ [79, 80].

Several cytokines have been found to downregulate the production of IFN-γ by APCs, including IL-4 and IL-10. Thus, as in T cells, the production of IFN-γ is positively regulated by IL-12 and IL-18, and negatively regulated by IL-4 and IL-10 [81, 82].

IL-4 is a cytokine produced by Th2 cells. It has an immunomodulating function, inducing complex MHC I and II to express antigen, suppressing IFN-γ, and inducing the expression of iNOS in response to stimulation by LPS [83]. Activated macrophages can release IL-12 with other pro-inflammatory cytokines together [84].

High concentrations of IL-4 at the beginning of a fungal infection can delay the immune response of the host through suppression of the Th1 response by inhibition of IFN-γ production. Conversely, at advanced stages of the disease, the presence of IL-4 limits the development of fungal infections [74, 85].

The greatest advances in experimental fungal immunology concern the important regulatory role of the cytokines in the innate and adaptive immune responses during fungal infections. Although some cytokines show clearly the characteristics of a Th1 or Th2 profile, many of them have pleiotropic effects that depend on their dose and secretion time for the development of immune response [85, 86]. This points to the importance of the delicate balance between the Th1 and Th2 cytokines for the development of an effective immune response.

We investigated the involvement of NO and the Th1/Th2 cytokine response in systemic infection, utilizing *S. schenckii* exoantigen [87]. We found low levels of iNOS and NO production in the initial (1st and 2nd weeks) and final (9th and 10th weeks) periods of the infection, contrasting with high levels from weeks 4 to 7. The variations in IFN-γ and IL-12 levels mirrored with those of NO/iNOS, showing the presence of a cellular immune response throughout the infectious process. In experimental models of fungal infection, the extent and efficacy of the protection by cell-mediated immunity varies with the host genetic background, the route and site of infection, the regional immune response, and the type of infecting organism. The production of IFN-γ, alone or in combination with other cytokines, resulting in the production of reactive nitrogen intermediates, is likely to suppress T cells in the micro-environment [88]. The results of this research showed that the release of IL-12 coincides with that of IFN-γ, at a time when IL-12 is a powerful inducer of the production of IFN-γ by T and NK cells. As IFN-γ induces NO production and greater production of NO was observed between the 4th and 6th weeks, we suggest that IL-12 and IFN-γ also play a part in the immunosuppression generated by fungal infection. More importantly, IL-12 plays a critical role in the development of Th1 cells from naive T cells. Thus, IFN-γ enhances the antigen-presenting activity of macrophages, resulting in the expansion of the Th1 cell population. However, IL-4 release increased after the 5th and 6th weeks, suggesting the participation of the Th2 response in this period as well. Regarding these results, the study demonstrated that in experimental sporotrichosis infection the cellular immune response participated throughout the period of the proposed NO-dependent mechanism. In contrast, the Th2 response began in the 5th week, suggesting the participation of the humoral immune response only in the advanced stages of sporotrichosis.

Conclusions

Every line of study has a past, present, and future, and our study of experimental sporotrichosis has shown that in the past, the pathway by which the organism of the host reacts to this infection was very unclear. In the early studies, we demonstrated that cellular immunity was the key to the containment of this fungal infection. After years of investigation, we now

know that both Th1 and Th2 responses participate in experimental sporotrichosis. Making use of what we now know to plan our future research, today we believe that the mechanism of the immune response will be understood better by investigating receptors present on the cells that participate effectively in the control of this infection. As a first result, the receptor TLR-4 is involved in the induction of the oxidative burst in the presence of the fungal lipid extract (LEY); further questions, which without any doubt will lead to new ways of approaching the study of sporotrichosis, are left to the future.

The study of sporotrichosis is justified by the fact that it is an endemic disease in several countries. An important point to be stressed is that the environment contributes significantly to the development of infectious diseases and more research should be carried out in this respect, contributing to the general improvement of public health worldwide.

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